

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Biologia Vegetal

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**ALTERAÇÕES NO PERFIL ANATÔMICO, HISTOQUÍMICO E CITOLÓGICO EM
GALHAS FOLIARES DE *ACERIA LITCHII* (KEIFER) (ERIOPHYIDAE) EM *LITCHI*
CHINENSIS SONN. (SAPINDACEAE)**

Belo Horizonte

2024

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RESUMO

Litchi chinensis Sonn. é uma planta frutífera e ornamental de origem chinesa suscetível ao ácaro da erinose (*Aceria litchii* (Keifer, 1943)), cuja atividade leva à indução de tricomas, denominados de eríneas, que protegem os ovos e ninfas. O ataque do ácaro da erinose leva também ao intumescimento e enrolamento da lâmina foliar no local afetado e, em infestações mais severas, causam prejuízos comerciais. Este trabalho mapeou o campo cecidogenético da erinose de modo a traçar seu perfil anatômico, histoquímico e citológico, argumentando o potencial impacto de suas alterações na fotossíntese das folhas hospedeiras. Amostras de folhas com e sem erinose foram coletadas em um pomar no campus da Universidade Federal de Viçosa (UFV), fotografadas em estereomicroscópio. Cortes transversais para análise anatômica e histoquímica foram realizados à mão livre. A largura das células epidérmicas, o comprimento e altura das galhas, a espessura da epiderme e do mesofilo foram medidos e submetidos a análise estatística. Células da epiderme abaxial, sítio de desenvolvimento das eríneas, foram analisadas em microscopia eletrônica de transmissão. As galhas jovens são caracterizadas por intumescimento e enrolamento da lâmina foliar devido aos processos de hipertrofia e hiperplasia. As eríneas se desenvolvem durante a maturação das folhas, quando células da epiderme abaxial sofrem desdiferenciação, originando conjuntos de células de natureza meristemática. A senescência do campo cecidogenético se caracteriza pela oxidação das eríneas. Análises histoquímicas revelaram o acúmulo de metabolitos primários e secundários nos tecidos da galha, os quais são diferentes em termos morfométricos da folha não galhada ($p < 0,001$). As células que formam as eríneas apresentam degeneração do núcleo e das organelas, o que implica na perda da funcionalidade citológica e a queda de produtividade dos cultivares afetados.

Palavras-chave: Erinose, *Litchi chinensis*, *Aceria litchii*, anatomia, histoquímica, morfometria

ABSTRACT

Litchi chinensis Sonn. is a plant native to China that is affected by galls induced by *Aceria litchii* (Keifer), which feed on the young leaves, leading to the emergence of trichomes, named erineae, which protect the eggs and nymphae. They also lead to the folding and bulging of the leaf, and more severe infestations greatly compromise sales. We hypothesized that the erinosis is indeed a complex gall and, as such, traced the anatomical, histochemical, and cytometric profiles of non-galled *L. chinensis* leaves and *A. litchii* leaf galls to map the cecidogenetic field and its effect on host plant fitness. Samples were collected at the Federal University of Viçosa (UFV), and fresh leaves were photographed, cross-sections were made for anatomical and histochemical analyses, gall height and width, mesophyll and epidermis thickness, and epidermal cell width were measured and subjected to statistical analysis, and transmission electron microscopy was performed on the abaxial epidermis. Young galls are characterized by the bulging or folding of the leaf blade due to hypertrophy and hyperplasia processes; maturation starts with the emergence of erineae and a meristem-like layer caused by cell dedifferentiation and redifferentiation of abaxial epidermis cells; on senescence, erineae oxidize. Histochemical analyses revealed the relocation of resources to the gall site. Morphometric results attest that the erinosis significantly affects the leaf blade ($p < 0.001$). Cytological analyses revealed a progressive overtaxing of cell machinery as the gall matures, leading to nucleus depletion and organelle degeneration. These results help explain how the gall impacts the plant metabolism, compromising host plant fitness, and add to the argument that erinosis is indeed a complex gall.

Key words: Erinosis, *Litchi chinensis*, *Aceria litchii*, anatomy, histochemistry.

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INTRODUÇÃO GERAL

Popularmente conhecida como lichia, *Litchi chinensis* Sonn. (Sapindaceae) é uma planta de origem chinesa cultivada há mais de dois mil anos (Hastie, 1994; Yamanishi *et al.*, 2005; Ramos, 2019). A lichia é considerada a rainha das frutas devido à sua beleza, fragrância e gosto delicado (Martins, 2005), sendo a lichieira capaz de crescer a até 20 m de altura (Castillo-Olvera *et al.*, 2022). Seus frutos são drupas que, quando maduros, possuem casca vermelha e polpa branca. Além do uso ornamental, diversas partes da planta têm seu valor comercial, como as folhas – usadas para óleos essenciais e medicina tradicional, o tronco – usado na indústria madeireira; e a casca, frutos, sementes e polpa – que possuem alto valor nutricional (Castillo-Olvera *et al.*, 2022).

A lichia é cultivada em diversos países (Carrillo *et al.*, 2020; Castillo-Olvera *et al.*, 2022; Revynthi *et al.*, 2022), sendo introduzida no Brasil no final da década de 1810 (Raga *et al.*, 2010; Azevedo, 2013; Navia *et al.*, 2013; Picoli & Vieira, 2013). O cultivo teve seu início no estado de São Paulo (Yamanishi *et al.*, 2005; Raga *et al.*, 2010; Azevedo, 2013; Castro e Castro *et al.*, 2018) e, posteriormente, foi expandido para outros estados de clima tropical e temperado. Os estados de Minas Gerais e Paraná (Raga *et al.*, 2011; Castro e Castro *et al.*, 2018) se destacam no cultivo da lichieira em virtude da boa aclimação da espécie ao clima tropical típico da região Sudeste (Carvalho, 2021; Fialho e Santos, 2022; Liu *et al.*, 2020) e ao clima subtropical da região Sul do Brasil, que se assemelham ao clima subtropical úmido, típico do sul da China. As três regiões compartilham perfis climáticos semelhantes segundo a classificação de Köppen e Geiger (1928), com temperaturas moderadas, períodos chuvosos bem estabelecidos, e invernos amenos com pouca ou nenhuma pluviosidade (climate-data.org, 2025). Em 2022, a área de plantio foi de 1781 ha e a média de produção foi de 1,87 t ha⁻¹ (Martins & Patino, 2023). A variedade mais cultivada no Brasil é a Bengal (Azevedo, 2013; Sebrae, 2016; Martins & Patino, 2023), cujos frutos são colhidos entre janeiro e novembro (Martins *et al.*, 2001; Yamanishi *et al.*, 2001). A

alta produtividade e custo-benefício, além do valor ornamental, fazem da lichia uma planta convidativa para o pequeno produtor (Sebrae, 2016).

No entanto, como a maioria das plantas de interesse econômico, a lichia é alvo de diversas pragas, dentre elas o *Aceria litchii* (Keifer) (Eriophyidae), popularmente conhecido como ácaro-da-erínose, ou ácaro-da-erínose-da-lichia (Keifer, 1943; Keifer & Knorr, 1978). Também de origem chinesa, a espécie foi introduzida em diversos países junto à planta hospedeira e se tornou uma praga agrícola que causa grandes prejuízos, principalmente na Austrália, Havaí, Índia, Paquistão, Taiwan e EUA (Keifer, 1943; Keifer & Knorr, 1978; Raga *et al.*, 2011; Carrillo *et al.*, 2020; Reyvinthi *et al.*, 2022; Castillo-Olvera *et al.*, 2022). O primeiro registro de *A. litchii* no Brasil data de 2007, quando o ácaro foi listado como praga quarentenária A1 (Raga *et al.*, 2010; 2011), o que atesta o seu potencial destrutivo como invasora. Dentro dos pomares, a dispersão do ácaro se dá entre hospedeiras afetadas (Raga *et al.*, 2011; Carrillo *et al.*, 2020), enquanto longas distâncias são percorridas com a ajuda de abelhas, nas quais o ácaro se fixa no momento da polinização (Waite, 1999).

A infestação se inicia quando uma fêmea adulta de *A. litchii* oviposita na superfície abaxial de folhas jovens de *L. chinensis*, induzindo a emergência de eríneas (Raga *et al.*, 2010). As eríneas consistem de uma variante de tricomas que não possuem as características de tricomas glandulares nem tectores, e servem apenas como reserva de compostos ou nutrientes para os ácaros. Quando os ovos eclodem, as ninfas se alimentam das eríneas, estimulando a sua constante produção, sendo o sítio de obtenção de alimento e proteção (Raga *et al.*, 2011; Reyvinthi *et al.*, 2022). A indução pode ocorrer no meio da folha, com intumescências na lâmina foliar, ou próxima à borda, com o enrolamento na direção da superfície abaxial, cobrindo as eríneas. Em estágios mais avançados, a erínose se expande, podendo cobrir toda a superfície abaxial da folha e atingir outros órgãos vegetais, como ramos, flores e frutos (Nishida & Holdaway, 1955; Raga *et al.*, 2011; Reyvinthi *et al.*, 2022). Devido a essas alterações estruturais nos órgãos responsáveis

pela atividade fotossintética, a produtividade dos cultivares pode cair em até 80% (Raga *et al.*, 2011; Azevedo, 2013; Carrillo *et al.*, 2020; Reyvinthi *et al.*, 2022), já que a cobertura da superfície abaxial compromete as trocas gasosas para a realização da fotossíntese (Patankar *et al.*, 2011; Guedes *et al.*, 2023). Os meios mais comuns de controle das infestações são a remoção de ramos afetados, controle químico com produtos à base de enxofre e controle biológico feito com ácaros da família Phytoseiidae (Khan *et al.*, 2007; Azevedo, 2013; Ramos, 2019; Reyvinthi *et al.*, 2022).

