

UNIVERSIDADE FEDERAL DE UBERLÂNDIA  
INSTITUTO DE BIOLOGIA  
CURSO DE CIÊNCIAS BIOLÓGICAS

Empirical evidence of color changing in galls: under the light of light

Phabliny Martins Silva Bomfim

Trabalho de Conclusão de Curso apresentado à  
Coordenação do Curso de Ciências Biológicas, da  
Universidade Federal de Uberlândia, para a  
obtenção do grau de Bacharel em Ciências  
Biológicas

Uberlândia - MG

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Uberlândia, 15 de dezembro de 2017.

BANCA EXAMINADORA

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Prof. Dr. Denis Coelho de Oliveira  
Presidente da Banca - Orientador

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Profa. Dra. Ana Sílvia Franco Pinheiro Moreira  
Membro

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Prof. Dr. Vinícius Coelho Kuster  
Membro

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## **Abstract**

Several studies have hypothesized about coloration in galls, including change in gall metabolism, galling–natural enemies interactions, gall maturation stage, influence of galling exogenous cytokinins and aposematism. However, none of these were confirmed so far. On the other hand, the vibrant colors of some galls may be explained simply by the effect of light exposure. This may lead to anthocyanin (Anth) accumulation, functioning as a mechanism of defense against the effects of sun light. Nevertheless, no studies provided systematic quantification/observation of this phenomenon. Herein, we ask whether gall tissues accumulate Anth like a mechanism of defense against the effects of sun light and which consequences may occur in photosynthetic rate. We demonstrate that adaxial galls induced by Cecidomyiidae (Diptera) in *Qualea parviflora* (Vochysiaceae) are exposed to higher light incidence, presenting more Anth accumulation and therefore red coloration. On the other hand, the abaxial galls receive less light compared with adaxial ones, with less Anth and green coloration. In addition, in galls from angled leaves, the greater the angulation of the leaf, the higher the non-equality between Anth on sun and shade sides of galls. This explains the intermediate color pattern found in galls from high angulated leaves, presenting green and red, respectively in shade and sun sides. We did not find any differences according to the concentration of photosynthetic pigments. However, concerning photosynthesis parameters,  $(F_m' - F')/F_m'$  and  $F_v/F_m$  were higher in bellow galls, indicating a better photosynthetic performance compared with above galls. In the other hand, due to high light exposure, the NPQ and  $R_{fd}$  were higher in above galls, indicating that the Anth functions as a photoprotective pigment, maintaining the high tissue vitality in galls exposed to the stress condition. We demonstrate empirical evidence that the variation of light regimes may create differential Anth accumulation and influence coloration in galls.

**Keywords:** Gall; Coloration; Anthocyanin; Light; Photosynthesis

## **Empirical evidence of color changing in galls: under the light of light**

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### **Introduction**

Galls are neoformed structures induced on host plant tissues by the feeding activity of some different agents like viruses, bacteria, nematodes, and insects as the most representative group (Shorthouse *et al.* 2005; Shorthouse & Rohfritsch 1992)<sup>1</sup>. The interaction between galling insect-host plant is species-specific, resulting in a diversity of gall morphotypes (Isaias *et al.* 2013). These structures showed several characteristics like projections, trichomes, bizarre shapes and conspicuous colors (Stone & Schönrogge 2003), which lead galls to be visually clearly distinguishable from other background host plant organs (Lev-Yadun 2016). This conspicuousness occurs generally by the red color, caused principally by the reflection of anthocyanins in the gall tissues (e.g. Dias *et al.* 2013).

Anthocyanins (Anths) are water-soluble pigments that have several key roles in plants, including anticancer (Bowen-Forbes *et al.* 2010) and antiviral (Valcheva-Kuzmanova *et al.* 2003). However, the main functions attributed to these pigments are that of antioxidant (Wiczowski *et al.* 2013) and protection against light stress caused by UV radiation. In this last case Anths can work like a sunscreen (Solovchenko & Schmitz-Eiberger 2003; Karageorgou & Manetas 2006). This strategy of accumulating Anths has been observed in flowers (Smith & Goldberg 2015), fruits (Lekkman *et al.* 2016), roots (Maloney *et al.* 2014) and leaves (Huang *et al.* 2014).

