ENVIRONMENTALLY INDUCED DIFFERENCES IN PLANT TRAITS: CONSEQUENCES FOR SUSCEPTIBILITY TO A LEAF-CUTTER ANT

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Abstract. The effects of different light and soil nutrient conditions on foliar chemistry and acceptability of Inga oerstediana seedlings to leaf-cutter ants were investigated. I determined differences among (1) leaves that were initiated and matured under identical conditions but subsequently were subjected to different light and fertilization treatments and (2) leaves that were initiated and matured under different soil, light, and fertilization treatments. I also assessed the relationship between environmentally induced differences in plant growth and the production of carbon-based secondary chemicals (specifically, tannins). Finally, I determined whether increases in tannins corresponded to decreases in the acceptability of leaves to leaf-cutter ants.

Once a leaf matured the concentrations of tannins did not change as a function of light and soil nutrient conditions despite very large differences in growth rates among the treatments. Leaves that expanded to maturity under different soil, light, and fertilization treatments did differ in tannin chemistry, and treatment differences in plant growth rates appeared to dictate tannin chemistry. When growth was light limited, tannin concentrations were low, but when growth was nutrient limited, tannin concentrations were high.

Only leaves that expanded to maturity under different environmental conditions differed in their acceptability to leaf-cutter ants. Decreases in tannins did not result in parallel increases in acceptability. Despite higher concentrations of tannins, leaf-cutter ants preferred the leaves of seedlings that were grown at 20% light over leaves of seedlings grown at 2% light. However, fertilization increased the acceptability of leaves when seedlings were grown at 20% light, a result consistent with the avoidance of very-high-tannin leaves.

From these results I suggest that spatial variation in resource availability in lowland tropical rainforests would result in differences in tannin chemistry, and in susceptibility to leaf-cutter ants. However, temporal changes in resource availability would only be important to tannin chemistry and susceptibility if the duration of the change were long enough to allow new leaves to be produced.

Key words: Atta cephalotes; attine ants; carbon–nutrient balance hypothesis; condensed tannins; Costa Rica; fertilization; host selection; Inga oerstediana; La Selva; light; nutrients; plant–herbivore interactions; soil quality; tannin production; tropical forests.

INTRODUCTION

Light and soil nutrient conditions in lowland tropical rainforests are highly variable and can result in conspecific seedlings and saplings growing under different conditions (Lugo et al. 1973, Haines 1978, Salick et al. 1983, Clark and Clark 1985, Vitousek and Denslow 1986, 1987, Denslow 1987). For example, light conditions in the forest understory are typically ten to twenty times lower than in treefall gaps (Chazdon and Fetcher 1984). Soil conditions are also variable; the root tip-up zone can have eight times less phosphorus than the bole and crown zones (Vitousek and Denslow 1986), and the abandoned nests of termites and leaf-cutter ants are high in nutrients (Lugo et al. 1973, Haines 1978, Salick et al. 1983). Seedlings and saplings are also likely to experience changes in light and nutrient conditions as they grow (Hartshorn 1978, Chazdon 1986, 1988, Cuevas and Medina 1986, Denslow 1987). Gaps in the forest canopy open and close (Hartshorn 1978), and tree growth may alter the distribution of sunflecks, which are important for understory plant growth (Chazdon 1986). Seasonal fluctuations in litterfall can create periods of higher soil nutrient availability (Cuevas and Medina 1986).

Light and soil nutrient conditions influence the physical, secondary chemical, and nutritional traits of leaves (Chandler and Goosom 1982, Waltz 1984, Larsson et al. 1986, Chapin et al. 1987, Denslow et al. 1987, Mole et al. 1988, Price et al. 1989), and the susceptibility of plants to herbivores and pathogens (Augspurger 1984, Harrison 1987, Núñez-Farfán and Dirzo 1988, Ernest 1989, Marquis and Clark 1989). While it is commonly understood that foliar traits can vary among leaves that were produced under different environmental conditions, there also may be chemical changes within a leaf

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following changes in its environment (Swain 1960, Mooney et al. 1981). Studies simultaneously comparing the influences of environmental changes on the chemistry of (1) leaves that were initiated and matured under different environments and (2) leaves that had matured prior to environmental manipulations, are rare. These chemical differences may be important since quantitative differences in foliar chemistry can alter the susceptibility of plants to their enemies (Larsson et al. 1986, Bryant et al. 1987b).

Tannins and other phenolics are carbon-based metabolites having a putative defensive function; their foliar concentrations are known to vary with light and soil nutrient conditions (Chandler and Goosem 1982, Gershenzon 1984, Waterman et al. 1984, Larsson et al. 1986, Denslow et al. 1987, Mole et al. 1988). It has been hypothesized that environmentally induced differences among plants in the concentrations of phenolics are related to the availability of resources for plant growth (Bryant et al. 1983, Tuomi et al. 1984, Coley et al. 1985, Bryant et al. 1987b, Price et al. 1989). The effects of light and soil nutrient conditions on growth and chemistry are summarized in the carbon-nutrient balance hypothesis (Bryant et al. 1983, Price et al. 1989). Whether a change in light and soil nutrient conditions will alter the concentrations of tannins within a leaf after it has reached maturity appears uncertain (Swain 1960, Baldwin and Schultz 1983, Coley 1988).

Leaf-cutter ants of the genus *Atta* are a dominant herbivore of the neotropics. They are selective in the plant species they harvest (Rockwood 1976, Howard 1987, 1988, Rockwood and Hubbell 1987). They also exhibit preferences for individual plants within a species and for specific leaves of an individual (Cherrett 1968, Rockwood 1976, Nichols-Orians and Schultz 1989, 1990, Howard 1990). I hypothesized that this intraspecific variation in the susceptibility of plants is, in part, due to environmentally induced changes in leaf chemicals, like tannins. Variation in leaf chemistry could affect the growth of the symbiotic fungus of the ants, which is the sole food source of the developing larvae (Quinlan and Cherrett 1979). Although some herbivores appear adapted to tannins (Bernays 1981, Schultz 1989), I hypothesized that tannins would deter leaf-cutter ants because tannins, especially condensed tannins, are strong inhibitors of fungi and their enzymes (Friend 1979, Zuckcr 1983, Seaman 1984, Cherrett et al. 1989).