O termo erinose deriva da junção das palavras gregas *érion* – que significa lã – e *nósos* – que significa doença. Ele é usado para descrever as emergências pilosas – denominadas eríneas – nas galhas foliares induzidas por ácaros (Nishida & Holdaway, 1955; Jeppson *et al.*, 1975; Karioti *et al.*, 2011). Essas galhas são caracterizadas pela hipertrofia e hiperplasia bem pronunciadas e localizadas na epiderme das folhas afetadas, dando origem a emergências parenquimáticas e pilosidades (Mani, 1964). Esse morfotipo é muitas vezes considerado uma galha simples (Chen & Qiao, 2012), ou seja, com pouca ou nenhuma neoformação de tecidos, ou mesmo como uma pseudogalha, por não desenvolver uma câmara larval ou ninfal (Wool, 2004; Floate, 2010). Entretanto, há trabalhos que argumentam a legitimidade da erinose como uma galha, como aqui considerado, devido ao fato de a hospedeira apresentar os processos de hipertrofia, principalmente nas células epidérmicas, e hiperplasia para a formação de tricomas (Mani, 1964; Álvarez *et al.*, 2013; Formiga *et al.*, 2015; Jorge *et al.*, 2016; Ferreira *et al.*, 2016; Ferreira *et al.*, 2019). O primeiro capítulo deste trabalho tem o objetivo de traçar o perfil anatômico e histoquímico das folhas não galhadas e galhas foliares de *A. Litchii* em *L. Chinensis*, mapeando as mudanças ao longo do gradiente cecidogenético.

O segundo capítulo trata das alterações induzidas na epiderme abaxial, onde ocorre o processo de hiperplasia e a formação de eríneas. As folhas de *L. chinensis* são hipoestomáticas e o desenvolvimento da erinose é restrito a essa mesma face epidérmica (Nishida & Holdaway,

1955). Hipotetizamos que a produção desses tricomas, além de comprometer a fotossíntese, altera as funções primárias e estrutura das células para sustentar o processo de crescimento e maturação da erinose. A análise citológica complementa o perfil anatômico das eríneas, evidenciando a perda de funcionalidade relacionada à alteração estrutural.

Anatomical and histochemical profile of *Aceria litchii* (Keifer) leaf galls on *Litchi chinensis* Sonn. (Sapindaceae)

Abstract

Litchi chinensis Sonn. is a plant native to China that hosts galls induced by *Aceria litchii* (Keifer, 1943) (Eriophyidae), or erinosis mites, leading to the emergence of numerous trichomes, named erineae, which protect the eggs and nymphae. They also lead to the folding and bulging of the leaf, and more severe infestations that greatly compromise sales. We traced the anatomical, histochemical, and cytometric profiles of non-galled *L. chinensis* leaves and *A. litchii* galls to map the amplitude of the cecidogenetic field caused by the erinosis. Fresh samples were collected at the Federal University of Viçosa (UFV) and photographed on a stereomicroscope. Cross-sections were made for anatomical and histochemical analyses. Gall height and width, mesophyll and epidermis thickness, and epidermal cell width were measured for cytometric comparative analyses. Young galls are characterized by the bulging or folding of the leaf blade due to epidermal and mesophyll hyperplasia; maturation starts with the emergence of erineae and the dedifferentiation of mesophyll cells, forming meristem-like cells. By senescence, the erineae oxidizes. Histochemical analyses revealed the accumulation of resources in the gall developmental site. The histometrical data supports the establishment of a cecidogenetic field from the abaxial to the adaxial erinosis surface and relates to the impairment of the photosynthetic apparatus.

Key words: Erinosis, *Litchi chinensis*, *Aceria litchii*, morphometry, cecidogenetic field.

Introduction

Galls result from interactions between a host plant and a galling organism (Mani 1964; Meyer, 1987; Ferreira *et al.* 2019). Due to their majorly parasitic nature, they tend to negatively impact the host plant fitness (Mani 1964; Stone & Schönrogge 2003; Raman *et al.*, 2006; Raman, 2012), as observed for *Litchi chinensis* cultivars in Brazil, with roughly 80% of productivity reduction (Navia *et al.*, 2013; Santos *et al.*, 2021). Lychee galls are induced by *Aceria litchii* (Keifer) (Eryophyidae), popularly known as the erinosis mites, or lychee erinosis mites (Raga *et al.*, 2010; Ramos, 2019), who feed from photoassimilates of young leaves. This feeding habit leads to the leaf blade folding and the emergence of trichomes, named erineae, which protect the eggs and mite individuals and give to the affected host organs a velvety texture (Nishida & Holdaway, 1955; Nobrega *et al.*, 2023). In more severe infestations, the stem, flowers, and fruits can also be compromised (Álvarez *et al.*, 2009; Carrillo *et al.*, 2020; Revynthi *et al.*, 2022). It has been observed that the affected fruits develop brownish stains, likely due to an accumulation of red pigments in the erineae (Karioti *et al.*, 2011), which compromises sales due to a reduction of product quality. Fruit mummification has also been reported (Azevedo, 2013; Ramos, 2019) as well as increased orchard maintenance costs and the loss of cultivars (Carrillo *et al.*, 2020). The most common means of infestation control are the removal of affected branches, chemical control with sulfur-based products, and biological control by acari of the Phytoseiidae family (Azevedo, 2013; Ramos, 2019), which has been little explored. In addition, erinosis is often considered a simple gall (Chen & Qiao, 2012), with only epidermal cells undergoing the processes of hypertrophy and hyperplasia, or as pseudo galls (Wool, 2004; Floate, 2010), due to the absence of a larval or nymphal chamber. However, others argue that these galls are indeed true galls seeing as they undergo the key processes of hypertrophy of epidermal cells, hyperplasia in the differentiation of trichomes, and parenchyma homogenization (Mani, 1964; Álvarez *et al.*, 2013; Formiga *et al.*, 2015; Jorge *et al.*, 2016; Ferreira *et al.*, 2017; Ferreira *et al.*, 2019).

Herein, we traced the anatomical, histochemical, and morphometric profiles of the non-galled *L. chinensis* leaves and *A. litchi* leaf galls to map the amplitude of the cecidogenetic field caused by the erinosis on the epidermal and mesophyll cells as well as to shed new light on the discussion whether this morphotype should be regarded as a true or pseudogall.

Materials and Methods

Sampling

Samples of non-galled *L. chinensis* leaves (NGL), young (YG), mature (MG), and senescent (SG) *A. litchi* leaf galls (n = 5 per stage from 5 individuals) were collected from an orchard at the Federal University of Viçosa (UFV) campus in the municipality of Viçosa, Minas Gerais state, Brazil (20°45'14" S; 42°52'54" O) on March and September, 2022. This orchard has the main purpose of teaching and academic activities, such as to allow a follow-up of the development and management of the lychee plants. The region has an altitude tropical climate, similar to that of southern China (climate-data.org), the native biome of *L. chinensis*. The samples were fixed in formaldehyde, acetic acid, and ethanol (FAA50) 1:1:18 v/v (Kraus & Arduin, 1997) for anatomical analysis and morphometry. In addition, fresh samples of non-galled leaves and leaf galls (n = 5 individuals per stage) were collected for macroscopical and histochemical analyses.

We did a preliminary triage of the affected individuals and considered the YG to be those with signs of leaf blade alterations but no erineae, MG to be those with erineae but restricted to the induction site, and the SG those in which the erinosis overtakes most, if not the entirety of the abaxial face of the leaf.

Macroscopical and microscopical analyses

The fresh galls (n = 15 affected leaves per stage, with 5 gall sites measured per leaf for a total of 45) were photographed with a Leica® S9i stereomicroscope and measured (gall length, width, and height) with the LAS X software. The samples fixed in FAA (n = 5 per stage) were transferred to 70% ethanol and hand-made cross-sectioned, followed by immersion in 50% sodium hypochlorite, v/v, for clarification. After clarification, the sections were washed and stained with Astra blue and safranin 9:1, v/v, then mounted on glass slides with jelly glycerin 50% (Kraus and Arduin 1997). The sections were analyzed and photographed on a light microscope (Leica® DM500) with a coupled digital camera (Leica® ICC50 HD). Macroscopical measurements were taken on the erinosis sites for height and width considering an adaxial-abaxial plane (n = 30). Additionally, microscopical measurements were taken on non-galled leaves and leaf galls for mesophyll and epidermis thickness, and epidermal cell width (n = 30). The macroscopical measurements were taken on a Leica® S90i stereomicroscope, and the microscopical measurements were taken on the AxioVision 7.4 software (Carl Zeiss® Microscopy GmbH, Jena, Germany). Finally, ANOVA (two-factor) was performed on Microsoft Excel® comparing each gall stage to the non-galled leaves.

Histochemical analyses

The histochemical profile of the NGL, YG, MG, and SG was traced by performing hand-made cross-sections for each stage and submitting to the specific reagents and protocols for primary and secondary metabolite detection (Table 1).

