The *Azteca–Cecropia* Association: Are Ants Always Necessary for Their Host Plants?[1]

ABSTRACT

We assessed the effects of *Azteca alfari* presence on herbivory and growth of saplings for two Amazonian *Cecropia* species. For both species, rates of herbivory were low and did not differ between ant-removed and ant-maintained plants. Plant growth, measured over six months, was also similar among treatments. This is the first experiment to show that in its native mainland habitat, *Cecropia* may suffer low incidence of attack by insect herbivores in the absence of associated ants.

RESUMO

Nós testamos os efeitos da presença de *Azteca alfari* sobre a herbivoria e crescimento em arvoretas de duas espécies de *Cecropia*, na Amazônia. Para ambas espécies, as taxas de herbivoria foram baixas e não diferiram entre plantas que tiveram as formigas removidas ou mantidas. O crescimento das plantas, medido após seis meses, também foi similar entre os tratamentos. Este é o primeiro experimento que mostra que em seu hábitat natural, *Cecropia* pode sofrer baixa incidência de ataque por herbívoros na ausência das formigas associadas.

ONE OF THE MOST CONSPICUOUS ANT–PLANT ASSOCIATIONS IN THE NEOTROPICS is that between *Azteca* and *Cecropia*. Most members of the plant genus *Cecropia* are myrmecophytic and display obvious adaptations to house ants, including large hollow internodes, thin spots in the internode wall via which ants gain access, and a modified petiole base (trichilium) from which food bodies are continuously produced and harvested by associated ants (Bailey 1922, Wheeler 1942, Rickson 1971, Berg 1978). *Cecropia* trees, in turn, may benefit from their *Azteca* in several ways. *Azteca* often chews and kills the shoot tips and tendrils of vines that attempt to climb *Cecropia* trunks, thus acting as an allelopathic agent of the plant (Janzen 1969, Schupp 1986). In addition, these ants may provide supplemental nutrients to *Cecropia* trees (Sagers *et al.* 2000, *cf.* Putz & Holbrook 1988). There has been a lack of consensus, however, on

whether or not *Azteca* effectively protect their *Cecropia* host plants against herbivores. One study in Ecuador showed that damage by Coleoptera, but not homoptera (Hemiptera) and cecidomyiid gall flies, was lower on ant-occupied plants (Schupp 1986). Ant-occupied *Cecropia* were also found to suffer fewer attacks from leaf-cutter ants than unoccupied plants (Jolivet 1990, Vasconcelos & Casimiro 1997); however, contrasting results have been found (see counter examples by Wheeler 1942 and Andrade & Carauta 1982), and as Janzen (1969) has pointed out, "it is commonplace to encounter occupied *Cecropia* with foliage heavily eaten by insects, and healthy unoccupied *Cecropia* are apparently not rare in some habitats."

The genus *Cecropia* ranges from Mexico to southern Brazil, where it occupies almost every terrestrial habitat (Berg 1978). In spite of the ubiquitous nature and diversity of *Cecropia* in the Neotropical region, surprisingly few studies have used an experimental approach to investigate the relationship between these plants and their associated ants. Here, we present the results of an experiment designed to determine the influence of *Azteca alfari* on herbivory and growth of two Central Amazonian *Cecropia* species: *Cecropia concolor* and *C. purpurascens*.

Observations and experiments were performed in an abandoned pasture area located *ca* 80 km north of Manaus, in Brazilian Amazonia (2°25′S, 59°50′W). This area was used as pasture for cattle grazing for about ten years, and during this period it was burned every two to three years. At the time of our study (about nine years after pasture abandonment), many pioneer trees had established, forming an uneven and broken canopy up to 10 m high. The vegetation was dominated by *Vismia* spp. (Clusiaceae) and *Cecropia* spp. (Cecropiaceae; Williamson *et al.* 1998). The average temperature in Manaus is 26.7°C and the mean annual precipitation is 2100 mm, with a distinct drier season between June and October. The soils are classified as Yellow Latosols, which typically have a high clay content and are poor in nutrients (Chauvel 1983). For further information about the study site, see Bierregaard and Gascon (2001).