To determine how light and soil nutrient conditions influence plant growth, foliar chemistry, and susceptibility to leaf-cutter ants, I cultivated seedlings of *Inga oerstediana* Benth. (formerly *Inga edulis* var. *minutula* Schery) (Fabaceae: Mimosoideae), a tropical rainforest tree, under various light and soil nutrient conditions. I chose *I. oerstediana* because *Inga* spp. produce high concentrations of tannins (Koptur 1985). Furthermore, *Inga* spp. relative to other tree species are not readily attacked by leaf-cutter ants (Arkell 1984); this may indicate that tannins dictate the susceptibility of *Inga* spp. to leaf-cutter ants. If so, environmentally induced decreases in tannins could result in increased susceptibility to leaf-cutter ants.

To determine how light and soil conditions influence foliar leaf traits and susceptibility to leaf-cutter ants, I assayed leaves that (1) were initiated and matured under different soil, light, and fertilization treatments and those that (2) were initiated and matured under identical conditions but subsequently were subjected to different light and fertilization treatments. Specifically, I determined how the chemical traits of these two leaf types differed following a reduction in available light (to simulate gap closure) and/or a simultaneous increase in soil nutrient availability (to simulate litterfall). In addition to measurements of foliar nutrients and tannin chemistry, I measured leaf toughness, because toughness can influence the susceptibility of leaves to leaf-cutter ants (Waller 1982, Nichols-Orians and Schultz 1989, 1990).

### The System

#### Study site

The study was conducted from January to July 1987 at the Organization for Tropical Studies' La Selva Biological Station (10°26' N, 83°59' W) in the Atlantic lowland near Puerto Viejo de Sarapiqui, Costa Rica. This forest is a tropical wet–very wet premontane forest (Holdridge et al. 1971), and receives a mean annual rainfall of 4000 mm (Hartshorn 1983), with a short dry season from late January to April (La Selva Biological Station, unpublished records).

Light conditions in the forest understory are typically 1–2% full sunlight, and 20% light is common in treefall gaps (Chazdon and Fetcher 1984). Soil conditions also are highly variable within La Selva. There are two major soil types, nutrient-rich alluvial and nutrient-poor residual soils (Vitousek and Denslow 1987), and even within these soils, conditions are variable (Vitousek and Denslow 1986).

#### Plant species

*I. oerstediana* is a canopy tree that is attacked naturally by leaf-cutter ants at La Selva (C. M. Nichols-Orians, personal observation), produces large concentrations of tannins (Nichols-Orians and Schultz 1990), and is abundant as seedlings within the La Selva property. Previous work with this plant species suggests that the condensed tannins in *I. oerstediana* can deter leaf-cutter ants (Nichols-Orians and Schultz 1990). Thus far surveys indicate that tannins are the only secondary compounds in several *Inga* species (Koptur 1985).

#### Leaf-cutter ants

Colonies of the leaf-cutter ant, *Atta cephalotes* (L.), are common within La Selva, and workers forage in
environments of varying light and soil nutrient availability. Chemical and physical properties of leaves, which may vary quantitatively among environments, can affect leaf selection by *A. cephalotes* in at least five ways. First, before ant workers inoculate the harvested leaf fragments with fungus, they deposit fecal fluid containing previously ingested fungal enzymes, mostly pectinases and proteases, onto the leaf fragments to initiate leaf digestion and enhance subsequent fungal growth (Boyd and Martin 1975, Martin et al. 1975). Poor in vitro fungal growth has been attributed to the absence of these enzymes (Mudd and Bateman 1978). Therefore secondary chemicals, like tannins, that inhibit fungal enzyme activity, could cause leaves to be rejected by *Atta* foragers. Second, some secondary chemicals are directly fungicidal and appear to cause leaves to be rejected (Hubbell et al. 1983). Third, workers appear to obtain a large portion of their metabolic needs by ingesting plant sap (Quinlan and Cherrett 1979). Therefore secondary leaf chemicals in the sap could also cause leaves to be rejected or accepted (Stradling 1978, Howard et al. 1988). Fourth, leaf-cutters may select leaves high in nutrients (Berish 1986) because they would increase the quality of sap and/or enhance the growth of the fungus. Finally, leaf toughness may also deter leaf-cutter ant foragers. High leaf toughness may prevent the acquisition of chemically acceptable leaf material (Waller 1982, Nichols-Orians and Schultz 1989, 1990).

**METHODS**

**Plants**

All leaf material was collected from *I. oerstediana* seedlings cultivated in shadehouses. In March of 1987 I collected seedlings (≈20–30 cm tall), beneath three isolated mother trees growing in alluvial soil, and planted them into a moist sand bench to promote root growth. The seedlings were subsequently transplanted into pots containing either nutrient-rich recent alluvial soil or nutrient-poor residual soil (84 seedlings were potted in alluvial and 84 in residual soils). The nutrient-poor residual soil is especially deficient in phosphorus (Vitousek and Denslow 1987), and phosphorus is known to influence the foliar concentrations of tannins and other phenolics (Gershenzon 1984). I mixed soil from three locations to ensure a general representation of each soil type.

Initially, seedlings planted in each soil type were kept in a shadehouse that allowed 20% of full sunlight (20% light) (Fig. 1). After 13 wk half were moved to a shadehouse allowing only 2% light, and grown for an additional 7 wk. At the time of light manipulation I initiated fertilization treatments for seedlings growing in each soil type within each light treatment. Seedlings received either 20 mL of a complete fertilizer mixture (3.53 g NH₄Cl + 4.35 g KH₂PO₄ + 0.74 g CaCl₂·2H₂O + 2.22 g MgSO₄·7H₂O + 5 mL of a micronutrient solution containing Bo, Zn, Cu, Fe, and Mo in 1 L distilled water, Denslow et al. 1987) or 20 mL of distilled water weekly. Thus, there were eight treatments (Fig. 1) with 21 plants in each. Three mother trees provided seedlings for the experiment, and each treatment included progeny of each mother: 7 or 8 from mother tree no. 1, 8 or 9 from mother no. 2, and 4 or 5 from mother no. 3.

**Soil quality**

At the end of the experiment I randomly collected three soil samples from each of the eight treatments and dried them at room temperature. They were later analyzed for N, P, K, Mg, Ca, and pH. The analysis was done by Pennsylvania State University's Merkle Soils Testing Laboratory (see Dahnke [1980] for methods employed).

**Leaf material**

All leaves were harvested at the end of the seventh week (± 4.5 days) of light and nutrient manipulation. Two leaf types were collected, those that were initiated and matured during the first 13 wk and during the following 7 wk experienced different light and fertilization treatments (PRE leaves), and those that, during the last 7 wk were initiated and matured under one of the eight different light and soil nutrient conditions (POST leaves) (Fig. 1). I defined mature leaves as those that had reached their maximum size and ultimate color (dark green). The number of leaves per leaf type per seedling ranged from two to four. At harvest, leaves were cut at the petiole, frozen within 10 min, and lyophilized (Hagerman 1988) at La Selva before being transported back to Pennsylvania State University for chemical analyses.

