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# Myrmecophytic *Cecropia*: antiherbivore defenses under different nutrient treatments

**Abstract** In greenhouse experiments, we compared putative biotic, chemical, physical and phenological defenses of six myrmecophytic *Cecropia* species cultivated under high and low nutrient regimes. We tested the intraspecific predictions of the C:N balance hypothesis for a broader range of defenses than included in other studies to date. Treatment effects on defenses appear to depend on the nutrient constituents of those defenses. Only strictly carbon-based defenses such as tannins and phenolics reached higher concentrations at the lower nutrient level. The production of glycogen-rich and membrane-bound Müllerian body ant rewards (MBs) increased with greater levels of both nutrients (this study) and light (Folgarait and Davidson 1994). In contrast, lipid- and amino acid-rich pearl body food rewards (PBs) were produced in greater numbers under conditions of high nutrient levels (this study) and low light (Folgarait and Davidson 1994), both of which should have contributed to a relative excess of nitrogen. Nutrient effects on toughness and leaf expansion rates (perhaps serving as phenological defenses) were inconsistent with the predictions of the C:N balance hypothesis. Mature leaves are protected principally by chemical and physical defenses, and new leaves, by biotic defenses. As in a previous study, interspecific comparisons agreed with the resource availability theory of plant defense. Plant investment in immobile defenses (tannins and phenolics, and leaf toughness), and in a defense with high initial construction costs (trichilia differentiated to produce MBs) were greater in each of three comparatively slow-growing "gap" *Cecropia* typical of small openings in primary forest, than in closely related and fast-growing "pioneer" species of large-scale disturbances (riparian edge and land slips). In contrast, both production of PBs (with negligible initial construction costs) and leaf expansion rates were greater in pioneers than in gap species. Rapid onset of biotic defenses during new leaf development (earlier in pioneers) may reduce new leaf herbivory in all species.

**Key words** Antiherbivore defenses · *Cecropia* · C:N balance · Interspecific comparisons · Nutrients

## Introduction

Much of the variation in kinds and amounts of plant defenses has been attributed either to the absolute and/or relative availabilities of key plant resources, especially carbon and nitrogen, or to (often correlated) leaf life spans (McKey 1984; Bryant et al. 1985; Coley et al. 1985; Schupp and Feener 1991; Davidson 1993). Specifically, for interspecific comparisons, environments poor in resources are correlated with high levels of defense investment (resource availability hypothesis, Coley et al. 1985), while in intraspecific comparisons, ecological conditions promoting an unbalanced C:N ratio within the plant give rise to greater levels of those defenses composed of the resource present in excess (C:N balance hypothesis, Bryant et al. 1983). Myrmecophytes (antplants) offer especially appropriate and interesting model systems for testing these hypotheses. First, plant investment in "biotic defense" is comparatively easy to quantify by measuring the amount and composition of ant rewards (Rickson 1976; O'Dowd 1980; Lanza 1988). Second, the adaptive significance of these defenses can be understood by studying their deployment in the context of other putatively defensive traits (McKey 1984; Waterman et al. 1984; Coley and Aide 1991). Third, tests of both the C:N balance hypothesis (Bryant et al. 1985) and the resource availability hypothesis (Coley 1983; Coley et al. 1985) can be extended to include kinds of defensive arsenals, such as biotic defenses or mechanical de-

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fenses, not commonly examined (see also Davidson and Fisher 1991; Schupp and Feener 1991; Folgarait and Davidson 1994).

This study continues prior investigations of the defenses of six myrmecophytic *Cecropia* species native to south-eastern Peru (Davidson and Fisher 1991; Folgarait and Davidson 1994). In earlier studies (Folgarait and Davidson 1994), we compared interspecific and intraspecific differences in growth parameters and defensive traits within each of three pairs of closely related *Cecropia* species cultivated under two light regimes. Within each pair, one member (called a "gap" species) typically grows in forest light gaps, and a closely related congener (or "pioneer" species) occupies more open habitats along river banks or landslips. Results of these previous studies can be summarized as follows: in interspecific comparisons of closely related gap and pioneer *Cecropia*, gap species showed significantly slower growth, a tendency for longer leaf life spans, and significantly higher investment in some defensive traits but not others. Compared to pioneers, gap species exhibited earlier onset of myrmecophytism and greater investment in Müllerian bodies (MBs, or glycogen-rich ant rewards). Levels of tannins and total phenolics in mature leaves of gap species were either similar to, lower than, or higher than those of pioneers. These results parallel estimates of increasing time since close relatives (within species pairs) diverged from their common ancestors (D. Davidson, unpublished morphological and molecular sequence data). In intraspecific comparisons, all of these traits were enhanced under the high light treatment, and they appear to be strongly based on a plant's carbon resources. Two other putatively defensive traits, however, failed to respond to light treatment. Of these, toughness of mature leaves tended to be greater (though not significantly so) in gap species, while pioneers exhibited significantly higher production rates of the lipid-rich ant rewards known as pearl bodies (PBs).

A major goal of this study is to examine plant responses to different nutrient treatments by systematically altering the balance of carbon to potentially limiting nutrients. In so doing, we hope to test the C:N balance hypothesis (Bryant et al. 1983) for a broader range of carbon- and nitrogen-based defenses than included in other studies to date, and to determine why some defensive traits behave according to predictions of the C:N balance hypothesis (Bryant et al. 1983), but others do not. We expect that levels of strictly carbon-based defenses, such as tannins and phenols, should increase with an excess of carbon under the low nutrient treatment, where the ratio of available carbon to nitrogen is greater. A similar prediction should hold for leaf toughness, which has been ascribed traditionally to fiber content and lignin of cell walls. Both types of ant rewards, elicitors of biotic protection, have a substantial carbon component in the form of glycogen (especially in MBs) and lipids (especially in PBs) (Rickson 1976). However, both rewards also depend on nitrogen, either as trapped enzymatic protein and protein constituents of binding membranes (MBs,

Rickson 1973, 1976), or as amino acids in the food bodies themselves (PBs, P. Folgarait, unpublished results of a modified cadmium-nynhidrin assay, *fide* W. Gray, personal communication). According to predictions of the C:N balance hypothesis, previous work (Folgarait and Davidson 1994) suggests that MBs behave as though carbon-based, and PBs, as though nitrogen-based.

In two ways, our study also extends prior work on interspecific differences among myrmecophytic *Cecropia*. Biotic, chemical and physical defenses are examined independently for young and mature leaves, and the study includes phenological traits relevant to the protection of new leaves (Aide and Londoño 1988; Kursar and Coley 1991).

## Methods

### Growth and monitoring

Seeds of six *Cecropia* species from western Amazonian Peru (Madre de Dios), were collected from beneath maternal parents in 1987 (at Reserva Tambopata, 12° 50′ S, 69° 17′ W, elevation 290 m), and in 1988 (at the Estación Biológica de Cocha Cashu, 11° 55′ S, 77° 18′ W, elevation 400 m). Collections included *C. polystachya, C. membranacea,* and *C. engleriana,* three pioneer species growing naturally along major rivers or streams, or on landslips (*C. polystachya* only). Also represented were *C. ficifolia, C.* sp. B, and *C.* sp. A, the respective close congeners of these pioneers, but residents of light gaps in primary forest. [Davidson et al. (1991) and Davidson and Fisher (1991), referred to *C.* sp. B as *C. tessmannii,* but this name is presently in doubt.] Species-specific nutrient regimes typical under field conditions overlap strongly between closely related pioneer and gap species (D. Davidson and D. Yu personal communication). The three pairs of closely related species (pair 1: *C. polystachya* – *C. ficifolia;* pair 2: *C. membranacea* – *C.* sp. B; pair 3: *C. engleriana* – *C.* sp. A; see photographs in Folgarait and Davidson 1994) were identified by morphological criteria prior to greenhouse studies, and subsequently confirmed by both a specialist in *Cecropia* systematics (C.C. Berg) and phylogenetic analysis of morphological traits (D. Davidson, unpublished). Our experiments were based either on seeds of single parents (*C. ficifolia, C.* sp. A, and *C. engleriana*), or on mixed progenies (*C. polystachya, C.* sp. B. and *C. membranacea*). Because *Cecropia* are dioecious, progeny of individual parents are likely to be genetically diverse. Moreover, maternal effects probably cannot account for interspecific differences reported in this or previous studies, since there were very few significant differences in the variables measured in comparisons between family groups (P. Folgarait, unpublished). Many of the patterns found in this study have also been observed in field populations (Davidson et al. 1991).

Seeds were germinated in early July of 1990, and housed in climatically-controlled tropical greenhouse bays in Salt Lake City, Utah (~40° N latitude, 1500 m). Progeny of each parent were paired by size, and one member of each pair was assigned haphazardly to either high (HN) or low nutrient (LN) treatments. Actual sample sizes for analyses depended on seedling availability but were further reduced due to the exclusion (except in *C. ficifolia*) of plants whose leaves had been harvested occasionally for chemical analyses, and to time constraints for certain types of analyses. Typical sample sizes per species and treatment (HN/LN) were: *C. ficifolia* 6-7/7-8; *C. polystachya* 10-11/6-8; *C.* sp. B 11-12/12-14; *C. membranacea* 10-12/11-12; *C.* sp. A 8-11/6-9; *C. engleriana* 8-12/8-12. Ranges of sample sizes for each variable measured (≥6 for all but leaf areas, where $n=3$ plants) appear in tables of results.

Plants were fertilized either twice weekly or once every 2 weeks with a standard nutrient solution. Peter's General Purpose 20-20-20 (N-P-K) fertilizer was dissolved in tap water at a concentration of 200 ppm per nutrient. Nitrogen was supplied by

5.61% nitrate, 3.94% ammonia, and 10.93% urea. Each fertilization consisted of watering plants until ~10 cm of fertilizer solution had accumulated above the soil. Plants were watered simultaneously and heavily three times each day. Before the largest plants had outgrown their pots, all seedlings and saplings were transplanted successively and simultaneously to 150-ml, 12-l, 20-l and then 40-l pots.

