Is the oxidative stress caused by *Aspidosperma* spp. galls capable of altering leaf photosynthesis?

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**Abstract**

The generation of ROS (reactive oxygen species) in plant galls may induce the degradation of the membrane systems of a plant cell and increase the number of plastoglobules. This numerical increase has been related to the prevention of damage to the thylakoid systems, and to the maintenance of photosynthesis rates. To investigate this hypothesis in gall systems, a comparative study of the ultrastructure of chloroplasts in non-galled leaves and in leaf galls of *A. australis* and *A. spruceanum* was conducted. Also, the pigment composition and the photosynthetic performance as estimated by chlorophyll fluorescence measurements were evaluated. The ultrastructural analyses revealed an increase in the number and size of plastoglobules in galls of both species studied. The levels of total chlorophylls and carotenoids were lower in galls than in non-galled tissues. The chlorophyll a/b ratio did not differ between the non-galled tissues and both kinds of galls. The values of maximum electron transport rate (ETR<sub>MAX</sub>) were similar for all the samples. The occurrence of numerous large plastoglobules in the galled tissues seemed to be related to oxidative stress and to the recovery of the thylakoid membrane systems. The maintenance of the ETR<sub>MAX</sub> values indicated an existence of an efficient strategy to maintain similar photosynthetic rates in galled and non-galled tissues.

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**1. Introduction**

Plant galls induced by insects have typical tissues [1–3] that are formed by cell redifferentiation in their host organ. Consequently, the neoformed tissues alter the host organ and the functions of the gall structure itself. The attack of gall inducers may cause either negative [4–7] or positive effects on the photosynthesis of the host organs [8,9]. In the case of galls caused by aphids, these effects may be neutral [6]. In most previous studies, the levels of photosynthetic rates in galls were estimated by measuring gas exchange. However, more recent studies have found significant alterations in photosynthetic quantum yield in galls, with damage to the PSI in addition to alterations in gas exchange and reduction in pigment contents [7–13].

The induction and development of galls expose plant tissues to high oxidative stress [14–17]. From the cytological point of view, the oxidative stress can stimulate the degradation of the thylakoid system and the formation of plastoglobules [18–20]. The plastoglobules are subcompartments of the chloroplasts, and can contain several kinds of lipids and proteins. Their functions are associated with the storage of molecular components, with the recovery of the thylakoid membrane systems [21–25], and with the scavenging of reactive oxygen species in chloroplasts [26,27]. The involvement of oxidative stress with the synthesis and storage of plastoglobulines and other molecules, such as tocopherol, has been correlated with the protection of the membranes from photodegradation and the PSI from photoinhibition [28,29]. Thus, because of the high oxidative stress, it is probable that the differentiation of plastoglobules occurs in gall tissues as a strategy to maintain photosynthesis in this neoformed organ. This differentiation has not been investigated previously. The presence of ROS (reactive oxygen species) in galls of *Aspidosperma australis* and *A. spruceanum* was detected in previous studies [16,17], and inspired an approach to check if there were more plastoglobules in the cells where the ROS were detected. This may indicate a strategy to prevent damage to the thylakoid systems. In addition, the feeding activity of *Pseudophacopteron* sp. in *A. australis* differs from that of the Cecidomyiidae in *A. spruceanum*. *Pseudophacopteron* is a phloem-sucking insect while the Cecidomyiidae possesses scraper feeding habit, which provokes greater damage to plant cells. Thus, the feeding habit of the gall inducing herbivore can influence the photosynthetic activity of the gall. To investigate these hypotheses, a comparative study of the ultrastructure of the chloroplasts in non-galled leaves and in leaf galls of *A. australis* and *A. spruceanum*. 

**Keywords:** Apocynaceae, Chlorophyll fluorescence, Plant gall, Plastoglobules, ROS (reactive oxygen species)
was conducted. The results were related to their pigment composition, and to the photosynthetic performance as estimated by measurements of chlorophyll fluorescence.