Seedlings that were assayed for acceptability to leaf-cutter ants were first transported in their pots to the study colony, where a minimal amount of leaf material was removed for assays with the ants. The remaining leaf material was harvested and preserved as above.

**Non-chemical analyses**

1. **Seedling growth.**—I estimated the leaf area of 21 seedlings per treatment at the time of light and nutrient manipulations and again at the end of the experiment with paper leaflets of ten known sizes. Because leaves of *I. oerstediana* are compound, every leaflet was matched with the most similar paper leaflet, and standard sizes were summed to estimate the total leaf area. The growth of each seedling was calculated to be the percentage of total leaf area produced during the 7 wk of light and nutrient manipulation.

2. **Leaf toughness and water content.**—I determined the leaf toughness of 4–5 leaves per leaf type (PRE and POST leaves) per treatment (4–5 different seedlings) using a penetrometer (Schultz and Baldwin 1982). Three measurements were taken per leaf and averaged, and the values (in grams per unit area) were converted to
kilopascals. The water content of 10 leaves per leaf type per treatment (ten different seedlings) was determined by weighing the leaves before and after lyophilization.

**Foliar chemical analyses**

I did chemical analyses on 10 seedling per treatment. Lyophilized leaf samples were ground in a cyclone sample mill (UDY Corporation, Fort Collins, Colorado). To measure tannin chemistry, ≈300 mg leaf powder was weighed, washed with ether, and extracted with 70% acetone for 3 h in a 40°C water bath. Acetone was removed under reduced pressure and all extracts were diluted to 10 mL with distilled water. Extracts were analyzed for total phenolics (Folin-Denis assay, Swain and Hillis 1959), proanthocyanidin condensed tannins (butanol/HCl method, Bate-Smith 1977), leucoanthocyanin condensed tannins (vanillin method, Broadhurst and Jones 1978, Butler et al. 1982), and protein binding capacity using hemoglobin as the substrate (Schultz et al. 1981). Concentrations are expressed as the percentage of tannic acid (Sigma, Lot no. 11F-0559) equivalents per milligram of dry mass for total phenolics and protein binding capacity, and as the percentage of wattle tannin (*Acacia* sp., from Leon Monnier, Inc., Peabody, Massachusetts, USA) equivalents per milligram of dry mass for condensed tannins (leucoanthocyanins and proanthocyanidins).

To estimate relative protein concentration, I extracted ≈20 mg of leaf powder in 10 mL of 0.1 mol/L NaOH for 2 h in a boiling water bath. This extraction technique is designed to measure the concentration of protein in leaves high in tannin (Jones et al. 1989). Protein was measured as the percentage of bovine serum albumin equivalents per milligram of dry mass (%BSAE) with the Coomassie blue reagent (Bio-Rad) (Snyder and Desborough 1978, Compton and Jones 1985).

The non-structural carbohydrate concentration was determined by extracting 20 mg of freeze-dried leaf powder three times with 3 mL of 80% methanol. The supernatant was diluted to 10 mL and the concentration determined by using the phenol–sulfuric acid method (Southgate 1976). Non-structural carbohydrate concentration is expressed as the percentage of glucose equivalents per milligram of dry mass.

The amount of leaf powder available for chemical analyses was limited, especially for unfertilized seedlings growing in residual soil. Therefore I had to combine the leaf powder from two or three seedlings of the same mother, within a treatment, to obtain chemical measures for the phenolic traits and protein concentration. (Measures of leaf toughness, water content, and non-structural carbohydrate were obtained from single individuals). When combining was necessary, equal amounts of leaf powder from each seedling were used. Combining samples for some treatments, but not for others, can violate the assumption of constant variance in an analysis of variance. I resolved this problem by employing a weighted analysis (Neter et al. 1985: 167), in which each chemical measure was weighted by the number of seedlings that contributed to the mean. (Details of the particular models employed are presented below in Statistical analyses.)

**Bioassays**

Differential acceptability of seedlings to *A. cephalotes* was determined by monitoring the removal of leaf discs by foraging ants (the “pickup” assay) (Howard 1987, Nichols-Orians and Schultz 1990). Previous studies have employed a “cutting” assay as well to determine if leaf toughness alters overall susceptibility (Howard 1988, Nichols-Orians and Schultz 1990). I have found that larger differences in toughness among mature leaves than those measured here do not alter the susceptibility of leaves (C. M. Nichols-Orians, unpublished data). Therefore, I did not employ the cutting assay.

In the pickup assay two leaf discs of a given leaf type were produced with a standard paper punch and placed with two discs of a highly preferred control, *Hamelia patens* (Rubiaceae), beside active trails of *A. cephalotes*.

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**Fig. 1.** Schematic diagram of treatments (n = 8) applied to *Inga oerstediana* seedlings before exposure to *Atta cephalotes*. F = fertilized, NF = not fertilized.
The use of *H. patens* as a control yields very consistent results (Nichols-Orians and Schultz 1990, Nichols-Orians 1991). When one disc was removed it was replaced by a disc of the same type. The chemical acceptability of each leaf type was expressed as the number of test leaf discs removed when 20 control discs had been removed, averaged over two replicate trials.

All assays were completed in a 9-d period in July 1987. Treatments were tested at random, but both PRE and POST leaves of each plant were tested on the same day. I conducted pickup assays on a single colony, using a subset of plants (*N* = 22), in order to conserve leaf material for subsequent chemical analyses. I repeated the assay five times for plants grown at 2% light and six times for those grown at 20% light. I used a single colony because I have found that the relative chemical acceptability of *I. oerstediana* leaves from different light environments is constant among *A. cephalotes* colonies (C. M. Nichols-Orians, unpublished data).

The use of a control indicated the willingness of ants to pick up leaf discs and standardized for fluctuations in ant activity between assay replications. *H. patens* was used as the control because of its high acceptability to leaf-cutter ants and its abundance in the clearing adjacent to the study colony. Only leaf discs from fully expanded mature leaves, located at nodes two to four from the branch terminus, of two different *H. patens* individuals were used as controls. I collected control leaves by cutting the branch beneath the terminal leaf cluster and then placing the branch in a floral pic filled with water. It has been suggested that the acceptability of leaves to herbivores depends upon the particular control species used (Richardson and Whittaker 1982). In this system I have obtained the same preferences when I do not use a control (Nichols-Orians and Schultz 1990, Nichols-Orians 1991).