Forty individuals of *C. concolor* and 37 of *C. purpurascens* were located and marked in January 1999. Most of these were found along the margins of a dirt road. Given the inherent difficulties of working with taller plants, only saplings between 1 and 2.5 m tall were selected. All marked plants were inhabited by *A. alfari*. Ant occupation was determined by vigorously shaking the plant stem. In occupied plants, ants swarmed out of the plant stem. Ants were experimentally removed from about half of the plants (*C. concolor* = 21 plants; *C. purpurascens* = 17 plants), whereas the remaining plants served as controls (ant-maintained). Assignment of marked plants into the control and experimental categories was made randomly. To remove ants, we used a syringe to apply a few milliliters of a contact insecticide (Dimmy®, Serv-San, Cajamar, SP, Brazil) into every plant internode that was colonized by ants (*i.e.*, those having an open prostoma). We checked the plants at regular intervals, and in case of recolonization, insecticide was applied again. The contact insecticide used here has a very short residual time and thus does not affect the colonization of herbivores in ant-removed plants, as has been already shown in previous ant-removal experiments (Vasconcelos 1991, Izzo & Vasconcelos 2002).

For a period of six consecutive months (February–July 1999), we monitored all plants at 15-day intervals. On each observation, we measured the height of each plant (height being measured as the length of the trunk, from the ground to the apex of the terminal internode), individually marked all new leaves produced, and recorded which, if any, of the previously marked leaves abscised. For each plant individual, the rate of leaf emergence and leaf mortality were calculated, respectively, as the number of leaves produced or lost over the course of the experiment. Leaf turnover rates were calculated as: (mortality rate + emergence rate)/2 (Phillips & Gentry 1994).

At 15-day intervals, we measured the total area and the area damaged of all leaves emerging after the beginning of the experiment. Many of these leaves (*ca* 50%) were already fully or almost fully expanded when first marked and measured, given the rapid leaf expansion rates of *Cecropia* leaves (less than two weeks for some pioneer species; Folgarait & Davidson 1994). The remaining leaves experienced a growth increment of up to 1500 percent. For these latter, we were unable to discriminate between missing tissue resulting directly from herbivore consumption and loss due to leaf expansion around damaged areas; however, as pointed out earlier (Schupp 1986), this distinction is unnecessary since the measurements show that the total loss of potential photosynthetic surface was ultimately due to herbivore feeding.

Herbivory rates were calculated as the difference in the percentage of damage between the initial leaf measurement and the final measurement, divided by the time interval (in days) between these measure-

ments. For determination of the damaged area, we used a transparent plastic grid (precision of 1 cm²). For determination of the total leaf area, we used the following equations, derived after measuring the maximum leaf width, maximum leaf length, and total area of 25 randomly collected *C. concolor* leaves and 21 *C. purpurascens* leaves, which included both young, developing leaves, as well as fully developed leaves. For *C. concolor*, leaf area $= -95.84 + 0.728*$(leaf width*leaf length) ($R^2 = 0.984$, $N = 25$, $P < 0.001$), whereas for *C. purpurascens*, leaf area $= 6.26 + 0.609*$(leaf width*leaf length) ($R^2 = 0.993$, $N = 21$, $P < 0.001$).

Treatment differences in herbivory rate, leaf emergence, mortality, and turnover were assessed using the Mann–Whitney U-test, as the data did not meet the assumption of normality. For analysis of differences in relative height increment (calculated as the difference between initial and final height, divided by the initial height), we used a t-test. All analyses were performed using Systat 8.0 (Wilkinson 1998).

Of a total 77 *Cecropia* saplings, only 49 were alive at the end of the experiment. Most (20 out of 28) of the dead saplings died due to attack by tapirs (*Tapirus terrestris*). *Azteca alfari* is ineffective against these animals because tapirs first knock down the plant to eat its young leaves and buds. There was no difference in plant mortality among treatments (*C. concolor*, nine treatment and ten control plants dead; *C. purpurascens*, five and four plants, respectively).

Herbivory rates were low and did not differ between control and experimental plants either for *C. concolor* ($U = 135$, $P = 0.40$) or *C. purpurascens* ($U = 105$, $P = 0.19$; Table 1). Since many *Cecropia* leaves did experience little or no growth over the course of the experiment—and therefore were presumably less dependent on ants for their defense—we further analyzed our data considering only leaves with a growth increment of over 20 percent. Here again, no treatment differences were detected either for *C. concolor* ($U = 87$, $P = 0.08$) or *C. purpurascens* ($U = 105$, $P = 0.28$).

