

Gall-forming and free-feeding herbivory along vertical gradients in a lowland tropical rainforest: the importance of leaf sclerophylly

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In contrast to most insect guilds, gall-forming insects are thought to reach highest diversity on sclerophyllous vegetation, such as Neotropical savannas and Mediterranean vegetation types. The water and nutrient stress endured by meristems of canopy trees in tall wet tropical rainforests may cause leaf sclerophylly. Hence, the upper canopies of such ecosystems may represent a suitable habitat for gall-forming insects. At the San Lorenzo Protected Area, Panama, we estimated free-feeding herbivory and gall densities within five sites in 2003 and 2004, by surveying leaves in vertical and horizontal transects. In each sample, we recorded leaf density (mature and young foliage), free-feeding herbivore damage and number of galls, including the presence of live larvae, parasitoids or fungi. We surveyed 43 994 leaves, including 231 plants and 73 tree and liana species. We collected 5014 galls from 17 host-plant species, including 32 gall species of which 59% were restricted to the canopy (overall infestation rates: 2.4% in 2003, 5.5% in 2004). In 2003, 16% of the galls were occupied by live larvae, against 5% in 2004. About 17–20% of leaves surveyed suffered from free-feeding herbivory. Leaf sclerophylly increased significantly with sampling height, while free-feeding herbivory decreased inversely. Conversely, the number of live galls collected in the canopy was 13–16 times higher than in the understorey, a pattern consistent across sites and years. Hence, the probability of gall survivorship increased with increasing leaf sclerophylly as death by fungi, parasitoids or accidental chewing were greater in the understorey. Increasing harsh ecophysiological conditions towards the upper canopy appear favourable to galls-forming population maintenance, in support of the hypothesis of harsh environment. Hence, gall diversity and abundance in the upper canopy of tall tropical forests are perhaps among the highest in the world.

To date, the biogeographical distribution of herbivory has generated much literature (reviews in Landsberg and Ohmart 1989, Coley and Aide 1991, Lowman 1995, Coley and Barone 1996, Fox et al. 1999, Ribeiro 2003, Rinker and Lowman 2004). However, most studies focus on damage generated by chewing insects. A neglected form of insect herbivory is gall tumours. Gall-forming insects oviposit in leaves or other tissues, and then cause cell tumours with increased nutrient content near developing larvae and increased levels of tannins and fibres in the outer layers of galls (Mani 1964). Studies of plant galls may

result in comprehensive analyses of herbivore ecology, as gall-forming insects are sedentary, and can be recorded as alive, dead, or hatched. Gall-forming insects rank among the most specialized insect guilds and are well studied in Mediterranean-type vegetation, where plant galls are conspicuous (Price et al. 1998, Mendonça 2001).

Fernandes and Price (1988, 1992) proposed the hypothesis of harsh environment (hereafter HHE) to explain patterns of gall distribution. Price et al. (1998) further documented the association of high gall species diversity with sclerophyllous vegetation worldwide.

Sclerophylly was assumed to be the main ecological factor behind the pattern of higher gall species diversity in xeric than mesic habitats (Fernandes and Price 1988). Furthermore, Fernandes and Price (1992) suggested that differential mortality (mainly by parasitoids and fungi) was ultimate the cause of this pattern.

Recently, Ribeiro (2003) questioned the pattern reported by previous articles on the basis that xeric canopy vegetation, low and easy to reach, was mainly compared with mesic understorey vegetation (Fernandes and Price 1988, 1991, 1992, Fernandes et al. 1997, Price et al. 1998). Galls have rarely been surveyed in the upper canopy of mesic forests (Price et al. 1998, Medianero et al. 2003). Against this background, litter-fall data suggested higher gall diversity in the canopy than in the understorey of mesic forests (Faria and Fernandes 2001). Further, Medianero and Barrios (2001) and Medianero et al. (2003) collected more gall species in the canopy than in the understorey while comparing young and mature trees in wet and dry forests of Panama.

A key assumption of Fernandes and Price (1988, 1992) is that xeric foliage is more sclerophyllous than mesic foliage. However, few workers actually measured this trait in the tropics or in the upper canopy of wet forests (Espírito-Santo and Fernandes 1998, Madeira et al. 1998, Ribeiro et al. 1999). One noticeable exception is Bell et al. (1999) study in southern Cameroon. These authors noticed convergence in leaf defensive traits and crown construction between upper canopy trees of a wet forest and chaparral shrub vegetation. Traits described were much alike sclerophyllous traits evolved in response to drought in Mediterranean vegetation, or to low nutrients in the Brazilian cerrado (Salatino 1993, Turner 1994, Ribeiro et al. 1999, Ribeiro 2003).