**STATISTICAL ANALYSES**

Statistical analyses were done using the SYSTAT statistical package (Wilkinson 1988) and SAS (SAS Institute 1982). SAS was used to do weighted analyses. When necessary, Tukey’s HSD was used to compare treatment means.

Preliminary analyses indicated that there were no differences among maternal progenies for leaf traits of PRE or POST leaves from any of the treatments (*P* ≥ .21). Therefore progeny effects were not included in any of the models.

**Soil quality**

Differences in soil nutrient levels were determined across soil types with a three-way fixed-effects ANOVA, with soil, light, and fertilization as the main effects (SOIL/LIGHT/FERT model).

**Seedling growth**

Differences in leaf area at the time of light and nutrient fertilization manipulations were determined with a three-way ANOVA (SOIL/LIGHT/FERT model). Treatment-related differences in growth (the percentage of total leaf area produced during the period of light and fertilization manipulations) were determined using the same ANOVA model following arcsine square-root transformation of the data. Subsequently, the data were split by soil type, so that I could compare the relationship between growth and chemistry more closely (see justification for reduced model below). Here I used two-way ANOVA models (LIGHT/FERT model).

**Leaf traits**

Water content data (percentages) were arcsine square-root transformed. Leaf toughness and all chemical data were square-root transformed because of positive correlations between means and standard deviations.

Two fundamentally different questions were being investigated in this study. First, I determined whether the chemistry of leaves (PRE leaves) of plants growing in two different soil types would change if light and soil nutrient levels were altered (soil type was not altered). Second, I determined whether leaves (POST leaves) produced by plants growing in different soil types, light levels, and soil nutrient environments would differ in chemistry. Consequently, I analyzed the two leaf types separately.

First, I evaluated the full model (SOIL/LIGHT/FERT model) for both PRE and POST leaves. One of the goals of this study was to determine whether differences among treatments in growth rates influenced chemical leaf traits in ways consistent with the carbon–nutrient balance hypothesis. I found that the growth of seedlings in alluvial soil was primarily light limited and the growth of seedlings in residual soil was primarily nutrient limited (see Results below). Therefore, in order to test the carbon–nutrient balance hypothesis, I also separated the POST leaf data by soil type and analyzed them separately (LIGHT/FERT model).

Statistical analyses on PRE and POST leaves were done similarly. Unweighted two- and three-way ANOVA models were used to quantify the effect of soil, light, and fertilization on leaf toughness and water content. Weighted ANCOVA models (two- and three-way) with total leaf area as the covariate were used to compare differences in tannin chemistry and protein concentration. A weighted analysis was used because leaf samples were combined, and total leaf area was used as a covariate because there were often significant correlations between total leaf area and the leaf trait in question. Unweighted ANCOVA models (two- and three-way) with total leaf area as the covariate were used to compare differences in the concentration of non-structural carbohydrate because leaf samples were not combined for this leaf trait.

**Bioassays**

Treatment differences in the number of leaf discs removed during the pickup assays were determined for
TABLE 1. Levels of soil nutrients in each of the four treatments in alluvial and residual soils. N = 3 in all cases. A three-way ANOVA model with soil, light, and fertilization as the main effects was used to determine which effects were significant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil characteristics</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alluvial soil</td>
<td>Nutrient concentration (μg/g)</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>2% light</td>
<td>Fertilized</td>
<td>0.42</td>
<td>26.0</td>
<td>246</td>
<td>1220</td>
<td>204</td>
<td>5.6</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>0.37</td>
<td>20.3</td>
<td>174</td>
<td>1253</td>
<td>204</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>20% light</td>
<td>Fertilized</td>
<td>0.40</td>
<td>27.2</td>
<td>241</td>
<td>1320</td>
<td>220</td>
<td>5.8</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>0.41</td>
<td>16.8</td>
<td>163</td>
<td>1207</td>
<td>200</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

Residual Soil

| 2% light | Fertilized | 0.32 | 15.8 | 216 | 220 | 60 | 4.0 |
| Not fertilized | 0.30 | 4.3 | 126 | 220 | 60 | 4.4 |
| 20% light | Fertilized | 0.34 | 8.0 | 126 | 200 | 44 | 4.4 |
| Not fertilized | 0.33 | 3.7 | 81 | 220 | 48 | 4.5 |

Source of variation

<table>
<thead>
<tr>
<th>Soil (S)</th>
<th>Light (L)</th>
<th>Fert. (F)</th>
<th>S x L</th>
<th>S x F</th>
<th>L x F</th>
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<tr>
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<td>NS</td>
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<tr>
<td>Fert. (F)</td>
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<tr>
<td>S x F</td>
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<td>L x F</td>
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<td>S x L x F</td>
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<td>NS</td>
<td>NS</td>
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*P ≤ .05, **P ≤ .01, NS = not significant.

PRE and POST leaves separately. I was unable to test all leaf types on the same day, so, as a covariate, I included the day of the assay. A three-way ANCOVA model was used. Since there were significant interactions effects between light and fertilization, I split the data between 2% and 20% light and determined the nature of the fertilization effect at each light level.

RESULTS

Soil quality

Alluvial and residual soils were very different in nutrients and pH (Table 1). Fertilization caused increases in soil P and K and a slight decrease in pH for both soils but there were no changes in N, Ca, or Mg. The availability of K was higher under 2% light, perhaps because K was assimilated by the plant less rapidly at low light. Fertilization did not increase soil nutrient levels beyond those found naturally at La Selva (Vitousek and Denslow 1987, P. M. Vitousek, personal communication).

Non-chemical responses

1. Seedling growth.—Prior to light and nutrient manipulations, all seedlings within a given soil type had similar leaf area (Fig. 2A and B; P ≥ .41). Those in the nutrient-rich alluvial soil were larger than those in the nutrient-poor residual soil (P < .05). The treatments differentially influenced subsequent leaf-area production (Fig. 3A and B; P ≤ .05). For seedlings grown in alluvial soil, higher light resulted in more growth (Fig. 3A), indicating that growth was light limited at 2% light. Fertilization only increased growth when seedlings were growing at 20% light, indicating that growth on the nutrient-rich alluvial soil was only nutrient limited at 20% light.

Fertilization of seedlings grown in residual soil enhanced growth under both light regimes. Light itself did not increase growth (P = .14), although there was a tendency for fertilized seedlings to grow more at 20% light. We are able to test this response by splitting our data between 2% and 20% light and determining the nature of the fertilization effect at each light level.