Ambient light was reduced by shade cloth to 30% full sun, corresponding to the "high light treatment" in a prior experiment (Folgarait and Davidson 1994). Greater light intensities burned the leaves of these plants during long temperate summer days, and the reduced light environments used here did not noticeably alter natural plant growth forms. Pot positions were rotated every other week until September, 1991, and monthly thereafter.

Monitoring of plants began during the second week of November 1990, and continued for 45 weeks (untill September 1991). Especially at LN, the onset of myrmecophytism occurred much later in C. polystachya than in other species. Therefore, in order to make simultaneous measurements after myrmecophytism in this species and its close congener, C. ficifolia, we continued monitoring saplings of these two species (pair 1) until September 1992. Since these extra measurements were taken only for LN plants, we were not able to test for the effect of nutrient treatments (in C. ficifolia and C. polystachya) because the variables in question were measured during different years. In many cases, we did not make intraspecific comparisons for the first year's data set because of the scarcity of data for LN plants during the first year of the experiment. Data quantifying antiherbivore defenses were collected only after the plants had developed their myrmecophytic traits, or in May through July of 1991. For pair 1 we waited until August-September 1991 to take some measurements of defenses. Some of these measurements for pair 1 at LN were repeated or gathered for the first time between June and September 1992 (see results in tables). Measurements of each variable in year 2 were gathered over the same months during which each variable was quantified in the first year. MBs for energy analyses were collected throughout both years for LN plants of pair 1.

## Growth parameters

To quantify growth, we measured total plant height (every 2 weeks) and total production and loss of marked leaves (weekly). Median leaf life spans were calculated from these data.

## Biotic defenses

MBs (ant rewards, specific to Cecropia) are produced on specialized structures called trichilia, located at the bases of petioles. PBs (generalized ant rewards, found in many other plants, e.g., Schupp and Feener 1991) are produced directly on the leaves throughout ontogeny. To assess the developmental onset of myrmecophytism, we recorded the date at which the first leaf with trichilia appeared. (All subsequent leaves produced trichilia.) As indicators of investment in biotic defenses, we measured rates of production of MBs and PBs, and the median duration of food body production by individual leaves.

MB production was quantified in all plants that had developed at least five active trichilia (those producing MBs) under the LN treatment by the time of each of these censuses. At the start of each census, we removed all MBs from each trichilium. Thereafter, we returned at 24-h intervals for three days to count and remove food bodies. Because preliminary observations (Davidson and Fisher 1991) revealed strong peaks of MB production just after dusk, we censused in early morning before watering.

Investment in MB production was assessed as energy content per trichilium, per leaf area, per time interval. We estimated energetic content of MBs by burning 0.1-g MB pellets in a semimicro oxygen bomb calorimeter (Parr 1261, Moline, IL). To calculate MB production per unit of leaf area, we measured the traced areas of six leaves per plant on a leaf area meter (Li-Cor-3050A, Lin-

coln, Neb.). The active lifetimes of individual trichilia, or duration of MB production, were estimated by noting the initiation and termination of MB production. Termination was defined as the production date immediately preceding 1 month of inactivity.

We enumerated PBs on abaxial surfaces of the four leaves immediately below the newest leaves of individual plants that had already developed trichilia. To quantify production rates, we counted PBs from a fixed leaf area (16 cm$^2$), removed them from the entire leaf, and enumerated them again one month later on the same leaf areas. We added the two counts per plant before analyzing the data. Duration of PB activity was estimated for individual leaves by noting the dates on which PB production began and ended. Termination was again defined as the census date preceding 1 month with no production.

## Secondary compounds

To quantify foliar secondary compounds, we harvested healthy young leaves (i.e., those completely outside of stipules but not fully expanded), and mature leaves (i.e., those that had reached their final size). Fresh leaf material was placed immediately in liquid nitrogen and transfered to a −70° C freezer, where it was held until analysis. Other details of sample collection, preparation and analysis are reported in Folgarait and Davidson (1994). We measured total phenolics by a modified Prussian blue assay (Price and Butler 1977), with gallic acid as the standard. Levels of condensed tannins were determined by both a modified protein precipitation assay, using BSA as the complexing protein (Hagerman and Butler 1978), and a proanthocyanidin assay, using purified quebracho tannin as the standard (Porter et al. 1986). In preliminary tests, the interspecific rankings of concentrations of condensed tannins were the same whether expressed as quebracho equivalents or as purified plant tannins (Folgarait and Davidson 1994).

Fresh young and mature leaves were also assayed qualitatively for alkaloids (Schall and Ressel 1991), saponins (Dearing and Schall 1992) and cyanogenic glycosides (Williams 1979). We found no evidence for these chemical compounds in leaves of either age class or either nutrient treatment, and no further analyses were undertaken.

## Physical defenses

Leaf toughness which correlates with fiber content (Stamp and Bowers 1990), was estimated for young and mature healthy leaves as the mean force needed to punch a 3-mm diameter rod (precision Chatillion punchameter) through the leaf ($n$=3 trials, avoiding major veins).

## Phenological defenses

Putatively defensive aspects of new leaf production were quantified in two ways and compared across treatments and species. First, because faster expansion of individual leaves should permit more rapid onset of fiber deposition and leaf toughening, we determined the number of days to full leaf expansion after both first partial and complete leaf emergence from protective stipules. Leaves were taken to be fully expanded when repeated (alternate day) measurements of the main vein revealed a constant length over at least 1 week. Second, as an index of the probable onset of biotic protection for individual leaves, we monitored leaves daily and recorded the timing of first MB and PB production. Finally, to assess the vulnerability of new leaves to herbivory during the window of time between emergence and biotic protection, we estimated a leaf-area-based "exposure index" at two different developmental stages: at the onsets of MB and PB production. The exposure index was calculated as $[S_1+(S_2-S_1/2)]\times T_{1-2}$, where $S_1$ is the leaf area at complete leaf emergence from stipules or first possible measurement, $S_2$ is leaf area at the onset of MB or PB production, and $T_{1-2}$ is the number of days between the complete release of

leaves from stipules and the onset of biotic protection by MBs or PBs. Leaf areas were estimated from measured lengths of main veins using regressions of leaf area on these lengths for leaf traces from each species × treatment combination (Folgarait 1993).

### Nitrogen content of leaves and MBs

To determine how experimental nutrient regimes translate into differences in tissue nitrogen levels, we measured nitrogen concentrations in both young and mature leaves, and in MBs. Nitrogen content (expressed as a percentage of dry mass) was determined on a CHN Perkins-Elmer autoanalyzer for 2 mg samples of ground leaf tissue or MBs.

### Data analysis

To test various functional hypotheses about the relationship between resources and plant allocation to growth versus defense, we restricted interspecific comparisons of gap and pioneer species to pairs of close relatives (see Folgarait and Davidson 1994). This practice helps to reduce the problem of non-independence of data points, as well as phylogenetic noise (often present in comparative studies), and allows a powerful test of functional hypotheses (Jarman 1982; Felsenstein 1985; Huey 1987).

Because data were not normally distributed, even after transformation, the effects of species and treatment on myrmecophytic and chemical traits, growth parameters and other measures were analyzed using non-parametric tests (Siegel 1974). Therefore, results are reported as medians, and ranges are given as variance estimates. Median leaf lifetimes and duration of MB and PB production were calculated with the LIFETEST procedure (SAS 1988). All statistical tests incorporated Bonferroni's adjusted $\alpha$ levels for multiple comparisons in a priori tests at an experimentwise $\alpha=0.05$ or less. Although we grew the six species simultaneously, each experiment consisted of a pair of close relatives with four relevant comparisons: two interspecific (one for each nutrient regime) and two intraspecific (one for each closely related species). Therefore, we adjusted the $\alpha$ levels for each comparison to $\alpha=0.0125$ (0.05/4) and $\alpha=0.0025$ (0.01/4) (Neter et al. 1985). Interspecific comparisons at each nutrient level were performed to address the evolved differences in plant responses to resource availability, while the intraspecific comparisons were designed to study plasticity in the pattern of defense allocation within the context of the C:N balance hypothesis.

## Results

### Growth rates and leaf life spans

Because final plant heights and leaf production rates were not generally correlated with initial seedling heights and leaf numbers (for species × treatment groups), we used final height and total area of leaves produced during the experiment as indices of growth rate (Table 1). All species grew significantly taller under HN than LN (Table 1). Final heights attained by pioneer species exceeded those of their gap counterparts, with the single exception of pair 1 under LN. All species produced significantly higher total leaf areas under HN than LN (Table 1). Each pioneer species produced significantly greater leaf area than did its gap counterpart.

In three of five possible statistical comparisons, leaves produced under LN were significantly longer-lived than those of HN plants (Table 1). Under both nutrient regimes, leaf life spans were significantly longer in gap species than in their pioneer counterparts, with the single exception being the reversal of this result in pair 1 under LN (Table 1).

### Biotic defenses

#### Development of trichilia and duration of MB production

In four of six intraspecific comparisons, trichilia appeared significantly earlier (Table 2) under the HN treatment. In interspecific comparisons under both nutrient regimes, each gap species produced trichilia significantly earlier in time (Table 2), earlier in development (i.e., at lower leaf numbers), and at smaller plant heights (Folgarait 1993) than did its pioneer counterpart.

Measures of duration of MB production for individual trichilia revealed no consistent treatment effects (Table 2). In four of six comparisons, gap species exhibited significantly longer duration of MB production than did their pioneer counterparts. However, because of the shorter leaf lifetimes of pioneers, leaves of closely related pioneer and gap *Cecropia* produced MBs over comparable fractions of their life spans (Folgarait 1993).

