

Epiphyll deterrence to the leafcutter ant *Atta cephalotes*

Ulrich G. Mueller and Bettina Wolf-Mueller

Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

Received May 24, 1990 / Accepted in revised form September 20, 1990

Summary. Epiphyll growth on leaves of the grapefruit *Citrus paradisi* and the understory cyclanth *Cyclanthus bipartitus* repelled the fungus-growing, leafcutter ant *Atta cephalotes* from harvesting leaves of these tropical plants. Experimental removal of epiphylls from leaves resulted in 2–3 times more herbivore damage by leafcutter ants as compared to matched leaves with epiphylls. Because of the protection from herbivore damage, host plants may derive a partial fitness benefit from association with epiphylls.

Key words: Antiherbivore protection – *Atta cephalotes* – Epiphylls – Herbivory – Repellency

Epiphylls are communities of leafy liverworts, crustose lichens, free-living bacteria, algae, and fungi growing on the surfaces of leaves (Richards 1964, 1984; Smith 1982), but the main structural component of epiphyll communities are leafy liverworts in the family Lejeuneaceae (Pócs 1978; Bien 1982). In rainforests of the neotropics, epiphylls are found on understory plants from a diverse spectrum of plant taxa. Average epiphyll load, however, varies considerably among host species and is determined primarily by two factors: (a) leaf age, since epiphyll load increases with leaf age, and (b) leaf surface properties (e.g. waxiness) that influence the rate of drainage from a leaf and thus affect the likelihood of epiphylls colonizing a leaf (Richards 1964, 1984; Gregory 1971; Pócs 1978). Theoretically, therefore, host plants may exert some control over the rate of epiphyll colonization by altering the surface properties of their leaves.

A priori, it may seem that epiphylls reduce the fitness of the host plant by (a) reducing the light energy absorbed by leaves, (b) trapping leaf pathogens (Gregory 1971), (c) directly injuring leaf tissue or cuticle (Berrie and Eze 1975), or (d) absorbing nutrients or water from the host

leaf (Berrie and Eze 1975). The relationship between epiphylls and their host plant would thus be parasitic. Host plants, on the other hand, may derive a benefit from their associated epiphylls. Potential benefits to the host plant include (a) transfer of nutrients (Bentley and Carpenter 1980, 1984), (b) physical protection (Riedl 1977), or (c) reduction of herbivore damage. If these benefits outweigh the costs of bearing epiphylls, the relationship between epiphylls and host plants would be mutualistic.

To test the hypothesis that epiphyll growth reduces herbivory on host plants, we manipulated epiphyll load on leaves of two tropical plant species. The manipulations showed that epiphylls reduce herbivore damage by the fungus-growing, leafcutter ant *Atta cephalotes*. Host plants may therefore benefit from associations with epiphylls by gaining partial protection against herbivores.

Methods

Study site and experimental design

We conducted all experiments between 18–27 February and 29–31 March 1990 at La Selva Biological Station of the Organization for Tropical Studies, Puerto Viejo de Sarapiquí, Costa Rica. The effect of epiphylls on leaf herbivory was studied in two host plant species, the introduced grapefruit *Citrus paradisi* (Rutaceae) growing on the grounds of the station, and the native cyclanth *Cyclanthus bipartitus* (Cyclanthaceae) growing in the understory of primary rainforest near the intersection of the CES and CEN trails. Leafcutter ants (*Atta cephalotes*) at La Selva prefer to cut leaves from these two plant species (Cherrett and Jutsum 1982, Wetterer 1988, 1990, personal observation).

In the following series of experiments, we randomly allocated leaves to two treatments, complete removal of epiphylls (Removal Condition) and intact epiphyll load (Epiphyll Condition). Leaves in the Removal Condition were cleaned from all epiphyll vegetation by wiping the upper surface with a moistened cloth. Leaves of the Epiphyll Condition were sham-treated by handling them with the same cloth for the same amount of time, squeezing them gently, yet being careful not to wipe off the epiphylls. With the exception of the first experiment, we selected only leaves that visually showed intermediate loads of epiphylls. To select leaves with intermediate loads,

We first gained an impression of the entire range of epiphyll loads, then excluded the extremes during the selection process. Epiphylls on leaves of both *Ci. paradisi* and *Cy. bipartitus* consisted primarily of leafy liverworts and crustose lichens.