![Figure 2A](image-url)  
![Figure 2B](image-url)  

Fig. 2. Leaf area (X̄ ± 1 se) of Inga oerstediana seedlings when light and fertilization treatments were initiated. (A) alluvial soil; (B) residual soil. Lack of significance of differences was determined using a two-way ANOVA model with light and fertilization as the main effects.
light than at 2% light (Fig. 3B). Clearly, growth in the nutrient-poor residual soil was primarily nutrient limited, even at 2% light.

2. Leaf toughness.—Leaf toughness varied among treatments (Table 2). Toughness was generally higher at 20% light. There were no soil or fertilization effects. For POST leaves (see Methods: Leaf material) there was a significant soil × light × fertilization (S × L × F) interaction. Fertilization reduced leaf toughness at 20% light and increased toughness at 2% light for seedlings growing in alluvial soil.

3. Water content.—The water content of PRE leaves (see Methods: Leaf material) of plants in residual soil was lower than in leaves of plants growing in alluvial soil (Fig. 4A). There was no effect of soil type on leaf water content of POST leaves (Fig. 4B). Reduced light availability resulted in an increase in the water content of PRE and POST leaves (Fig. 4A, B).

**Foliar chemical responses**

**PRE leaves.**—Differences in soil type were primarily responsible for differences in chemical traits of PRE leaves (Table 3). Tannin chemistry (total phenolics, condensed tannins, and protein binding capacity) and nutritional traits were higher in seedlings grown in residual soil (Table 4). A change in light and soil nutrient conditions did not change tannin chemistry or the concentration of protein in PRE leaves (Table 3). However, the concentration of non-structural carbohydrate was lower in the leaves of seedlings grown at 2% light in both soil types.

**POST leaves.**—The chemistry of POST leaves reflected the experimental treatments. Up to 78% of the variance in tannin chemistry was accounted for by the model (Table 3). The main effects (soil type, light, and fertilization) were significant for all tannin measures, as were soil × fertilization effects. Fertilization had a greater affect on seedlings growing in the nutrient-poor

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**Table 2.** Toughness (pressure required to puncture leaves of cultivated *Inga oerstediana*). Data are X ± 1 se; N = 4 or 5. A three-way ANOVA model with soil, light, and fertilization as the main effects was used to determine which effects were significant.

<table>
<thead>
<tr>
<th>Plant environment</th>
<th>Leaf type†</th>
<th>PRE leaves</th>
<th>POST leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alluvial soil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>202.8 ± 20.2</td>
<td>184.8 ± 16.8</td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
<td>190.8 ± 14.3</td>
<td>155.2 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>20% light</td>
<td></td>
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</tr>
<tr>
<td>Fertilized</td>
<td>195.5 ± 7.3</td>
<td>201.2 ± 21.6</td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
<td>252.2 ± 7.3</td>
<td>248.0 ± 16.3</td>
<td></td>
</tr>
<tr>
<td>Residual soil</td>
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</tr>
<tr>
<td>2% light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>178.5 ± 11.4</td>
<td>173.8 ± 9.4</td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
<td>185.9 ± 19.8</td>
<td>199.3 ± 20.1</td>
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<tr>
<td>20% light</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized</td>
<td>210.1 ± 10.8</td>
<td>223.7 ± 17.1</td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
<td>203.8 ± 10.2</td>
<td>244.7 ± 17.7</td>
<td></td>
</tr>
</tbody>
</table>

**Source of variation**

| Soil (S)          | NS | NS |
| Light (L)         | ** | ** |
| Fert. (F)         | NS | NS |
| S × L             | NS | NS |
| S × F             | NS | NS |
| L × F             | NS | NS |
| S × L × F         | NS | NS |

*P < .05, **P < .01, NS = not significant.
†Leaves initiated before (PRE) and after (POST) light conditions were altered by removal of leaf discs by foraging ants (the “pickup” assay). See also Fig. 1.
residual soil (Tables 5 and 6). Light × fertilization effects were marginally significant for three of the four phenolic traits. The two nutritional traits (protein and non-structural carbohydrate) differed in their response to the main effects; protein concentration responded to soil type and fertilization, and non-structural carbohydrate concentration responded to changes in light availability (Table 3).

Leaves produced by seedlings grown in alluvial soil exhibited differences in chemistry depending upon the light and soil nutrient environment, but the effects of light were much greater than the effects of fertilization (Tables 5 and 6). The tannin chemistry and non-structural carbohydrate concentration of leaves produced at 2% light were lower than those produced at 20% light (Table 5). Protein concentration did not differ with light.

Leaves produced by fertilized seedlings in alluvial soil had slightly less condensed tannin; but total phenolics and protein binding capacity did not differ, nor did the concentrations of protein and non-structural carbohydrate (Table 6). With the exception of protein binding capacity, there were no light by fertilization effects (Table 6).

Leaves produced by seedlings grown in residual soil also exhibited significant differences in chemistry depending upon the light and soil nutrient environment. As above, leaves of seedlings in 2% light had lower concentrations of tannins (protein binding capacity did not differ; Table 5). Non-structural carbohydrate was also lower at 2% light, but protein concentration did not differ.

Fertilization of seedlings in residual soil resulted in a significant reduction in the concentrations of tannin (Tables 3 and 6). In fact, they responded to changes in nutrient availability (fertilization) more strongly than did seedlings in alluvial soil (Table 3: significant soil × fertilizer [S × F] interaction). Furthermore, fertilized seedlings at 20% light had concentrations similar to those of unfertilized seedlings at 2% light (Table 5). (Leaves of seedlings in alluvial soil, fertilized or unfertilized, always had higher concentrations at 20% light.) Protein concentration increased with fertili-
Table 4. Phenolic, protein, and non-structural carbohydrate concentrations [\( \bar{X} \pm 1 \text{ se} \)] of *Inga oerstediana* leaves initiated and matured prior to environmental manipulations (PRE leaves) for seedlings grown in alluvial and residual soils.