The role of red color on galls and what triggers the concentration of the Anth pigments in these tissues, has raised in the literature. Basically, the hypotheses related to gall

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<sup>1</sup> As normas de formatação seguidas são da revista científica Plant Species Biology.

pigmentation range from physiological to defensive (Lev-Yadun 2016). For example, Dias *et al.* (2013) suggested that the change of color is influenced by the interactions between galling insects and its natural enemies, which then interferes in gall metabolism. However, they did not find any evidences corroborating this. It was also suggested that the gall color is given as effect of the maturation stage, with older galls being red (Sáiz & Núñez 1997; White 2010; Lev-Yadun 2016). For instance, White (2010) proposed that the galling agent induces the gall to senesce early, releasing nutrients from gall dying tissues and changing its coloration. Despite this temporal correlation seems to be applicable to some systems, there are no studies testing any adaptive value of such ontogenetic changes (Lev-Yadun 2016).

Connor *et al.* (2012) proposed that red coloration is a “fabricational noise” of the gall development. These authors argue that gall-inducing insects produce exogenous cytokinins, leading to establishment of gall sink structure. Then, the exogenous cytokinins promote a cascade of effects, leading to Anth synthesis. Despite this hypothesis seems to be applicable in some systems, it was quickly criticized as an oversimplification by Gerchman *et al.* (2013). They argued that the link between cytokinins and pigmentation is not obligatory. In addition, since the galling habit has evolved independently several times, the role of gall coloration may vary.

Finally, the most classic is the aposematic gall hypothesis, which states that the vibrant colors added to the accumulation of unpalatable and toxic secondary metabolites protect galls against the attack of enemies (Inbar *et al.* 2010). Despite gall coloration is a common and in many cases possible an adaptive trait, the aposematic hypothesis has not been corroborated yet. However, accordingly the same authors that launched the hypothesis, to make any affirmations about the selective pression that leads to conspicuousness, is necessary initially to discard the gall exposition to solar radiation, which is the principal cause of Anths accumulation in vegetal tissues (Inbar *et al.* 2010). In fact, some reports in the literature

recognize the possibility of light exposure related to gall coloration (Wool 2004; Lev-Yadun 2016). An example of the possibility of interference from radiation exposure to gall color was speculated for *Eucalyptus camaldulensis* (Myrtaceae) galls (Protasov *et al.* 2007). Although the authors suggest that the incidence of light throughout the day (south, north, east and west) causes color variation of the galls, it was not systematically quantified or observed. In summation, there are many hypotheses in the literature trying to elucidate the phenomenon of gall pigmentation, which led to intense discussions in the last decade (reviewed in Lev-Yadun 2016). However, the few systems addressed are described mainly speculatively, as examples, without precise tests of causes and consequences. Thus, it's past time to test each one of the hypotheses methodologically in the field (Gerchman *et al.* 2013). For instance, the causal relationship between light exposure and Anths accumulation in galls has never been properly investigated. The same lack applies to the functions of the accumulation of these substances in front of the luminous stress to which the gall tissues are subjected. To test these possibilities, it is necessary a model system that presents galls under different light regimes, allowing the differential accumulation of Anths.

Galls induced by Cecidomyiidae (Diptera) on *Qualea parviflora* (Vochysiaceae) (Urso-Guimarães *et al.* 2003) occur in the abaxial and adaxial portions of the leaves, probably submitted to different light regimes. Preliminary observations suggest that adaxial galls (hereafter, above galls) are red, while abaxial galls (hereafter, below galls) are green (Fig. 1A, B). In addition, in some angled leaves, it is possible to observe intermediate galls containing the two colors (Fig. 1C). In this sense, this case represents an ideal system to test if light can generate the Anth accumulation in galls and consequently influence its coloration. So, we asked: (1) Can the variation of light regimes generate differential Anth accumulation in galls? and (2) how the coloration associated with light affect different parameters of photosynthesis?

## Materials and Methods

### Study Site

This study was carried out at Estação Ecológica do Panga, Uberlândia, Minas-Gerais, Brazil, in an area of stricto sensu cerrado. Climate in the region is seasonal, with rainy (October – March) and dry seasons (April – September) (AW in the Köppen-Geiger scale) (Kottek *et al.* 2006). Mean annual precipitation in Panga is 1482 mm<sup>3</sup> and temperature monthly average is 22,8 °C (Cardoso *et al.* 2009).

### Studied Species

*Qualea parviflora* Mart. (Vochysiaceae) is a deciduous tree, found in several Cerrado phytophysognomies. This plant is considered a super-host, harbouring several galling insects and their respective gall morphotypes (Araújo *et al.* 2013). The studied gall is globoid with projections, induced by an unidentified Cecidomyiidae (Diptera) (Urso-Guimarães *et al.* 2003).