In response to increasing hydraulic constraints and nutrient competition between terminal buds in mature trees, a vertical gradient in leaf sclerophylly should be expected in wet tropical forests. Although many phytochemical studies have reported higher secondary defences, particularly phenols, in sunny microhabitats of the upper canopy (Mole et al. 1988), most studies did not attempt to relate ecophysiological stress in the canopy to insect-plant interactions (Oldeman 1974, Sterck et al. 2001).

Likewise, few data exist on the relation between the distribution of leaf damage and that of insect herbivores or gall-forming insects in tall tropical canopies (Basset 1991). Leaf biomass may be not linearly related to food resource availability to different insect functional guilds. According to the HHE, sclerophyllous leaves in the upper canopy should be more suitable to gall-forming insect. Conversely, chewing insects should be more sensitive to leaf nutrient contents, and attack preferentially soft, young leaves, in lower canopy or understorey

(Elton 1973, Novotny et al. 2002), regardless of the decreasing amount of foliage in lower forest layers. To our knowledge, the present study represents one of the first standardized accounts of leaf availability/quality and herbivore damage and distribution along vertical profiles in a tropical forest.

The present investigation was part of the IBISCA (Investigating Biodiversity of Insects in Soil and Canopy) programme, which aims at studying the beta diversity and vertical distribution of arthropods in a lowland tropical forest in Panama, using comprehensive and complementary sampling protocols (Didham and Fagan 2003). Here, we use a new protocol allowing a comparative study of the distribution of leaf traits, gall-forming and free-feeding herbivory from the understorey to the upper canopy.

We test the hypothesis that gall density responds positively to increasing sclerophylly, while free-feeding herbivory responds negatively. We predict that sclerophylly increases with height within the canopy, regardless of host plant species and location, and that height also affects gall distribution and gall insect survivorship. Furthermore, we investigated whether gall host plant species in the Panamanian wet forest would bear any relation to gall-rich plant communities elsewhere.

Materials and methods

Study sites and canopy access

Field work took place in a lowland wet forest in the San Lorenzo Protected Area (Colón Province, Republic of Panama), in the vicinity of a tower crane yielding easy access to 0.92 ha of canopy (Wright et al. 2003). This location averages 3139 mm of annual rainfall and an annual air temperature of 26.0°C (1998–2002 data). The climate is wet all year-round, with a comparatively drier season between January and mid-April. The forest has been mostly free of severe disturbance for the past 150 yr and is evergreen, with <3% loss in canopy cover by the end of the dry season (Condit et al. 2000, 2004).

The IBISCA programme (Didham and Fagan 2003) focused on twelve study sites, each 20 × 20 m in area, in which woody plants >1 cm dbh were tagged and identified. These sites were all situated within a 2 km radius around the canopy crane and three of them were inside the crane perimeter. Our sampling protocol allowed us to survey galls and free-feeding herbivory at five of these sites (Table 1), where we performed quantitative comparisons in equivalent canopy and understorey volumes, hereafter called pin-cylinder transects.

Table 1. Main characteristics of sites (20 × 20 m) surveyed for galls and free-feeding herbivory at San Lorenzo.

Site code	Latitude (deg. N)	Longitude (deg. W)	No. stems > 10 mm	No. tree species	Canopy access (1)	Replication (2)	Notes and flora (3) Largest trees/most common spp.
B1	9°17.133	79°59.106	207	51	Bubble+SRT	3/1	Younger forest, disturbed site Sap, Cal/Psy, Ing
C1	9°16.774	79°58.495	138	70	Canopy crane	3/1	Bro, And/Prb, Geo
C2	9°16.793	79°58.499	102	43	Canopy crane	3/1	Voc, Bro/Prb, Mar
C3	9°16.779	79°58.468	121	48	Canopy crane	3/1	Bro, Jac/Tov, Geo
I1	9°16.617	79°58.377	123	59	SRT	3/1	Ape, Cal/Soc, Tov

(1) SRT = single rope technique, see text for other devices.

(2) No. of pin transects in the canopy/understorey.

(3) And = *Andira inermis* (W. Wright) DC.; Ape = *Apeiba membranacea* Spruce ex Benth.; Bro = *Brosimum utile* (Kunth) Pittier; Cal = *Calophyllum longifolium* Willd.; Geo = *Geonoma congesta* H. Wendl. ex Spruce; Ing = *Inga sertulifera* DC.; Jac = *Jacaranda copaia* (Aubl.) D. Don.; Mar = *Marila laxiflora* Rusby; Prb = *Perebea xanthochyma* H. Karst; Psy = *Psychotria horizontalis* Sw.; Sap = *Sapium* sp. "broadleaf"; Soc = *Socratea exorrhiza* (Mart.) H. Wendl.; Tov = *Tovomita stylosa* Hemsl.; Voc = *Vochysia ferruginea* Mart.