<table>
<thead>
<tr>
<th>Plant environment</th>
<th>N</th>
<th>VN</th>
<th>PA</th>
<th>TP</th>
<th>PBC</th>
<th>PRT</th>
<th>NSC†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alluvial soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% light</td>
<td>9</td>
<td>12.9 ± 0.30</td>
<td>18.8 ± 0.46</td>
<td>5.7 ± 0.13</td>
<td>4.9 ± 0.08</td>
<td>22.1 ± 0.22</td>
<td>6.4 ± 0.43</td>
</tr>
<tr>
<td>Fertilized</td>
<td>10</td>
<td>12.6 ± 0.18</td>
<td>16.2 ± 0.30</td>
<td>5.3 ± 0.12</td>
<td>4.9 ± 0.06</td>
<td>20.8 ± 0.37</td>
<td>6.9 ± 0.38</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>10</td>
<td>10.8 ± 0.20</td>
<td>14.8 ± 0.36</td>
<td>5.0 ± 0.12</td>
<td>4.6 ± 0.09</td>
<td>22.2 ± 0.29</td>
<td>7.1 ± 0.59</td>
</tr>
<tr>
<td>20% light</td>
<td>9</td>
<td>12.3 ± 0.28</td>
<td>19.8 ± 0.82</td>
<td>5.5 ± 0.12</td>
<td>4.7 ± 0.07</td>
<td>23.1 ± 0.33</td>
<td>7.4 ± 0.53</td>
</tr>
<tr>
<td>Fertilized</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
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<td></td>
</tr>
<tr>
<td><strong>Residual soil</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2% light</td>
<td>5</td>
<td>16.4 ± 2.58</td>
<td>34.0 ± 5.32</td>
<td>6.5 ± 1.12</td>
<td>7.7 ± 0.92</td>
<td>29.2 ± 1.13</td>
<td>7.7 ± 1.17</td>
</tr>
<tr>
<td>Fertilized</td>
<td>6</td>
<td>18.2 ± 2.92</td>
<td>38.0 ± 1.58</td>
<td>7.8 ± 0.51</td>
<td>9.4 ± 0.67</td>
<td>29.9 ± 1.11</td>
<td>8.5 ± 1.68</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>8</td>
<td>17.2 ± 1.80</td>
<td>39.9 ± 4.21</td>
<td>7.2 ± 0.58</td>
<td>8.6 ± 0.68</td>
<td>29.7 ± 1.13</td>
<td>10.3 ± 1.22</td>
</tr>
<tr>
<td>20% light</td>
<td>7</td>
<td>20.8 ± 0.94</td>
<td>46.8 ± 3.20</td>
<td>8.8 ± 0.42</td>
<td>10.8 ± 0.72</td>
<td>28.9 ± 0.85</td>
<td>11.0 ± 0.41</td>
</tr>
<tr>
<td>Fertilized</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
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<td></td>
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</tr>
</tbody>
</table>

* VN = leucoanthocyanins, PA = proanthocyanidins, TP = total phenolics, PBC = protein binding capacity, PRT = protein, NSC = non-structural carbohydrate; BSAE = bovine serum albumin equivalents. Units, for TP and PBC: %tannic acid equivalents/mg dry leaf; for VN and PA: %wattle tannin equivalents/mg dry leaf; for PRT: % BSAE/mg dry leaf; for NSC: %glucose equivalents/mg dry leaf.
† NSC: N = 3 or 4.

Bioassays

Leaf-cutter ants accepted fewer leaf discs from PRE leaves of seedlings grown in residual soil than from leaves of seedlings grown in alluvial soil (Fig. 5A, Table 7). Light, fertilization, and the interaction between light and fertilization had no effect on acceptability (Table 7). However, compared to the POST leaves, PRE leaves were much less acceptable (Fig. 5A vs. 5B) \((P < .01)\), indicating a distinct effect of leaf age on acceptability to *A. cephalotes*.

Soil type and light treatment affected the acceptability of POST leaf discs (Table 7, Fig. 5B). *A. cephalotes* preferred leaves from seedlings grown in alluvial soils over those in residual soils, and those grown at 20% light over those at 2% light (Fig. 5B). The effects of fertilization, however, were not independent of the effects of light (Table 7: significant L \(\times\) F). There were no differences in acceptability of fertilized and unfertilized seedlings at 2% light (Fig. 6A), but at 20% light, fertilized seedlings were selected over unfertilized ones (Fig. 6B; \(P < .05\)).

**DISCUSSION**

The results of these experiments suggest that habitats differing in light and soil nutrient availability, or changes

Table 5. Phenolic, protein, and non-structural carbohydrate concentrations [\( \bar{X} \pm 1 \text{ se} \)] of *Inga oerstediana* leaves initiated and matured after light conditions were altered (POST leaves) for plants grown on alluvial and residual soils.

<table>
<thead>
<tr>
<th>Plant environment</th>
<th>N</th>
<th>VN</th>
<th>PA</th>
<th>TP</th>
<th>PBC</th>
<th>PRT</th>
<th>NSC†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alluvial soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2% light</td>
<td>8</td>
<td>8.2 ± 0.22</td>
<td>6.0 ± 0.32</td>
<td>3.5 ± 0.06</td>
<td>3.7 ± 0.10</td>
<td>25.0 ± 0.50</td>
<td>5.4 ± 0.46</td>
</tr>
<tr>
<td>Fertilized</td>
<td>8</td>
<td>8.4 ± 0.18</td>
<td>6.2 ± 0.22</td>
<td>3.2 ± 0.11</td>
<td>3.1 ± 0.12</td>
<td>21.0 ± 0.35</td>
<td>4.7 ± 0.33</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>10</td>
<td>12.2 ± 0.16</td>
<td>14.2 ± 0.32</td>
<td>4.9 ± 0.06</td>
<td>4.5 ± 0.07</td>
<td>25.4 ± 0.19</td>
<td>7.4 ± 0.66</td>
</tr>
<tr>
<td>20% light</td>
<td>9</td>
<td>13.4 ± 0.25</td>
<td>19.4 ± 1.05</td>
<td>5.2 ± 0.13</td>
<td>4.7 ± 0.06</td>
<td>24.7 ± 0.48</td>
<td>7.7 ± 0.43</td>
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<td>Fertilized</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
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<td></td>
</tr>
<tr>
<td><strong>Residual soil</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2% light</td>
<td>5</td>
<td>8.8 ± 0.92</td>
<td>12.8 ± 1.90</td>
<td>3.3 ± 0.37</td>
<td>4.4 ± 0.55</td>
<td>28.7 ± 1.42</td>
<td>4.9 ± 0.67</td>
</tr>
<tr>
<td>Fertilized</td>
<td>3</td>
<td>10.8 ± 1.43</td>
<td>17.7 ± 2.68</td>
<td>4.2 ± 0.55</td>
<td>7.1 ± 0.27</td>
<td>27.2 ± 0.83</td>
<td>6.2 ± 1.54</td>
</tr>
<tr>
<td>Not fertilized</td>
<td>8</td>
<td>10.1 ± 0.68</td>
<td>16.9 ± 1.48</td>
<td>4.1 ± 0.26</td>
<td>5.0 ± 0.46</td>
<td>30.1 ± 1.41</td>
<td>7.1 ± 0.84</td>
</tr>
<tr>
<td>20% light</td>
<td>4</td>
<td>16.6 ± 1.10</td>
<td>28.9 ± 1.83</td>
<td>6.4 ± 0.56</td>
<td>7.8 ± 0.45</td>
<td>25.5 ± 0.56</td>
<td>9.2 ± 0.67</td>
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<td>Fertilized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not fertilized</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* VN = leucoanthocyanins, PA = proanthocyanidins, TP = total phenolics, PBC = protein binding capacity, PRT = protein, NSC = non-structural carbohydrate; BSAE = bovine serum albumin equivalents. Units, for TP and PBC: %tannic acid equivalents/mg dry leaf; for VN and PA: %wattle tannin equivalents/mg dry leaf; for PRT: % BSAE/mg dry leaf; for NSC: %glucose equivalents/mg dry leaf.
† NSC: N = 2, 3, or 4.
Table 6. Significance of effects for the chemistry of *Inga oerstediana* leaves initiated and matured after light conditions were altered (POST leaves) in an analysis of covariance model with total leaf area (Total) as the covariate. Alluvial and residual soils analyzed separately. All values were square-root transformed before statistical analyses.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Chemical leaf trait†</th>
<th>VN</th>
<th>PA</th>
<th>TP</th>
<th>PBC</th>
<th>PRT</th>
<th>NSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alluvial soil</td>
<td>Light</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Fertilization</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>L × F</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Residual soil</td>
<td>Light</td>
<td>*</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Fertilization</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>L × F</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* P < .05, ** P < .01, NS = not significant.
† VN = leucoanthocyanins, PA = proanthocyanidins, TP = total phenolics, PBC = protein binding capacity, PRT = protein, NSC = non-structural carbohydrate.