### Field Procedures

During March 2017, we selected the first 22 individuals of *Q. parviflora* found (mean height  $\pm$  SD = 1.94  $\pm$  1.18 m; range = 0.56 – 4.30 m). In these plants, we measured height and counted the number of leaves and galls, regarding their position as below and above. Below (n = 29) and above galls (n = 21) had their occurrence height in plant, as well as height and width measured (using a Digimess® 100.256 digital calliper, 0.01 mm readability).

The amount of light incidence (taken as  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) on abaxial and adaxial surfaces of leaves was measured using the quantometer (LI-250A Light Meter). For this purpose, we selected ten leaves positioned straight (leaf angulation < 5 degrees) per plant.

Leaves angles were taken by using a protractor. Each leaf has its light incidence taken on upper and lower surfaces, approximately at the occurrence region of respective galls. The same leaves were measured during three periods of the day by the same person (between 8.00 – 10.00 h, 11.00 – 13.00 h and 14.00 – 16.00h). Plants were measured in the same sequential order during all periods.

### **Anth analyses**

We used spectral reflectance patterns to assess the Anth content on plant tissues by calculating the Anthocyanin reflectance index (ARI) (*sensu* Gitelson *et al.* 2001). The ARI is determined by the difference between the inverse reflectances of 550 (Anth absorption peak) and 700 nm (subtraction of chlorophyll effect) (Gitelson *et al.* 2001). However, as some leaves and galls presented low amounts of Anth, the subtraction of chlorophyll could generate very small values, including negative. Thus, as suggested by Gitelson *et al.* (2001), we did not discount the values regarding 700 nm. Reflectance measurements were obtained by using the JAZ spectrometer (Ocean Optics®). First, we measured 25 below and 15 above galls coming from leaves positioned straight (angulation < 5 degrees). The spectrometer's light was positioned on the top of the gall and the mean of three measurements was extracted. We also calculate the ARI of the respective leaf each gall was (mean of three measurements), considering the abaxial surface for below galls and the adaxial for above galls.

In addition, reflectance on galls from angled leaves (> 5 degrees) was measured, taking 65 below and 63 above. For this case, each gall was divided in the middle into a sun (side facing the light incoming) and shade portion (side opposite to the sun). Each side and respective leaf were measured three times (mean extracted). In addition, each gall had its occurrence height cataloged in the field.

### Photosynthetic Pigment analyses

Pigment contents were extracted using fresh above ( $n = 10$ ) and below galls ( $n = 9$ ) coming from different individuals. Each gall was weighed in laboratory and immersed in 80% acetone. Samples were macerated, centrifuged at 1000 rpm for 5 minutes (centrifuge Centribio Mod. 80-2B) and the supernatant was analyzed in spectrophotometer (Biospectro Mod. SP-220 UV-Vis) at the wavelengths of 470, 646 and 663nm. Using the equations proposed by Lichtenthaler and Wellburn (1983), we calculated the concentrations of pigments. The results of carotenoids, chlorophyll *a*, *b*, and total are expressed in  $\mu\text{g}$  of pigments per gram of sample.

### Chlorophyll fluorescence analyses

We selected above and below galled leaves (10 samples each) from different individuals for chlorophyll fluorescence patterns. All measurements were conducted in the field between 8.00 – 10.00 AM using a Handy FluorCam – PSI (Photon Systems Instruments<sup>®</sup>, Czech republic), after the samples being adapted to the dark during 30 min. The Fluorcam7<sup>®</sup> software as well as its quenching protocol were used for data acquisition and image processing. We evaluated the following parameters:  $F_0$  = minimal fluorescence in dark-adapted state;  $F_m$  = maximum fluorescence in dark-adapted state;  $(F_m' - F')/F_m'$  = PSII operating efficiency;  $F_v/F_m$  = maximum quantum yield; NPQ = non-photochemical quenching during light adaptation; and  $R_{fd}$  = fluorescence decline ratio in steady-state.

### Statistical analyses

We compared the number of above and below galls by fitting a GLMM (generalized linear mixed model) adjusting a Poisson distribution for count data. Number of leaves, plant height, and the mean light incidence on upper and lower surfaces per individual were taken as

random effects. We investigated the factors influencing the difference between the number of above and below galls by using a gamma GLM. The ratio between the number of above and below galls on each plant was taken as response variable. Since the gamma distribution is not applicable to values of zero (Zuur *et al.* 2009), a constant of 0.001 was added to the response variable. Plant height and light incidence on the lower surface of leaves were not included in the analysis due to its high correlation, respectively with number of leaves in plant (correlation = 0.9) and light incidence on upper surface (correlation = 0.93). The full model comprised these two latter (correlation = 0.45) and their interaction as predictor variables. In order to examine differences in gall volume according to position (upper or lower), a gamma GLMM was applied having gall occurrence height and plant identity (number of individual) as random effects. Gall height and width measurements were used to calculate volume based on an oblate ellipsoid.