Removal of ants also did not affect the increment in height of *Cecropia* saplings (*C. concolor*, $t = 1.80$, $P = 0.097$; *C. purpurascens*, $t = 0.17$, $P = 0.86$; Table 1). Finally, we did not detect a significant effect of the ant removal treatment on any parameter of leaf dynamics analyzed, including leaf emergence rates, mortality, and turnover (Mann–Whitney U-test, $P > 0.05$ in all cases; Table 1).

In spite of our negative results, it is premature to conclude that *A. alfari* does not defend its host plants against herbivores. If, in fact, *C. concolor* and *C. purpurascens* relied on ants for their defense, and *A. alfari* was ineffective in protecting them, one would have expected to find high levels of herbivore damage on both ant-removed and ant-maintained plants; however, that was not the case. Estimated rates of herbivory for *C. concolor* were 0.065 %/day, and for *C. purpurascens*, 0.053 %/day (data combining both control and treatment plants). These rates are two to ten times lower than those reported for *C. insignis* and *C. obtusifolia* in Panama (Coley 1983). In absolute values, herbivory rates in both *C. concolor* and *C. purpurascens* averaged 0.001 cm²/day, while Del Val and Dirzo (2003) reported values ranging between 0.18 and 0.26 cm²/day for *C. peltata* in Mexico. Our observed rates of herbivory in *C. concolor* and *C. purpurascens* are more similar to those found in plants that either (1) have a great amount of secondary compounds in their leaves (*e.g.* mature leaves of persistent trees in tropical forests having an estimated herbivory rate of 0.03–0.05 %/day; Coley 1983), (2) have strong biotic defenses (*e.g.*, young leaves of ant-occupied *Hirtella mirmecophyla*; herbivory rates of 0.039 %/day; Izzo & Vasconcelos 2002), or (3) presumably have no defenses but grow in areas in which specialist herbivores are absent (*e.g.*, introduced *C. peltata* in Malaysia; herbivory rates of 0.046 %/day; Putz & Holbrook 1988).

Since we removed all possible biotic defenses (ants) from our treatment plants, only two alternatives remain to explain why herbivory was so low in *C. concolor* and *C. purpurascens*. One possibility is that herbivore abundance was also low. In fact, a seedling-transplant experiment in our study area indicated that herbivore abundance in disturbed habitats, such as those where our *Cecropia* were located, was much lower than in nearby primary forest (Benitez-Malvido 1995). Alternatively or in addition, it is possible that *Cecropia* saplings were investing in chemical defenses and therefore were avoided by herbivores. Tannins are probably the major class of secondary compounds in *Cecropia*, and large variation exists in the amount of tannins produced by a given *Cecropia* individual (Coley 1986) as well as among *Cecropia* species (Folgarait & Davidson 1994). Tannin concentrations are also affected by light and are usually much greater in plants growing in full sun than in forest gaps or in the forest understory (Newbery & de Foresta 1985, Nichols-Orians 1991, Folgarait & Davidson 1994). Since most *Cecropia* individuals studied were in full sun, it is likely that their leaves had a greater concentration of tannins (Folgarait &

TABLE 1. *Effects of ant removal on herbivory, growth, and leaf dynamics for two Amazonian* Cecropia *species. There was no significant effect of the ant-removal treatment for any of the variables measured in these two plant species.*

Variable	Treatment	Cecropia concolor				Cecropia purpurascens			
		$\bar{x}$	Median	Range	N	$\bar{x}$	Median	Range	N
Average leaf damage (%/d)	With ants	0.05	0.04	0–0.11	17	0.05	0.02	0–0.29	19
	Without ants	0.08	0.05	0.01–0.31	19	0.05	0.02	0–0.27	15
Relative height increment (%)	With ants	15.6	13.8	5–29.1	9	10.0	7.7	1.4–30.4	16
	Without ants	9.5	9.3	3.4–21.4	12	10.5	6.2	0–28.0	12
Rate of leaf emergence (leaves/d)	With ants	0.056	0.057	0.04–0.07	8	0.033	0.036	0.02–0.04	16
	Without ants	0.047	0.045	0.03–0.06	12	0.041	0.039	0.02–0.08	12
Rate of leaf mortality (leaves/d)	With ants	0.024	0.021	0.01–0.04	8	0.012	0.012	0–0.02	16
	Without ants	0.026	0.027	0–0.05	12	0.010	0.006	0–0.02	12
Leaf turnover	With ants	2.6	2.4	1.7–4.5	8	2.5	2.0	1.5–4	16
	Without ants	1.9	1.8	1.1–3.7	12	3.7	2.9	2–6	12

Davidson 1994) and therefore were less palatable to herbivores than, for example, individuals growing in forest gaps.