#### Müllerian body production

Within species, the higher nutrient level led to significantly higher rates of MB production per trichilium (Fig. 1, Table 2). However, the energetic content of MBs was significantly reduced under HN in the four species of pairs 2 and 3. In interspecific comparisons, both MB numbers per trichilium (with one exception), and energy investment in MBs per unit of leaf area, were significantly greater in gap species than in their pioneer counterparts. The latter result held despite significantly smaller MB sizes (except in pair 2) and leaf areas (Folgarait 1993) in gap species, and the lower energetic content of MBs in gap species (excepting pairs 2 and 3 under LN). MB production declined steeply with leaf age (Fig. 1).

#### Pearl body production and duration of PB production

Among conspecifics, PB production was significantly greater under HN in five of six comparisons (Table 3 and Fig. 2). *Cecropia* sp. B was alone in exhibiting no difference. Under both nutrient regimes, pioneer species of pairs 1 and 3 produced significantly more PBs per unit of leaf area than did their gap counterparts. PB production declined with leaf age, but much less steeply than did MB production (Fig. 2).

Measures of duration of PB production for individual leaves revealed no consistent treatment effects among species (Table 3). In comparisons of close relatives, pioneer and gap species did not differ consistently in this trait. However, if expressed as a percentage of leaf life-

**Table 1A** Plant growth parameters and leaf life spans for three pairs (1, 2, and 3) of closely related *Cecropia* species under two nutrient treatments. Measurements extended over 2 years for pair 1 only. Final plant height, total leaf area, and leaf lifetimes based on all leaves produced during the experiment. Values are medians (and ranges)

| Growth parameters | Nutrient level | Year | PAIR 1 C. ficifolia (Gap sp.) | PAIR 1 C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | PAIR 2 C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | PAIR 3 C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|---|
| Final height (m) | High | 1 | 1.71 (1.37–2.05) | 2.13 (1.58–2.68) | 0.68 (0.44–0.92) | 2.62 (2.06–3.18) | 1.73 (1.53–2.05) | 2.75 (2.23–3.27) |
|  |  | 2 | – | – | – | – | – | – |
|  | Low | 1 | 0.48 (0.31–0.64) | 0.47 (0.16–0.77) | 0.40 (0.33–0.46) | 1.12 (0.84–1.39) | 0.77 (0.63–0.91) | 1.32 (0.96–1.68) |
|  |  | 2 | 1.98 (1.56–2.39) | 1.68 (0.94–2.28) | – | – | – | – |
| Total leaf area produced (m²) | High | 1 | 2.12 (1.92–2.31) | 3.58 (3.11–4.04) | 1.22 (1.04–1.39) | 2.11 (1.88–2.33) | 0.92 (0.84–1.01) | 2.62 (2.32–2.91) |
|  |  | 2 | – | – | – | – | – | – |
|  | Low | 1 | 0.46 (0.42–5.10) | 1.65 (1.44–1.85) | 0.39 (0.36–0.42) | 0.45 (0.41–0.49) | 0.37 (0.33–0.40) | 0.59 (0.48–0.70) |
|  |  | 2 | 1.09 (1.00–1.17) | 3.71 (3.33–4.08) | – | – | – | – |
| Leaf lifetimes (weeks) | High | 1 | 20.0 (17.5–22.5) | 18.0 (15.5–20.5) | 19.0 (17.0–21.0) | 14.0 (12.0–16.0) | 19.0 (16.5–21.5) | 17.0 (15.5–18.5) |
|  |  | 2 | – | – | – | – | – | – |
|  | Low | 1 | 18.0 (15.5–20.5) | – | 20.0 (15.0–25.0) | 16.0 (14.5–17.5) | 22.0 (20.0–24.0) | 20.0 (17.5–22.5) |
|  |  | 2 | 19.0 (17.0–21.0) | 23.0 (17.0–29.0) | – | – | – | – |

**Table 1B** Statistical comparisons between pairs of close relatives and between nutrient treatments (*HN* high, *LN* low) for data from Table 1A. Results under HN/LN. $n=7–14$ plants per species X treatment category

| Growth parameters | Comparison | Year | PAIR 1 C. ficifolia (Gap sp.) | PAIR 1 C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | PAIR 2 C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | PAIR 3 C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|---|
| Final height[a] (m) | Between spp. | 1 | */NS |  | **/** |  | **/** |  |
|  |  | 2 | –/NS |  | –/– |  | –/– |  |
|  | HN vs. LN | 1 | ** | ** | ** | ** | ** | ** |
|  |  | 2 | – | – | – | – | – | – |
| Total leaf area produced[a] (m²) | Between spp. | 1 | **/** |  | **/** |  | **/** |  |
|  |  | 2 | –/** |  | –/– |  | –/– |  |
|  | HN vs. LN | 1 | ** | ** | ** | ** | ** | ** |
|  |  | 2 | – | – | – | – | – | – |
| Leaf lifetimes[b] (weeks) | Between spp. | 1 | **/– |  | **/** |  | **/* |  |
|  |  | 2 | –/** |  | –/– |  | –/– |  |
|  | HN vs. LN | 1 | * | – | NS | ** | ** | ** |
|  |  | 2 | – | – | – | – | – | – |

* $P<0.0125$, ** $P<0.0025$, NS=not significant
[a] Statistical comparisons by Mann-Whitney U-test
[b] Statistical comparisons by LIFETEST Proc (SAS)

time, duration of PB production was longer in pioneers than in their gap counterparts (Folgarait 1993).

Chemical and physical components of mature leaves

*Tannins and phenols*

In all cases where we found significant differences, greater concentrations of condensed tannins and phenolics were produced under LN than HN (Table 4).

Pairs of gap and pioneer species did not differ consistently in foliar concentrations of condensed tannins and phenols (Table 4). In pair 1, all significant results showed that the gap species produced greater levels of these compounds than did its pioneer counterpart. In pair 2, close relatives were statistically indistinguishable in their levels of chemical defenses. In contrast, under both nutrient regimes and with a single exception, measures of condensed tannins and phenols were significantly greater in the pioneer of pair 3, than in its gap counterpart.

*Toughness*

Leaves were tougher under HN than LN in all cases where we found significant differences (Table 5). In all four comparisons in which differences were significant, leaf toughness was greater in gap species than in their pioneer counterparts (Table 5). The same pattern was found for leaf specific weights (Folgarait 1993).

**Table 2A** Week numbers of appearance of first trichilia (numbered relative to start of experiment), Müllerian body (*MB*) production and related calculations for three pairs of closely related *Cecropia* species under two nutrient treatments. Measurements made once in year 2 for pair 1. Durations of MB production are based on leaves with trichilia. Values are medians (and ranges)

| Myrmeco-phytic traits | Nutrient level | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | *C. ficifolia* (Gap sp.) | *C. polystachya* (Pioneer sp.) | *C.* sp. B (Gap sp.) | *C. membranacea* (Pioneer sp.) | *C.* sp. A (Gap sp.) | *C. engleriana* (Pioneer sp.) |
| Week no. for first trichilium | High | 1 | 31.0 (25.5–36.5) | 39.5 (34.5–44.5) | 22.0 (16.5–27.5) | 35.0 (31.0–39.0) | 31.0 (26.0–36.0) | 36.0 (34.0–38.0) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 32.0 (23.5–40.5) | 56.0 (53.0–59.0) | 21.0 (17.0–25.0) | 44.0 (36.0–52.0) | 35.0 (30.0–40.0) | 41.0 (32.5–45.5) |
| | | 2 | – | – | – | – | – | – |
| Duration of MB production (weeks) | High | 1 | 18.0 (16.0–20.0) | 15.0 (11.5–18.5) | 13.0 (10.0–16.0) | 14.0 (11.5–16.5) | 12.0 (10.5–14.5) | 10.0 (8.0–12.0) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 17.0 (15.0–19.0) | – | 15.0 (12.0–18.0) | 13.0 (10.0–16.0) | 12.0 (10.0–14.0) | 10.0 (8.0–12.0) |
| | | 2 | 18.0 (15.5–20.5) | 15.0 (11.5–18.5) | – | – | – | – |
| No. MBs per trichilium per day | High | 1 | 69.6 (53.5–85.7) | 34.3 (20.0–48.5) | 41.5 (15.0–67.9) | 24.6 (10.6–38.5) | 71.6 (47.1–96.0) | 24.6 (17.9–31.9) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 23.3 (12.8–33.7) | – | 10.6 (0.0–21.2) | 5.3 (0.0–11.5) | 28.3 (6.4–50.1) | 8.8 (3.9–13.7) |
| | | 2 | 82.0 (61.0–102.9) | 32.3 (18.0–46.5) | – | – | – | – |
| Joules per g of MB[a] (×10³) | High | 1 | 21.3 (19.9–22.7) | 24.2 (19.4–29.1) | 23.3 (22.7–23.9) | 23.6 (23.1–24.1) | 19.8 (18.4–21.3) | 21.6 (20.7–22.5) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | – | – | 25.1 (24.2–25.9) | 24.7 (24.1–25.4) | 24.9 (24.7–25.5) | 22.7 (22.1–23.3) |
| | | 2 | 19.3 (18.4–20.1) | 23.6 (22.2–25.1) | – | – | – | – |
| Investment in MBs (joules of MBs per trichillium, per m² leaf, per day) | High | 1 | 11.6 (10.9–12.4) | 5.1 (4.8–5.4) | 60.7 (21.8–99.6) | 13.4 (5.7–20.9) | 24.1 (15.9–32.2) | 6.8 (4.9–8.7) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 10.8 (10.3–11.3) | – | 32.8 (0.3–65.3) | 7.0 (4.3–9.7) | 21.9 (17.5–26.4) | 4.8 (2.2–7.5) |
| | | 2 | 38.1 (36.5–39.7) | 5.2 (4.8–5.5) | – | – | – | – |