Epiphylls on trees of *Citrus paradisi*

On 23 January 1990, quadruplets of leaves growing adjacent to each other on the same branch were selected on three trees of *Ci. paradisi*. Leaves of a quadruplet were of the same age, free of detectable herbivore damage, and approximately equal in size. Within each quadruplet, two leaves were randomly assigned to the Epiphyll, and two to the Removal conditions. Leaves in either condition were code marked with loops of thin, colored wire around the petiole. Twelve such quadruplets were prepared on each of the three trees of *Ci. paradisi*. On each tree, six quadruplets consisted of young leaves with few or no epiphylls, and six of older leaves with average loads of epiphylls.

Seventy days later, percent leaf area damaged by herbivores on all experimental leaves was scored blindly by an independent judge on a scale with 10% increments. Within each quadruplet, we calculated the mean damage by averaging the scores for the two leaves in the same treatment condition. If epiphyll growth reduced herbivory, we predicted that (a) Removal leaves would show significantly more herbivore damage than Epiphyll leaves, and that (b) the protective effect of epiphylls would be least pronounced in younger leaves with minimal epiphyll loads, but substantial in older leaves with average epiphyll loads.

Leaves presented on foraging trails

Leaves with epiphylls were presented directly to the fungus-growing, leafcutter ant *A. cephalotes* by placing branches with manipulated leaves on foraging trails of the ants. Branches with from 1 to 4 pairs of leaves were cut from trees of *Ci. paradisi*. Within each pair, leaves were of the same age, free of herbivore damage, and of similar size. One leaf per pair was assigned randomly to the Epiphyll and the other to the Removal condition. Fifty pairs of leaves on 27 branches were presented to leafcutter ants.

Leaves of *Cy. bipartitus* are bifid (composed of two leaflets) and much larger than leaves of *Ci. paradisi*. We therefore cut a 20 cm segment from each of the two leaflets of 25 leaves of *Cy. bipartitus* (=50 leaflets), leaving 10 cm of the midvein intact beyond the 20 cm of the blade, to serve as a support during presentation. Each leaflet was treated by removing epiphylls from a randomly selected half to either side of the leaflet's midvein, that is either the half originally medial or lateral in the bifid leaf. Within a pair of leaflets cut from the same leaf, epiphylls were removed from the medial half of one leaflet, and from the lateral half of the other leaflet. This controlled for differences in leaf properties between medial and lateral halves.

Branches of *Ci. paradisi* and sections of *Cy. bipartitus* were stuck vertically into the substrate of foraging trails of five colonies of *A. cephalotes*. Since leafcutter ants at La Selva forage primarily during the night (Wetterer 1988, 1990), we set out all experimental leaves between 17:00–18:00, before the onset of main foraging activity. It was important to present leaves before dusk, because leaves set out later were less likely to be cut by the ants during several pilot trials.

After 12 h of presentation, the area cut by ants was calculated and its proportion of the original area determined from tracings of the leaves made before presentation. Leaf area before and after presentation was measured using a leaf area digitizer (LI-COR Inc., Model 3100). To meet statistical independence, the percent damage on the Epiphyll and Removal leaf areas of *Cy. bipartitus* was averaged across the two leaflets within each of the 25 leaves presented.

Leaf-disc choice experiment

Pairs of small leaf-discs (13 mm diameter) were cut from leaves of *Ci. paradisi*, one disc in each pair from one randomly selected half where epiphylls were wiped off, the other from the sham-treated half of the same leaf. Leaf-discs of *Cy. bipartitus* were cut in the same manner, except that both Removal and Epiphyll discs were cut from adjacent portions of the same half of leaflets.

We conducted choice experiments between 20:00–2:00 on five foraging trails of *A. cephalotes*. Discs were presented by placing 10 pairs of discs in two rows (5 mm spacing) along the midline of trails, alternating the sides of Epiphyll and Removal discs between successive pairs. Ten such trials were run for each plant.