in resource availability via changes in canopy structure and/or simultaneous fluctuations in soil nutrient levels, could affect the growth and foliar chemistry of *I. oerstediana* seedlings, and their subsequent acceptability to leaf-cutter ants. Based on the carbon–nutrient balance hypothesis (C–N hypothesis), I predicted that environmentally induced changes in growth would dictate the production of tannins (phenolics) and subsequent selection by leaf-cutter ants. However, I found the relationships among environment, chemistry, and acceptability more complicated than predicted.

**Seedling growth**

According to the C–N hypothesis, the concentration of carbon-based secondary chemicals, such as tannins, should depend upon whether plant growth is limited by light or nutrients (Bryant et al. 1983, Price et al. 1989). The C–N hypothesis leads to the prediction that tannins will accumulate in leaves when growth is more limited by nutrient availability than by photosynthetic activity. With this scenario, high light and low nutrient availability should lead to higher concentrations of phenolics than in environments where nutrients are readily available. Conversely, when plants are shaded (i.e., under carbon stress), nutrients should accumulate and the concentrations of phenolics should decline.

In my experiments the growth of seedlings was often limited by light or nutrients. Growth in the nutrient-rich alluvial soil was primarily light limited; there were no differences in growth between fertilized and unfertilized plants at 2% light (Fig. 2B). However fertilization of plants grown at 20% light caused an increase in growth, suggesting that plants at 20% light were nutrient limited (Fig. 3A). Growth by plants in residual soil appeared mostly nutrient limited because growth increased dramatically when fertilized at both light lev-

els (Fig. 3B). In fact, light level had no significant effect on growth, although fertilized seedlings at 20% light tended to grow more than those at 2% light. Because, in these experiments, plant growth was limited by light and/or nutrients, I predicted that tannin chemistry would vary as well.

**Growth and chemistry**

Although light manipulation and fertilization produced differences in growth, tannin levels in PRE leaves of seedlings growing in nutrient-rich alluvial or nutri-

![Fig. 5](image-url)
TABLE 7. Significance of effects for the acceptability to the leaf-cutter ant *Atta cephalotes* of *Inga oerstediana* leaves initiated and matured before (PRE leaves) and after (POST leaves) light and nutrient conditions were altered, as measured by removal of leaf discs by foraging ants (the "pickup" assay) in an analysis of covariance model with the day of assay as the covariate. Leaf types were analyzed separately. All values were square-root transformed before statistical analyses.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>PRE leaves</th>
<th>POST leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (L)</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Soil (S)</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Fertilization (F)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L \times S interaction</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>L \times F interaction</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>S \times F interaction</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L \times S \times F interaction</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Day (covariate)</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

* P < .05, ** P < .01, NS = not significant.

Researchers measure nitrogen concentration, not protein or carbohydrate concentration.

The response of seedlings grown in residual soil to light manipulation was similar to that of those grown in alluvial soil (Table 3: nonsignificant S \times L). Again a reduction in light caused a decrease in all leaf traits except protein binding capacity and protein concentration (Table 5). Fertilization, however, affected tannin chemistry of seedlings in residual soil differently than those in alluvial soil (Table 3: significant S \times F).

![Graph A](image1.png)

A) 2% light

![Graph B](image2.png)

B) 20% light

**Fig. 6.** The acceptability (\(\bar{x}\) and 1 se) to the leaf-cutter ant *Atta cephalotes* of *Inga oerstediana* leaves initiated and matured after (POST leaves) light and nutrient conditions were altered, as measured by removal of leaf discs by foraging ants (the "pickup" assay). Distinction between PRE and POST leaves is shown in Fig. 1. (A) 2% light; (B) 20% light. Significance of differences was determined using a two-way ANOVA model with light and fertilization as the main effects. Bars with different letters are significantly different at P < .05 by Tukey’s HSD.
Fertilization caused a much stronger decrease in tannin chemistry (Table 6). This result is as expected because phosphorous levels are low in residual soil (Table 1; Vitousek and Denslow 1987). Gershenzon (1984) found that when phosphorous availability is limited the concentrations of tannins and other phenolics are higher. The strong reduction in tannins in leaves of fertilized seedlings is also consistent with the C-N hypothesis, since growth was nutrient limited for these plants (Fig. 3B). However, the concentrations of condensed tannins increased under higher light conditions, especially in unfertilized seedlings (Tables 5 and 6), even though growth was not light limited (Fig. 3B). This suggests that part of the effect of light on the production of tannins is independent of seedling growth, and therefore is not predicted by the C-N hypothesis. Enzymes, like phenylalanine ammonia-lyase, which are involved in the synthesis of phenolics, are light-activated (Schröder et al. 1976, Schütte 1978, Schopfer 1984). Perhaps the very high concentrations of tannins found in leaves of seedlings grown at high light are in part due to this light activation.