[a] MB collection extended over 2 years for low nutrient plants in pair 1

**Table 2B** Statistical comparisons by Mann-Whitney *U*-tests between pairs of close relatives and between nutrient treatments for data from Table 2A. Results under HN/LN

| Myrmecophytic traits | Comparison | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | *C. ficifolia* (Gap sp.) | *C. polystachya* (Pioneer sp.) | *C.* sp. B (Gap sp.) | *C. membranacea* (Pioneer sp.) | *C.* sp. A (Gap sp.) | *C. engleriana* (Pioneer sp.) |
| Week no. for first trichilium[a] | Between spp. | 1 | | */** | | **/** | | **/* |
| | | 2 | | –/– | | –/– | | –/– |
| | HN vs. LN | 1 | NS | ** | NS | ** | * | ** |
| | | 2 | – | – | – | – | – | – |
| Duration of MB production (weeks)[b] | Between spp. | 1 | | **/– | | NS/** | | */NS |
| | | 2 | | –/** | | –/– | | –/– |
| | HN vs. LN | 1 | NS | – | * | ** | NS | NS |
| | | 2 | – | – | – | – | – | – |
| No. MBs per trichilium per day[c] | Between spp. | 1 | | **/– | | NS/** | | **/** |
| | | 2 | | –/** | | –/– | | –/– |
| | HN vs. LN | 1 | ** | – | ** | ** | ** | ** |
| | | 2 | – | – | – | – | – | – |
| Joules per g of MB (×10³)[d] | Between spp. | 1 | | **/– | | */NS | | **/** |
| | | 2 | | –/** | | –/– | | –/– |
| | HN vs. LN | 1 | – | – | ** | ** | ** | ** |
| | | 2 | – | – | – | – | – | – |
| Investment in MBs (joules of MBs per trichillium, per m² leaf per day)[c] | Between spp. | 1 | | **/– | | **/** | | **/** |
| | | 2 | | –/** | | –/– | | –/– |
| | HN vs. LN | 1 | NS | – | NS | * | NS | * |
| | | 2 | – | – | – | – | – | – |

* $P < 0.0125$, ** $P < 0.0025$, NS = not significant
[a] $n = 9$–13 per species X treatment category
[b] Statistical comparisons by LIFETEST Proc (SAS); $n = 9$–14 per species X treatment category

[c] $n = 6$–14 plants per species X treatment category
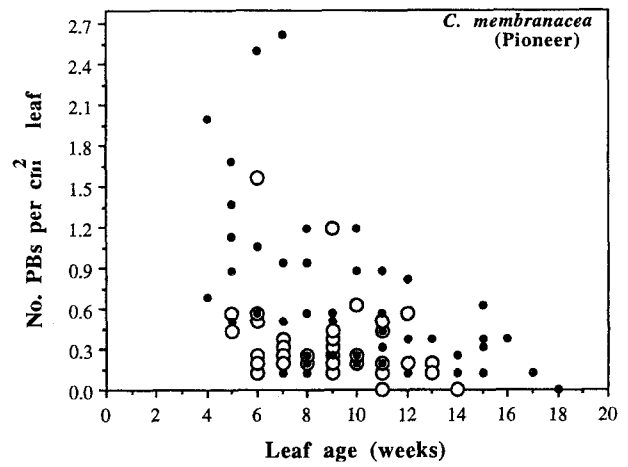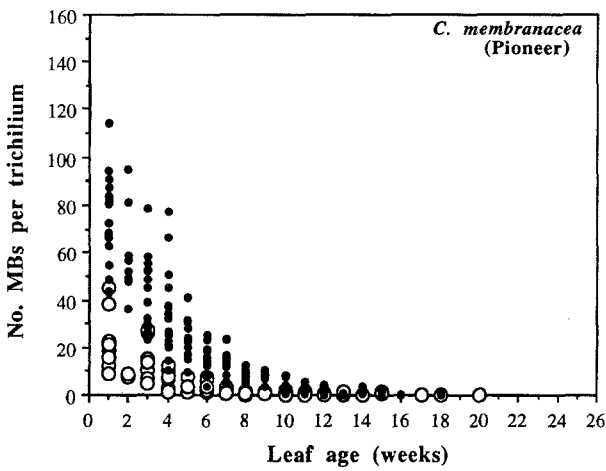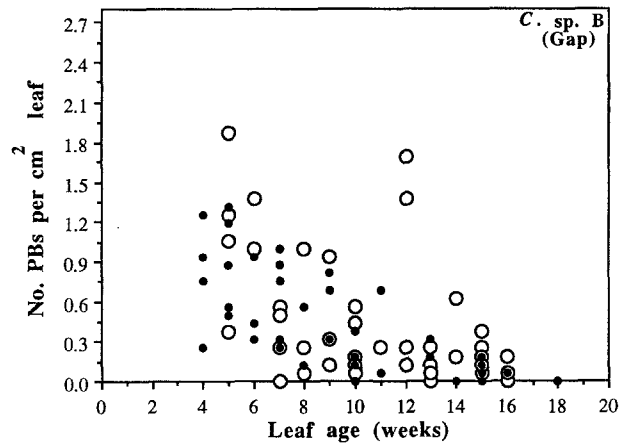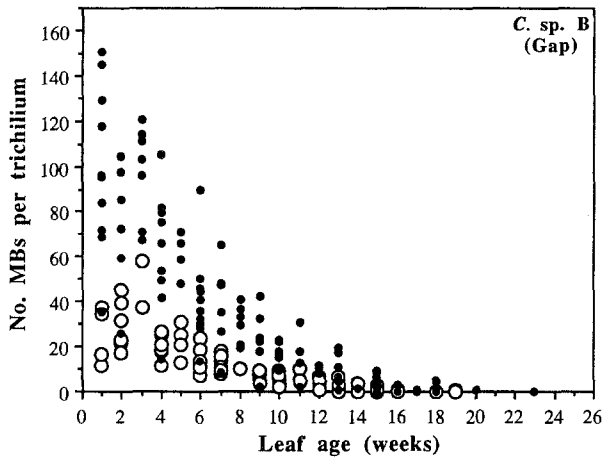[d] $n = 7$–14 plants per species X treatment category

**Fig. 1** Relationship between leaf age (weeks) and Müllerian body (*MB*) production per trichilia per day for one representative pair of *Cecropia* species (*open circles* low nutrient treatment, *solid circles* high nutrient treatment)

**Fig. 2** Relationship between leaf age (weeks) and pearly body (*PB*) production per cm² of leaf area, 1 month after removal, for one representative pair of *Cecropia* species (*open circles* low nutrient treatment, *solid circles* high nutrient treatment)

**Table 3A** Pearl body (*PB*) densities on abaxial leaf surfaces, and duration of PB production, for three pairs of closely related *Cecropia* species under two nutrient treatments. Measurements made once in year 2 for pair 1. Duration of PB production, based on all leaves produced during the experiment. Values are medians (and ranges)

| Pearl bodies | Nutrient level | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | *C. ficifolia* (Gap sp.) | *C. polystachya* (Pioneer sp.) | *C.* sp. B (Gap sp.) | *C. membranacea* (Pioneer sp.) | *C.* sp. A (Gap sp.) | *C. engleriana* (Pioneer sp.) |
| No. of pearl bodies/cm² | High | 1 | 1.24 (0.76–1.71) | 2.02 (0.63–3.41) | 0.83 (0.11–1.55) | 1.47 (0.49–2.44) | 2.48 (0.38–4.57) | 4.48 (1.50–7.45) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 0.50 (0.38–0.61) | 1.47 (0.84–2.09) | 0.92 (0.00–2.23) | 0.65 (0.00–1.71) | 0.89 (0.50–1.28) | 2.30 (1.16–3.44) |
| | | 2 | 1.61 (1.04–2.18) | 1.77 (0.38–3.15) | – | – | – | – |
| Duration of PB production (weeks) | High | 1 | 17.0 (15.0–19.0) | 16.0 (13.0–19.0) | 13.0 (9.00–15.0) | 12.0 (10.0–14.0) | 17.0 (14.5–19.5) | 16.0 (13.5–18.5) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 16.0 (13.5–18.5) | – | 15.0 (12.5–17.5) | 13.0 (11.5–15.5) | 18.0 (16.0–20.0) | 19.0 (16.0–22.0) |
| | | 2 | 16.0 (12.0–18.0) | 22.0 (16.5–27.5) | – | – | – | – |

**Table 3B** Statistical comparisons between pairs of close relatives and between nutrient treatments for data from Table 3A. Results under HN/LN

| Pearl bodies | Comparison | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
| No. of pearl bodies/cm²ᵃ | Between spp. | 1 | **/** | | NS/NS | | */** | |
| | | 2 | –/NS | | –/– | | –/– | |
| | HN vs. LN | 1 | ** | ** | NS | * | ** | ** |
| | | 2 | – | – | – | – | – | – |
| Duration of PB productionᵇ (weeks) | Between spp. | 1 | NS/– | | NS/* | | */* | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | – | * | NS | * | ** |
| | | 2 | – | – | – | – | – | – |

* P<0.0125, ** P<0.0025, NS=not significant
ᵃ Statistical comparisons by Mann-Whitney U-tests; n=9–13 per species X treatment category

ᵇ Statistical comparisons by LIFETEST Proc (SAS); n=9–14 plants per species X treatment category