Sampling protocol – the pin-cylinder transect

Our protocol involved sampling galls on all leaves within a volumetric space of 1 m in diameter, from three meters above the ground up to the upper canopy (canopy-pin transect) or along an equivalent horizontal transect of 30 m (the average height of this forest) parallel to the soil (understorey-pin transect). This method is similar to the pin-quadrat method used in phytosociological surveys of grasslands (Borges and Brown 1999), but instead of counting leaf touches, we counted leaves and galls found inside this cylindrical volume (see below). The pin-transect methodology was applied to five sampled sites, and at each we performed three canopy-pin and one understorey-pin transects repeatedly in two seasonally distinct periods: October 2003 (mid wet season, sum of precipitation 252 mm) and May 2004 (transition dry-wet season, 637 mm), yielding a total of 30 canopy-pin and 10 understorey-pin transects (Table 1).

The very same transects were used in 2003 and 2004. At each site, the three canopy pin-transects were positioned randomly within a range of accessible sites, in distant places within the 400 m² plot. The pin cylinder volume was visualized and positioned using a wooden frame (1 m large) and a compass. At the three canopy crane sites, a random positioning of canopy-pin transects was achieved with a one-person crane basket, whereas randomisation was possible within a range of safe anchoring points for transects surveyed with single rope technique at other sites. Understorey-pin transects deliberately avoided seedlings and plants smaller than 10 cm, and focused exclusively on dicotyledonous plants. The higher sampling effort in the canopy than in the understorey (Table 1) was deliberate, aiming a representative sampling in terms of plant diversity and site variability in the canopy. Although canopy foliage is denser than that in the understorey, canopy transects typically included only 4–5 plant species locally,

against the more speciose understorey transects, which spread along a larger horizontal forest area.

A "sample" consisted of branches or continuous groups of leaves within a pin-cylinder transect, whose height was recorded. For each sample we further recorded: 1) number of mature leaves and active meristems/young leaves; 2) gall densities; and 3) number of leaves with >10% of leaf area lost to chewing insects. Since this figure represents global average leaf area loss in tropical wet forests, leaves scored above this average may be qualified as "substantially damaged" (Coley and Aide 1991). Leaf area and traits were estimated or measured from a subsample of three mature leaves obtained from each sample, or from each species when a sample included more than one plant species. Leaf area was measured using a hand scanner (CI-202 Portable Area Meter). Leaves were then dried out at 60°C in a laboratory oven. An estimation of specific mass per leaf area unit, related to leaf sclerophylly (Cooke et al. 1984), was obtained by dividing leaf mass by area, using mature leaves collected in 2003. To estimate gall densities, all leaves in a sample with galls, or any gall-like imperfection, were collected and frozen for future analyses. We considered the following gall categories: a) all galls (including galls with live and dead insects; hereafter "live" and "dead" galls for sake of brevity); b) only galls with live larvae; c) recently hatched galls; and d) galls with larvae that died recently of fungal attack (recognized by rotten larvae or presence of mould) or parasitoid attack (identified by the presence of the parasitoid ecdysis or several small asymmetric apertures in the gall, along with wastes inside).

Statistical analyses

We used a mixed ANCOVA model to examine the spatial distribution of galls, with gall density as the dependent variable and expressed either as a) the total

number of galls per sample, b) the mean number of galled leaves per total number of leaves in samples, or c) the mean number of galls per number of infected leaves in samples. The dependent variable was $\ln(x + 1)$ transformed, to satisfy the assumptions of normality (the proportion of dead galls was arc-sin transformed). We considered sites as a random factor, canopy and understorey as fixed levels of a habitat factor, and sample height and mean leaf sclerophylly per sample as covariates in the model. ANCOVA mixed model analysis were developed in SPSS 13.0.

