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Photochemical performance and source-sink relationships in galls induced by *Pseudophacopteron longicaudatum* (Hemiptera) on leaves of *Aspidosperma tomentosum* (Apocynaceae)

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Abstract

The establishment of the galling insect generates a biotic stress that leads to tissue transformation. However, galls can maintain chlorophyll (Chl) and consequently photosynthesize. Herein, we evaluated the consequences of the biotic stress generated by the galling insect *Pseudophacopteron longicaudatum* on photosynthetic rate during the leaf galls development on *Aspidosperma tomentosum*. In addition, we quantified polysaccharides and water content in order to evaluate the capacity of gall tissues to drain photoassimilates. The non-galled leaves contained more Chl and carotenoids per gram of fresh mass. Galls had lower values of maximum PSII quantum yield, fluorescence decline ratio, and nonphotochemical quenching compared to non-galled tissue. These results showed a significant reduction of photosynthesis photochemistry in galled tissues although total soluble sugar did not differ between gall and non-galled leaves. Water-soluble polysaccharides, relative water content, and leaf specific mass were significantly higher in galls and increased through their development, suggesting a sink relationship.

Additional key words: gall photosynthesis; green island; herbivory; Kautsky effect; oxidative stress.

Introduction

The induction and establishment of galling insects generate a biotic oxidative stress leading to imbalance of redox homeostasis in the host plant tissues (Isaias *et al.* 2015) and triggering the developmental processes of galls (Oliveira *et al.* 2014). Gall development on host plant organs has shown convergent morphogenetic steps such as cell hypertrophy and tissue hyperplasia, which create a coordinated gradient of cell expansion and tissue growth (Oliveira and Isaias 2010, Isaias *et al.* 2011, Carneiro *et al.* 2014a, Ferreira and Isaias 2014, Oliveira *et al.* 2016). Thus, galls can be considered to be new plant organs induced by galling organisms, especially by insects (Shorthouse *et al.* 2005). The metabolism of these neoformed plant organs is deeply changed, especially in terms of Chl and carotenoid content, enzymatic activity, nutrient composition, carbon and water allocation, as well as photosynthetic performance (El-Akkad 2004, Oliveira and Isaias 2010, Castro *et al.* 2012, Huang *et al.* 2014, Oliveira *et al.* 2017, Rezende *et al.* 2018).

Photosynthetic activity is a basic metabolic process in plants which, in addition to performing carbon fixation, helps with the control of redox homeostasis in both non-galled tissues (Foyer and Noctor 2005) and galls (Isaias *et al.* 2015). In addition, the maintenance of photosynthetic activity in gall tissues can increase O_2 production and CO_2 consumption, which reduce tissue hypoxia and hypercarbia (Pincebourde and Casas 2016, Oliveira *et al.* 2017). However, the galling insects can also damage the

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Abbreviations: Chl – chlorophyll; DM – dry mass; F' – measurement of the light-adapted fluorescence signal; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_m' – fluorescent signal when all PSII centers are closed in the light-adapted state; F_v – variable fluorescence; F_v/F_m – maximum PSII quantum yield in dark-adapted state; $(F_m' - F')/F_m'$ – PSII operating efficiency in light-adapted steady state; LSM – leaf specific mass; NPQ – steady-state nonphotochemical quenching in the light; R_{fd} – fluorescence decline ratio in steady state; RWC – relative water content; TM – turgid mass.

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photosynthetic apparatus of both non-galled and gall tissues (Oliveira *et al.* 2011, 2017; Huang *et al.* 2014, Kmieć *et al.* 2018), thus reducing the efficiency of the photosystems and consequently the photosynthesis. The photosynthetic performance of gall tissues depends on many factors, including the content of photosynthetic pigments (Nabity *et al.* 2008) and the level of oxidative stress generated by the insect's activity in gall tissues. In this context, the Chl *a* fluorescence parameters may reveal a photochemistry pattern which may be used to assess stress in host plants (Nabity *et al.* 2008, Kalaji *et al.* 2016) and in green gall systems (Oliveira *et al.* 2011, 2017). For instance, the high values of F_0 (minimum fluorescence of PSII in dark-adapted state) indicate that the primary electron acceptor is impaired, and the reaction center of the PSII is damaged (Bolhàr-Nordenkampf *et al.* 1989), a fact that can be used as an indicator of biotic stress (Kmieć *et al.* 2018). In contrast, a decrease of F_v/F_m values (maximum PSII quantum yield in dark-adapted state) can be interpreted as a result of stress factors since the decrease of this parameter implies PSII damage that reduces the photochemical capacity (Roháček 2002, Bączek-Kwinta *et al.* 2011). R_{fd} (fluorescence decline ratio in steady state) may also be used as a diagnostic parameter of stress in plants (Lichtenthaler and Miehé 1997), with low values representing the loss of tissue vitality.

Galls induced by chewing and scraping-sucking insects can develop a typical nutritive tissue around the larval chambers that accumulate primary metabolites (*i.e.*, proteins, carbohydrates, and lipids). On the other hand, galls induced by phloem-sucking insects usually do not develop a true nutritive tissue (Bronner 1992). These phloem-sucking insects impose a strong physiological demand on host plant tissues, acting as a true sink of photoassimilates (Mani 1964, Castro *et al.* 2013, Huang *et al.* 2014). These changes in source–sink relationships induced by galling insects can impact the photosynthetic rate of both non-galled and galled tissues (Zangerl *et al.* 2002, Nabity *et al.* 2008, Oliveira *et al.* 2017). However, despite the increase of sink demand for gall tissues, some species of galling insects do not cause significant effects on the photosynthetic performance of tissue surrounding the gall or of the gall tissues themselves, as reported for galls induced by aphids on *Rhus glabra* L. and by psyllids on *Aspidosperma australe* Müll. Arg. leaves (Larson 1998, Oliveira *et al.* 2011).