**Table 4A** Carbon-based secondary compounds in mature and young leaves harvested after the onset of myrmecophytism, for three pairs of closely related Cecropia species under two nutrient treatments. Measurements made once in year 2 for pair 1. Values are medians (and ranges)

| Carbon-based secondary compounds | Nutrient level | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
| MATURE LEAVES | | | | | | | | |
| Condensed tanninsᵃ (Proanthocyanidin assay, mg/g) | High | 1 | 752.8 (208–1298) | 618.5 (250–987) | 408.0 (21–795) | 444.4 (170–719) | 98.8 (34–164) | 471.7 (158–785) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 613.1 (273–953) | 711.6 (440–983) | 765.9 (557–974) | 628.9 (249–1009) | 527.4 (373–682) | 966.9 (870–1063) |
| | | 2 | 2110.7 (1375–2845) | 845.6 (457–1234) | – | – | – | – |
| Condensed tanninsᵃ (Protein precipitation assay, mg/g) | High | 1 | 85.4 (68–103) | 53.3 (27–80) | 72.8 (34–107) | 70.6 (48–93) | 12.0 (5–19) | 61.9 (41–82) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 63.9 (21–106) | 76.5 (57–96) | 68.9 (0–168) | 66.7 (16–117) | 53.7 (40–67) | 64.2 (57–71) |
| | | 2 | 111.5 (67–156) | 46.1 (33–59) | – | – | – | – |
| Total phenolsᵇ (Prussian blue assay, mg/g) | High | 1 | 43.4 (23–64) | 42.0 (25–59) | 32.2 (9–55) | 36.3 (8–65) | 7.7 (1–14) | 38.1 (26–50) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 30.0 (17–43) | 48.1 (33–63) | 52.6 (37–68) | 41.2 (17–65) | 27.5 (17–38) | 62.9 (51–75) |
| | | 2 | 54.7 (34–76) | 35.6 (22–49) | – | – | – | – |
| YOUNG LEAVES | | | | | | | | |
| Condensed tanninsᵃ (Proanthocyanidin assay, mg/g) | High | 1 | 234.2 (0–831) | 209.8*(0–720) | 128.8*(0–279) | 138.7*(0–434) | 16.6 (0–54) | 429.9 (179–680) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 390.5 (183–598) | 687.8 (210–1165) | 683.5 (234–1133) | 614.0 (223–1005) | 276.9*(114–440) | 567.7*(281–854) |
| | | 2 | 2168.4 (1631–2705) | 510.8*(175–846) | – | – | – | – |
| Condensed tanninsᵃ (Protein precipitation assay, mg/g) | High | 1 | 31.9*(0–66) | 19.3*(0–63) | 24.1*(12–36) | 22.2*(0–54) | 5.9 (3–8) | 47.8 (14–81) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 45.6 (31–60) | 73.8 (44–104) | 88.6 (13–164) | 63.9 (25–102) | 30.9*(18–43) | 49.5*(17–81) |
| | | 2 | 128.9 (108–150) | 18.6*(1–36) | – | – | – | – |
| Total phenolsᵇ (Prussian blue assay, mg/g) | High | 1 | 9.9 (0–35) | 25.9 (0–83) | 12.5*(5–20) | 15.9*(1–31) | 4.2 (2–6) | 22.2 (0–44) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 19.7*(11–28) | 48.9 (17–81) | 44.1 (13–75) | 33.1 (10–56) | 13.1*(4–22) | 30.2 (18–42) |
| | | 2 | 61.0 (32–90) | 16.4*(7–26) | – | – | – | – |

ᵃ Condensed tannins in quebracho equivalents, or mg of quebracho/g dry leaf mass
ᵇ Total phenols in gallic acid equivalents, or mg of gallic acid/g dry leaf mass.

* P<0.0125 for comparisons between young and mature leaves by Mann-Whitney U-tests

**Table 4B** Statistical comparisons between close relatives and between nutrient treatments by Mann-Whitney $U$-tests for data from Table 4A. Results under HN/LN; $n=6$–11 plants per species X treatment category

| Carbon-based secondary compounds | Comparison | Year | PAIR 1 C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|---|
| **MATURE LEAVES** | | | | | | | | |
| Condensed tannins (Proanthocyanidin assay, mg/g) | Between spp. | 1 | NS/NS | | NS/NS | | */** | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | NS | ** | * | * | ** |
| | | 2 | – | – | – | – | – | – |
| Condensed tannins (Protein precipitation assay, mg/g) | Between spp. | 1 | */NS | | NS/NS | | **/NS | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | * | NS | NS | ** | NS |
| | | 2 | – | – | – | – | – | – |
| Total phenols (Prussian blue assay, mg/g) | Between spp. | 1 | NS/NS | | NS/NS | | */** | |
| | | 2 | –/* | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | NS | ** | NS | ** | ** |
| | | 2 | – | – | – | – | – | – |
| **YOUNG LEAVES** | | | | | | | | |
| Condensed tannins (Proanthocyanidin assay, mg/g) | Between spp. | 1 | NS/* | | NS/NS | | */** | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | * | NS | ** | ** | * | NS |
| | | 2 | – | – | – | – | – | – |
| Condensed tannins (Protein precipitation assay, mg/g) | Between spp. | 1 | NS/* | | NS/NS | | */NS | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | * | ** | ** | ** | NS |
| | | 2 | – | – | – | – | – | – |
| Total phenols (Prussian blue assay, mg/g) | Between spp. | 1 | NS/* | | NS/NS | | */* | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | NS | ** | ** | ** | NS |
| | | 2 | – | – | – | – | – | – |

$*$ $P<0.0125$, $**$ $P<0.0025$, NS=not significant

## Nitrogen content

### Leaves

In all four cases where treatment effects were significant, nitrogen concentrations in mature leaves were greater under HN (Table 6). Foliar nitrogen levels did not differ consistently between close congeners.

### MBs

In four of six *Cecropia* species, nitrogen levels were significantly greater in MBs of plants cultivated under HN (Table 6). In species of pairs 2 and 3, nitrogen concentrations of MBs were significantly greater for gap species than for their pioneer counterparts. The reverse was true for pair 1.

## Defensive and other characteristics of new leaves

### Biotic defenses

In all six species, MB and PB production were highest in young leaves, though relative to MB production, a greater fraction of PB production is accounted for by mature leaves (Figs. 1 and 2).

## Secondary compounds

For every species in each assay, young leaves produced higher levels of condensed tannins and phenolics under the LN treatment; 55.5% of these differences were significant (Table 4). Foliar concentrations of carbon-based compounds did not differ consistently between close relatives. However, in each assay, measures were significantly greater for pioneers than for gap counterparts in pair 3 (both nutrient regimes) and pair 1 (low nutrient treatment only). In the latter case, however, the pattern was reversed by the end of year two, when the majority of leaves on *C. polystachya* plants had developed active trichilia. In pair 2, differences between close relatives were not significant for any assay. With only two exceptions, levels of carbon-based secondary compounds were lower in young leaves than in mature leaves (significantly so in 50% of cases, Table 4).

### Physical defenses

In comparisons between nutrient treatments and between close relatives, differences in toughness of young leaves were generally not significant (Table 5). Nevertheless,

**Table 5A** Toughness of mature and young leaves for three pairs of closely related *Cecropia* species under two nutrient treatments. Values are medians (and ranges)

| Leaf toughness | Nutrient level | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|
| | | C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
| Mature leaves (newtons) | High | 2.65 (2.18–3.11) | 2.30 (1.92–2.67) | 2.48 (1.95–3.00) | 1.34 (0.99–1.68) | 2.55 (2.34–2.76) | 2.27 (1.81–2.73) |
| | Low | 2.16 (1.62–2.70) | 1.47 (1.23–1.70) | 1.93 (1.67–2.19) | 1.04 (0.78–1.30) | 2.66 (2.15–3.16) | 1.96 (1.71–2.20) |
| Young leaves (newtons) | High | 2.07 (1.71–2.43) | 1.93 (1.32–2.53) | 2.40 (1.53–3.26) | 1.11*(0.92–1.29) | 1.69*(1.30–2.08) | 1.91 (1.24–2.58) |
| | Low | 2.27 (1.58–2.95) | 1.18 (0.75–1.60) | 1.88 (1.46–2.30) | 1.34 (0.50–2.17) | 2.29 (1.45–3.12) | 1.83 (1.37–2.29) |

* $P<0.0125$ for comparisons between young and mature leaves by Mann-Whitney $U$-tests

**Table 5B** Statistical comparisons by Mann-Whitney U tests between pairs of close relatives and between nutrient treatments for data from Table 5A. Results under HN/LN; $n=6–7$ per species and treatment

| Leaf toughness | Comparison | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|
| | | C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
| Mature leaves (newtons) | Between spp. | | NS/* | | **/** | | NS/** |
| | HN vs. LN | NS | ** | * | ** | NS | NS |
| Young leaves (newtons) | Between spp. | | NS/* | | **/* | | NS/NS |
| | HN vs. LN | NS | NS | NS | NS | NS | NS |

* $P<0.0125$, ** $P<0.0025$, NS=not significant

toughness was significantly greater for gap species than for their pioneer counterparts in all three comparisons for which interspecific differences were significant. In 10 of 12 comparisons, toughness of mature leaves exceeded that of young leaves. However, these differences were significant in only two cases (Table 5).

### Nitrogen in leaves

Young leaves produced under HN exhibited significantly greater nitrogen concentrations than did those of conspecifics under LN (Table 6). Foliar nitrogen levels did not differ consistently between close congeners. In all but *C.* sp. A, the median values for plants at HN lie within the range of field results for individual species; in all six species, LN values are lower than medians from field results (Table 6). Foliar nitrogen concentrations were uniformly higher in young than in mature leaves, and these differences were significant in 8 of 12 cases (Table 6).

### Phenological defenses

*Leaf expansion times* It took 0–7 days for leaves to emerge fully from their protective stipules. Nutrient levels did not affect leaf expansion times. Leaves of pioneers were significantly larger at full expansion than were leaves of related gap species (P. Folgarait, unpublished work), and in 8 of 12 comparisons, the former expanded to full size significantly more quickly than the

latter after both partial and complete release from stipules (Table 7).