Photons incidence differences between upper and lower parts of leaves were compared by using gamma GLMM with log link, having leaf identity, plant identity and period of the day as random effects. To compare Anth concentration between upper and lower galls from straight angled leaves, we fitted a gamma GLMM with log link having the ARI of galls as response variable. Position, ARI of leaves and the interaction term were treated as fixed while plant identity as random effects.

In galls coming from angled leaves, we used the ratio between shade and sun sides as response variable. Values close to one imply a similar Anth deposition on both sides. On the other hand, the lower the shade-sun ratio, for instance, the higher the Anth difference between sides, meaning a higher accumulation on the side facing the sun. We investigated factors influencing the shade-sun ratio by fitting a GLMM with gamma adjusted distribution. Leaf angulation, position, ARI of leaves and all possible interactions were treated as fixed while plant identity and height of gall as random effects. We considered multicollinearity among the

continuous explanatory variables to be not problem in this model, presenting a low correlation of 0.082.

Concerning pigment parameters, we used Welch's t-tests to analyze differences between above and below galls according to (1) chlorophyll *a*, (2) chlorophyll *b*, (3) total chlorophyll, (4) ratio of chlorophyll *a*/chlorophyll *b*, (5) carotenoids and (6) total chlorophyll/carotenoids. We used the *p.adjust* function in R software stats package to apply the Benjamini and Hochberg (BH) (1995) correction and avoid type I error due to multiple testing.

We analyzed the different chlorophyll fluorescence parameters according to gall position by building a LMM (linear mixed-effects model) for each one of them (BH corrected). The values obtained for leaves in a given parameter were treated as random effect. All models were log corrected to improve residuals distribution, except for that testing Fm.

All models (LMM, GLM and GLMM) were built using the package *lme4* (Bates *et al.* 2014). In addition, the likelihood ratio (LR) test was applied to attain p values for fixed factors (i.e. comparing two models, one with and other without a given factor to assess its significance) (Zuur *et al.* 2009). Tests were validated by inspecting homogeneity of fitted vs. residual values plots, histograms, quantile-quantile plots and Cook's distance (Zuur *et al.* 2009). Whenever a factor was find significative, we determined its marginal  $R^2$  (*sensu* Nakagawa & Schielzeth 2013) using the package *r2glmm* (Jaeger 2016). Analyses were carried out in R statistical environment version 3.4.1 (R Core Team 2017).

## Results

We found that number of below galls ( $51.5 \pm 43.09$ ) in the field was, on average, 4.44 times higher than that of above galls ( $11.59 \pm 13.26$ ) ( $\chi^2 = 600.07$ ;  $df = 1$ ;  $p < 0.001$ ;  $R^2 = 0.33$ ; Fig 2A). There was no relationship between the above-below galls ratio and the number

of leaves ( $\chi^2 = 3.69$ ;  $df = 2$ ;  $p = 0.27$ ), light incidence on leaves upper surface ( $\chi^2 = 2.98$ ;  $df = 2$ ;  $p = 0.35$ ) or the interaction term ( $\chi^2 = 0.54$ ;  $df = 1$ ;  $p = 0.54$ ). Considering volume, there was no difference between below ( $294.97 \pm 84.58$ ) and above galls ( $213.68 \pm 93.55 \text{ mm}^3$ ) ( $\chi^2 = 0.19$ ;  $df = 1$ ;  $p = 0.66$ ).

Light incidence measurements revealed that upper surface (mean  $\pm$  SD:  $166.04 \pm 259.77$ ) received more light than lower surface of leaves ( $7.29 \pm 10.09$ ) ( $\chi^2 = 1585.6$ ;  $df = 1$ ;  $p < 0.001$ ;  $R^2 = 0.50$ ; Fig 2B). On average, the former received 22.78 times more light, presenting also a wider range (from 4.13 to 1071.9) when compared to the lower surface (0.059 to 83.42).

Concerning Anth concentration on galls coming from straight angled leaves, we found significative differences according to position, with upper galls ( $0.44 \pm 0.12$ ) showing an average ARI 6.47 times higher when compared to the lower ( $0.068 \pm 0.024$ ) ( $\chi^2 = 76.39$ ;  $df = 2$ ;  $p < 0.001$ ;  $R^2 = 0.86$ ; Fig 2C). Anth content on leaves (ARI of leaves:  $\chi^2 = 1.17$ ;  $df = 2$ ;  $p = 0.56$ ) or the interaction term (ARI of leaves\*Position:  $\chi^2 = 1.02$ ;  $df = 1$ ;  $p = 0.31$ ) had no effect on Anth concentration on galls.