The objective of the present study was to assess the cascade of photochemical apparatus responses caused by the induction of galls on *Aspidosperma tomentosum* Mart. (Apocynaceae) leaves by *Pseudophacopteron longicaudatum* (Malenovský, Burckhardt, Queiroz, Isaias & Oliveira) (Hemiptera: Psyllidae), a phloem-sucking galling insect (Malenovský *et al.* 2015, Oliveira *et al.* 2016). The *P. longicaudatum* galling insect can induce galls on two host plants, *Aspidosperma tomentosum* Mart. and *Aspidosperma macrocarpon* Mart. (Malenovský *et al.* 2015). In both host plants, the first-instar nymph of the galling insect induces galls on the abaxial leaf surface and develops an intralaminar globoid morphotype (Isaias *et al.* 2013). For the current study, we analyzed the photochemical efficiency of gall tissues in three stages of gall development (*i.e.*, young, mature, and senescent). We also assessed the photosynthetic pigments, carbohydrate content, leaf specific mass, tissue water storage, and relative water content in order to understand the photosynthetic and source–sink relationships occurring during the leaf gall development on *A. tomentosum*.

Materials and methods

Study area and plant sampling: The samples of non-galled leaves and galls on *A. tomentosum* (Fig. 1*A*) were collected in the Cerrado *stricto sensu* in Minas Gerais, Brazilian southeast (18°58'57"S, 48°17'45"W). The climate is megathermic Cwa with well-defined dry and wet seasons (Alvares *et al.* 2013). This host species is deciduous, losing its leaves mainly in October. During leaf sprouting, the *P. longicaudatum* galling insect induces the gall on the abaxial leaf surface in a highly synchronized and adjusted behavior with its host plant phenology (Malenovský *et al.* 2015). The galls and non-galled leaves were sampled in young (November to December), mature (February to April), and senescent stages (June to September). The gall stages were defined based on the gall size and color: young galls (first white green callus induced on young leaves), mature galls (larger and dark green protuberance, sampled on mature leaves), and senescent galls (opened galls). For the senescent stage we considered green and opened galls without the galling insects. In order to evaluate the physiological parameters, all samples were collected between 6 and 8 h during the first hours of daylight, stored in ice, and analyzed within 1 h after harvesting to avoid pigment deterioration and metabolic changes.

General features of the gall system: The *P. longicaudatum* galls induced on leaves of *A. tomentosum* are green and intralaminar (Fig. 1*B–D*). The mature galls occur on mature leaves (Fig. 1*B*), while the senescent galls usually occur when the leaf starts losing Chl during its own senescence; however, the galls continue being green with Chl in tissues in a process denominated as 'green islands' (Fig. 1*C*). The gall has a single larval chamber in the center of its structure, surrounded by a parenchymatous cortex and Chl tissue on both gall surfaces (Fig. 1*D*).

Photosynthetic pigment content and Chl *a* fluorescence: For the extraction of photosynthetic pigments (Chl and carotenoids), samples from fresh non-galled leaves and galled tissues of 15 individuals were collected in the three developmental stages (young, mature, and senescent) with a 0.9-cm^2 disk cutter, weighed and stored in 5 ml of 80% acetone (v/v) for 48 h. During this period, the samples were stored in amber containers protected from light. Subsequently, they were ground and suspended in a 10 ml of 80% acetone, followed by centrifugation at 3,000 rpm for 3 min. The supernatant was analyzed with a UV-Vis spectrophotometer (*Digital spectrophotometer Biospectro*, 200–1,000 nm, Brazil) at wavelengths of 470, 646, and 663 nm. The Chl *a*, Chl *b*, and carotenoid concentrations were obtained by the method of Lichtenthaler and Wellburn

(1983) and the results are reported as μg(pigment) per gram of fresh mass.

The transitory fluorescence of Chl *a* between light and dark adaptation was obtained using the *Handy FluorCam* (*Photon Systems Instruments*, Czech Republic) with the *FluorCam 7* software (quenching protocol). The measurements were made on 15 non-galled leaves and galls in the three developmental stages, with gall and non-galled measurements taken on the same leaf. The fluorescence tests were performed in the field, and the leaves were removed from the plant with subsequent and immediate analysis. The Kautsky effect (Stirbet 2011) was calculated in order to determine the Chl relaxation to excitation cycle. The following photosynthetic parameters associated with the Kautsky effect were calculated and used in the current study: F_0 – minimum fluorescence of PSII in dark-adapted state; F_m – maximum fluorescence of PSII in dark-adapted state; F_v/F_m –maximum PSII quantum yield in dark-adapted state, where $F_v = F_m - F_0$; $(F_m' - F')/F_m'$ – PSII operating efficiency in light-adapted steady-state, where F_m' is the fluorescent signal when all PSII centers are closed in the light-adapted state and F' is the measurement of the light-adapted fluorescence signal; NPQ – steady-state nonphotochemical quenching in light; and R_{fd} – fluorescence decline ratio in steady state (Genty *et al.* 1989, Oxborough 2004).

Carbohydrate content: The content of water-soluble polysaccharides, total soluble sugars, and starch was analyzed in leaves ($n = 10$) and galls ($n = 10$) in all developmental stages using the phenol-sulfuric acid colorimetric method with 20 mg of dry sample (Chow and Landhäusser 2004). For extraction of the total soluble sugars (TSS), a mixture of methanol:chloroform: water (12:5:3, v/v) was used (Bielski and Turner 1966), followed by resuspension of the residue in 10% ethanol to extract the content of water-soluble polysaccharides (WSP) (Shannon 1968). The last extraction for measurement of the starch content was done with the resuspension of the pellet in a solution of 30% perchloric acid (McCready *et al*. 1950). The same protocol was used on glucose as control. The experiments were performed in triplicate for each sample and the results are reported as mg carbohydrate per gram of dry mass.

Leaf specific mass (LSM), succulence, and relative water content (RWC): All measurements were made with a 0.64-cm^2 discs cut from three non-galled leaves and galls in all developmental stages from five individuals ($n = 15$). Fresh samples were weighed (fresh mass, FM) after soaking for 24 h in distilled water (turgid mass, TM), and after drying in an oven at 50°C for 24 h (dry mass, DM) (Turner 1981). LSM was calculated dividing DM by disc area (Witkowski and Lamont 1991). Succulence was determined using the (TM – DM)/(DM) formula from Ogburn and Edwards (2012), and the (FM – DM)/(TM – DM) equation in order to obtain RWC.

Statistical analyses: Factorial analysis of variance (*ANOVA*) followed by the *Tukey*'s test was used to analyze differences between non-galled leaves and galls in all stages of development. The following parameters were tested: Chl *a*, Chl *b*, total Chl, Chl *a*/*b* ratio, carotenoids (Car), total Chl/Car, carbohydrate content (total soluble sugars, water-soluble polysaccharides, and starch), as well as each of the Chl *a* fluorescence parameters. The Chl *a* fluorescence values obtained for each plant sample were treated as random effects to determine the dependence between galls and non-galled leaf tissues. All tests were carried out using the *R Stats* (R Core Team 2017) and *R Studio*. All models were corrected when needed to improve residual distribution, with the level of significance set at 5%.