There is a particular interest in examining the relative importance of sclerophylly along height gradient and actual height effects (i.e. understorey vs canopy micro-habitat particularities) on gall distribution. We therefore explored the interrelation of height and sclerophylly. Variance inflation factor (VIF = 1.25; VIF critical value = 2.0) and collinearity condition index (tolerance = 0.80; critical index values > 15) showed that median sample height and sclerophylly neither caused inflating effects nor were strongly collinear. Both tests allowed the use of these variables complementarily in a same ANCOVA. Nevertheless, we also performed partial correlations between these variables controlled by gall parameters (total galls, galls per infected leaves, live galls). We detected a substantial (81%) change in the correlation between sclerophylly and live galls after removing sample height from the regression (in a zero-order correlation approach). Therefore, the ANCOVA was performed only with sclerophylly as covariate for testing live galls, and height was analyzed separately by using a simple linear regression model on live gall insects, and on proportion of dead gall insects. For testing these covariates against total galls and galls per infected leaves, the decrease in correlation with sclerophylly after removing height factor was only 24 and 8%,

respectively. Then, both factors were used as co-variables in a same model. Relationships among height, leaf sclerophylly, mature and young leaves, free-feeding herbivory, and gall survivorship were explored with simple linear regressions.

In 2003, all transects surveyed included galls. In 2004, galls occurred in fewer transects and, for example, were absent in sites C2 and I1. In order to avoid unbalance analyses but still test the consistency in the gall distribution patterns among seasons, we opted for two separate statistical analyses for each year, using different site numbers (B1, C1, C2, C3 and I1 for 2003 and C1, C3 and B1 in 2004; Table 1). For each site, the three canopy transects were averaged and compared to the understorey transect.

Results

Properties of plant samples

A total of 73 tree and liana species, including 50 plant genera and 31 families, were identified out of 231 samples collected in the canopy and the understorey (Table 2). Comparing resource distribution, 7.3 more plant individuals were on average sampled in the understorey than in the canopy, but foliage was denser in the canopy. Higher numbers of plant individuals and species were found in the understorey of sites closer to light gaps (B1 and I1). Still, for all sites canopy transects yielded on average 8.5 times more leaves than understorey transects (Table 2).

Regardless of plant taxonomy, leaf sclerophylly increased significantly with sampling height (Fig. 1). The regression supporting such pattern was not affected by sites, or by the distribution of foliage at different canopy strata ($p > 0.05$, stepwise regression).

Table 2. Plant properties of pin transects obtained in the canopy and understorey of each study site measured in 2003: number of plant individuals (Ind) and species (Spp) censused; leaf density (mean number of leaves per plant individual in the understorey or mean numbers per canopy leaf strata); and sclerophylly (specific leaf mass in g mm^{-2}). Canopy values are the mean (SE) of 3 transects.

Site and habitat	Ind	Spp	Leaf density	Sclerophylly (g mm^{-2})
B1 – understorey	47	21	25.1 (17.2)	0.0072 (0.0114)
B1 – canopy	3.7 (0.58)	3.7 (0.58)	286.7 (799.2)	0.0103 (0.0072)
C1 – understorey	27	Na	21.6 (26.0)	0.0059 (0.0015)
C1 – canopy	2.7 (0.58)	2.7 (0.58)	153.2 (113.4)	0.0121 (0.0057)
C2 – understorey	38	7	17.8 (15.5)	0.0052 (0.0022)
C2 – canopy	5.3 (0.58)	5 (1.0)	99.05 (147.4)	0.0120 (0.0037)
C3 – understorey	9	9	25 (27.3)	0.0054 (0.0014)
C3 – canopy	4.3 (2.3)	4 (1.7)	375.5 (877.9)	0.0088 (0.0034)
I1 – understorey	18	12	25.8 (18.9)	0.0051 (0.0014)
I1 – canopy	3.7 (1.5)	3.7 (1.5)	183.4 (204.6)	0.0093 (0.0036)

Na = the high number of juvenile specimens difficult to track taxonomically in understorey transects at site C1 precluded to estimate accurately species number.

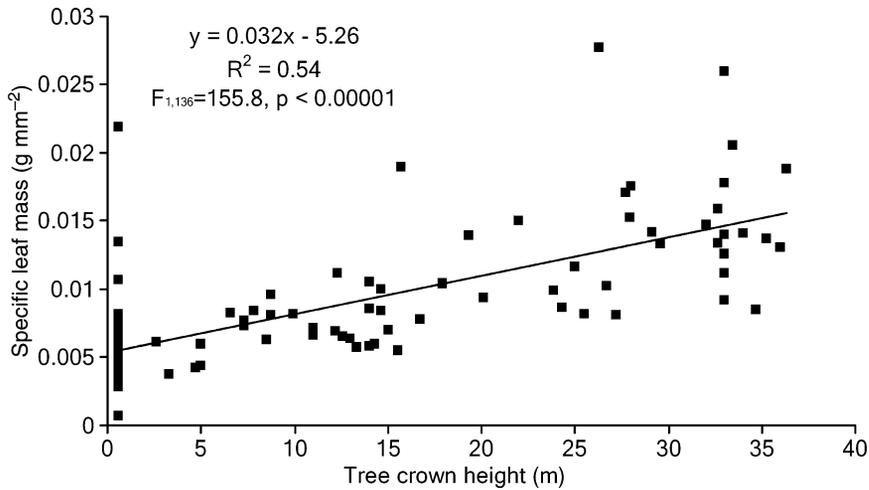


Fig. 1. Relationship between leaf sclerophylly (specific leaf mass) and sampling height (median height of samples) during the 2003 sampling period.