Considering the factors determining the shade-sun ARI ratio in galls coming from non-straight angled leaves, angulation had a significant effect ( $\chi^2 = 31.38$ ;  $df = 5$ ;  $p < 0.001$ ;  $R^2 = 0.07$ ; slope = 0.65; intercept = 2.20; Fig 2D). The greater the angle of the leaf, the higher the non-equality between Anth on sun and shade sides of galls. We did not found effects concerning the ARI of leaves ( $\chi^2 = 3.70$ ;  $df = 5$ ;  $p = 0.59$ ), position ( $\chi^2 = 9.45$ ;  $df = 5$ ;  $p = 0.09$ ), or any interaction term (Angulation\*Position:  $\chi^2 = 0.08$ ;  $df = 2$ ;  $p = 0.96$ ; Angulation\*ARI of leaves:  $\chi^2 = 1.41$ ;  $df = 2$ ;  $p = 0.50$ ; Position\*ARI of leaves:  $\chi^2 = 0.47$ ;  $df = 2$ ;  $p = 0.79$ ; Angulation\*Position\*ARI of leaves:  $\chi^2 = 0.004$ ;  $df = 1$ ;  $p = 0.95$ ).

We did not find any differences according to the concentration of photosynthetic pigments or the ratios between them (Table 1). There are no differences in the minimum ( $F_0$ )

and maximum ( $F_m$ ) fluorescence of PSII in the dark-adapted state between above and below galls (Table 2). However,  $(F_m' - F')/F_m'$  and  $F_v/F_m$  were respectively 13 and 5.5 % higher in bellow galls when compared with above galls (Fig 3A, B; Table 2). On the other hand, NPQ and  $R_{fd}$  were 86.5 and 76.7 % higher in above galls, respectively (Fig 3C, D; Table 2).

## Discussion

### *Anths accumulation in different regimes of light*

The variation of light regimes generated differential Anth accumulation in galls. We observe that there is a relation between the incidence of light and the accumulation of Anths in galls in the system Cecidomyiidae – *Q. parviflora*, in the same way more previously showed for galls of *S. wertheimae* as in one experiment than young galls exposed to sunlight become red and by young leaves (Wool 2004, Karageorgou and Manetas 2006). This makes sense because the Anths work like a sunscreen then tend to accumulate in areas that require protection (in this case protection against the damages caused by the light stress) (e.g. Neill & Gould 2003, Solovchenko & Schmitz-Eiberger 2003). The different concentrations of Anths in galls from straight angled leaves might be explained by the upper galls are more expose to the sun light. In this way these galls suffer more with light stress and hence accumulate more Anths to avoid or to soften the negative effect of this stress. The lack of relationship between Anth in leaves and their respective galls demonstrate that galls have their own Anth metabolism, independently of the quantity present in leaves. The number of bellow galls was bigger than the above galls, this may represent one preference of the gall insect for the position abaxial of the leaves to avoid the luminous stress.

According with Inbar *et al.* (2010), to corroborate the aposematic hypothesis is necessary to discard first the influence of the gall exposition to solar radiation. In galls of *Q. parviflora* is evident that the sun light influences the pattern of color (tuning red the gall

exposed to the sun light or keeping green those not exposed) being unlikely the occurrence of the aposematic hypothesis for this case. In the Connor *et al.* (2012) hypothesis for the coloration to be a “fabricational noise”, it was necessary that all the galls have the same or similar color, because this secondary effect caused exogenous cytokinins would occur simply due to innate factors of the galling insects. Once in the literature there are several hypotheses of coloration in galls not corroborated, this study is important to elucidate one interesting case in which the sun light in different regimes cause the accumulation of Anths, changing the color of the gall expose to it. Its possible that these hypotheses can explain specific cases being necessary more studies to understand more about how the recruitment process of Anth works.