Results

Photosynthetic pigments and Chl *a* fluorescence: The Chl-containing tissues occurred especially on the outer regions of the gall, on both adaxial and abaxial surfaces (Fig. 1*D*). There was a clear difference in the content of photosynthetic pigments between non-galled leaves and galls in all developmental stages (Table 1). In general, non-galled leaf tissues contained more Chl and Car per gram of sample ($F_{1,84} = 70.5$, $p<0.001$; $F_{1,84} = 153.4$, $p<0.001$, respectively) (Table 1). Total Chl was reduced in both gall tissues and non-galled tissues during development ($F_{2,84} = 123.9$, $p<0.001$). The Car content increased from young to mature galls and decreased from mature to senescent galls ($F_{2,84} = 25.7$, $p<0.001$). In non-galled tissues, Car increased from the young to the mature stage, but the content remained the same through senescence. The Chl *a*/*b* ratios of young and mature stages were similar in gall and leaf; however, in senescence, the gall Chl ratio was higher ($F_{2,83} = 29.9$, $p<0.001$). The ratio between Chl and Car was equal in mature galls and leaf tissues, but during senescence it became higher in galls then in leaves ($F_{2,81} = 22.8$, $p<0.001$).

The Chl *a* fluorescence transient experiment during light excitement and dark relaxation (Kautsky effect) showed similar patterns in young and mature non-galled and galled tissues (Fig. 2). However, when senescent non-galled and galled tissues were compared, fluorescence was maintained in the galls through the Kautsky effect, while it was drastically reduced in the leaves (Fig. 2*C*). The minimum fluorescence in the dark-adapted state (F_0) increased in mature and senescent galls ($z = 6.4$; $p<0.001$ and $z = 10.6$; $p<0.001$, respectively), while it remained stable at low values in non-galled tissues (Table 2). The maximum fluorescence in the dark-adapted state (F_m) was lower in young galls than in non-galled tissues ($z = 6$; $p<0.001$), but similar in mature tissues and higher in senescent gall tissues ($z = 5$; $p<0.001$). The maximum PSII quantum yield (F_v/F_m) was significantly higher in non-galled leaves ($t = 66.8$; $p<0.001$) and was reduced in both non-galled leaves and galls during senescence. The PSII operating efficiency in light-adapted steady state [$(F_m' - F')/F_m'$] was higher in young and mature non-galled tissues than that in galls. However, $(F_m' - F')/F_m'$ was higher in senescent galls than that in senescent non-galled leaves. The fluorescence decline ratio in steady state (R_{fd}), used to measure plant vitality, was lower in young

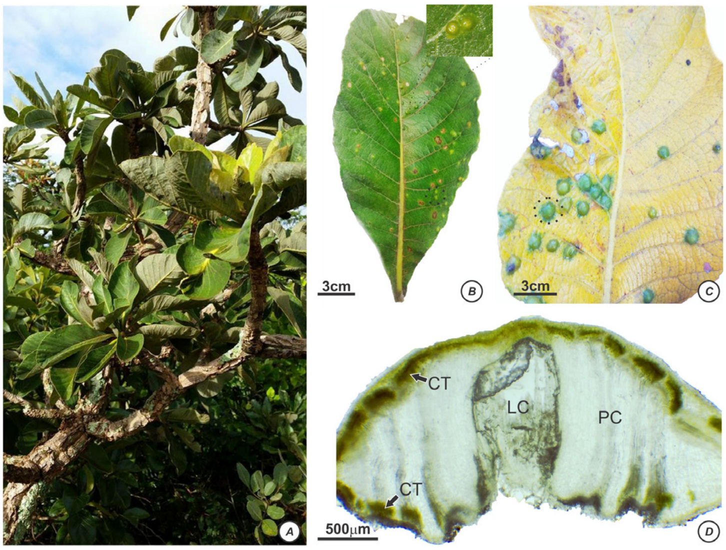

Fig. 1. Galls induced on *Aspidosperma tomentosum* (Apocynaceae) leaves by the galling insect *Pseudophacopteron longicaudatum.* (*A*) General view of *A. tomentosum*; (*B*) mature leaf with mature galls; (*C*) senescent leaf with green galls, showing the 'green island' effect; (*D*) transversal section of a mature gall, showing chlorophyllous tissues near to epidermis. CT – chlorophyllous tissues; PC – parenchymatous cortex; LC – larval chamber.

Table 1. Photosynthetic pigment contents in non-galled leaves of *Aspidosperma tomentosum* and galls induced by *Pseudophacopteron longicaudatum*. The leaves and galls (*n* = 15) were analyzed considering three different stages of maturation: young, mature, and senescent. The data compared together non-galled and galled tissues, and their developmental stages, using factorial *ANOVA* with posterior *Tukey*'s HSD test considering differences below 5%. FM – fresh mass.

Developmental	Total Chl [μg g^{-1}(FM)]		Carotenoids [μg g^{-1}(FM)]		Chl *a*/*b*		Total Chl/carotenoids	
stages	Non-galled leaves	Galls	Non-galled leaves	Galls	Non-galled leaves	Galls	Non-galled leaves	Galls
Young	1.82 ± 0.43^a	1.01 ± 0.39^b	0.21 ± 0.06^b	0.13 ± 0.05^c	2.43 ± 0.23^c	2.42 ± 0.37^c	8.28 ± 1.06^a	7.36 ± 0.90^a
Mature	1.28 ± 0.22^b	0.51 ± 0.12^c	0.36 ± 0.05^a	0.20 ± 0.03^b	7.30 ± 0.29^a	5.60 ± 2.08^a	3.51 ± 0.29^b	2.65 ± 0.68^b
Senescent	0.40 ± 0.29^c	0.18 ± 0.11^d	0.32 ± 0.09^a	0.10 ± 0.02^c	1.91 ± 0.90^c	4.11 ± 0.69^b	1.05 ± 0.75^d	1.74 ± 0.87^c

and mature galls than that in non-galled tissues (z = 6.3; $p<0.001$ and z = 5.6; $p<0.001$, respectively), except during senescence when the values were similar. The steady-state nonphotochemical quenching (NPQ) was similar in young galls and non-galled tissues, while in mature galls it was lower than that in leaves (z = 5.6; $p<0.001$). No difference in NPQ (z = 2; $p>0.05$) was observed in senescent galls and leaves.