*Onset of MB production* In intraspecific comparisons, nutrient enrichment did not have a significant effect on the onset of MB production by individual leaves (Table 7).

Although pioneer and gap species did not differ consistently in the temporal onset of MB production (Table 7), this onset came at significantly earlier stages of leaf development (in proportion to leaf life span) in gap species than in pioneer counterparts (Folgarait 1993). The most striking discrepancies in these measures occurred between species pairs; species in pair 2 initiated MB production much earlier in time and in leaf development than did species of pairs 1 and 3.

*Onset of PB production* Treatment effects on the onset of PB production were not significant (Table 7) nor in a consistent direction. In all three cases where interspecific differences were significant, pioneers initiated PB production earlier in time than did gap species (Table 7).

*Exposure of new leaves to herbivory* An index of new leaf exposure to herbivory (Table 7) accounts for both the length of the "vulnerability window" (the interval between complete leaf emergence and onset of biotic protection) and the amount of leaf tissue exposed during that period. There was a tendency for greater leaf exposure to herbivory under HN (with one exception). There were seven cases of zero exposure due to the immediate production of food bodies after complete release of the

**Table 6A** Nitrogen content of MBs and of mature and young leaves for three pairs of closely related *Cecropia* species under two nutrient treatments. Leaf nitrogen content also for young leaf samples from saplings in their characteristic habitats in the field. Values are medians (and ranges)

| Nitrogen content | Nutrient level | PAIR 1 C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|
| MBs (% dry mass) | High | 2.49 (2.30–2.67) | 3.15 (2.97–3.46) | 4.60 (4.16–5.03) | 3.99 (3.68–4.30) | 3.03 (2.84–3.27) | 2.63 (2.31–2.94) |
|  | Low | 2.31 (2.13–2.48) | 2.96 (2.75–3.17) | 4.17 (3.68–4.30) | 3.90 (3.59–4.21) | 2.76 (2.54–2.97) | 2.35 (2.10–2.60) |
| Mature leaves (% dry mass) | High | 1.65 (1.31–1.99) | 2.20 (1.52–2.87) | 2.22 (2.02–2.42) | 1.92 (1.77–2.07) | 3.52 (3.33–4.53) | 2.01 (1.56–2.45) |
|  | Low | 1.35 (0.99–1.71) | 1.38 (1.20–1.56) | 1.32 (1.07–1.56) | 2.10 (1.91–2.29) | 1.65 (1.44–2.09) | 1.53 (1.34–1.71) |
| Young leaves (% dry mass) | High | 2.60*(2.14–3.05) | 2.65 (1.99–3.30) | 3.18*(2.77–3.59) | 2.94 (2.48–3.39) | 4.45*(3.95–4.94) | 2.52*(2.13–2.91) |
|  | Low | 1.70 (1.42–1.98) | 1.74*(1.52–1.95) | 1.71*(1.37–2.05) | 2.27*(2.02–2.51) | 2.33*(1.95–2.71) | 1.75 (1.53–1.97) |
| Field leaf samples (% dry mass) |  | 2.53 (2.31–2.75) | 2.36 (1.74–2.97) | 2.77 (2.25–3.28) | 3.36 (2.75–3.97) | 3.07 (2.46–3.38) | 2.75 (2.17–3.33) |

* $P<0.0125$ for comparisons between young and mature leaves by Mann-Whitney $U$-tests

**Table 6B** Statistical comparisons by Mann-Whitney $U$-tests between pairs of close relatives and between nutrient treatments for data from Table 6A. Results under HN/LN

| Nitrogen content | Comparison | PAIR 1 C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|
| MBs[a] (% dry mass) | Between spp. | **/** |  | **/** |  | **/** |  |
|  | HN vs LN | * | NS | ** | NS | ** | * |
| Mature leaves[b] (% dry mass) | Between spp. | NS/NS |  | **/** |  | */NS |  |
|  | HN vs LN | NS | ** | ** | NS | ** | * |
| Young leaves[b] (% dry mass) | Between spp. | NS/NS |  | NS/* |  | **/** |  |
|  | HN vs LN | ** | ** | ** | ** | ** | ** |
| Field leaf samples[c] (% dry mass) | Between spp. | NS |  | * |  | * |  |

* $P<0.0125$, ** $P<0.0025$, NS=not significant
[a] $n=6$–18 per species X treatment category
[b] $n=6$–7 per species X treatment category

[c] Young leaf samples collected from their characteristic habitats in the field in December 1992; $n=4$–6

leaf from the stipule (see also Folgarait 1993). Based on the onset of biotic protection by MBs, the exposure index was significantly lower in gap species than in pioneers for three of the four comparisons within pairs 1 and 3, but identical in species of pair 2. In contrast, when evaluated with respect to the onset of PBs, exposure tended to be lower in pioneers than in gap species (5 of 6 comparisons). However, this trend held in just two of the three cases for which interspecific differences were significant. Relative to both types of biotic protection (whichever began first), the exposure index again was lower in pioneers than in gap species of pairs 1 and 3.

## Discussion

### Treatment effects and the carbon:nutrient balance

Our two nutrient treatments produced significantly different nitrogen levels in mature leaves of four of the six species, and in the young leaves of all species (Table 6). These plant responses to nitrogen treatments can be used to test the C:N balance hypothesis (Bryant et al. 1985). In its traditional form, the C:N balance hypothesis predicts a decline in carbon-based defenses (and an increase in nitrogen-based defenses) as carbon becomes more limiting relative to nitrogen (i.e., under HN). To what extent do the various defenses of *Cecropia* match predictions of this hypothesis? Before answering this question it is necessary to mention that the effects of nutrients on plant performance and defenses may occur by both direct and indirect routes. Nutrients contribute directly to plant defenses when they are constituents of those defenses. Whether constituents or not, nutrients may affect defenses indirectly, e.g., by pairing with carbon to siphon it away from defensive functions and into growth (Bryant et al. 1983), or possibly by enhancing carbon fixation (see MBs below). The effects of nutrient treatments on particular defenses might then reflect complex and even species-specific rules for the allocation of carbon and nutrients among various plant functions.

**Table 7A** Phenological traits for three pairs of closely related *Cecropia* species under two nutrient treatments. Measurements made once in year 2 for pair 1. Time to full expansion after first partial emergence and complete emergence from protective sti- pules. Onset of MB and PB production following first partial leaf emergence from the stipule. Results of the index estimating expo- sure to herbivory during the vulnerability window (see text for de- tails). Values are medians (and ranges)

| Phenological trait | Nutrient level | Year | PAIR 1 | | PAIR 2 | | PAIR 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
| Leaf expansion times Partial (days) | High | 1 | 16 (14–19) | 12 (6–18) | 15 (13–19) | 12 (10–14) | 19 (16–22) | 17 (16–19) |
| | | 2 | – | | – | – | – | – |
| | Low | 1 | 20 (17–23) | – | 17 (14–21) | 13 (10–16) | 18 (16–21) | 17 (13–21) |
| | | 2 | 20 (15–25) | 14 (9–19) | – | – | – | – |
| Leaf expansion times Complete (days) | High | 1 | 16 (14–19) | 9 (5–15) | 14 (12–18) | 10 (8–12) | 15 (13–17) | 13 (11–15) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 17 (14–20) | – | 14 (10–18) | 12 (10–15) | 13 (10–16) | 10 (8–11) |
| | | 2 | 20 (15–25) | 12 (6–18) | – | – | – | – |
| Onset of MB production (days) | High | 1 | 9 (7–11) | 9 (6–12) | 3 (0–5) | 2 (0–3) | 9 (6–12) | 12 (9–17) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 10 (5–15) | – | 4 (2–5) | 3 (0–7) | 11 (6–15) | 13 (8–18) |
| | | 2 | 11 (8–14) | 9 (6–12) | – | – | – | – |
| Onset of PB production (days) | High | 1 | 9 (6–11) | 2 (0–5) | 4 (1–7) | 4 (1–7) | 7 (5–10) | 5 (3–7) |
| | | 2 | – | – | – | – | – | – |
| | Low | 1 | 8 (5–11) | – | 5 (2–8) | 4 (2–6) | 9 (4–13) | 4 (1–7) |
| | | 2 | 5 (0–9) | 1 (0–2) | – | – | – | – |
| Exposure to herbivory (considering only MBs, $\times 10^3$) | High | 1 | 1.89 (0.2–0.4) | 7.71 (3.3–12.2) | 0.00 (0.0–0.1) | 0.00 (0.0–0.2) | 0.71 (0.1–1.3) | 3.19 (1.4–4.9) |
| | | 2 | – | | – | – | – | – |
| | Low | 1 | 0.33 (0.0–0.8) | – | 0.00 (0.0–0.1) | 0.00 (0.0–1.3) | 0.50 (0.1–0.9) | 0.85 (0.3–1.4) |
| | | 2 | 0.83 (0.2–1.3) | 2.76 (1.1–4.4) | – | – | – | – |
| Exposure to herbivory (considering only PBs, $\times 10^3$) | High | 1 | 1.37 (1.7–2.6) | 0.00 (0.0–1.3) | 100.00 (0.0–0.3) | 0.00 (0.0–8.9) | 0.45 (0.1–0.8) | 0.13 (0.0–0.5) |
| | | 2 | – | | – | – | – | – |
| | Low | 1 | 0.26 (0.0–0.6) | – | 64.00 (0.0–2.6) | 206.00 (0–603) | 0.31 (0.0–0.7) | 0.00 (0.0–0.2) |
| | | 2 | 0.06 (0.0–0.2) | 0.00 (0.0–0.0) | – | – | – | – |

We assume that condensed tannins and phenolics function as plant defenses (Reese et al. 1982; Lindroth and Batzli 1984; Bernays et al. 1989), and at least the former compounds appear to reduce herbivory rates on *Cecropia* in the field (Coley 1986). Because tannins and phenolics contain no nitrogen, their relationship to nitro- gen must be wholly indirect. If more nitrogen is avail- able, it may be paired with carbon to promote faster growth or enhance nitrogen-based defenses, and less car- bon should be directed toward these carbon-based sec- ondary compounds. Almost uniformly (though not in mature leaves of *C. ficifolia*), and in agreement with the C:N balance hypothesis, tannins and total phenolics reached lower levels under HN (Table 4).