#### *Association between coloration and parameters of photosynthesis*

The photosynthetic activity on gall tissues has been discussed based on two main perspectives, (i) avoiding hypoxia and hypercarbia in the high compact gall tissues (Pincebourde & Casas 2016, Oliveira *et al.* 2017) and (ii) maintenance of redox homeostasis (Isaias *et al* 2015, Oliveira *et al.* 2017). In the system of *Q. parviflora*, the galls are exposed to different light conditions based on the position on the leaf organ. Consequently, these galls showed distinct patterns of coloration and photosynthesis performance. We found low values of maximum quantum efficiency of PSII ( $F_v/F_m$ ) and maximum PSII operating system,  $(F_m' - F')/F_m'$ , (Maxwell & Johnson, 2000) that indicates a decrease of photosynthetic performance in above gall tissues. Despite this, above galls showed high levels of Anth when compared to below galls. Anths are produced in vegetative organs under stress conditions as high-light, temperature and pathogen attack (Dixon & Paiva 1995, Chalker-Scott 1999, Albert *et al.* 2009). This substance is a potent antioxidant that acts as a reactive oxygen species scavenger

and protecting the photosynthetic tissues from photoinhibition (Neill & Gould 2003, Merzlyak *et al.* 2008).

The decrease in photochemistry in galls should lead to an increment in non-photochemical quenching (NPQ), which indicates how the tissues convert the absorbed energy into heat (Pavlovic 2012), an efficient mechanism of energy dissipation. NPQ dissipation increase in galls induced by *Tetraneura ulmi* and *Eriosma ulmi* on leaves of *Ulmus glabra* (Sansone *et al.* 2012) and galls induced by *Bystracoccus mataybae* on leaves of *Matayba guianensis* (Oliveira *et al.* 2017). Herein, the decrease of  $F_v/F_m$  and  $(F_m' - F')/F_m'$  on galls of *Q. parviflora* occur concomitant to the increase of NPQ and  $R_{fd}$  in above galls. Although above galls are exposed to high-light conditions, the  $R_{fd}$  (empiric parameter used to assess plant vitality) increase. This result is a clear indicative of the role of Anth as photoprotective pigments, maintaining the high tissue vitality in galls exposed to stress condition. Even though the photosynthetic activity is not capable of supporting gall development, it is important to maintain tissue stability and the aerobic gall metabolism in both above and below galls of *Q. parviflora*. Once that the consumption of molecular oxygen by the respiration of the plant cells and of the galling insect ends up in CO<sub>2</sub> production, which is consumed in the Calvin-Benson cycle of photosynthesis avoiding hypercarbia (Pincerboune & Casas 2016, Oliveira *et al.* 2017).

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## Tables

Table 1. Results of photosynthetic pigment analysis. Values of above and below galls are expressed as mean  $\pm$  SD and  $p$  values are Benjamini–Hochberg corrected.

|                            | Gall              |                   | Statistics |      |      |
|----------------------------|-------------------|-------------------|------------|------|------|
|                            | Above             | Below             | t          | df   | $p$  |
| Chl <i>a</i>               | 0.015 $\pm$ 0.007 | 0.013 $\pm$ 0.003 | -0.99      | 13.5 | 0.44 |
| Chl <i>b</i>               | 0.017 $\pm$ 0.007 | 0.012 $\pm$ 0.003 | -2.20      | 12.6 | 0.19 |
| Chl tot                    | 0.033 $\pm$ 0.013 | 0.025 $\pm$ 0.006 | -1.66      | 13.0 | 0.24 |
| Chl <i>a</i> /Chl <i>b</i> | 0.88 $\pm$ 0.23   | 1.06 $\pm$ 0.16   | 2.00       | 16.0 | 0.19 |
| Carot                      | 0.058 $\pm$ 0.014 | 0.051 $\pm$ 0.018 | -0.93      | 15.2 | 0.44 |
| Chl tot/Carot              | 0.56 $\pm$ 0.18   | 0.52 $\pm$ 0.11   | -0.65      | 15.2 | 0.52 |

Table 2. Results of photosynthesis parameters analysis. Values of above and below galls are expressed as mean  $\pm$  SD. Significant  $p$  values (Benjamini–Hochberg corrected) are expressed in bold. df = 1 in all cases.

|                                        | Gall             |                  | Statistics |                  |                |
|----------------------------------------|------------------|------------------|------------|------------------|----------------|
|                                        | Above            | Below            | $\chi^2$   | $p$              | R <sup>2</sup> |
| F <sub>0</sub>                         | 45.4 $\pm$ 11.2  | 49 $\pm$ 6.8     | 1.24       | 0.26             | –              |
| F <sub>m</sub>                         | 184.9 $\pm$ 61.3 | 225.6 $\pm$ 37.3 | 3.28       | 0.084            | –              |
| (F <sub>m</sub> '-F')/F <sub>m</sub> ' | 0.62 $\pm$ 0.04  | 0.70 $\pm$ 0.03  | 15.90      | <b>&lt;0.001</b> | 0.54           |
| F <sub>v</sub> /F <sub>m</sub>         | 0.73 $\pm$ 0.05  | 0.77 $\pm$ 0.02  | 5.53       | <b>0.028</b>     | 0.26           |
| NPQ                                    | 0.97 $\pm$ 0.21  | 0.52 $\pm$ 0.07  | 29.27      | <b>&lt;0.001</b> | 0.76           |
| R <sub>fd</sub>                        | 1.29 $\pm$ 0.25  | 0.73 $\pm$ 0.08  | 31.36      | <b>&lt;0.001</b> | 0.78           |