Carbohydrate content: The total soluble sugars increased throughout the developmental stages in both non-galled tissues and galls ($F_{2,54}$ = 149.1; $p<0.001$), with no difference between them (Table 3). The water-soluble polysaccharides were higher in young galls than that in young leaves and increased throughout development ($F_{2,52}$ = 71.5; $p<0.001$). The starch content differed between gall and non-galled leaf tissues ($F_{2,53}$ = 32.2; $p<0.001$); no difference of starch content was in mature galls and non-galled leaves, but senescent galls showed higher starch accumulation compared to all other tissues.

Relative water content, leaf specific mass, and succulence: The relative water content differed between non-galled and gall tissues ($F_{2,72}$ = 10.2; $p<0.001$) (Table 4). Galls and non-galled leaves had similar values during the young and senescent stages, but a faster increase in the water content occurred in galls than that in non-galled leaves throughout the developmental stages, with the content remaining high at the senescent stage. The leaf specific mass was significantly higher in gall tissues ($F_{2,72}$ = 39.0; $p<0.001$), increasing throughout their development. The specific mass of non-galled leaf tissues increased to maturity, but decreased during senescence. Galls had also higher succulence than non-galled leaf tissues ($F_{2,73}$ = 27.4; $p<0.001$) (Table 4). In both, galls and non-galled tissues, the mature stage exhibited the lowest succulence.

Discussion

The *P. longicaudatum* feeding activity changes the *A. tomentosum* leaf cell and functions during gall development (Martini *et al.* 2020, in press), leading to a loss of primary functions in host leaves. The Chl concentrations decreased in gall tissues, promoting a photochemical imbalance. As expected, the low photosynthetic efficiency of galls did not reduce the carbohydrate resources. On the other hand, there was a significant increase in carbohydrates along gall development, especially starch. We also detected an increase of starch even in the senescent galls. This result may reflect the formation of the 'green island', described here for the first time in *P. longicaudatum* galls.

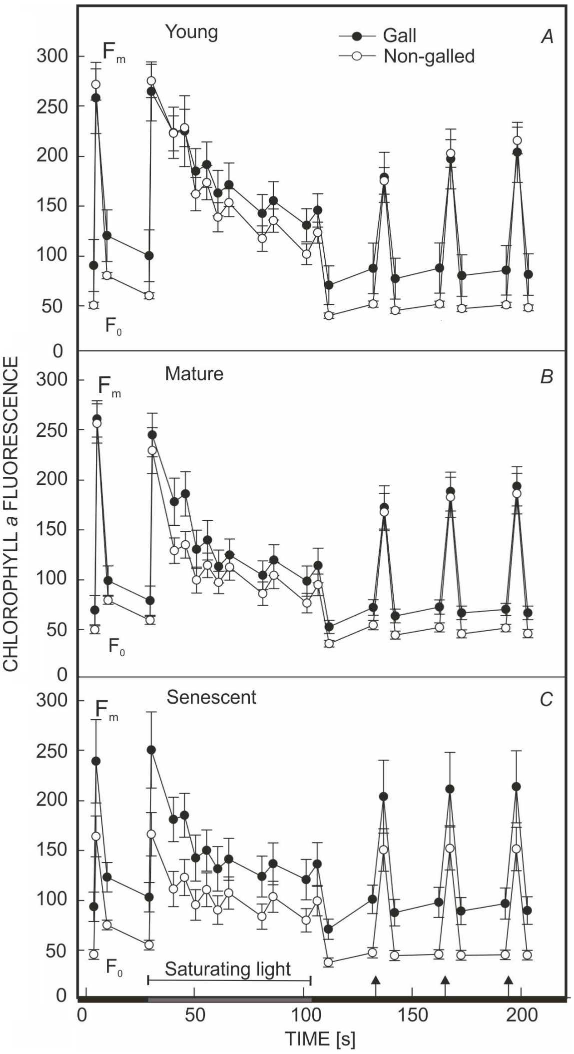

Fig. 2. Chlorophyll *a* fluorescence transient experiment during light excitement and dark relaxation (Kautsky effect) in *Pseudophacopteron longicaudatum* galls (*solid circle*) and *Aspidosperma tomentosum* non-galled leaves (*open circle*). (*A*) Young phase; (*B*) mature phase; (*C*) senescent phase. Far-red light pulses (*black arrow*). F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state.

The 'green island' effect seems to break the pattern of resource mobilization usually reported for the process of senescence, maintaining carbohydrates and succulence in the galls until the galling insect comes out.

The galling insects create functional compartments in galls, changing the normal structure, chemistry, and physiological features of the host plant for their own benefit (Oliveira *et al.* 2014, Bragança *et al.* 2016). In this context, the maintenance of photosynthesis in galls seems to be an adaptive advantage for the galling insect. Photosynthesis provides tissue oxygenation and carbon dioxide consumption in gall tissues, preventing hypoxia and hypercarbia (Castro *et al.* 2012, Pincebourde and Casas 2016). The occurrence of green and photosynthetic galls has been reported in the neotropical region, as shown for intralaminar leaf galls on *Aspidosperma spruceanum* Benth. and *Aspidosperma australe* Müll. Arg (Oliveira *et al.* 2011), intralaminar leaf galls on *Matayba guianensis* Aubl. (Oliveira *et al.* 2017), and horn-shaped galls on leaflets of *Copaifera langsdorffii* Desf. (Castro *et al.* 2012). In the current study, the non-galled leaves had a higher concentration of Chl (*i.e.*, total Chls and Car) than that of *P. longicaudatum* galls. The reduction of Chl is a usual phenomenon in galls (Oliveira *et al.* 2011, Dias *et al.* 2013, Kmieć *et al.* 2018, Rezende *et al.* 2018) and has been associated with cellular volume extension of galls and consequently pigment dilution (Oliveira *et al.* 2011, Dias *et al.* 2013). Chl dilution in galls was supported by the increase of relative water content and succulence compared to non-galled leaves at mature and senescent stages, causing a reduction in pigment concentration. As a special case, *P. longicaudatum* galls did not lose all Chl and Car contents during senescence, showing it as so called 'green island'. The 'green island' effect promotes a delay in senescence by the parasite stimulus (Kaiser *et al.* 2010) and may maintain the insect and gall tissues alive until the complete nymph development and adult comes out, despite the death of the surrounding leaf tissue.