Although there was a high variation in sclerophylly (some understorey leaves being as tough as canopy leaves), canopy leaves were nearly twice more sclerophyllous than understorey leaves (Table 2).

Patterns of herbivory

Galls were present only in 47 samples and 16 identified host species (Table 3). Overall, 32 gall species were

Table 3. Host species, gall incidence, and biogeographical distribution of species or genera, considering particularly co-occurrence or co-generic species in the Brazilian cerrado.

Plant family	Species	Sampled plants	Individuals with galls	Total sampled galls	Species distribution	Genus or species present in the cerrado
Moraceae	<i>Perebea xanthochyma</i> H. Karst.	5	1	1	Meso-America and Amazon	No
Moraceae	<i>Brosimum utile</i> (Kunth) Oken ex J. Presl	4	2	80	Neotropical	Genus
Clusiaceae	<i>Calophyllum longifolium</i> Willd.	2	1	3	Neotropical	Subspecies
Meliaceae	<i>Carapa guianensis</i> Aubl.	1	1	27	Neotropical	No
Polygonaceae	<i>Coccoloba parimensis</i> Benth.	1	1	16	Meso-America and Amazon	Genus
Dilleniaceae	<i>Doliocarpus dentatus</i> (Aubl.) Standl.	6	1	15	Neotropical	Species
Moraceae	<i>Ficus insipida</i> Willd.	2	1	9	Neotropical	Species
Olacaceae	<i>Heisteria acuminata</i> (Humb. and Bonpl.) Engl.	1	1	5	Meso-America and Amazon	Genus
Olacaceae	<i>Heisteria concinna</i> Standl.	1	1	3	Meso-America and Amazon	Genus
Humiriaceae	<i>Humiriastrum diguense</i> (Cuatrec.) Cuatrec.	1	1	55	Meso-America and Amazon	Genus
Clusiaceae	<i>Marila laxiflora</i> Rusby	4	1	7	Meso-America and Amazon	No
Rubiaceae	<i>Psychotria horizontalis</i> Sw.	4	3	9	Meso-America and Amazon	Genus
Anacardiaceae	<i>Tapirira guianensis</i> Aubl.	8	4	558	Neotropical	Species
Combretaceae	<i>Terminalia amazonia</i> (J.F. Gmel.) Exell	2	1	18	Meso-America and Amazon	Genus
Hyppocrataceae	<i>Tontelea ovalifolia</i> (Miers) A.C. Sm.	2	1	2	Meso-America and Amazon	Genus
Myristicaceae	<i>Virola sebifera</i> Aubl.	3	1	71	Neotropical	Species

Source – Missouri Botanical Gardens W³ Tropicos database and Sano and Almeida (1998).

Table 4. Herbivore properties of pin transects obtained in the canopy and understorey of each study site in 2003 (values for 2004 followed similar pattern but with reduced numbers in C2 and I1): galls numbers are average per strata in each site/habitat, and leaf damage is the proportion of leaves damaged above 10% of area lost. Canopy values are the mean (SE) of 3 transects.

Site and habitat	All galls	Live galls (%)	Leaf damage
B1 – understorey	21.68 (96.27)	1.23 (5.69%)	0.44 (0.23)
B1 – canopy	6.13 (17.90)	2.5 (41.3%)	0.27 (0.25)
C1 – understorey	1.81 (7.8)	0	0.57 (0.33)
C1 – canopy	9.54 (16.21)	9.54 (100%)	0.33 (0.27)
C2 – understorey	0	0	0.40 (0.22)
C2 – canopy	6.78 (17.18)	6.05 (89.3%)	0.26 (0.27)
C3 – understorey	0.11 (0.31)	0.11 (100%)	0.31 (0.25)
C3 – canopy	1.0 (4.0)	0	0.26 (0.28)
I1 – understorey	2.0 (4.91)	0.83 (41.67%)	0.55 (0.27)
I1 – canopy	1.8 (5.20)	1.4 (77.78%)	0.29 (0.20)

collected, including 59% of which occurred exclusively in the canopy, 28% only in the understorey, and 12.5% found in both habitats. We noted that 81% of our gall host plants at San Lorenzo have either congenics (50%) or are present (31%) in the Brazilian cerrado (Table 3), where gall diversity is one of the highest in the world.