2. Materials and methods

2.1. Plant material collection

Trees of *Aspidosperma australe* Müell. Arg. (*n* = 10) and *A. spruceanum* Benth. ex Müell. Arg. (*n* = 10), remnants of a semi-deciduous forest, were marked on the campus of the Universidade Federal de Minas Gerais (43°57′51″W and 19°52′11″S). Non-galled leaves and mature galls of *A. australe* induced by *Pseudophacopteron* sp. (Hemiptera), and of *Aspidosperma spruceanum* induced by an unidentified species of Diptera: Cecidomyiidae were collected for cytological and pigment studies, and analyzed in situ for chlorophyll fluorescence measurements.

2.2. Histometric analysis of chlorophyll parenchyma

The areas of chlorophyll parenchyma were measured in cross-sections of non-galled leaves (*n* = 40) and galls (*n* = 40) with the Axio-Vision 4.7® software. The data were expressed as the percentage of chlorophyll tissue within the total tissue, per unit surface area. Numerical data were submitted to an ANOVA, followed by Tukey’s test (*P* ≤ 0.05) using Graphpad Prism® software.

2.3. Cytological analyses

The samples were fixed in 4% Karnovsky for 24 h [30], modified by the addition of 0.1 M phosphate buffer (pH 7.2), post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2), dehydrated in an ethanol series [31], and embedded in Araldite® [32]. The material was cross-sectioned in a Reichert-Jung Ultracut ultramicrotome,
phylls calculated by the Axio-vision software. The contents of chloro-
acetone (v/v) after maceration and centrifugation. All samples were
and the area of their tissues were calculated by the Axio-vision software. The contents of chloro-
 acetone (v/v) after maceration and centrifugation. All samples were

2.4. Pigment content and chlorophyll fluorescence

Pigments of non-galled leaves non-galled tissues of galled leaves (disks of 1 cm²), and mature galls (n = 10) were extracted in 80%

The cytological features of A. spruceanum are similar to those of A. australe. The non-galled tissues have chloroplasts with strong lamellation associated with primary starch grains (Fig. 4a and b). The galls also have starch grains, plastoglobules mainly at the periphery of the chloroplasts (Fig. 4c and d). The disorganization of the thylakoid membrane system, and the loss of the integrity of the grana are also apparent (Fig. 4c and d).

3.4. Photosynthetic pigments and chlorophyll a fluorescence

The photosynthetic pigment contents (total chlorophylls and carotenoids), and the ratios of chlorophyll a/b and total carotenoids/chlorophyll were similar in both the non-galled leaves and in the non-galled portion of the galled leaves of both species, either on area or weight basis (Table 1). However, the total contents of chlorophylls and carotenoid in the galls were lower than those in the non-galled leaves of A. spruceanum, and A. australe. The carotenoid/chlorophyll ratio was lower in the galls of A. spruceanum. The chlorophyll a/b ratio did not differ among the samples of both species.

The values of Fv/Fm at midday were higher than 0.8 (data not presented), and photoinduction was not detected either in non-galled or in galled leaves (in non-galled portions and in the galls) of both species. The values of ETR were similar for all the samples of both host plant species (Table 1). The galls of A. australe had values of ETRMAX of 64.0 ± 11.9 μmol m⁻² s⁻¹, while the non-galled leaves, and the
Fig. 3. Transmission electron micrographs of the cells of the mesophyll in non-galled leaves and galls of Aspidosperma australae. (a and b) Detail of a chloroplast in non-galled leaf. Intense lamellation and associated starch grains. (c) Cells of the outer gall cortex with plastids and plastoglobules. (d) A chloroplast with disaggregation of the membrane system and plastoglobules grouped at its periphery. (e) Detail of a plastoglobule in a chloroplast. ch – chloroplast, cw – cell wall, gra – grana, m – mitochondria, p – plastoglobule, s – starch, v – vacuole.

non-galled tissues of galled leaves had values of 74.6 ± 29.7 and 73.6 ± 30.6 μmol m⁻² s⁻¹, respectively. The galls of A. spruceanum showed values of ETRMAX of 62.1 ± 30.1 μmol m⁻² s⁻¹, while in non-galled leaves and in non-galled tissues of galled leaves, these values were about 50.3 ± 16.3 and 42.7 ± 16.6 μmol m⁻² s⁻¹, respectively. The light curves showed no statistical differences between PARSAT and PAR in 1/2ETRMAX. A. australae showed a saturating PAR between 606.3 ± 22.1 and 676.8 ± 181.8 μmol m⁻² s⁻¹, while A. spruceanum PAR ranged between 452.7 ± 128.7 and 524.2 ± 128.7 μmol m⁻² s⁻¹.