**Figure**

Fig 1. Above (A) and below galls (B). Below gall on a leaf angled 90 degrees (C). The white arrow indicates sun side and the black arrow the shade side. Scale bar: 5 mm.

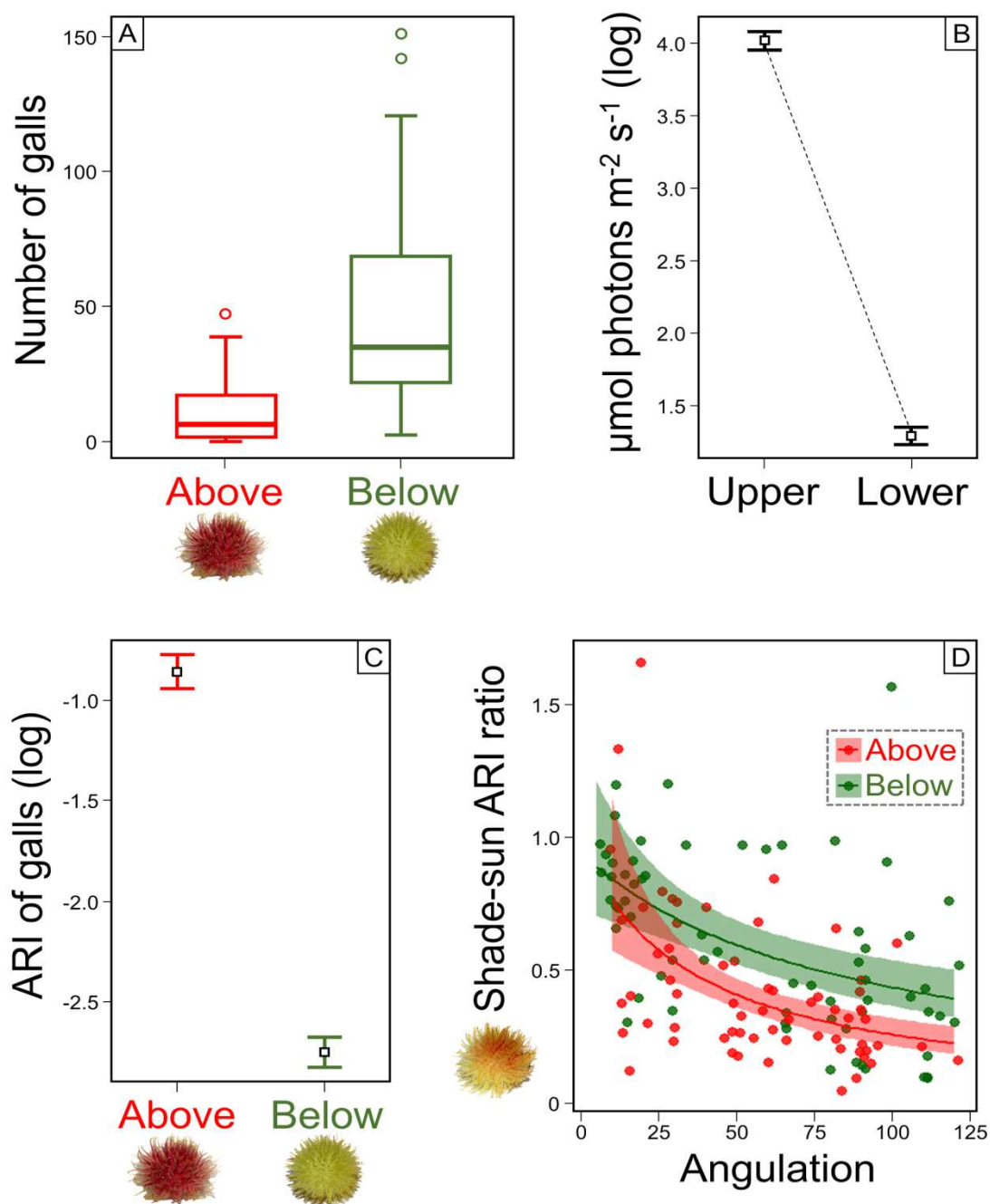


Fig 2. Number of galls according to the possible position on leaf: above or below (A). Box plots show median, quartiles and extreme values. Photons incidence on upper and lower surface of leaves (B). White squares and line segments indicate respectively means and 95% CI. ARI of galls according to galls position (C). Shade-sun ARI ratio of galls in relation to leaf angulation (D). Curves (gamma adjusted) are separated according to position to demonstrate that the same pattern occurs in both above and below galls.