Scaling down to leaf level, we surveyed 22 155 leaves in 2003, of which 2.4% bore galls. In 2004, similar transects yielded 21 839 leaves, with twice the rate of gall infection (5.5%), although restricted to fewer trees. In 2003, a total of 2540 galls were counted, with 15.6% of galls with live larvae, against 2474 galls recorded with 5.4% live larvae in 2004. The mean number of galls per infected leaf was 4.8 in 2003 and 2.05 in 2004. Leaves substantially damaged by free-feeding herbivory were 20 and 17% of leaves surveyed in 2003 and 2004, respectively. Distribution of galls and chewed leaves between habitats and sites are showed in Table 4.

Leaf sclerophylly affected insect herbivory more significantly than foliage density, but in contrasting directions for gall-forming and free-feeding chewing insects. Although the absolute number of chewed leaves was proportional to the total number of leaves available, thus increasing with height, the proportion of chewed leaves decreased significantly with height. This pattern was similar in both sampling seasons (Fig. 2). Nevertheless, the mean number of young leaves per sample did not affect free-feeding herbivory (simple linear regression, $p > 0.05$). On the other hand, sclerophylly affected gall density positively, an effect more important than sample height (Table 5), and more relevant to gall survivorship than oviposition patterns, as examined below.

In 2003, total gall densities were not significantly affected by forest habitat (canopy vs understorey transects), both when investigating number of galls per total leaves or per total infected leaves (Table 5).

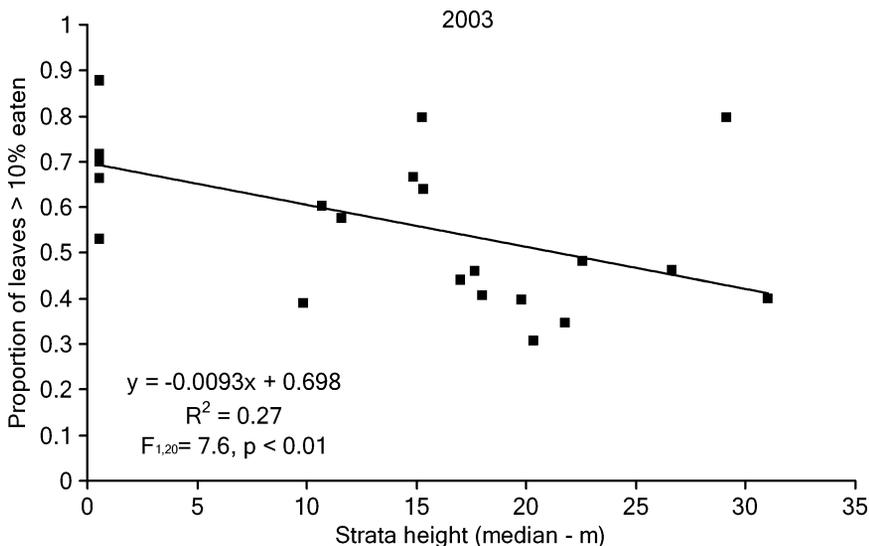


Fig. 2. Relationship between the proportion of damaged leaves (>10% leaf area lost – mean per sample, see methods for details) and sampling height showed for 2003 (patterns similar in 2004 and not shown here).

Table 5. Results of ANCOVA models with average gall densities per sample in 2003 as the dependent variable and expressed as follows: (a) all galls (live and dead) per total no. of leaves surveyed; (b) all galls (live and dead) per no. of infected leaves only; and (c) live galls per total no. of leaves surveyed.

Source of variation	Type III sum of squares	DF	Mean square	F	p
(a) All galls per total no. of leaves					
Sclerophylly (covariate)	20.17	1	20.18	8.05	0.01
Error	50.08	20	2.5		
Sample height (covariate)	0.25	1	0.25	0.1	0.75
Error	50.08	20	2.5		
Habitat	2.53	1	2.53	0.89	0.37
Error	29.5	10.38	2.84		
Site (random block)	24.45	3	8.15	2.5	0.23
Error	10.2	3.14	3.25		
Habitat × Site	9.81	3	3.27	1.3	0.30
Error	50.08	20	2.5		
(b) All galls per no. of infected leaves					
Sclerophylly (covariate)	15.46	1	15.46	8.15	0.01
Error	37.93	20	1.9		
Sample height (covariate)	2.14	1	2.14	1.13	0.30
Error	37.93	20	1.9		
Habitat	0.15	1	0.15	.09	0.76
Error	27.28	17.12	1.59		
Site (random block)	7.29	3	2.43	2.0	0.28
Error	4.02	3.3	1.2		
Habitat × Site	3.6	3	1.2	0.63	0.60
Error	37.93	20	1.9		
(c) Live galls per total no. of leaves					
Sclerophylly (covariate)	0.39	1	0.39	0.41	0.53
Error	19.98	21	0.95		
Habitat	19.45	1	19.45	26.30	0.002
Error	4.68	6.32	0.74		
Site (random block)	1.75	3	0.58	0.86	0.54
Error	2.27	3.35	0.68		
Habitat × Site	2.0	3	0.67	0.70	0.56
Error	19.98	21	0.95		