After discs were laid out, we observed whether discs were picked up by ants and carried away along the foraging trail, or were moved off the trail and discarded. If a disc was picked up and carried away, we recorded the time when the disc was carried across an imaginary line 20 cm distant from the rows of discs. We assumed that discs carried for at least 20 cm were carried all the way to the nest, since ants seemed to discard discs only in the immediate vicinity of the location of presentation. Trials with discs of *Ci. paradisi* lasted 10 min. Because removal rates were much lower with discs cut from *Cy. bipartitus*, we extended these trials to 20 min.

For each trial and for each plant species, we calculated the proportion of discs in either condition that were carried away, and the average latency until removal. If epiphyll growth reduced herbivory by *A. cephalotes*, we predicted that discs without epiphylls were more likely to be carried away by leafcutter ants, and with shorter latencies, than discs with epiphylls.

Results

Epiphylls on trees of *Citrus paradisi*

Six Epiphyll and five Removal leaves were shed naturally from the trees of *Ci. paradisi* during the 70 days of experimentation. Herbivore damage on the remaining 66 Epiphyll and 67 Removal leaves could be classified into two categories: (a) Damage due to leafcutter ants as indicated by semi-circular cuts characteristic for herbivory by these ants, and (b) damage due to other herbivores, that is leaf damage appearing not semi-circular. Weevils (Coleoptera) and moth larvae (Lepidoptera) inhabited leaves of the three experimental trees and may have caused this second type of leaf damage.

Cuts from leafcutter ants were evident in 14 of the 36 experimental quadruplets, 7 comprised of young, and 7 of old leaves. We assumed that all leaves of these 14 quadruplets (28 Epiphyll and 28 Removal) had been contacted by foraging leafcutter ants and potentially could have been cut. Overall, Removal leaves showed significantly greater damage by leafcutter ants (71.9%, $SD=39.52$) than Epiphyll leaves (50.7%, $SD=44.80$; Wilcoxon signed-rank test $z=2.14$, $p=0.016$).

The effect of epiphyll removal on leaf herbivory was dependent on leaf age, confirming the predicted interaction between leaf age and experimental treatment (Mann-Whitney two-sample test $z=2.907$, $p<0.002$, comparing within-pair-differences in herbivory between the samples of young and old leaves; this comparison represents a nonparametric test for the interaction between two independent variables in a paired design). Damage by leafcutter ants on the subset of young leaves (7 quadruplets) was not significantly different between

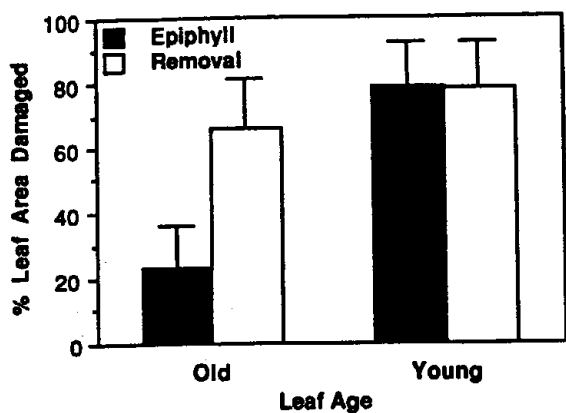


Fig. 1. *Citrus paradisi*. Percent leaf area damaged within 70 days due to herbivory by the leafcutter ant *Atta cephalotes* on paired, experimental leaves with epiphylls (Epiphyll) and with epiphylls removed (Removal). Epiphyll growth significantly reduced herbivory in old leaves ($p=0.009$), but not in young leaves ($p=0.296$). Error bars represent one standard error

the Removal (77.9%, SD = 38.47) and the Epiphyll leaves (78.5%, SD = 35.77; Wilcoxon signed-rank test $z=0.535$, $p=0.296$; Fig. 1). This is not surprising, since young leaves are almost free of epiphylls and manipulation of such minimal epiphyll load is therefore not expected to result in differential herbivory by leafcutter ants. For older leaves with average levels of epiphylls (7 quadruplets), however, removal of all epiphyll vegetation greatly increased the damage by leafcutter ants (65.5%, SD = 41.15) as compared to untreated leaves (23.1%, SD = 35.21; Wilcoxon signed-rank test $z=2.37$, $p=0.009$; Fig. 1). This represents a reduction in herbivory by a factor of almost three.