In general, the reduction of Chl pigments can be associated with changes and/or damage to the photosynthetic apparatus (Baker and Oxborough 2004), supporting the photochemical imbalance found in *P. longicaudatum* galls. The minimum fluorescence in dark-adapted state (F_0) is a stress indicator in plants since it reflects the status of the PSII reaction center. In non-galled tissue, the reaction centers are open, and fluorescence is low, as shown in different developmental stages of non-galled tissues of *A. tomentosum*. However, in mature and senescent gall phases, higher F_0 values indicate that most of the PSII reaction center remains closed and the electron transporter chain oxidized (Bolhàr-Nordenkampf *et al.* 1989). Thus, using the F_0, we can propose that higher values in galls, compared with non-galled leaves, may represent an oxidative stress in gall tissues. The higher values of maximum fluorescence in the dark-adapted state (F_m) of young and mature non-galled leaves suggests that gall induction leads to a reduction of PSII numbers, which can be recovered after the mature gall stage. The reduction of F_m values after gall induction has been described in many other gall systems (Oliveira *et al.* 2011, 2017; Carneiro *et al.* 2014b) and seems to be another good indicator of changes in gall tissues. As a result of the senescence processes, we can note a reduction of maximum PSII quantum yield (F_v/F_m) and PSII operating efficiency [$(F_m' - F')/F_m'$] both in *A. tomentosum* non-galled tissue and *P. longicaudatum* galls. Similar results have been reported in the literature, *e.g.*, for *Eugeniamyia dispar* Maia. on the leaves of *Eugenia uniflora* L. (Rezende *et al.* 2018), *Bystracoccus mataybae* Hodsgon. on leaflets of *M. guianensis* (Oliveira *et al.* 2017), and many others (*e.g.*, Larson 1998, Florentine *et al.* 2005), indicating that this is a usual response.

Car are related to energy dissipation during photosynthesis, protecting the photosynthetic apparatus against photodamage (Niyogi 2000). Moreover, the high concen-

Table 2. Chlorophyll *a* fluorescence analysis in *Aspidosperma tomentosum* leaves and *Pseudophacopteron longicaudatum* galls. Leaves and galls (n = 15) were analyzed under paired experiment which measured galls and non-galled tissue in the same leaf, considering the three different stages of maturation: young, mature, and senescent. F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximum PSII quantum yield in dark-adapted state; F' – measurement of the light-adapted fluorescence signal; F'_m – fluorescent signal when all PSII centers are closed in the light-adapted state; $(F_m' - F')/F_m'$ – PSII operating efficiency in light-adapted steady state; NPQ – steady-state nonphotochemical quenching in light; R_{fd} – fluorescence decline ratio in steady state. The data compared together non-galled and galled tissues (in the row), and their developmental stages, using a factorial nested *ANOVA* with posterior *Tukey*'s HSD test considering differences below 5%.

Parameter	Non-galled leaves			Galls		
	Young	Mature	Senescent	Young	Mature	Senescent
F_0	45.31 ± 2.86^c	50.55 ± 4.30^c	49.00 ± 4.22^c	53.44 ± 4.51^c	75.20 ± 8.50^b	89.20 ± 17.90^a
F_m	225.28 ± 14.47^a	256.60 ± 21.70^a	179.40 ± 17.20^b	147.54 ± 15.05^b	249.80 ± 29.90^a	222.10 ± 41.60^a
$(F_m' - F')/F_m'$	0.63 ± 0.01^a	0.59 ± 0.01^a	0.40 ± 0.01^c	0.50 ± 0.05^b	0.48 ± 0.02^b	0.50 ± 0.05^b
F_v/F_m	0.79 ± 0.08^a	0.80 ± 0.01^a	0.72 ± 0.01^b	0.69 ± 0.02^b	0.69 ± 0.03^b	0.59 ± 0.07^c
NPQ	1.70 ± 0.12^b	2.22 ± 0.16^a	0.69 ± 0.18^c	1.69 ± 0.26^b	1.33 ± 0.20^b	0.92 ± 0.33^c
R_{fd}	2.55 ± 0.10^a	2.01 ± 0.40^b	1.22 ± 0.11^d	1.93 ± 0.35^b	1.57 ± 0.24^c	1.20 ± 0.35^d

Table 3. Carbohydrate contents in *Aspidosperma tomentosum* leaves and *Pseudophacopteron longicaudatum* galls. The leaves and galls (n = 10) were analyzed considering three different stages of maturation: young, mature, and senescent. The data compared together non-galled leaves and galls, and their developmental stages, using factorial *ANOVA* with posterior *Tukey*'s HSD test considering differences below 5%. DM – dry mass.

Developmental stages	Total soluble sugars [mg g^{-1}(DM)]		Water-soluble polysaccharides [mg g^{-1}(DM)]		Starch [mg g^{-1}(DM)]	
	Non-galled leaves	Galls	Non-galled leaves	Galls	Non-galled leaves	Galls
Young	94.4 ± 38.5^c	84.4 ± 28.6^c	11.4 ± 9.1^d	30.2 ± 14.7^c	235.7 ± 67.1^c	294.9 ± 102.5^c
Mature	333.9 ± 96.7^b	258.7 ± 96.6^b	65.1 ± 26.4^b	51.3 ± 12.1^b	183.4 ± 37.2^d	162.2 ± 55.2^d
Senescent	444.6 ± 112.6^a	507.6 ± 116.0^a	146.8 ± 63.4^a	114.0 ± 36.7^b	360.9 ± 72.5^b	410.7 ± 35.3^a

Table 4. Relative water content (RWC), leaf specific mass (LSM), and succulence (SU) in *Aspidosperma tomentosum* leaves and *Pseudophacopteron longicaudatum* galls in all development stages. The data compared together non-galled leaves and galled tissues, and their developmental stages, using factorial *ANOVA* with posterior *Tukey*'s HSD test considering differences below 5%. DM – dry mass.