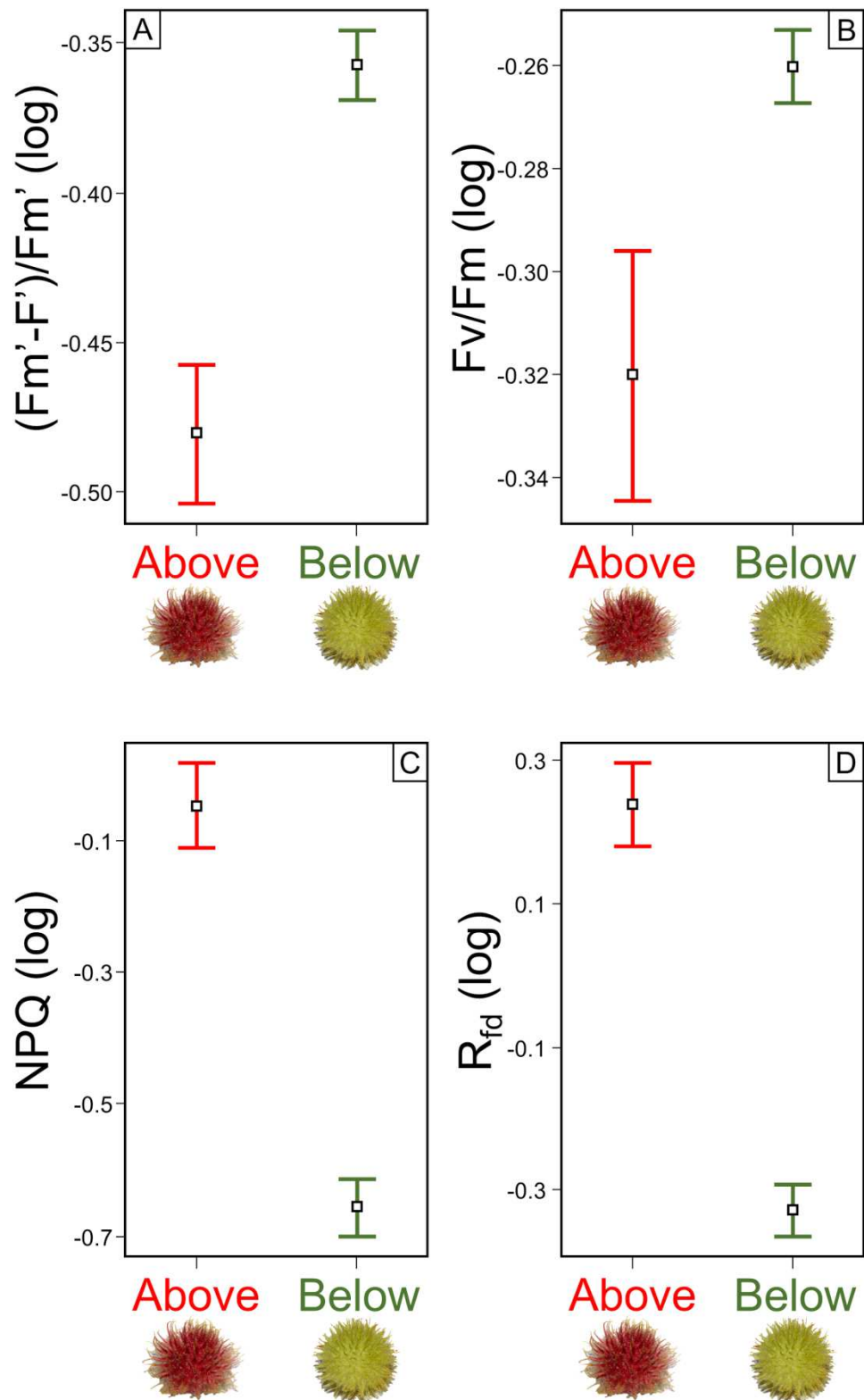


Fig 3.  $(F_m' - F')/F_m'$  (A),  $F_v/F_m$  (B), NPQ (C) and  $R_{fd}$  (D) photosynthetic parameters. White squares and line segments indicate respectively means and 95% CI.