4. Discussion

4.1. Histometric analysis of chlorophyll tissue and pigment contents

The galls of Aspidosperma spp. contained smaller amounts of photosynthetic pigments, as previously observed by Yang et al. [10] in galls of Marchilus thunbergii induced by a cecidomyiid. Also, the chlorophyll tissues were limited to the outer cortex, in both A. australae and in A. spruceanum. This position is several cell layers away from the feeding site of the galling larvae, which is the area of the gall with the highest level of oxidative stress [16,17]. Thus, it is plausible that the alterations in the pigment contents were not due to a reduction in the synthesis or degradation of pigments, but to an increase of non-chlorophyll tissues, as observed in the galls of A. australae and A. spruceanum. Also, there is no indication that the galls alter the phenology of their host plants [39], which proves that the reduction in pigment contents is not associated to leaf senescence.

4.2. Chlorophyll a fluorescence and chloroplast ultrastructure

The increase in number and size of plastoglobules at the periphery of the chloroplasts is cytological evidence of the increase of oxidative stress in gall tissues. The plastoglobules contain, among other proteins, tocopherol cyclase, an enzyme whose activity increases with oxidative stress. Tocopherol cyclase protects the thylakoid membranes and the proteins related to photosynthesis from damage caused by the ROS [40,41]. Also, this enzyme is proposed as a molecule that protects the membrane lipids from photooxidation, and photosystem II from photoinactivation [28]. The plastoglobules are functionally coupled, and structurally linked to each other and to the membranes of the thylakoids. This coupling allows the free exchange of lipophilic molecules such as plastoquinones, carotenoids, and tocopherol (VTE1) between the thylakoids and the plastoglobules, which is the site of synthesis.
and storage of these substances. These molecules serve as electron corridors, protecting the photosynthetic apparatus from damage caused by the free radicals [19]. Also, tocopherol cyclase catalyzes the penultimate stage in the process of tocopherol synthesis (vitamin E) [19,41]. The production of carotenoids and tocopherol is the mechanism of scavenging of the ROS in chloroplasts [26,27]. In the galls of *A. australe* and *A. spruceanum*, there was no variation in the ratio of carotenoids/total chlorophyll, which may suggest that the increase in number and size of plastoglobules did not result in changes in the pool of carotenoids. Nevertheless, the functioning of the PSI was not affected by gall induction.