Test/Reagent - Substance	Concentrations/reaction times	Reference
Lugol's reagent – starch Positive results – dark blue	1% potassium iodine–iodide solution for 5 min	Johansen, 1940
Sudan IV - total lipids Positive results – red stains	0.3% Sudan in 70% ethanol for 5 min	Pearse, 1972
Bromophenol blue – proteins Positive results – blue stains	0.1g bromophenol blue-10g mercury chloride in ethanol 95% for 15 min	Mazia <i>et al.</i> 1953
Fehling – reducing sugars Positive results – brown stains	Equal parts of “A” (II copper sulfate 6.93% w:v) and “B” (sodium potassium tartrate 34.6% and 12% sodium hydroxide m:m:v) solutions heated to a pre-boiling temperature	Sass, 1951
Ferric chloride – phenolics Positive results – black stains	1% ferric chloride solution for 5 min	Johansen, 1940
DMACA – flavonoids Positive results – blue stains	Fixation: 0.5% caffeine sodium benzoate in 90% butanol for 1–2 h. Reaction: 1% p-dimethylaminocinnamaldehyde for up to 30 min.	Feucht <i>et al.</i> 1986
Dragendorff – alkaloids Positive results – dark brown stains	25 mL of “I” (12.5% bismuth nitrate-25% acetic acid in H ₂ O) and 10 mL of “II” (40% potassium iodate) for 5-10 min	Figueiredo <i>et al.</i> 2005
Wiesner – total lignins Positive results – purple stains	1 part of “A” (2% phloroglucin in 95% ethanol) and 2 parts of “B” (25% chlorine acid v:v) for 5 min	Johansen, 1940
Maule – types of lignins Positive results – purple for S-type/brown for G- type	1% KMnO ₄ for 5 min then wash in distilled water; 10% HCl until the sections become dark brown: 5–10 min then wash in distilled water 2x; mount on slides with concentrated ammonium dioxide	Mäule, 1901

Results

Host leaf and gall morphology

The non-galled leaves (NGL) are dark brilliant green on the adaxial surface and matte green on the abaxial surface (Fig. 1A). The galls occupy the abaxial leaf surface (Fig. 1B). When young, the galls (YG) show early signs of leaf blade intumescence and bending, restricted to the induction site and no erinea. The intensity of the alterations varies between different sites on the same leaflet, in different leaflets and leaves, and among different leaf ages. Mature galls (MG) are characterized by the development of erinea, sometimes locally displayed, sometimes occupying large areas with dark spots but not covering the midrib. The leaf edges tend to fold downward, enveloping the erinea. The folding of senescent galls (SG) is prominent in most sites, with a large density of erinea covering the affected tissues and assuming a local velvety aspect. In addition, exuviae of insects and other associated guild components, such as fungi and larvae, may be observed interspersed with the erinea. In extreme cases, the erinea were observed on both leaf surfaces and the rachis.

Host leaf and gall anatomy

NGL (Fig. 2A, B, C) are hypostomatic and have an unstratified epidermis covered by a thick cuticle on both leaf surfaces, dorsiventral mesophyll, and interspersed stomata (Fig. 2C). Gall induction promotes alterations already evidenced in the induction site YG (Fig. 2D, E, F) showing alterations in mesophyll organization, with lignification of cell walls of the spongy parenchyma (Fig. 2E), periclinal cell divisions on the adaxial epidermis forming a subepidermal additional layer (Fig. 2F), and loss of intercellular spaces. MG (Fig. 2G, H, I) has more conspicuous alterations, such as the bending of the leaf blade and the emergence of erinea on the

abaxial leaf surface (Fig. 2H). Abaxial epidermal cells dedifferentiate assuming meristematic features (Fig. 2H), and redifferentiate into the erineae, the feeding site of the galling mites. In addition, cell division changes from the anticlinal to the periclinal axis (Fig. 2I), and sites of meristem-like cells emerge below the epidermis. SG (Fig. 2J, K, L) shares the alterations described in MG. The gall site becomes darker (Fig. 2J), the mesophyll is completely overtaken by meristem-like cells (Fig. 2K), and due to the large number and density of erineae, the abaxial epidermis cells are indistinguishable from the neoformed cells in the gall site (Fig. 2K, L). The erineae are uniseriate and can be uni or multicellular (Fig. 2L).

Histochemical profile

The non-galled leaves (NGL) of *L. chinensis* accumulate starch grains in the pith parenchyma (Fig. 3A) and abaxial epidermal cells (Fig. 3B). They have a lipidic cuticle, but also accumulate lipids on the cell walls of the adaxial epidermis, and droplets in the vacuoles of epidermal and parenchyma cells (Fig. 3C). Proteins accumulate in the vessel element and sieve tube lumen, and in the symplast of the parenchyma and vascular bundles (Fig. 3D). Reducing sugar precipitates accumulate in the symplast of the sieve tubes, epidermis, and parenchyma (Fig. 3E). Phenolics are detected in the symplast of the epidermis and parenchyma cells (Fig. 3F). Flavonoids are detected in the vacuoles of the epidermis, phloem, and parenchyma cells (Fig. 3G). Alkaloids are detected as precipitates on the symplast, as well as in the cell walls of the epidermis, phloem, parenchyma, and vascular bundles (Fig. 3H). Lignins were detected in the secondary walls of the vessel elements (Fig. 3I) and fibers of the vascular sheath (Fig. 3J). They are predominantly Syringyl (type-S) – detected in the vascular sheath and vessel elements in pink - and sparsely Guaiacyl (type-G) – detected in the vessel elements in brown.

YG starts to deviate from NGL, accumulating lipids on the hypodermis cell walls (Fig.

4A) and not accumulating starch on the pith parenchyma, but also has similar results concerning other tested metabolites. MG share traits with both YG – lipids in the cell walls of the adaxial epidermis, and as droplets in the vacuoles of the epidermis and parenchyma cells - and NGL - starch on the pith parenchyma (Fig. 4B), but the neoformed meristem-like layer and hypodermis accumulate primary and secondary metabolites, such as proteins (Fig. 4C, D), reducing sugars (Fig. 4E), alkaloids (Fig. 4F), phenolics (Fig. 4G), and flavonoids (Fig. 4H).

SG does not accumulate starch in the pith parenchyma, accumulates lipids in the adaxial epidermis cell walls, and metabolites on the meristematic layer and multistratified epidermis, such as proteins (Fig. 4C, D), reducing sugars (Fig. 4E), alkaloids (Fig. 4F), phenolics (Fig. 4G), and flavonoids (Fig. 4H). Lignins were detected in the secondary walls of xylem cells (Fig. 4I) and fibers of the vascular sheaths (Fig. 4J), and in the secondary lignification in the galls (Fig. 4K, L). On the vascular sheaths, lignins are type syringyl (S), and in the xylem and secondary lignification, type guaiacyl/hydroxyphenyl (G). The proportion of G lignins is higher on the galls than in non-galled leaves.

The neoformed erineae (Fig. 5), like YG and SG, do not accumulate starch, but they accumulate primary metabolites on the basal cells – such as lipids (Fig. 5A) and proteins (Fig. 5B) – and in the symplast – such as reducing sugars (Fig. 5C). Secondary metabolites are either accumulated on the basal cells – such as flavonoids (Fig. 5D) and phenolics (Fig. 5E, F) - or not detected – such as alkaloids.

Morphometric data

The minimum measured gall length was 1.07 μm in YG, 1.49 μm in MG, and 1.91 μm in SG. The maximum measured length was 22.98 μm in YG, 17 μm in MG, and 36.85 in SG.

The minimum measured gall width was 0.3 μm in YG and MG and 1.19 μm in SG. The maximum measured width was 19.2 μm in YG, 8.73 μm in MG, and 13.09 in SG. The minimum measured gall height was 0 μm in YG and SG, and 0.2 μm in MG. The maximum measured height was 7.88 μm in YG, 7.14 μm in MG, and 8.95 μm in SG (Table 2).

Averages and standard deviations were similar between all three gall stages, except for the standard deviation in SG, of 9.07, which was discrepant from the other stages (Table 2, Fig. 6A).

	Length (μm)			Width (μm)			Height (μm)		
	Min	Max	Average + std dev.	Min	Max	Average + std dev.	Min	Max	Average + std dev.
YG	1.07	22.98	7.14 ± 5.63	0.30	19.20	4.81 ± 4.83	0.00	7.88	1.71 ± 2.01
MG	1.49	17.00	7.37 ± 4.20	0.30	8.73	4.47 ± 2.08	0.20	7.14	3.93 ± 1.98
SG	1.91	36.85	13.17 ± 9.07	1.19	13.09	6.57 ± 3.3	0.0	8.95	3.57 ± 2.55

The minimum measured thickness for the mesophyll was 436.98 μm in NGL, 186.25 μm in YG, 445.35 μm in MG, and 362.28 μm in SG. (Fig. 6A). The maximum measured thickness was 739.99 μm in NGL, 829.87 μm in YG, 1098.7 μm in MG, and 1187.49 in SG. The minimum measured thickness for the adaxial epidermis was 29.23 μm in NGL, 18.2 μm in YG, 50.78 μm in MG, and 27.43 μm in SG. The maximum measured thickness was 63.76 μm in NGL, 117.07 μm in YG, 157.14 μm in MG, and 105.4 μm in SG. The minimum measured thickness was 15.5 μm in NGL, 9.42 μm in YG, 21.2 μm in MG, and 20.04 μm in SG. The maximum measured thickness was 40.44 μm in NGL, 81.61 μm in YG, 49.57 μm in MG, and 60.06 μm in SG (Fig. 6B). The minimum measured width for the epidermal cells was 15.5 μm in NGL, 9.42 μm in YG, 21.12 μm in MG, and 20.04 μm in SG. The maximum measured width was 40.44 μm in NGL, 81.61 μm in YG, 49.57 μm in MG, and 60.06 μm in SG (Fig. 6C).