Herbivores other than leafcutter ants never injured more than 5% of the leaf area. Fourteen Epiphyll leaves and 13 Removal leaves showed such damage. In the time span of this experiment, therefore, removal of epiphylls significantly reduced herbivory by *A. cephalotes*. Overall damage by other herbivores, however, was not large enough to evidence the presence or absence of epiphyll deterrence to these herbivores.

Leaves presented on foraging trails

After 12 h of presentation on active trails of *A. cephalotes*, leaves of trees of *Ci. paradisi* showed an average damage of 72.9% (SD = 36.74, $n=50$) when being free of epiphylls, but only 43.8% (SD = 42.36, $n=50$) when bearing epiphylls (Wilcoxon signed-rank test $z=3.74$, $p<0.0001$; Fig. 2). Similarly for *Cy. bipartitus*, 35.1% (SD = 26.63, $n=25$) of the area of Removal leaves was cut by leafcutter ants, but only 14.6% (SD = 21.75, $n=25$) of the Epiphyll leaves (Wilcoxon signed-rank test $z=4.27$, $p<0.0001$). Thus, epiphylls on leaves of both *Ci. paradisi* and *Cy. bipartitus* reduced, but did not prevent, herbivore damage by leafcutter ants.

Leaf-disc choice experiments

In both *Ci. paradisi* and *Cy. bipartitus*, a greater proportion of Removal discs was carried off by leafcutter ants than of Epiphyll discs (Fig. 3A). In none of the ten trials

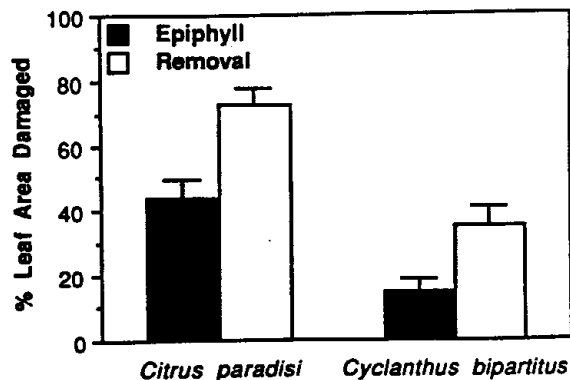


Fig. 2. Percent leaf damage on paired leaves with epiphylls (Epiphyll) and without epiphylls (Removal) presented to the leafcutter ant *Atta cephalotes* on foraging trails for 12 h. Leafcutter ants preferentially harvested leaves without epiphylls in both *Citrus paradisi* ($p<0.0001$) and *Cyclanthus bipartitus* ($p<0.0001$). Error bars represent one standard error