By the larger number and size of the plastoglobules in the galls of *Aspidosperma* spp, there should be an increase in tocopherol and plastoglobulins synthesis [19,29]. In *Arabidopsis* and tobacco leaves, the increase in plastoglobulines increased the tolerance to light stress, while plants with reduced levels of this protein were weakly tolerant to light stress.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Total chlorophylls (µg cm⁻² FM)</th>
<th>Carotenoids (µg cm⁻² FM)</th>
<th>Chl a/b</th>
<th>Carot/chl</th>
<th>ETRMAX (µmol m⁻² s⁻¹)</th>
<th>PAR sat (µmol m⁻² s⁻¹)</th>
<th>PAR 1/2ETRMAX (µmol m⁻² s⁻¹)</th>
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<tbody>
<tr>
<td><strong>A. australe</strong></td>
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<tr>
<td>Non-galled leaves</td>
<td>76.8 ± 24.9²</td>
<td>3.7 ± 1.2²</td>
<td>16.6 ± 4.1²</td>
<td>0.8 ± 0.2²</td>
<td>3.0 ± 0.15 ± 0.02²</td>
<td>74.6 ± 29.7 ± 0.22²</td>
<td>621.1 ± 229.6 ± 0.22²</td>
</tr>
<tr>
<td>Non-galled tissues</td>
<td>51.6 ± 17.1²</td>
<td>3.0 ± 1.0²</td>
<td>12.0 ± 3.4²</td>
<td>0.7 ± 0.22</td>
<td>3.7 ± 0.82 ± 0.12²</td>
<td>73.7 ± 30.6 ± 0.12²</td>
<td>606.3 ± 222.1 ± 0.12²</td>
</tr>
<tr>
<td>Galls</td>
<td>35.5 ± 19.7²</td>
<td>0.9 ± 0.5²</td>
<td>7.9 ± 3.9²</td>
<td>0.2 ± 0.1³</td>
<td>3.4 ± 1.4³ ± 0.07²</td>
<td>64.0 ± 12.0³ ± 0.07²</td>
<td>676.8 ± 181.8³ ± 0.07²</td>
</tr>
<tr>
<td>P</td>
<td>0.0008</td>
<td>0.0006</td>
<td>0.042</td>
<td>0.030</td>
<td>0.536 ± 0.969</td>
<td>0.722 ± 0.808</td>
<td>0.808 ± 0.808</td>
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<td><strong>A. spruceanum</strong></td>
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<tr>
<td>Non-galled leaves</td>
<td>51.5 ± 13.6²</td>
<td>1.9 ± 0.5²</td>
<td>13.6 ± 2.7²</td>
<td>0.5 ± 0.12</td>
<td>4.04 ± 1.71 ± 0.03²</td>
<td>50.4 ± 16.31 ± 0.03²</td>
<td>473.6 ± 117.61 ± 0.03²</td>
</tr>
<tr>
<td>Non-galled tissues</td>
<td>48.8 ± 10.8²</td>
<td>1.8 ± 0.4²</td>
<td>8.4 ± 2.7²</td>
<td>0.31 ± 0.12</td>
<td>3.92 ± 1.02 ± 0.072</td>
<td>42.7 ± 16.62 ± 0.072</td>
<td>452.7 ± 128.72 ± 0.072</td>
</tr>
<tr>
<td>Galls</td>
<td>10.5 ± 3.5²</td>
<td>0.6 ± 0.2³</td>
<td>1.7 ± 0.8³</td>
<td>0.1 ± 0.05⁵</td>
<td>3.55 ± 0.75 ± 0.05⁵</td>
<td>62.1 ± 30.12 ± 0.05⁵</td>
<td>524.2 ± 128.72 ± 0.05⁵</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.774 ± 0.050</td>
<td>0.300 ± 0.057</td>
<td>0.557 ± 0.057</td>
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</table>

P < 0.0001, 0.05 < P < 0.001, 0.01 < P < 0.05
strongly photoinhibited [29,42,43]. This would constitute an alternative strategy to protect the photosynthetic apparatus against the damage caused by oxidative stress during gall formation. This suppression was corroborated by the similarity between ETR values in the non-galled and galled tissues.

4.3. Feeding habit × photosynthesis

In A. australis, the galls induced by Pseudopachycentron sp, a sucking insect, did not present a nutritive tissue [16]. However, its feeding activity could increase the sinking of photosynimates towards the gall site. In A. spruceanum, a typical nutritive tissue, spatially separated from the chlorophyll tissue by layers of lignified cells, was differentiated [17]. The development of this specific site for the feeding of the galling herbivore should explain the low damage caused to the photosynthetic cells and, consequently, the maintenance of the photosynthetic rates. According to Crawley [44], the photosynthetic rates may be increased in the host plants in function of additional sinks induced by sucking insects. Similarly, insect galls that do not cause mechanical damages to the photosynthetic cells can increase the photosynthetic rates in their host plant [45,46], or simply do not alter these rates as was observed in A. australis. Although the symptoms detected during the development of the galls of A. australis and A. spruceanum indicated an increase in the oxidative stress, and damages to the membrane system of the chloroplasts, the photosynthetic performance assessed by chlorophyll fluorescence remained unaffected. Also, plant cells may respond to galling stimuli by differentiating plastoglobules, which additionally can explain the maintenance of the photosynthetic performance in its host plant tissues.

4.4. Conclusions

Even though structural changes and sites of oxidative stress were detected during the development of the galls of A. australis and A. spruceanum [16,17], the damage to the chloroplast membrane system was not accompanied by a reduction in photosynthetic performance. Our results indicate that the two systems have distinct plant anatomical responses to the feeding activity of their associated galling herbivores. Nevertheless, the plastoglobules as ROS scavengers may generate an equilibrium in the physiology of the cells converging to the maintenance of the photosynthetic performance. This is the first evidence of a similar cytological strategy in response to two distinct galling herbivores attack in galls of Neotropical plants.

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References