The average thickness for mesophyll and adaxial epidermis was similar between NGL and YG and between MG and SG. And the epidermal cell width was similar between NGL and all three gall stages (Table 3).

	Mesophyll thickness (µm)			Epidermis thickness (µm)			Epidermal cell width (µm)		
	Minimum	Maximum	Average + std dev.	Minimum	Maximum	Average + std dev.	Minimum	Maximum	Average + std dev.
NGL	436.98	739.99	559.68 ± 96.88	29.23	63.76	44.89 ± 9.88	15.50	40.44	30.40 ± 6.33
YG	186.25	829.87	513.24 ± 107.76	18.20	117.07	46.96 ± 22.25	9.42	81.61	35.02 ± 12.84
MG	445.35	1098.70	863.60 ± 181.33	50.78	157.14	84.10 ± 24.36	21.20	49.57	37.01 ± 6.69
SG	362.28	1187.49	815.35 ± 225.34	27.43	105.40	66.01 ± 18.29	20.04	60.06	33.62 ± 90.50

The p-value for ANOVA was lower than 0.001, attesting to significant changes to the affected sites during gall maturation.

Discussion

The observed results evidence that the affected sites undergo profound and complex changes, affecting tissue layers other than the epidermis. Cell divisions are also affected, as the mature galls have an increased number of cells when compared to non-galled leaves. The anatomical analysis shows that the entire leaf blade undergoes cell hypertrophy, hyperplasia, and tissue neoformation processes for erineia formation on the abaxial face of the affected leaves, which fits the definition of a complex gall (Mani, 1964; Álvarez *et al.*, 2013). In addition, the histochemical and morphometric analyses show that the feeding activity of *A. litchii* significantly affects the structure of *L. chinensis*, relocating photoassimilates and metabolites to the gall site

and thus compromising the ordinary metabolism of the host leaves.

The center of the cecidogenetic field is located at the abaxial epidermal cells, the site of interaction of the colony of mites with the leaf surface. These cells dedifferentiate into meristem-like cells that undergo intense cell divisions leading to erineal formation, as seen in previous studies concerning mite galls (Petanovic´ & Kielkiewicz, 2010; Nobrega *et al.*, 2013; Carrillo *et al.*, 2020; Guedes *et al.*, 2023). These structural alterations become more conspicuous along gall maturation, up to the stage where the erineae overtake the abaxial epidermis, and the mesophyll undergoes a process of homogenization, common in several gall morphotypes (Petanovic´ & Kielkiewicz, 2010; Ferreira *et al.*, 2016), which starts with the emergence of a layer of meristem-like cells. Even distant from the feeding sites, the adaxial epidermal cells pass by a hyperplasia process, adding layers to the epidermis, visible at the early stages of gall development (Petanovic´ & Kielkiewicz, 2010; Guedes *et al.*, 2023). At the senescent stage, it is no longer possible to distinguish between palisade and spongy parenchyma, and the affected sites do not have stomata, which, as discussed by Guedes *et al.* (2023), incapacitates gas exchanges at the affected site as the structure assumes features inherent to those of the gall.

Histochemical results align with the structural development of the erinosis. They are similar to other host plant-gall inducer systems, in which the relocation and accumulation of resources to the gall site favor the growth and development of the galling organism to the detriment of the host plant, creating permanent sinks (Raman *et al.*, 2006; Álvarez *et al.*, 2009; Petanovic´ & Kielkiewicz, 2010; Carneiro *et al.*, 2014; Inbar *et al.*, 1995; Isaias *et al.*, 2014). Primary metabolites – such as starch, lipids, proteins, and reducing sugars – accumulate in tissue sites similar to the non-galled leaves, whereas secondary metabolites – phenolics, flavonoids, alkaloids, and lignins – were relocated to the gall site. The deposition of cutin in the multilayered epidermis offers a thicker layer of protection (Oliveira & Isaias, 2009) for the gall and the mite colony, and, as highly energetic molecules, may also provide maintenance for the meristem-like

cells (Oliveira *et al.*, 2010). The detection of proteins and reducing sugars in the meristematic layer is also related to the maintenance of the gall and its higher metabolic demand, as the primary functions of the neoformed cells are cell division and erineae formation. Similarly, the detection of reducing sugars in the erineae is related to the main source of nutrition for the nymphae, as seen in other Eriophyidae gall models (Petanovic´ & Kielkiewicz, 2010; Ferreira *et al.*, 2016; Guedes *et al.*, 2023; Nobrega *et al.*, 2023), as well as for sustaining the accelerated growth of the structure which also protects the mite colony (Bronner, 1992; Stone & Schönrogge, 2003; Guedes *et al.*, 2023; Nobrega *et al.*, 2023).

The relocation of phenolics indicates the mitigation of oxidative stress generated by constant cell divisions (Carneiro *et al.*, 2014; Isaias *et al.* 2015; Guedes *et al.*, 2023). In addition, the accumulation of phenolics at the base of the erineae might indicate unpalatability, both to predators (Bronner, 1992) and to the nymphae themselves, to prevent them from feeding on the basal cells and damaging the structure; these phenolics might also act as auxin “protectors” – molecules that prevent auxin degradation on the site in order to stimulate the abnormal growth and the erineae formation process (Karioti *et al.*, 2011). Similarly, flavonoid and alkaloid relocation to the erineae is justified by the high metabolic demand of the gall, as well as the maintenance of structural integrity, as they also act as antioxidants (Carneiro *et al.*, 2014; Guedes *et al.*, 2023) and chemical protection against natural enemies (Bronner, 1992; Álvares *et al.*, 2008), respectively. Furthermore, the higher proportion of G-type lignins indicates an alteration in the biosynthetic pathways of the host plant due to the gall, as changes in the ratio of lignin types are commonly associated with plant stress (Fukushima, 2001; Moura *et al.*, 2010; Bhardwaj *et al.*, 2013).

The leaf lamina undergoes a dedifferentiation process of chlorophyllous parenchyma cells, originating a homogeneous parenchyma with meristematic potential (Petanovic´ & Kielkiewicz, 2010). The photosynthetic status is impaired at the gall site, and consequently, the

source of photoassimilates to sustain gall metabolism must be relocated from other host leaf parts. In addition, a secondary layer of mesophyll cells lignifies, conferring mechanical support to the galled leaf, as seen in other mite gall models (Oliveira & Isaias, 2009; Petanovic' & Kielkiewicz, 2010; Guedes *et al.*, 2023). At later stages of gall development, cell divisions extend to the hypodermis.

The morphometric results align with the intense cell hypertrophy and hyperplasia of the epidermis and mesophyll, as reported for other host plant-gall inducer systems (Mani, 1964; Nyman *et al.*, 2000; Shorthouse *et al.*, 2005; Isaias *et al.*, 2014; Jorge *et al.*, 2016; Guedes *et al.*, 2023; Nobrega *et al.*, 2023). They also follow the cecidogenetic gradient observed in the anatomical profile, the measurements demonstrating, in numbers, the processes of hypertrophy, hyperplasia, and tissue neoformation near the mite colony with the thicker mesophyll and meristematic layer, and hypertrophy and hyperplasia at the adaxial epidermis with wider cells and a multistratified tissue. In addition, the statistical values demonstrate the heterogeneity of cell shapes at the induction sites and the variety of gall sizes, aligning with the rapid and disorderly growth of the erinosis (Nobrega *et al.*, 2023). These results add to the argument that erinosis is not restricted to the folding of the leaf blade and the emergence of erineae, but also causes conspicuous and significant changes to the leaf lamina.

Author's Contributions

AMAS collected and prepared the samples and wrote the first draft; RMSI designed and coordinated the experiment, analyzed the data, and revised the manuscript; EATP assisted in sample collection and data analysis.

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Conflict of Interest

The authors had no conflicts of interest during the production of this paper.

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Table and Figure Captions

Table 1: Reagents used for histochemical detection of primary and secondary metabolites in hand-made cross sections of non-galled *Litchi chinensis* Sonn. (Sapindaceae) and *Aceria litchii* (Keifer, 1943) (Eryophyidae) leaf galls.

Fig. 1: Non-galled *Litchi chinensis* Sonn. (Sapindaceae) leaf on abaxial and adaxial surfaces (A) and, from left to right, leaves of *Aceria litchii* (Keifer, 1943) (Eryophyidae) with young, mature, and senescent galls (B). Scale: 1.5 cm.

Fig. 2: Cross-sections of non-galled *Litchi chinensis* Sonn. leaves and young, mature, and senescent *Aceria litchii* (Keifer, 1943) leaf galls. (A) Non-galled leaf on adaxial surface view. (B-C) Anatomical aspect of leaf lamina on a transverse section. (D) Young *A. litchii* leaf gall on abaxial surface view. (E) Anatomical aspect of leaf lamina on a transverse section. (F) Adaxial epidermis on a transverse section. (G) Mature *Aceria litchii* leaf gall on abaxial surface view. (H) Anatomical aspect of leaf lamina on a transverse section. (I) Adaxial epidermis on transverse section. (J) Senescent *Aceria litchii* leaf gall on abaxial surface view. (K) Anatomical aspect of leaf lamina on transverse view. (L) Meristem-like cells and erinea on transverse view. PP = palisade parenchyma. SP = spongy parenchyma. VB = vascular bundle. MC = meristem-like cells. AdE = adaxial epidermis. AbE = abaxial epidermis. Black arrow = stomata. White arrows = anticlinal cell divisions. Black arrowhead = unicellular erinea. White arrowhead = multicellular erinea. Asterisk = spongy parenchyma lignification. Scale: 200 μm (A) and 50 μm (B, C).