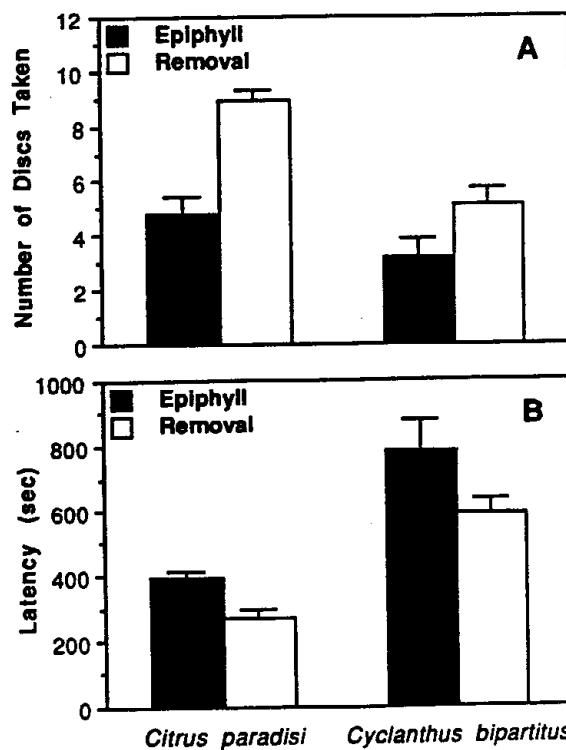


Fig. 3. A Number of leaf discs with epiphylls (Epiphyll) and with epiphylls removed (Removal) taken by leafcutter ants *Atta cephalotes* in a choice experiment, and B corresponding latencies (s) until disc removal by the ants. Error bars represent one standard error

for each of the two plant species were more Epiphyll discs taken than Removal discs. Only in one trial with *Cy. bipartitus* were an equal number of both types of discs taken (sign test for *Ci. paradisi* $p=0.001$, $n=10$; for *Cy. bipartitus* $p=0.0039$, $n=10$). This preference of leafcutter ants for discs without epiphylls is further underlined by the fact that Removal discs were picked up and carried off with shorter latencies in both plant species (Wilcoxon signed-rank test for *Ci. paradisi* $z=2.80$, $p=0.0026$; for *Cy. bipartitus* $z=2.84$, $p=0.0023$; Fig. 3B). Foragers of *A. cephalotes* therefore preferentially carried leaf fragments without epiphylls to their nest, over fragments with epiphylls.

Discussion

Leafcutter ants *Atta cephalotes* prefer to cut leaves free of epiphylls over leaves with epiphylls, and preferentially select to carry leaf fragments free of epiphylls to their nest. The presence of epiphylls therefore reduces herbivory on host plants by one of the most destructive herbivores in neotropical rainforests (Weber 1966, 1972).

Leafcutter ants may avoid leaves with epiphylls because (a) epiphylls protect leaves structurally by increasing the cutting effort of foragers at the site of harvesting, and the processing effort of "shredders" in the nest (Cherrett 1972; Waller 1982; Nichols-Orians and Schultz 1989); or (b) epiphylls protect leaves chemically by containing secondary compounds harmful to the ants themselves or the ants' fungus gardens (Hubbell et al. 1984, Howard and Wiemer 1986; Howard 1987, 1988, 1990). Both lichens and liverworts possess anti-fungal properties (Rundel 1978, Lawrey 1986, Chopra and Kumra 1988). Liverworts, for example, are extremely rich in terpenoids located in their oil-bodies (Markham and Porter 1978; Huneck 1983), and terpenoids are toxic to both leafcutter ants and their mutualistic fungus (Hubbell et al. 1983; Howard et al. 1988).

Because of the protective effect from herbivore damage, host plants may derive a partial fitness benefit from an association with epiphylls. Whether the costs of bearing epiphylls (reduced light levels, physical damage, trapping of pathogens, nutrient drain) are outweighed by the benefit of reduced herbivory, however, is unclear. It remains to be answered, also, whether the relationship between epiphylls and host plants represents a mutualistic liason shaped by a coevolutionary process, or a mere fortuitous association in the complex, ecological web of tropical rainforests. An analysis of leaf surface properties (Gregory 1971; Pócs 1978), demonstrating design (*sensu* Williams 1966) to enhance or reduce rates of epiphyll colonization, may distinguish between a mutualistic or fortuitous nature of the association between epiphylls and their host plants.

Acknowledgments. We wish to thank David Clark, Deborah Clark, Jack Schultz, Nat Wheelwright, and Jim Wetterer, but especially Bette Loiselle, Giselle Mora, and Mauricio Macho Quesada, for valuable suggestions regarding the design of the field bioassays. Special thanks also to the students of OTS 90-1. George Eickwort, Ann Herzog, Thomas Seeley, and two anonymous reviewers offered comments to improve the manuscript. The Organization for Tropical Studies and the staff at La Selva Biological Station provided invaluable, logistical support. This research was supported by a stipend from the Division of Biological Sciences at Cornell University, and by a Jesse Smith Noyes post-course research award from the Organization for Tropical Studies.

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