Both new growth and MBs have a nitrogen compo- nent (Table 6), and the evidence suggests direct and indi- rect effects of nutrient treatment on these traits. Al- though HN produced faster growth in every case (Table 1), growth increments were always greater in pioneers than in gap species. Moreover, only in pioneers did HN lead to earlier development of myrmecophytism and greater MB production per unit leaf area. (*C. polystachya* could not be tested for a treatment effect on the latter traits.) One possible explanation for these patterns is that comparatively fast-growing pioneers were better able to translate HN levels into rapid rates of carbon fixation and later into MBs. Based on the C:N balance hypothe- sis, we expected the glycogen-rich MBs to increase un- der LN, like other mainly carbon-based defenses. How- ever, their production increased under both HN (here) and high light (Folgarait and Davidson 1994) (see be- low).

For PBs, the direct contribution of nutrients may out- weigh any indirect effects. This suggestion is supported by the enhancement of PB production under HN (in five of six species, Table 3), the absence of strong differences in the magnitudes of treatment effects on gap versus pio- neer species, and the negative effect of light on PB pro- duction in previous experiments (Folgarait and Davidson 1994, and unpublished work). Therefore, the production of PBs supports the predictions of the C:N balance hy- pothesis for N-based defenses. Although exact determi- nations of the nitrogen content of PBs is precluded by the small quantities produced, our results show that PB pro- duction was limited more by the nitrogen than by carbon.

The interpretation of treatment effects is less straight- forward for two other putatively defensive traits. First, if toughness were strictly carbon-based, and dependent on- ly on fiber and cellulose, it should have decreased under HN. However, in all three cases of significant treatment

**Table 7B** Statistical comparisons between close relatives and between nutrient treatments, by Mann-Whitney $U$-tests for data from Table 7A. Results under HN/LN; $n$=7–12 per species X treatment category

| Phenological trait | Comparison | Year | PAIR 1 C. ficifolia (Gap sp.) | C. polystachya (Pioneer sp.) | PAIR 2 C. sp. B (Gap sp.) | C. membranacea (Pioneer sp.) | PAIR 3 C. sp. A (Gap sp.) | C. engleriana (Pioneer sp.) |
|---|---|---|---|---|---|---|---|---|
| Leaf expansion times Partial (days) | Between spp. | 1 | NS/– | | **/** | | NS/NS | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | – | * | NS | NS | NS |
| | | 2 | – | – | – | – | – | – |
| Leaf expansion times Complete (days) | Between spp. | 1 | */– | | **/** | | NS/** | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | – | NS | NS | NS | ** |
| | | 2 | – | – | – | – | – | – |
| Onset of MB production (days) | Between spp. | 1 | NS/– | | */NS | | */NS | |
| | | 2 | –/NS | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | – | NS | NS | NS | NS |
| | | 2 | – | – | – | – | – | – |
| Onset of PB production (days) | Between spp. | 1 | **/– | | NS/NS | | NS/* | |
| | | 2 | –/* | | –/– | | –/– | |
| | HN vs. LN | 1 | NS | – | NS | NS | NS | NS |
| | | 2 | – | – | – | – | – | – |
| Exposure to herbivory (considering only MBs) | Between spp. | 1 | **/– | | NS/NS | | */NS | |
| | | 2 | –/** | | –/– | | –/– | |
| | HN vs. LN | 1 | ** | – | NS | NS | NS | * |
| | | 2 | – | – | – | – | – | – |
| Exposure to herbivory (considering only PBs) | Between spp. | 1 | */– | | NS/* | | NS/** | |
| | | 2 | –/NS | | –/– | | –/– | |
| | HN vs. LN | 1 | ** | – | NS | * | NS | NS |
| | | 2 | – | – | – | – | – | – |

* $P<0.0125$, ** $P<0.0025$, NS=not significant

effects on toughness, mature leaves were tougher under HN, and young leaves showed no pattern at all (Table 5). Therefore, the pattern of variation in toughness with resource availability does not agree with the predictions of the C:N balance hypothesis. Second, leaf expansion times were significantly shorter under HN for only one species (Table 7). This result was surprising since leaf expansion is a growth process, and as such, should have shown a clear nutrient effect. Therefore, treatment effects for leaf expansion do not agree with predictions of the C:N balance hypothesis.

At least two hypotheses have been advanced to explain why some carbon-based defenses have failed to behave according to predictions of the C:N balance hypothesis. Most recently, Fajer et al. (1992) have suggested that environmental triggers might be necessary to activate certain enzymes or hormones for the further allocation of excess carbon into antiherbivore defenses. Earlier, Reichardt et al. (1991) argued that only static secondary metabolites (those with low turnover, like tannins, lignins, and triterpenes) exhibit detectable differences in secondary compound concentrations under different resource treatments. In contrast, dynamic secondary metabolites (e.g., monoterpenes, phenolic glycosides, some sesquiterpenes and phenols), which are produced more continuously, fail to behave as predicted by the C:N balance hypothesis (see Mihaliak et al. 1991). However, a review of the literature (P. Folgarait, unpublished work) suggests that, under similar suites of resource treatments (i.e., the same sets of environmental triggers), production of the same nitrogen- or carbon-based chemical compound (therefore equally dynamic or static), may either increase or decline, and therefore either support or refute predictions of the C:N balance hypothesis (e.g., James 1950; Verpoorte 1991, for alkaloids; Louda and Rodman 1983; Collinge and Louda 1988, for glucosinolates).

Our results suggest a different explanation for the lack of correspondence of our data to the C:N balance hypothesis. All of the data contradicting the hypothesis involve defenses with both a carbon and a nitrogen component. Recognition of this nitrogen contribution might help to explain not only the enhancement of MB production by both higher light and greater nutrient levels, but also the inconsistency of variation in toughness, and cases where toughness increased under HN. Nutrients might contribute directly and substantially to leaf toughness, for example, by enhancing production of pallisade cells. For defenses based on both carbon and nitrogen, tests of the C:N balance hypothesis may be further compounded by competition between various metabolic pathways and functions within the plant. Trade-offs between metabolic pathways leading to the production of alkaloids and phenolics (Prudhomme 1983), cyanogenesis versus condensed tannins (Cooper-Driver et al. 1977),

and support of symbionts versus defenses (Briggs 1990) are all suggestive of metabolic competition for resources. There may also be resource competition between defenses originating from different branches of the same pathway (Chew and Rodman 1979; Baldwin et al. 1987; Berenbaum and Zangerl 1988), as well as trade-offs between defense and growth or reproduction (Palo et al. 1985; Coley 1986; Lindroth et al. 1986; Lilov and Angelova 1987; Lorio 1988; Glyphis and Puttick 1989; Briggs and Schultz 1990; Björkman and Larsson 1991). Also arguing in favor of the multiple resource dependencies of defenses is the suggestion of Björkman et al. (1991) that resin acids in pines contradict predictions of the C:N balance hypothesis because their (C-based) synthesis is limited more by the size of resin ducts (dependent on nitrogen availability) than by the synthesis of resin acids.

## Differences between pioneer and gap species

### The resource availability hypothesis

The resource availability hypothesis of plant defense (Coley et al. 1985) predicts that plants typical of poor-resource environments are slow-growing species with long leaf life spans and high levels of immobile (Coley et al. 1985), non-reclaimable (McKey 1984), or static (Reichardt et al. 1991) defenses. Leaf life spans should be longer, and investment in immobile defenses greater in unfavorable environments, because resource limitation slows the replacement of lost tissues, and intrinsically slow growth reduces the opportunity costs of defense (Coley et al. 1985). In contrast, intrinsically fast-growing species of favorable resource environments (Janzen 1974; McKey et al. 1978; Bryant et al. 1983, 1985, 1991; Coley 1983; Coley et al. 1985; Reichardt et al. 1990; Shure and Wilson 1993) have comparatively short-lived leaves and low levels of mobile (= reclaimable or dynamic) defenses. Carbon-based secondary compounds, toughness, and MBs have been proposed as immobile defenses of *Cecropia*, while PBs have been argued to be mobile defenses (Folgarait and Davidson 1994; and see below).

Data presented here and elsewhere (Davidson and Fisher 1991; Folgarait and Davidson 1994) represent the first support for the resource availability hypothesis in myrmecophytic plants (Table 8). Because our gap species grow regularly with less light than do pioneers, they are predicted to have evolved slower growth rates, longer leaf life spans, and higher levels of immobile defenses. Under both nutrient treatments, growth was significantly slower in gap *Cecropia* than in closely-related pioneers (Table 1). Mean leaf lifetimes of gap species significantly exceeded those of pioneer relatives (Table 1). Compared with their pioneer counterparts, gap species became myrmecophytic significantly earlier in time (Table 2), earlier in ontogeny, and at shorter plant heights (Davidson and Fisher 1991; Folgarait and Davidson 1994).