Whether or not the relationship between *Azteca* and *Cecropia* is a mutualistic one has been a matter of debate, because some investigators have found evidence in favor of a protective role for *Azteca* (Janzen 1969, Schupp 1986, Vasconcelos & Casimiro 1997) while others did not (Wheeler 1942, Andrade & Carauta 1982). Longino (1997) has recently proposed that the question should be rephrased to when and where is it a mutualism, and we agree with that. His argument is based on the fact that *Azteca* species vary greatly in their behaviors toward intruders (herbivores and vines) and thus that the status of mutualism probably depends on which species of ant occupies the plant (Longino 1997). *Azteca alfari* is considered to be the least aggressive of all *Cecropia*-inhabiting *Azteca* (Longino 1991), and therefore, we cannot exclude the possibility that our negative results reflect the fact that all *Cecropia* we studied were inhabited by *A. alfari* and not by a more aggressive *Azteca* species; however, *A. alfari* workers in young *Cecropia* saplings can be quite aggressive, more than in mature plants (J. T. Longino, pers. comm.), and are efficient at removing at least some types of insect herbivores from their host plants (Jolivet 1990, Vasconcelos & Casimiro 1997). Therefore, we propose that the question of when and where the *Azteca–Cecropia* association is a mutualism should include not only the species of ant occupying the plant (as originally suggested by Longino 1997), but also the particular conditions under which the plant is growing. It is likely that *Azteca* ants (regardless of which particular species) will be crucial for *Cecropia* where herbivores and/or vines abound but not in sites in which these organisms are rare and/or at sites in which *Cecropia* invest more heavily in chemical defenses. In fact, the survival of *Cecropia* where associated *Azteca* ants do not occur, such as in the Caribbean islands and Malaysia (where *C. peltata* was introduced), has been attributed to the presumably low abundance of *Cecropia* herbivores at these sites (Janzen 1973, Putz & Holbrook 1988).

The results of our experiment strongly suggest that at least in parts of its native mainland habitat, *Cecropia* also suffers low incidence of attack by insect herbivores and therefore is able to survive in the absence of defensive ants. It must be stressed, however, that the habitat in which our experiments were performed (a large anthropogenic clearing) is a relatively novel habitat, very distinct from the ones in which the *Cecropia–Azteca* association probably evolved (forest gaps and river margins). Nevertheless, even though anthropogenic habitats are not a part of the evolutionary history of the *Cecropia–Azteca* association, they are now an important component of its contemporary ecology. If in these novel habitats, as shown here, *Azteca* is of little importance for its *Cecropia*, selection against the production of myrmecophytic traits may occur because production of these structures and associated food bodies is costly (Rickson 1977, Folgarait & Davidson 1994). Loss of myrmecophytic traits has been observed in introduced populations of *C. peltata* in Malaysia (Putz & Holbrook 1988), and the same could occur in large anthropogenic disturbances within the native range of *Cecropia*.

ANDRADE, J. C. DE, AND J. J. P. CARAUTA. 1982. The *Cecropia–Azteca* association: A case of mutualism? Biotropica 14: 15.

BAILEY, I. W. 1922. Notes on Neotropical ant-plants I. *Cecropia angulata*, sp. nov. Bot. Gaz. 74: 369–391.

BENITEZ-MALVIDO, J. 1995. The ecology of seedlings in Central Amazonian forest fragments. Ph.D. dissertation. University of Cambridge, Cambridge, England.

BERG, C. C. 1978. Cecropiaceae, a new family of Urticales. Taxon 27: 39–44.

BIERREGAARD, R. O., AND C. GASCON. 2001. The Biological Dynamics of Forest Fragments Project: The study site, experimental design, and research activity. *In* R. O. Bierregaard Jr., C. Gascon, T. E. Lovejoy, and R. Mesquita (Eds.). Lessons from Amazonia: The ecology and conservation of a fragmented forest, pp. 31–42. Yale University Press, New Haven, Connecticut.