Developmental stages	RWC [%]		LSM [mg(DM) cm^{-2}]		SU [mg(H_2O) g^{-2}(DM)]	
	Non-galled leaves	Galls	Non-galled leaves	Galls	Non-galled leaves	Galls
Young	62.51 ± 10.00^b	63.56 ± 8.20^b	9.20 ± 1.10^e	13.46 ± 1.70^c	2.52 ± 0.57^b	3.11 ± 0.76^a
Mature	64.29 ± 5.29^b	76.94 ± 4.15^a	14.60 ± 0.72^c	22.75 ± 2.71^b	0.90 ± 0.09^d	2.10 ± 0.28^b
Senescent	71.22 ± 4.25^a	75.39 ± 4.78^a	10.99 ± 1.28^d	28.07 ± 6.14^a	1.55 ± 0.18^c	3.23 ± 0.26^a

tration of Car in plants can lead to increased energy dissipation by heat (including NPQ values) from xanthophyll cycle (Demmig-Adams *et al.* 1996), something expected for *P. longicaudatum* galls. However, the steady-state NPQ values in all gall phases showed the same pattern for non-galled tissue. At first sight, these results seem to represent no change in nonphotochemical dissipation due to the low Car concentrations in the *P. longicaudatum* galls. But the low concentration of Car in galls is similar to the Chl dilution effect observed due the hyperplasia of gall tissues, and the total Chl/Car ratio showed that there were no changes in the proportional occurrence of Car. During senescence (when the 'green island' effect was observed), the Car even proportionally increased in galls.

Reactive oxygen species (mainly $^1O_2$ and H_2O_2) are responsible for damage to D1 protein at PSII reaction centers, and F_v/F_m values lower in gall tissue compared to non-galled tissue, as shown for *P. longicaudatum* galls, indicate photoinhibition (Lichtenthaler and Miehé 1997, Lüttge *et al.* 1998, Oliveira *et al.* 2017). Then, the decrease in F_v/F_m, as reported here for *P. longicaudatum* galls, implies PSII damage and reduced photochemical capacity (Maxwell and Johnson 2000, Bączek-Kwinta *et al.* 2011). The F_v/F_m ratio, as well as NPQ, help reduce 3Chl* (triplet chlorophyll), which otherwise can transfer energy to O_2 to generate the singlet oxygen (Pavlovič 2012), and then convert it into H_2O_2 (Bhattacharjee 2010). R_{fd} can also assess stress status from gall induction until its complete development. This is an empirical parameter used to determine plant vitality in which values higher than 1 can indicate photosynthesis efficiency despite of any damage. (Lichtenthaler and Miehé 1997). In non-galled leaves of *A. tomentosum* and in *P. longicaudatum* galls, the young stages were diagnosed as having better vitality, which became worse closer to senescence. In addition, there was a clear reduction of plant tissue vitality after gall induction and development. R_{fd} reduction after gall establishment has been reported for *E. dispar* on the leaves of *E. uniflora* (Rezende *et al.* 2018) and for *B. mataybae* on leaflets of *M. guianensis* (Oliveira *et al.* 2017).

Despite the loss of tissue vitality in galls, RWC, succulence, and LSM values were high. The term senescence in plants is used for deteriorative and degenerative physiological changes occurring during mitotic or replicative senescence and postmitotic senescence (Woo *et al.* 2019). In leaf senescence, there is a breakdown of chloroplasts and reallocation of nutrients and resources (Buchanan-Wollaston *et al.* 2003), in agreement with the lower total Chl content and reduced photochemical capacity of *P. longicaudatum* senescent galls. The increase of water content and succulence in *P. longicaudatum* galls is related to cell hypertrophy and tissue hyperplasia (Oliveira and Isaias 2010, Isaias *et al.* 2011, Ferreira and Isaias 2014, Oliveira *et al.* 2016) mediated by turgor pressure from vacuoles (Cosgrove 1986). The higher water content in galls may also be related to the absence or low number of stomata (Moura *et al.* 2008, Campos *et al.* 2010, Oliveira *et al.* 2017) or to the occurrence of nonfunctional stomata (Oliveira *et al.* 2006, Isaias *et al.* 2011). The maintenance of relative water content and succulence in *P. longicaudatum* senescent galls may be associated with the 'green island' effect (Kaiser *et al.* 2010), which maintains photosynthetic and watered tissues even during senescence. In addition, the higher LSM values in galls are a response to tissue growth and tissue density (Moreira *et al.* 2014) as a consequence of cell division and extension during gall formation.

P. longicaudatum is a phloem-sucking insect obtaining resources directly from phloem (Malenovský *et al.* 2015, Oliveira *et al.* 2016). Phloem-sucking insects do not develop a true nutritive tissue with proteins, lipids or carbohydrate accumulation (Bronner 1992), which led us to believe in a low carbohydrate reserve for *P. longicaudatum* galls. However, the *P. longicaudatum* young galls showed the content of water-soluble polysaccharides, total soluble sugar, and starch closely similar to that of young non-galled leaves, with all values increasing until gall senescence. These results demonstrate changes in the pattern of mobilization of carbohydrate resources described during senescence processes, probably as a result of the 'green island' effect. Since the insect is a phloem-sucking one, our data show the metabolic energy cost of maintaining the gall structure at the cellular level (Castro *et al.* 2012) and indicate that the 'green island' effect probably also requires a large allocation of energy to be maintained. Therefore, although the decrease of photosynthetic rate could weaken the sink demand (Retuerto *et al.* 2004), this is not true for galls induced by *P. longicaudatum*, which stored more resources compared to non-galled leaves, especially in the senescent stages.

Conclusion: The photosynthetic parameters showed that *P. longicaudatum* galls maintained photosynthesis throughout their development; however, the efficiency was lost compared to non-galled tissue of *A. tomentosum*. The pigment contents also decreased in galls (as a result of an increasing cell water status), reducing the performance of maximum quantum yield (F_v/F_m) and light-adapted [$(F_m' - F')/F_m'$] states. Moreover, the high values of carbohydrate resources in phloem-sucking *P. longicaudatum* galls were not expected, possibly related to the formation of the 'green island' effect, where gall needs resources to maintain tissue functions after leaf senescence.

References

Alvares C.A., Stape J.L., Sentelhas P.C. *et al.*: Köppen's climate classification map for Brazil. – Meteorol. Z. **22**: 711-728, 2013.