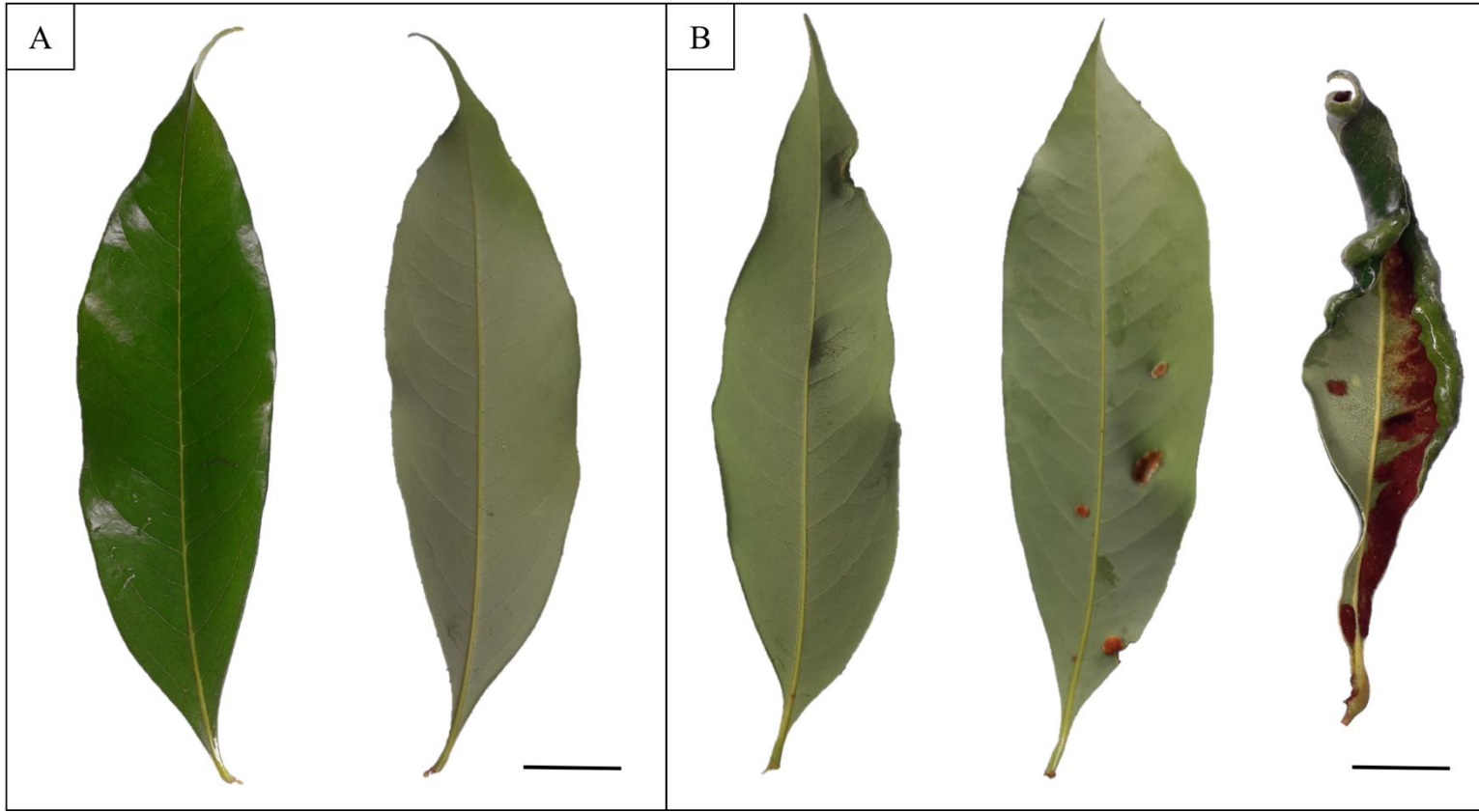
Fig. 3: Histochemistry of primary (A – E) and secondary (F – H) metabolites on non-galled *Litchi chinensis* Sonn. leaves. Starch grains positive detection in the pith (A) and abaxial epidermal cells (B). Positive reaction for lipid droplets in the abaxial epidermal cells and for the cuticle (C). Proteins accumulated in the vascular bundle and adaxial epidermal cells (D). Reducing sugars accumulated in the adaxial epidermal and palisade parenchyma cells (E). Phenolics accumulated in the vacuoles of adaxial epidermal and palisade parenchyma cells (F). Positive reaction for flavonoids in the vacuoles of adaxial epidermal and vascular bundle cells (G). Alkaloids accumulated as brown precipitates in the adaxial epidermal, palisade parenchyma, and vascular bundle cells (H). Red arrows = metabolite detection. PP = palisade parenchyma. SP = spongy parenchyma. VB = vascular bundle. AdE = adaxial epidermis. AbE = abaxial epidermis. Scale: 200 μm (A) and 50 μm (B - H).

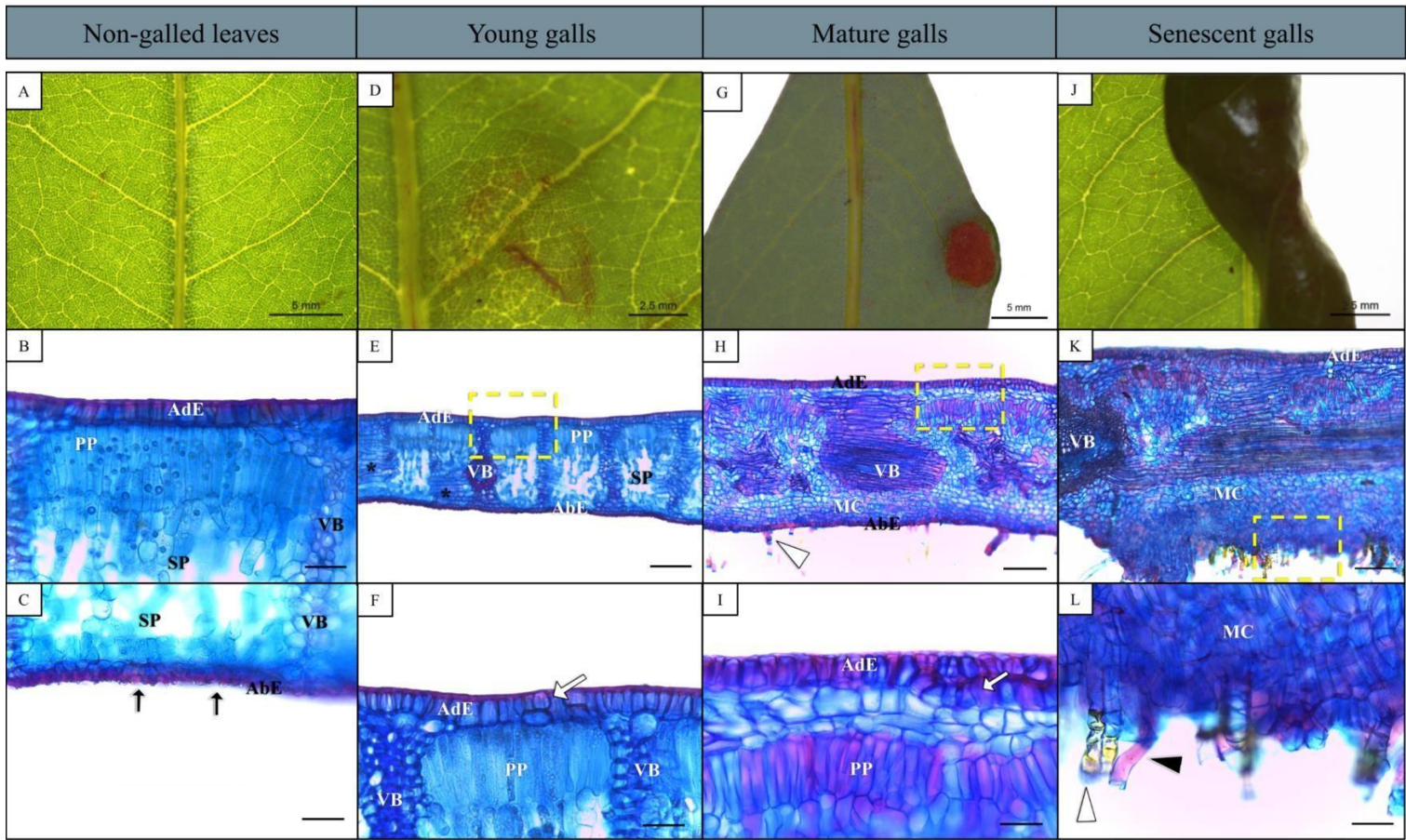
Fig. 4: Histochemistry of primary (A – D) and secondary (E – H) metabolites in the symplast, and lignin detection in the cell walls (I – L) on cross-sections of young (A, I, L), mature (B, C, D, E, H, I, J) and senescent (F, G, K) *Aceria litchii* (Keifer) (Eriophyidae) leaf galls. Lipids in the cuticle and as droplets on the adaxial epidermis cell walls (A). Positive detection of starch grains in the vacuoles of the pith parenchyma cells of mature galls (B). Proteins accumulated in the vacuoles of the pith, vessel element lumen, vascular sheath fibers, and meristem-like cells (C, D). Reducing sugar precipitates accumulated in the meristem-like cells (E). Flavonoid precipitates accumulated in the multistratified adaxial epidermis and meristem-like cells (F). Phenolics accumulated in the adaxial epidermis and meristem-like cells (G). Alkaloids in the meristem-like cells and mesophyll lignification (H). Type S and G lignins in the secondary cell walls of the midrib (I). Type G lignins in the secondary cell walls of the xylem in the vascular bundle (J). Type G and S lignins in the secondary cell walls of the mesophyll (K, L). PP = palisade parenchyma. SP = spongy parenchyma. VB = vascular bundle. MC = meristem-like cells. AdE