CHAUVEL, A. 1983. Os latossolos amarelos, alicos, argilosos dentro do ecossistemas das bacias experimentais do INPA e da região vizinha. Acta Amaz. 12: 47–60.

COLEY, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Monogr. 53: 209–233.

———. 1986. Costs and benefits of defense by tannins in a Neotropical tree. Oecologia 70: 238–241.

DEL VAL, E., AND R. DIRZO. 2003. Does ontogeny cause changes in the defensive strategies of the myrmecophyte *Cecropia peltata*? Plant Ecol. 169: 35–41.

FOLGARAIT, P. J., AND D. W. DAVIDSON. 1994. Antiherbivore defenses of myrmecophytic *Cecropia* under different light regimes. Oikos 71: 305–320.

IZZO, T., AND H. L. VASCONCELOS. 2002. Cheating the cheater: Domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. Oecologia 133: 200–205.

JANZEN, D. H. 1969. Allelopathy by myrmecophytes: The ant *Azteca* as an allelopathic agent of *Cecropia*. Ecology 50: 147–153.

———. 1973. Dissolution of mutualism between *Cecropia* and its *Azteca* ants. Biotropica 5: 15–28.

JOLIVET, P. 1990. Relative protection of *Cecropia* trees against leaf-cutting ants in tropical America. *In* R. K. Vander Meer, K. Jaffe, and A. Cedeno (Eds.). Applied myrmecology: A world perspective. pp. 251–254. Westview Press, Boulder, Colorado.

LONGINO, J. T. 1991. *Azteca* ants in *Cecropia* tress: Taxonomy, colony structure, and behaviour. *In* C. Huxley and D. F. Culver (Eds.). Ant–plants interactions, pp. 271–288. Oxford University Press, Oxford, England.

———. 1997. The *Cecropia–Azteca* association in Costa Rica. (http://www.evergreen.edu/ants/ANTPLANTS/CECROPIA/Cecropia.html).

NEWBERY, D. M., AND H. DE FORESTA. 1985. Herbivory and defense in pioneer, gap, and understory trees of tropical rain forest in French Guiana. Biotropica 17: 238–244.

NICHOLS-ORIANS, C. 1991. The effects of light on foliar chemistry, growth and susceptibility of seedlings of a canopy tree to an attine ant. Oecologia 86: 552–560.

PHILLIPS, O. L., AND A. H. GENTRY. 1994. Increasing turnover through time in tropical forests. Science 263: 954–958.

PUTZ, F. E., AND N. M. HOLBROOK. 1988. Further observations on the dissolution of mutualism between *Cecropia* and its ants: The Malaysian case. Oikos 53: 121–125.

RICKSON, F. R. 1971. Glycogen plastids in Müllerian bodies of *Cecropia peltata*, a higher green plant. Science 173: 344–347.

———. 1977. Progressive loss of ant-related traits of *Cecropia peltata* on selected Caribean islands. Am. J. Bot. 64: 585–592.

SAGERS C. L., S. M. GINGER, AND R. D. EVANS. 2000. Carbon and nitrogen isotopes trace nutrient exchange in an ant–plant mutualism. Oecologia 123: 582–586.

SCHUPP, E.W. 1986. *Azteca* protection of *Cecropia*: Ant occupation benefits juvenile trees. Oecologia 70: 379–385.

VASCONCELOS, H. L., 1991. Mutualism between *Maieta guianensis* Aubl., a myrmecophytic melastome, and one of its ant inhabitants: Ant protection against insect herbivores. Oecologia 87: 295–298.

———, AND A. B. CASIMIRO. 1997. Influence of *Azteca alfari* ants on the exploitation of *Cecropia* trees by a leaf-cutting ant. Biotropica 29: 84–92.

WHEELER, W. M. 1942. Studies of Neotropical ant-plants and their ants. Bull. Mus. Comp. Zool. 90: 1–262.

WILLIAMSON, G. B., R. C. G. MESQUITA, K. ICKES, AND G. GANADE. 1998. Estratégias de árvores pioneiras nos Neotrópicos. *In* C. Gascon and P. Moutinho (Eds). Floresta Amazônica: Dinâmica, regeneração e manejo, pp. 131–144. INPA, Manaus, Brasil.

WILKINSON, L. 1998. SYSTAT 8.0 for Windows. SPSS Inc., Chicago, Illinois.

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