However, when dead galls were excluded, a striking difference was observed between canopy and understorey transects (Table 5). The average number of live galls collected in the canopy was 13 times greater than in the understorey, a pattern consistent across sites and sampling years (Fig. 3). In 2003, there was a positive correlation between the average number of live galls and sampling height (simple linear regression, $y = 0.364 + 0.0715x$; $R^2 = 0.45$; $F_{1,28} = 22.7$, $p < 0.001$). Further, there was a negative relationship between leaf sclerophylly and the proportion of dead galls ($y = 1.102 - 55.16x$; $R^2 = 0.29$; $F_{1,28} = 11.14$, $p < 0.02$).

In 2004, only the density of live galls was significantly different between canopy and understorey transects (ANCOVA $F_{1,84} = 9.4$, $p < 0.003$). Densities were on average 16 times higher in the canopy than in the understorey (Fig. 3B). However, there was no significant effect of height on gall densities, either for all galls recorded or only for live galls (ANCOVA $F_{1,82} = 0.64$, $p > 0.05$ and $F_{1,82} = 2.5$, $p > 0.05$, respectively). This lack of significance appeared to be related to the high number of galls at intermediate heights, in the lower canopy.

Discussion

Leaf sclerophylly increased with sample height and significantly affected gall densities. In this study, both the density of live galls and the proportion of dead galls were explained by leaf sclerophylly. This pattern was robust and little affected by horizontal variance (sites). Hence, since gall-forming larvae were rather vulnerable to fungi, predators and accidental free-feeding herbivory in non-sclerophyllous leaves, it follows that leaf sclerophylly may promote gall survivorship. This interpretation and our results are thus consistent with the HHE hypothesis of Fernandes and Price (1992) and Price et al. (1998).

The present study further suggests that hydraulic and nutrient stress in large trees in a tropical wet forest may induce a harsh environment for insects in the upper canopy, which may however be particularly suitable to gall-forming insects. Recent data on the variation of stomata closure during day time in *Carinana legalis*, a canopy tree species of semi-deciduous tropical forests (Sanchez and Ribeiro unpubl.), indicated a pattern of closure and photosynthetic inhibition in the upper

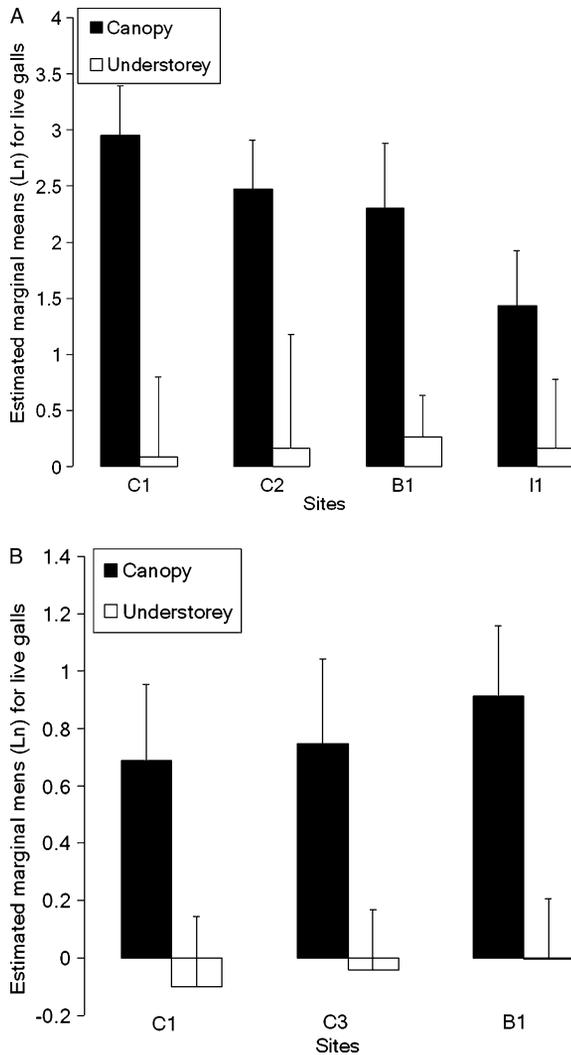


Fig. 3. Distribution of live galls in canopy and understorey transects, during the (A) 2003 and (B) 2004 sampling periods. Logarithmic scaled means as generated from the ANOVA model. See methods for details on estimated marginal means.