Others studying the effects of light and nutrient availability on the concentrations of carbon-based secondary chemicals in the leaves of tropical plants have observed similar environmentally induced differences in foliar chemistry (Chandler and Goosen 1982, Waterman et al. 1984, Mole et al. 1988). My findings suggest that these patterns could have been related, in part, to resource availability and growth limitation (e.g., Bryant et al. 1983, Price et al. 1989). When growth was limited by nutrients, nutrient-enhanced growth resulted in lower concentrations of tannins, but when growth was limited by light (Price et al. 1989), light-enhanced growth resulted in higher concentrations of tannins (Table 5). Thus there is a trade-off between growth and the production of phenolics when growth is nutrient limited but not when growth is light limited.

Implications.—To predict differences in the concentrations of carbon-based secondary chemicals among seedlings growing in different light and nutrient environments, it may be important first to identify whether growth is light or nutrient limited. It is not sufficient to assume that any seedling growing in low light (e.g., the forest understory) will have lower concentrations of phenolics than those growing in high light (e.g., treefall gaps); nutrient availability also influences the concentrations of phenolics. In this study, leaves of seedlings growing in residual soil at 2% light had higher concentrations of condensed tannins than those in alluvial soil at 2% or 20% light. Furthermore, when seedlings growing in residual soil at 20% light were fertilized, the resulting concentrations of tannins were similar to those found in unfertilized seedlings at 2% light (Table 5).

I suggest that spatial heterogeneity in soil nutrient and light conditions within a tropical forest (Vitousek and Denslow 1986, 1987; Denslow 1987) would be a major factor contributing to differences in foliar chemistry among I. oerstediana seedlings. Temporal heterogeneity in resource availability (Chazdon and Fitcher 1984, Cuevas and Medina 1986) only would be important to the tannin chemistry of leaves if the time scale were long enough to allow for the production of new (POST) leaves.

Seedling acceptability to leaf-cutter ants

Changing the light and soil nutrient conditions did not result in differences in the acceptability of PRE leaves to A. cephalotes (Table 7), despite changes in water content and slight changes in non-structural carbohydrate. Water content was lower at 20% light (Fig. 4A), and the concentration of non-structural carbohydrate was slightly higher at 20% light (Tables 3 and 4). The magnitude and type of changes appear to have been insufficient to cause a change in leaf acceptability.

Leaves produced under different soil, light, and nutrient conditions (POST leaves) showed large differences in chemistry, and also differed in their acceptability to A. cephalotes (Table 7, Fig. 5). A. cephalotes preferred sun leaves (20% light) over shade leaves (2% light) (Fig. 5B) and, at 2% light, leaves of seedlings in alluvial over residual soils (Fig. 6A). Fertilization did not increase the acceptability of leaves of seedlings growing in 2% light but did increase the acceptability of those growing in 20% light (Fig. 6).

Implications.—My results suggest that leaf-cutters would concentrate their foraging efforts in gaps, because they prefer sun leaves. Indeed, during the course of my experiments, I observed more damage to I. oerstediana seedlings that were growing in gaps or in full sun. Leaf-cutter ants also primarily attack Piper arietanum (Piperaceae) growing in gaps (R. Marquis, personal communication). The higher susceptibility of plants in gap disturbances is consistent with observations that leaf-cutter ants do more damage in early successional habitats (Jonkman 1977, Blanton and Ewel 1985), and are generally located close to gaps (Jaffe and Vilela 1989). Studies of other systems have shown that plants growing under higher light conditions often experience more damage than plants growing in the understory (Harrison 1987, Ernest 1989, R. Marquis, unpublished data; but see Coley 1983).

I would expect that plants growing in treefall gaps would be most susceptible to A. cephalotes when growing in nutrient-rich soils. For example, plants growing in the crown zone of a gap may be more susceptible than those in the root tip-up zone because the root tip-up zone has about eight times less nutrients (Vitousek and Denslow 1986). Work with Piper arietanum indicates that fertilized plants in gaps are more susceptible to leaf-cutter ants than unfertilized plants (R. Marquis, personal communication). However, in another system Marquis and Clark (1989) did not detect an increase in herbivore damage to Hampea appendiculata when they were fertilized.
It appears that small differences in nutrient availability would not influence the probability that understory seedlings would be attacked (Fig. 6A). Auerbach and Strong (1981) fertilized Heliconia, common understory plants at La Selva, and also found no increase in their susceptibility to hispine beetles. From these results I suggest that temporal increases in nutrient availability would enhance only the susceptibility of plants growing in gaps, provided that the duration of the increase were long enough to promote the production of new leaves.

Role of chemistry

Because condensed tannins appear to deter leaf-cutter ants and their fungus (Cherrett et al. 1989, Nichols-Orians and Schultz 1990), I hypothesized that increases in the concentrations of tannins would lead to parallel decreases in the susceptibility of seedlings. However, despite light-induced increases in the concentrations of condensed tannins (Table 5), ants preferred leaves of seedlings grown at 20% of full sunlight over those grown at 2% light (Table 7). This suggests that condensed tannins do not uniformly deter A. cephalotes. The preference for sun leaves over shade leaves may have been due to an increase in nutrient availability (Nichols-Orians 1991; Table 5). However, the preference for POST leaves over PRE leaves and leaves of fertilized over unfertilized seedlings (at 20% light) suggests that tannins may deter leaf-cutter ants at high concentrations. PRE leaves and leaves of unfertilized seedlings had much higher concentrations of condensed tannins (Tables 4 and 5).

Implications. — In some systems environmentally induced increases in the concentrations of carbon-based secondary chemicals have been consistent with the C-N hypothesis, and have been correlated with reduced acceptability of plants to herbivores (Larsson et al. 1986, Bryant et al. 1987a, b). My results indicate that such increases do not result in parallel decreases in acceptability to an attine ant; only high concentrations of tannins appear to deter leaf-cutter ants. In a separate study of herbivory at La Selva, Waltz (1984) also found that tannins deterred three orthopteran herbivores only when at high concentrations; at low tannin concentrations herbivory was most correlated with available nitrogen. Thus leaf selection by herbivores may involve a trade-off between maximizing the harvesting of nutrients while minimizing the harvesting of tannins.

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