**Table 8** Traits of pioneer and gap *Cecropia*

| Pioneer spp. | Gap spp. |
| --- | --- |
| Faster growth | Slower growth |
| Delayed onset of myrmecophytism | Accelerated onset of myrmecophytism |
| Invest more in pearl bodies | Invest more in Müllerian bodies |
| More leaves | Fewer leaves |
| Shorter leaf lifetimes | Longer leaf lifetimes |
| Larger leaf sizes | Smaller leaf sizes |
| Less tough leaves | More tough leaves |
| More rapid leaf expansion | Slower leaf expansion |
| Variable amounts of tannins and phenolics | Variable amounts of tannins and phenolics |

The production of MBs by gap plants, accounting for interspecific differences in both leaf area and the energy value of MBs, significantly exceeded that by pioneer relatives (Table 2). Although leaves of gap and pioneer *Cecropia* did not differ consistently in the relative ages at which MB production was curtailed, a greater fraction of the total MB production occurred in young leaves of gap species than in young leaves of their pioneer counterparts (unpublished data). Furthermore, as quantified by both punchameter measurements (Table 5) and leaf specific weights (Folgarait 1993) of mature leaves, toughness, an immobile defense, was higher in gap species than in pioneers. This study also affirms our earlier finding of faster leaf expansion rates and higher PB production rates in pioneers than in gap relatives (Tables 7 and 3; Folgarait and Davidson 1994). In other studies, leaf expansion rates, which may be related to phenological defense of new leaves (Aide and Londoño 1989; Kursar and Coley 1991), were inversely correlated with leaf toughness and may have been determined by it (see also Mooney et al. 1983). Closely related gap and pioneer species did not differ consistently in the length of PB production by individual leaves, though pioneers tended to produce PBs over a longer fraction of their leaf life spans (Folgarait 1993). If maintenance costs are greater and construction costs, lower in PBs than in MBs (Folgarait and Davidson in press), then this result supports the prediction (Coley et al. 1985) that mobile defenses should be most characteristic of fast-growing species, in this case, pioneers (see next two sections).

Interspecific comparisons of chemical defenses do not consistently agree with the predictions of the resource availability hypothesis. As in a previous experiment varying light intensity (Folgarait and Davidson 1994), pioneer and gap species did not differ regularly in levels of carbon-based secondary compounds, and the most evident differences involved greater production of these compounds by the pioneer species of pair 3 (Table 4). As in the light experiment, condensed tannins and phenolics were higher in the gap species than in the pioneer of pair 1. However, this result did not hold for the comparison of LN plants in year 1, perhaps because carbon was also being allocated to MB production by the gap species but not by the pioneer. Gap and pioneer species of pair 2 ex-

hibited indistinguishable levels of these carbon-based compounds in both experiments. In these interspecific comparisons, disparities in the patterns exhibited by the three species pairs may be correlated with differences in the degree of phylogenetic relatedness within the pairs (closeness for pair 2 > pair 3 > pair 1, Davidson, unpublished analyses based on morphological and molecular sequence data). Confirmed as sister species with minimal molecular sequence divergence, species in pair 2 may not have been isolated for sufficient time to allow for divergence in strategies of chemical defense allocation. An older but still recent divergence of the close relatives in pair 3 might explain why interspecific differences correspond more closely to the predictions of the C:N balance hypothesis. Finally, the oldest divergence of species in pair 1 might explain why interspecific differences correspond more closely to the predictions of the resource availability hypothesis.

*Plant defenses in relation to leaf life span*

Why are evolved disparities in the biotic defenses of gap and pioneer species not in accord for MBs and PBs? The economics of investment in different classes of defenses should vary in relation to leaf life spans (McKey 1984; Coley et al. 1985). Investment in carbon-based physical and chemical defenses (e.g., tannins and lignin) may be warranted only in species with comparatively long-lived leaves, whose initially high and "non-reclaimable" synthesis costs can be amortized over longer productive life spans. Other types of defenses (e.g., extrafloral nectar and mobile toxins) have comparatively low synthesis costs but greater maintenance costs that are continuous and constant for a given level of defense. Investment in these defenses is more economical for plants with short-lived leaves, since the resources on which they are based can be shunted easily from aging to younger leaves. The same reclaimable class of defenses should be used for young leaves, which are arguably analogous to short-lived leaves (McKey 1984).

Although McKey (1984) regarded biotic defenses (extrafloral nectar, in his study) as having low synthesis costs but relatively high maintenance costs, our results suggest that the temporal pattern of investment may vary among different types of biotic defenses, albeit in a manner consistent with the underlying assumptions of his model (Folgarait and Davidson 1994). Most of the MB production occurs early in the leaf life span (Fig. 1). Most importantly, however, MB production depends on specialized structures (trichilia) elaborated early in leaf development. This additional feature leads to a greater synthesis cost for MBs than for PBs. Overall then, defensive traits such as tannins, toughness (especially the lignin component), and MBs have comparatively high synthesis costs and low maintenance costs, and are most pronounced in gap species with relatively long-lived leaves. In contrast, with substantial maintenance costs but no dependency on specialized structures, PBs ap-

peared to be mobile defenses (see Folgarait and Davidson 1994), and PB production rates are higher in pioneers with short-lived leaves (Tables 1 and 3). Many other nitrogen-based defenses also behave as mobile defenses (McKey 1979; Coley et al. 1985). In apparent contradiction of McKey's model, MBs appear to be targeted more toward the defense of new leaves than are PBs. However, in accord with the view that biotic defenses are generally directed toward the protection of young leaves (e.g., Janzen 1967; Downhower 1975; McKey 1984; Koptur 1984; Beattie 1985; Fiala et al. 1989; Mackay and Whalen 1991), the production of both MBs and PBs falls off with leaf age (Figs. 1 and 2), and mature leaves are better protected than young leaves by tannins and toughness (Tables 4 and 5).

Defenses of new leaves

Low levels of putative physical and chemical defenses (Tables 4 and 5) and comparatively high foliar nitrogen content (Tables 6) should make young leaves more vulnerable to herbivory. Consistent with this expectation, field measurements show higher herbivory rates on young than mature leaves (D. Davidson and D. Yu, unpublished work). The exposure of new leaves to herbivores depends not only on how rapidly food body production (by MBs or PBs) is initiated after leaf emergence from stipules (i.e., on the length of the vulnerability window), but also on leaf size, or the amount of tissue exposed during this period. If our index is a good estimate of exposure to leaf herbivory during the vulnerability window, and if MBs and PBs elicit equal biotic protection of leaves, pioneer species of pairs 1 and 3 would appear to be less exposed than their gap counterparts to leaf herbivory during the vulnerability window. Selection for early protection of new leaves may be stronger in pioneers, both because the "reproductive value" of carbon exported from the leaf should peak earlier in species with shorter-lived leaves (Harper 1989), and because fast-growing species tend to be defended mainly by mobile defenses (Coley et al. 1985). Early protection of new leaves may also explain the differential persistence of pioneers (D. Davidson and D. Yu, unpublished work) in habitats where more dense and continuous stands of *Cecropia* are correlated with greater herbivore densities (Davidson and Fisher 1991).

In four of the six species, PBs form the earliest biotic protection for new leaves. Species in pair 2 are exceptional in their reliance on MBs, rather than PBs, for the protection of both new (Table 7) and mature leaves (Table 2 versus Table 3). What are the potential costs and benefits of producing MBs at this early stage? The structural costs of trichilia may be the principal factors elevating costs of MBs over those of PBs. If so, and if these costs are incurred early in leaf development in all species, MBs and PBs may not differ greatly in cost thereafter. One potential benefit of young leaf protection by MBs is that it may represent a more effective defense

against specialized herbivores of stems and petioles. Beetles (Chrysomelidae) are the most damaging herbivores of *Cecropia* in south-eastern Peru (Davidson and Fisher 1991). Adults mate on the plants, and females deposit their eggs through the prostoma into swollen *Cecropia* stems. Emerging larvae feed not only on leaves but on petioles; by stripping the lower surfaces of petioles, larvae cause leaves to wilt and dehisce. Located at the juncture of petiole and stem, trichilia are uniquely positioned to attract ant activity to these vulnerable sites, and ants are stationed at productive trichilia on new leaves almost constantly (D. Davidson, unpublished observations). After the establishment of their ant colonies, *Cecropia* appear to be immune to the destructive activities of these beetles (Schupp 1986; D. Yu and D. Davidson, unpublished observations). Interestingly, after myrmecophytism, the stems of species in pair 2 are wider than those of the other four species, and their prostomata are also larger (author's unpublished observations). These traits may have increased the susceptibility of the two *Cecropia* species to egg-laying by the beetles. In turn, these insects may have acted as selective pressures to accelerate the onset of MB production (Table 7). *Cecropia* sp. B and *C. membranacea* are unique too in exhibiting long, erect, urticant stem hairs, which may also have evolved as protection against chrysomelid herbivory.

## Importance of phylogeny

Myrmecophytic pioneer and gap *Cecropia* appear to have diverged repeatedly in ways consistent with resource-based models of plant defensive investment (Davidson and Fisher 1991; Folgarait and Davidson 1994; Table 8). The predicted patterns in defensive investment emerge more clearly when comparisons are made between pairs of closely related species. Had we instead compared all gap species against all pioneers, distinctions would have been found in week of first trichilia produced, but not in six other variables: final height, leaf life span, investment in MBs, PB production rates, tannin concentrations (from protein precipitation assay), and toughness of mature leaves. For four of these six traits, a pattern would have been apparent under one nutrient treatment only, and no pattern would have existed for comparisons of PB production and levels of condensed tannins. In contrast, comparisons between the three sets of close relatives reveal the same pattern of difference between gap and pioneer species for four of these seven traits (and under both nutrient treatments): final height, week of first trichilia, investment in MBs, and toughness. For the remaining variables (except tannin concentrations), differences between the two classes of species are consistent in two of the three species pairs and under both nutrient treatments. Overall then, our results provide a caution against making interspecific comparisons without regard for infrageneric relationships.

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