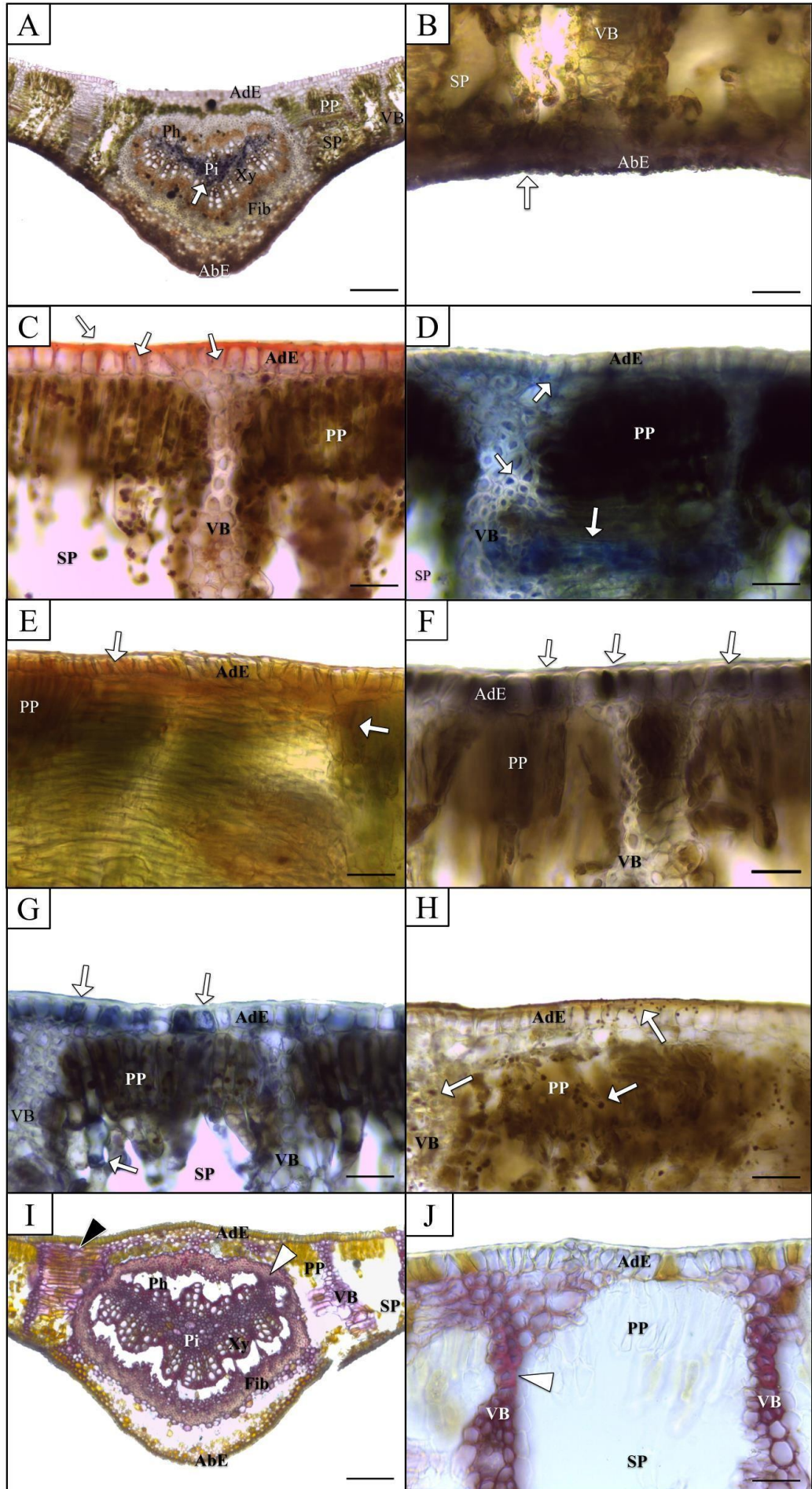
= adaxial epidermis. AbE = abaxial epidermis. White arrows = metabolite detection. White arrowheads = Type-S lignins. Black arrowheads = Type-G lignins. Scale: 200 μm (G, K) and 50 μm (A – F, H – J, L).

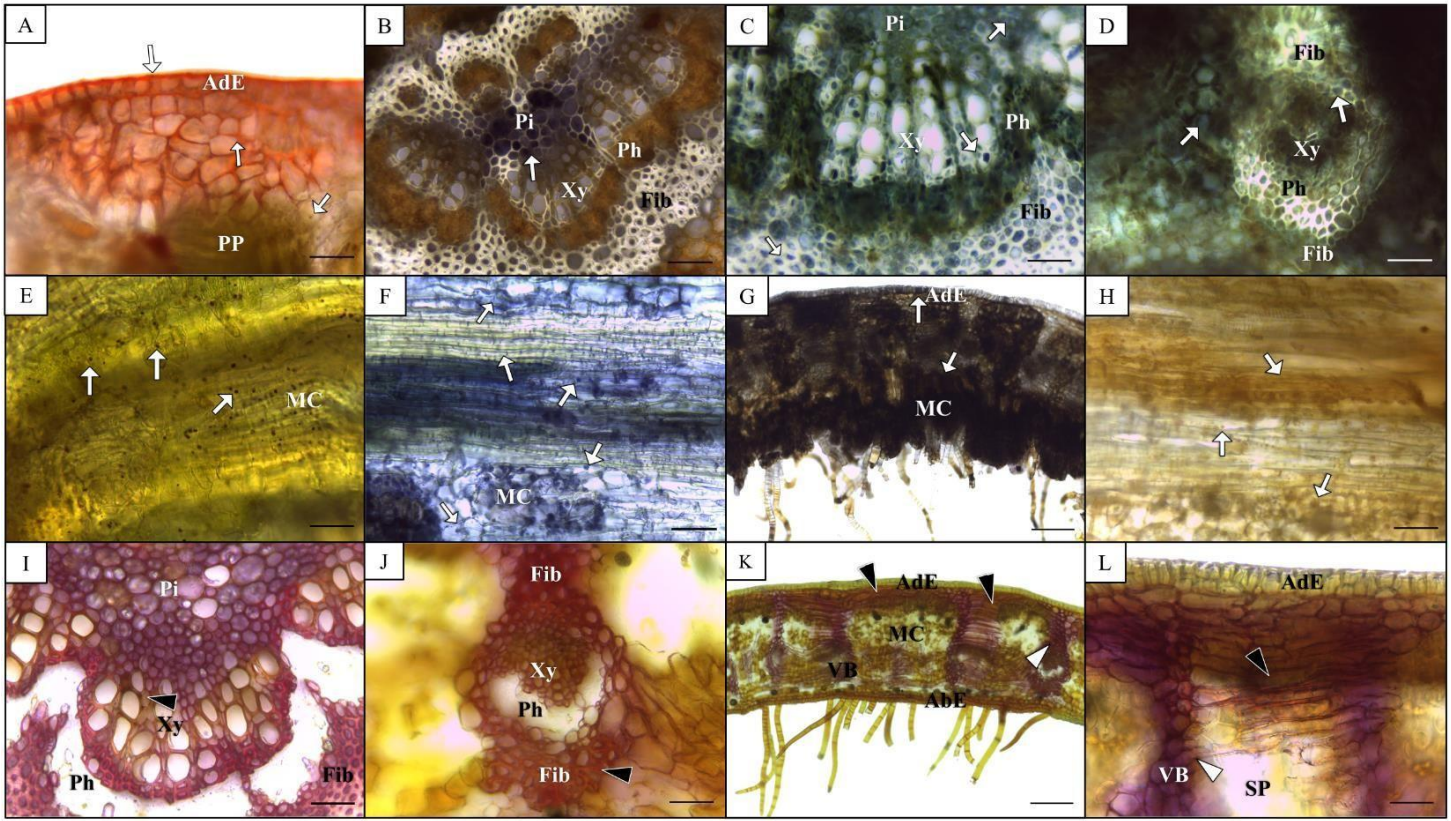
Fig. 5: Histochemistry of primary (A - C) and secondary (D - F) metabolites on the erineae of young (YG), mature (MG) and senescent (SG) *Aceria litchii* (Keifer) (Eriophyidae) leaf galls on *L. chinensis* Sonn. (Sapindaceae). Lipids detected on the meristematic layer of SG (A). Proteins detected on the meristematic layer and along the erineae of SG (B). Reducing sugars detected on the erineae of MG (C). Flavonoids detected on the erineae of MG (D). Phenolics detected on the meristematic layer and on the base of the erineae of SG (E). Alkaloid precipitates detected on the erineae of MG (F). White arrows = metabolite detection. Scale: 50 μm .