Bączek-Kwinta R., Kozieł A., Seidler-Łożykowska K.: Are the fluorescence parameters of German chamomile leaves the first indicators of the anthodia yield in drought conditions? – Photosynthetica **49**: 87-97, 2011.

Baker N.R., Oxborough K.: Chlorophyll fluorescence as a probe of photosynthetic productivity. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 65-82. Springer, Dordrecht 2004.

Bhattacharjee S.: Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. – In: Gupta S.D. (ed.): Reactive Oxygen Species and Antioxidants in Higher Plants. Pp. 1-30. CRC Press, Boca Raton 2010.

Bielski R.L., Turner L.A.: Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. – Anal. Biochem. **17**: 278-293, 1966.

Bolhàr-Nordenkampf H.R, Long S.P, Baker N.R. *et al.*: Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. – Funct. Ecol. **3**: 497-514, 1989.

Bragança G.P., Oliveira D.C., Isaias R.M.S.: Compartmentalization of metabolites and enzymatic mediation in nutritive cells of Cecidomyiidae galls on *Piper arboreum* Aubl. (Piperaceae). – J. Plant Stud. **6**: 11, 2016.

Bronner R.: The role of nutritive cells in the nutrition of Cynipids and Cecidomyiids. – In: Shorthouse J.D., Rohfritsch O. (ed.): Biology of Insect-Induced Galls. Pp. 60-86. Oxford University Press, Oxford 1992.

Buchanan-Wollaston V., Earl S., Harrison E. *et al.*: The molecular analysis of leaf senescence – a genomics approach. – Plant Biotechnol. J. **1**: 3-22, 2003.

Campos P.T., Costa M.C.D., Isaias R.M.S. *et al.*: Phenological relationships between two insect galls and their host plants: *Aspidosperma australe* and *A. spruceanum* (Apocynaceae). – Acta Bot. Bras. **24**: 727-733, 2010.

Carneiro R.G.S., Castro A.C., Isaias R.M.S.: Unique histochemical gradients in a photosynthesis-deficient plant gall. – S. Afr. J. Bot. **92**: 97-104, 2014b.

Carneiro R.G.S., Oliveira D.C., Isaias R.M.S.: Developmental anatomy and immunocytochemistry reveal the neo-ontogenesis of the leaf tissues of *Psidium myrtoides* (Myrtaceae) towards the globoid galls of *Nothotrioza myrtoidis* (Triozidae). – Plant Cell Rep. **33**: 2093-2106, 2014a.

Castro A.C., Oliveira D.C., Moreira A.S.F.P. *et al.*: Source-sink relationship and photosynthesis in the horn-shaped gall and its host plant *Copaifera langsdorffii* Desf. (Fabaceae). – S. Afr. J.

Bot. **83**: 121-126, 2012.

Castro A.C., Oliveira D.C., Moreira A.S.F.P., Isaias R.M.S.: Synchronism between *Aspidosperma macrocarpon* (Apocynaceae) resources allocation and the establishment of the gall inducer *Pseudophacopteron* sp. (Hemiptera: Psylloidea). – Rev. Biol. Trop. **61**: 1891-1900, 2013.

Chow P.S., Landhäusser S.M.: A method for routine measurements of total sugar and starch content in woody plant tissues. – Tree Physiol. **24**: 1129-1136, 2004.

Cosgrove D.: Biophysical control of plant cell growth. – Ann. Rev. Plant Physio. **37**: 377-405, 1986.

Demmig-Adams B., Gilmore A.M., Adams III W.W.: *In vivo* function of carotenoids in higher plants. – FASEB J. **10**: 403-412, 1996.

Dias G.G., Moreira G.R.P., Ferreira B.G., Isaias R.M.S.: Why do the galls induced by *Calophya duvauae* Scott on *Schinus polygamus* (Cav.) Cabrera (Anacardiaceae) change colors? – Biochem. Syst. Ecol. **48**: 111-122, 2013.

El-Akkad S.S.: Biochemical changes induced in *Populus nigra* leaves by galling aphid *Pemphigous populi*. – Int. J. Agric. Biol. **6**: 650-664, 2004.

Ferreira B.G., Isaias R.M.S.: Floral-like destiny induced by a galling Cecidomyiidae on the axillary buds of *Marcetia taxifolia* (Melastomataceae). – Flora **209**: 391-400, 2014.

Florentine S.K., Raman A., Dhileepan K.: Effects of gall induction by *Epiblema strenuana* on gas exchange, nutrients, and energetics in *Parthenium hysterophorus*. – BioControl **50**: 787-801, 2005.

Foyer C.H., Noctor G.: Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. – Plant Cell Environ. **28**: 1056-1071, 2005.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – BBA-Gen. Subjects **990**: 87-92, 1989.

Huang M.Y., Chou H.M., Chang Y.T., Yang C.M.: The number of cecidomyiid insect galls affects the photosynthesis of *Machilus thunbergii* host leaves. – J. Asia-Pac. Entomol. **17**: 151-154, 2014.

Isaias R.M.S., Carneiro R.G.S., Oliveira D.C., Santos J.C.: Illustrated and annotated checklist of Brazilian gall morphotypes. – Neotrop. Entomol. **42**: 230-239, 2013.

Isaias R.M.S., Oliveira D.C., Carneiro R.G.S.: Role of *Euphalerus ostreoides* (Hemiptera: Psylloidea) in manipulating leaflet ontogenesis of *Lonchocarpus muehlbergianus* (Fabaceae). – Botany **89**: 581-592, 2011.

Isaias R.M.S., Oliveira D.C., Moreira A.S.F.P. *et al.*: The imbalance of redox homeostasis in arthropod-induced plant galls: Mechanisms of stress generation and dissipation. – BBA-Gen. Subjects **1850**: 1509-1517, 2015.

Kaiser W., Huguet E., Casas J. *et al.*: Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. – P. Roy. Soc. Lond. B Bio. **277**: 2311-2319, 2010.

Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – Acta Physiol. Plant **38**: 102, 2016.

Kmieć K., Rubinowska K., Michałek W., Sytykiewicz H.: The effect of galling aphids feeding on photosynthesis photochemistry of elm trees (*Ulmus* sp.). – Photosynthetica **56**: 989-997, 2018.

Larson K.C.: The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. – Oecologia **115**: 161-166, 1998.

Lichtenthaler H.K., Miehé J.A.: Fluorescence imaging as a diagnostic tool for plant stress. – Trends Plant Sci. **2**: 316-320, 1997.