canopy similar to that observed in Brazilian cerrado plants (Franco 1998, Franco and Luttge 2002, de Mattos et al. 2002) or Mediterranean vegetation (Tenhunen et al. 1984). Thus, it is plausible that trees ecophysiological responses to water and illumination stress in the upper canopy of tall wet forests may result in a distinct, sclerophyllous environment, favourable to gall-forming insects (Bell et al. 1999).

The effects of sclerophylly (which is an important leaf trait related to nutrient stress) on insect herbivores has been suitably investigated (Moran 1981, Perevolotsky 1994, Madeira et al. 1998, Price et al. 1998, Mendonça 2001, Ribeiro and Brown 2006), as well as other bottom-up evolutionary mechanisms affecting herbivores (Bryant et al. 1983, Coley et al. 1985, Herms and

Mattson 1992). Sclerophylly is beneficial for gall-forming and adverse for free-feeding chewing insects. Nevertheless, its effects on galls cannot be adequately explained based on bottom-up principles only (Madeira et al. 1998, Ribeiro et al. 1999, Ribeiro 2003).

A gall represents a modified, nutrient-rich, tissue around the gall-forming larvae. However, this does not explain why nutritionally poor leaves are preferred by gall insects. Consider galls developing in nutrient-rich leaves: why should it not be beneficial for the larvae? One historical explanation for straight connection between gall insects and sclerophyllous plants has been proposed. The phylogenetic constraints hypothesis (Price 1994) predicts that gall diversity may be strictly dependent on historical factors, such as host plant biogeography and dispersal. The Brazilian cerrado has one of the highest world gall species diversity (Price et al. 1998). Interestingly, a substantial amount of gall host species in Panama belongs to species or genera largely distributed in this South American biome. When these tree species invaded the Panamanian isthmus in a climatically drier period, gall insects may have dispersed with their hosts. However, this reasoning transfers discussion to another geological era and does not clarify evolutionary mechanisms that relate gall insects to the leaf sclerophylly of such host trees.

In the present study, for the first time gall mortality and concomitant distributions of gall versus chewing insects in a tropical wet forest were analysed jointly. Both our distribution and mortality data support the hypothesis of Fernandes and Price (1992), namely their explanation on enemy free space in xeric and sclerophyllous habitats. However, we further support an additional and poorly investigated death cause. The probability of gall-forming insects to be eaten or damaged by chewing insects may decrease with leaf sclerophylly. Risks of gall accidental death by chewing insects may increase in the understorey, where low leaf density concentrate ovipositing activities of both gall forming and chewing insects on similar sites.

Enemy-free space thus appears to be a key factor for increasing ecological success of galls on hosts with sclerophyllous leaves, while the capability to cause the gall tumour should be the mechanism which guarantees a successful survivorship in nutrient-poor leaf, as galls drain resources from the host and induce nutrient-rich tissues.

Concluding remarks

A robust pattern of high gall diversity in harsh environments was documented by the HHE hypothesis. However, the mechanism behind preferences of gall-forming insects for sclerophyllous leaves is still open to discussion. In the present study, we demonstrated that

oviposition preference for sclerophyllous sites is stronger than for open sunny and heated sites. In addition, a noticeable greater larval performance on upper canopy based on sclerophyllous distribution (more importantly than height) corroborates a narrower hypothesis based on evolving choice for enemy free leaves rather than light or other strict environmental condition. Amongst important death causes, we propose accidental death by free-feeding chewing herbivores.

Price et al. (1998) reported 46 species as being the top diversity found for gall species in high altitude grassland in Brazil. Also, these authors recorded 30–40 species for the next six richest habitats. Thus, our conservative estimate of 32 species obtained during the present work in a wet rainforest is similar to the most sclerophyllous and richest habitats listed by Price et al. (1998). This corroborates the year-long results of Medianero and Barrios (2001), who collected 57 gall species in the wet San Lorenzo forest, as opposed to 47 gall species in the drier Parque Natural Metropolitano forest, in Panama city. Further, both the data of Medianero and Barrios (2001) and Medianero et al. (2003) confirm that the upper canopy of tall closed wet tropical forests may represent a suitable habitat for galls-forming insects, perhaps among the richest in the world.

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