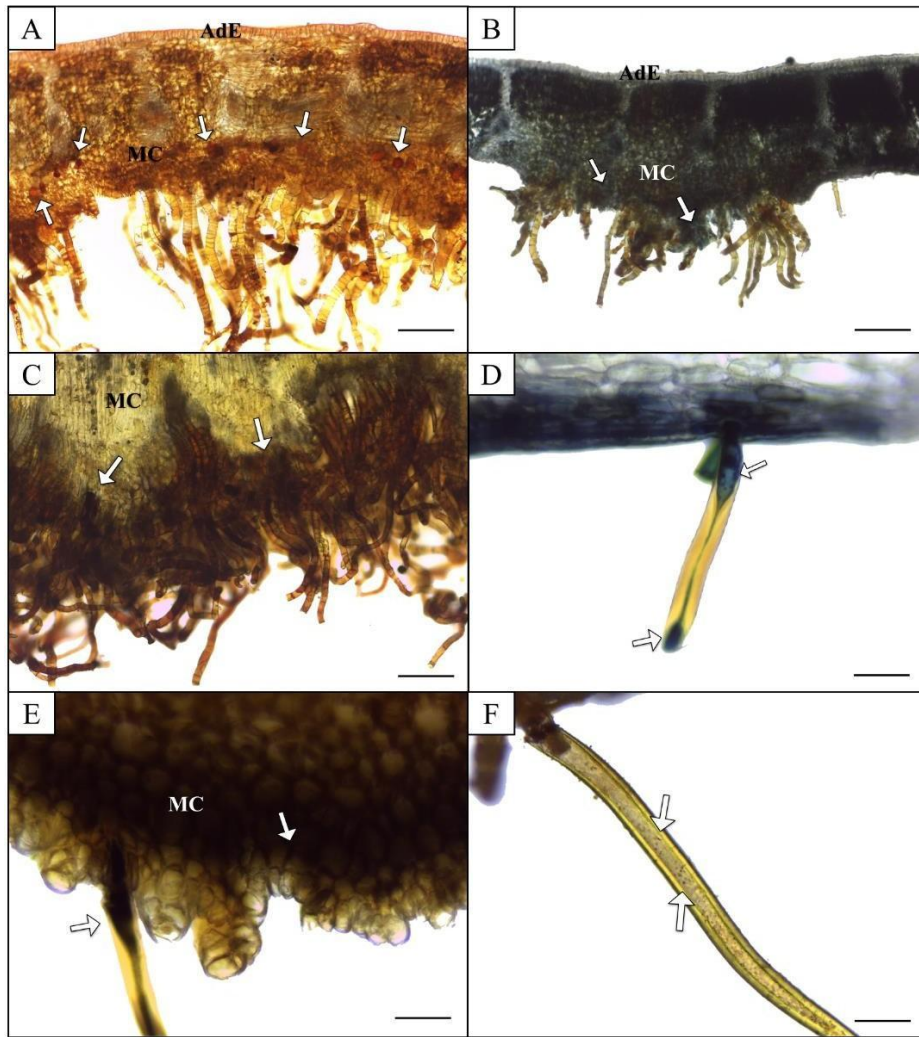
Fig. 6: Comparison of measurement dispersion for mesophyll thickness (A), adaxial epidermis thickness (B), and adaxial epidermal cell width (C) between non-galled *Litchi chinensis* Sonn. leaves (NGL) and young (YG), mature (MG) and senescent (SG) *Aceria litchii* (Keifer) (Eriophyidae) leaf galls. Values expressed in μm . Asterisks indicate significant statistical differences demonstrated by the t-test ($p < 0.05$).

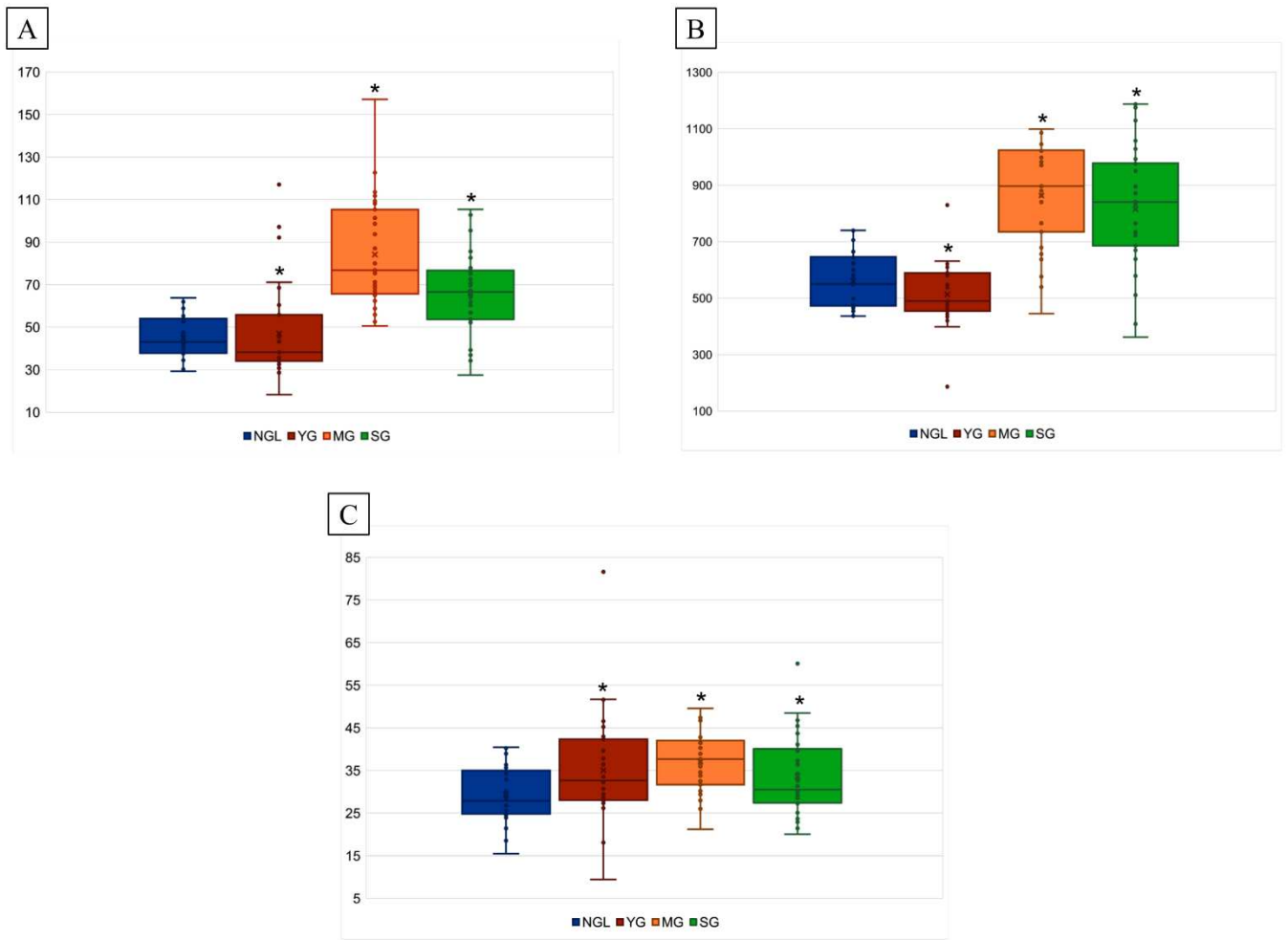












**Cytological alterations caused by *Aceria litchii* (Keifer) (Eriophyidae) leaf galls on
epidermal cells of *Litchi chinensis* Sonn. (Sapindaceae)**

Abstract

Litchi chinensis Sonn. is an ornamental plant that is subject to infestations by *Aceria litchii* (Keifer, 1943) (Eriophyidae), which leads to the emergence of erineae on the abaxial surface of the leaves, providing both nutrition and protection for the nymphae. The constant cell divisions stimulated by the feeding activity of the mites establish a high metabolic demand, which leads to host plant fitness and sales reduction. We comparatively analyzed the ultrastructure of the epidermal cells of non-galled *L. chinensis* leaves and *A. litchii* leaf galls, hypothesizing that the reduced fitness could be related to ultrastructural alterations of the cell machinery. Samples were collected at the Federal University of Viçosa (UFV) campus, Minas Gerais, Brazil, embedded in historesin for anatomical analyses and subjected to transmission electron microscopy for cytological analyses. The abaxial epidermis undergoes cell dedifferentiation, leading to the formation of a meristematic layer, whose cells redifferentiate into numerous erineae. The vacuoles enlarge and accumulate phenolics and other metabolites to sustain gall metabolism, but some organelles degenerate due to high oxidative stress. Metabolite relocation and accumulation are similar to other host plant-gall inducer systems and relate to the reduced productivity of the host plant as the gall matures.

Key words: Erinosis, *Litchi chinensis*, *Aceria litchii*, dedifferentiation, oxidative stress.

Introduction

The majority of gall morphotypes are known for forming an enclosed chamber around the inducer, offering protection from biotic and abiotic factors, as well as nutrition (Mani, 1964; Stone & Schönrogge 2003; Ferreira *et al.*, 2019), the latter coming from the neoformation of one or more layers of nutritive cells from which the gall inducer feeds (Mani, 1964; Jorge *et al.*, 2016; Ferreira *et al.*, 2016, 2019). Erinoses galls - a less common morphotype, induced mainly by Acarina and referred to by Mani (1964) as hairy galls or filzgalls - also benefit from a similar process, which is the formation of erinea – trichomes – in high quantity and density, which also confer protection for the growth and development of the nymphae (Mani, 1964; Bronner, 1992; Petanovic´ & Kielkiewicz, 2010). Unlike the more well-known gall morphotypes, however, these galls do not fully enclose the inducer inside a chamber (Nishida & Holdaway, 1955; Ferreira *et al.*, 2019), being very similar to galls induced by aphids and Thysanoptera (Mani, 1964; Ferreira *et al.*, 2019) in the sense that they are initially induced by a founding female (Mani, 1964; Wool, 2004; Ferreira *et al.*, 2019).