Lichtenthaler H.K., Wellburn A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. T. **11**: 591-592, 1983.

Lüttge U., Haridasan M., Fernandes G.W. *et al.*: Photosynthesis of mistletoes in relation to their hosts at various sites in tropical Brazil. – Trees **12**: 167-174, 1998.

Malenovský I., Burckhardt D., Queiroz D.L. *et al.*: Descriptions of two new *Pseudophacopteron* species (Hemiptera: Psylloidea: Phacopteronidae) inducing galls on *Aspidosperma* (Apocynaceae) in Brazil. – Acta Ent. Mus. Nat. Pra. **55**: 513-538, 2015.

Mani M.S.: Ecology of Plant Galls. Pp. 434. Springer, Dordrecht 1964.

Martini V.C., Fuzaro L., Goncalves L.A. *et al.*: *Pseudophacopteron longicaudatum* (Hemiptera) induces intralaminar leaf galls on *Aspidosperma tomentosum* (Apocynaceae): a qualitative and quantitative structural overview. – An. Acad. Bras. Ciênc., 2020, in press.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

McCready R.M., Guggolz J., Silveira V., Owens H.S.: Determination of starch and amylase in vegetables. Application to peas. – Anal. Chem. **22**: 1156-1158, 1950.

Moreira A.S.F.P., Queiroz A.C.L., Barros F.V. *et al.*: Do leaf traits in two *Dalbergia* species present differential plasticity in relation to light according to their habitat of origin? – Aust. J. Bot. **61**: 592-599, 2014.

Moura M.Z.D., Soares G.L.G., Isaias R.M.S.: Species-specific changes in tissue morphogenesis induced by two arthropod leaf gallers in *Lantana camara* L. (Verbenaceae). – Aust. J. Bot. **56**: 153-160, 2008.

Nabity P.D., Zavala J.A., DeLucia E.H.: Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. – Ann. Bot.-London **103**: 655-663, 2008.

Niyogi K.K.: Safety valves for photosynthesis. – Curr. Opin. Plant Biol. **3**: 455-460, 2000.

Ogburn R.M., Edwards E.J.: Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. – Plant Cell Environ. **35**: 1533-1542, 2012.

Oliveira D.C., Christiano J.C.S., Soares G.L.G. *et al.*: Reactions of chemical and structural defenses of *Lonchocarpus muehlbergianus* Hassl. (Fabaceae) to the action of the galler *Euphalerus ostreoides* Crawf. (Hemiptera: Psyllidae). – Rev. Bras. Bot. **29**: 657-667, 2006. [In Portuguese]

Oliveira D.C., Isaias R.M.S., Fernandes G.W. *et al.*: Manipulation of host plant cells and tissues by gall-inducing insects and adaptive strategies used by different feeding guilds. – J. Insect Physiol. **84**: 103-113, 2016.

Oliveira D.C., Isaias R.M.S., Moreira A.S.F.P. *et al.*: Is the oxidative stress caused by *Aspidosperma* spp. galls capable of altering leaf photosynthesis? – Plant Sci. **180**: 489-495, 2011.

Oliveira D.C., Isaias R.M.S.: Redifferentiation of leaflet tissues during midrib gall development in *Copaifera langsdorffii* (Fabaceae). – S. Afr. J. Bot. **76**: 239-248, 2010.

Oliveira D.C., Magalhães T.A., Ferreira B.G. *et al.*: Variation in the degree of pectin methylesterification during the development of *Baccharis dracunculifolia* kidney-shaped gall. – PLoS ONE **9**: e94588, 2014.

Oliveira D.C., Moreira A.S.F.P., Isaias R.M.S. *et al.*: Sink status and photosynthetic rate of the leaflet galls induced by *Bystracoccus mataybae* (Eriococcidae) on *Matayba guianensis* (Sapindaceae). – Front. Plant Sci. **8**: 1249, 2017.

Oxborough K.: Imaging of chlorophyll *a* fluorescence: Theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. – J. Exp. Bot. **55**: 1195-1205, 2004.

Pavlovič A.: The effect of electrical signals on photosynthesis and respiration. – In: Volkov A.G. (ed): Plant Electrophysiology: Signaling and Responses. Pp. 33-62. Springer-Verlag, Berlin 2012.

Pincebourde S., Casas J.: Hypoxia and hypercarbia in endophagous insects: larval position in the plant gas exchange network is key. – J. Insect Physiol. **84**: 137-153, 2016.

R Core Team: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 2017. *Available at*: https://www.R-project.org/

Retuerto R., Fernandez-Lema B., Obeso J.R.: Increased photosynthetic performance in holly trees infested by scale insects. – Funct. Ecol. **18**: 664-669, 2004.

Rezende U.C., Moreira A.S.F.P., Kuster V.C.. Oliveira D.C.: Structural, histochemical and photosynthetic profiles of galls induced by *Eugeniamyia dispar* (Diptera, Cecidomyiidae) on the leaves of *Eugenia uniflora* (Myrtaceae). – Rev. Biol. Trop. **66**: 1469-1480, 2018.

Roháček K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationship. – Photosynthetica **40**: 13-29, 2002.

Shannon J.C.: Carbon-14 distribution in carbohydrates of immature *Zea mays*. Kernels following $^{14}CO_2$ treatment of intact plants. – Plant Physiol. **43**: 1215-1220, 1968.

Shorthouse J.D., Wool D., Raman A.: Gall-inducing insects – Nature's most sophisticated herbivores. – Basic Appl. Ecol. **6**: 407-411, 2005.

Stirbet A.: On the relation between the Kautsky effect (chlorophyll *a* fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. – J. Photoch. Photobio. B **104**: 236-257, 2011.

Turner N.: Techniques and experimental approaches for the measurement of plant water status. – Plant Soil **58**: 339-366, 1981.

Witkowski E.T.F., Lamont B.B.: Leaf specific mass confounds leaf density and thickness. – Oecologia **88**: 486-493, 1991.

Woo H.R., Kim H.J., Lim P. O. *et al.*: Leaf senescence: Systems and dynamics aspects. – Annu. Rev. Plant Biol. **70**: 347-376, 2019.

Zangerl A.R., Hamilton J.G., Miller T.J. *et al.*: Impact of folivory on photosynthesis is greater than the sum of its holes. – P. Natl. Acad. Sci. USA **99**: 1088-1091, 2002.