Infestations by *Aceria litchii* (Keifer) on *Litchi chinensis* Sonn. induce the emergence of erinoses galls which may spread all over the leaf blade (Nishida & Holdaway, 1955; Carrillo *et al.*, 2020). The erinea emerge on the abaxial leaf surface, promoting the dedifferentiation of epidermal cells and their redifferentiation toward meristem-like cells (Petanovic´ & Kielkiewicz, 2010). The production of erinea increases along the maturation stage of the gall, and results in the reduction of plant fitness, as the location of the erinoses renders gas exchange impossible, thus impairing the host plant's photosynthetic status (Guedes *et al.*, 2023; Patankar *et al.*, 2011). The affected leaves become a permanent sink (Raman *et al.*, 2006; Álvarez *et al.*, 2009; Patankar *et al.*, 2011; Carneiro *et al.*, 2014; Isaias *et al.*, 2014), rerouting and accumulating photoassimilates and metabolites, making the erinea an optimal environment for the nymphae (Stone & Schönrogge, 2003). Ultimately, the reduced fitness and longevity of the affected

cultivars is reflected on the lesser quality of the final product, be it esthetic or for consumption (Azevedo, 2013; Ramos, 2019), and in the increased maintenance cost of orchards (Carrillo *et al.*, 2020).

The high metabolic demand and tissue neoformation have been reported to greatly affect the ultrastructure of the host plant cells (Oliveira *et al.*, 2010; Ferreira *et al.*, 2016; Bragança *et al.*, 2017; Costa *et al.*, 2022), but how these changes impact the cellular structure of the erinosis has yet to be explored. Thus, we analyzed the anatomy and cytology of non-galled *L. chinensis* leaves and *A. litchii* leaf galls focusing on the epidermis, the neoformed erineae, and the meristem-like cells occupying the mesophyll, to map the ultrastructural changes and any damage done to the cell machinery, which could explain the reduction of productivity reported.

Materials and Methods

Sampling

Samples of non-galled *L. chinensis* leaves (NGL), young (YG), mature (MG), and senescent (SG) *A. litchii* leaf galls (n = 5 per stage) were collected from an orchard at the Federal University of Viçosa (UFV) campus in the municipality of Viçosa, Minas Gerais state, Brazil (20°45'14" S; 42°52'54" O), and fixed in Karnovsky's solution (Karnovsky, 1965).

We did a preliminary triage of the affected individuals and considered the YG to be those with signs of leaf blade alterations but no erineae, MG to be those with erineae but restricted to the induction site, and the SG those in which the erinosis overtakes most, if not the entirety of the abaxial face of the leaf.

Anatomical analyses

The samples fixed in Karnovsky's solution ($n = 5$ per stage) were dehydrated in an ethanol series and then embedded in Leica® historesin. Sections ($6\ \mu\text{m}$) were made in a rotatory microtome (Leica® 2035 BIOCUT), then mounted on glass slides with water and stained with Toluidine blue. The sections were analyzed and photographed on a light microscope (Leica® DM500) with a coupled digital camera (Leica® ICC50 HD).

Cytological analyses

Samples of non-galled *L. chinensis* leaves and *A. litchii* young, mature and senescent leaf galls ($n = 2$ per stage) fixed in Karnovsky's solution were post-fixed in 1% osmium tetroxide (OsO_4) in $0.1\ \text{mol L}^{-1}$ PBS, dehydrated in an ethanol series (O'Brien and McCully, 1981), and embedded in Spurr® resin (Sigma-Aldrich) (Luft, 1961). The samples were sectioned ($50\text{--}70\ \text{nm}$) in an Ultramicrotome Reichert-Jung-Ultracut (Leica, Wetzlar, Germany), contrasted in uranyl acetate and lead citrate (Reynolds, 1963), and analyzed with a transmission electron microscope Tecnai™ G2-12 Spirit BioTwin (FEI, Hillsboro, USA; 120 kV) at the Centro de Microscopia of the Universidade Federal de Minas Gerais (CM-UFGM).

Results

The abaxial epidermal cells of the NGL (Fig. 1) are characterized by a thick and irregular cuticle interspersed with stomata (Fig. 1A, B, C), smooth cell walls and a conspicuous nucleus (Fig. 1C, D), healthy Golgi apparatus and endoplasmic reticulum (ER) (Fig. 1E, F), dense and abundant symplast, abundant mitochondria (Fig. 1F, G), and a scarce presence of phenolics in

the vacuoles (Fig. 2G).

The YG (Fig. 2) have signs of hyperplasia with a loss of intercellular spaces (Fig. 2A, B) and irregularly shaped cell walls (Fig. 2C, D). The symplast is more dense and concentrated next to the plasmalemma (Fig. 2D, E); the vacuoles are enlarged, and the Golgi apparatus and mitochondria are less abundant (Fig. 2F).

The MG (Fig. 3) has a multilayered meristematic tissue on the abaxial leaf surface (Fig. 3A, B). The cell types are covered by a thin cuticle. The meristem-like cells have dense symplast and enlarged vacuoles filled with phenolics and other metabolites, and the basal cells of the erineae are filled with phenolics (Fig. 3C, E). The nucleus is inconspicuous (Fig. 3D, E, F), mitochondria and chloroplasts are ruptured, and the ER is hypertrophic (Fig. 3D, F).

The SG (Fig. 4) no longer has distinguishable parenchyma cells (Fig. 4A), with the meristem-like cells overtaking the mesophyll (Fig. 4A, B). The basal cells of the erineae are covered by a thin cuticle (Fig. 4C, F), and the nucleus is inconspicuous (Fig. 4D, F). The symplast is degraded and concentrated next to the plasmalemma (Fig. 4C, D, F, G, H). Mitochondria and chloroplasts are less abundant and ruptured (Fig. 4E, G). The vacuoles are enlarged and filled with phenolics and other metabolites (Fig. 4C, F). The ER is hypertrophic and the nucleus is depleted (Fig. 4G, H).

Discussion

The first response of leaf cells of *L. chinensis* to the infestation of *A. litchi* is the enlargement of the vacuoles of the abaxial epidermal cells and the accumulation of phenolics inside them. Due to this enlargement, the symplast is pushed to the cell's periphery, as seen in insect galls (Carneiro & Isaias, 2018; Ferreira *et al.*, 2019; Costa *et al.*, 2022), and increases in

density, possibly due to the large amount of resources being produced and accumulated in the induction site, as discussed by Petanovic´ and Kielkiewicz (2010). During gall development, the numerous cell divisions indicate a high metabolism imposed by the activity of the colony of *A. litchi*, imposing oxidative stress on the gall environment, resulting in the breakdown of organelles such as mitochondria and chloroplasts (Cesarino & Bennet Jr, 1999), which was observed in mature erineae on *L. chinensis*.

Along gall maturation, abaxial epidermal cells dedifferentiate into meristem-like cells, as seen in typical Eriophyidae galls (Petanovic´ & Kielkiewicz, 2010; Ferreira *et al.*, 2016), with organelles such as ER and the Golgi apparatus becoming enlarged to support the increment in cell divisions and accelerated metabolism (Oliveira *et al.*, 2010; Carneiro *et al.*, 2014). By the time of senescence, the nucleus is depleted and inconspicuous, possibly due to repeated exposure to oxidative stress, as discussed in other cellular models (Cassarino & Bennet Jr, 1999; D'Angelo *et al.*, 2009). Due to the accelerated cell divisions, the cell walls become irregular, and the cuticle deposition over the meristem-like cells and erineae is thinner, which can be explained by the sequestering of the cell machinery by the gall, as well as the relocation of resources, as the cuticle is composed by highly energetic molecules (Muller & Riederer, 2005). In addition, thinning the cuticle might also be a strategy for evading plant defense, as damaging it is viewed as a signal of plant injury (Serrano *et al.*, 2014).

The erineae have an increasing development during the mature stage of the gall, at the cells of the abaxial epidermis, which have redifferentiated from the neoformed meristem-like cells (Petanovic´ & Kielkiewicz, 2010). Developing erineae have a denser symplast than mature erineae, which mainly accumulate metabolites to feed the mite colony, whereas senescent erineae oxidize due to compromised cell metabolism. The basal cells also accumulate metabolites, such as phenolics, in the enlarged vacuoles, which helps mitigate the oxidative stress caused by the constant cell divisions (Carneiro *et al.*, 2014; Isaias *et al.*, 2015, 2018), likely by capturing free

radicals (Castro *et al.*, 2021), but does not seem to prevent the breakdown of the cellular machinery. Other metabolites are also stored in the vacuoles to support the accelerated metabolism of the mature gall (Petanovic' & Kielkiewicz, 2010; Carneiro *et al.*, 2014; Ferreira & Isaias, 2014) and, similar to a typical nutritive tissue (Ferreira & Isaias, 2014; Ferreira *et al.*, 2016; Costa *et al.*, 2022), provide nutrition for the mite colony.

The main consequence of the relocation of resources and accelerated metabolism is the overtaxing on the cell machinery, which leads to progressive organelle degeneration as the gall matures and subsequent plant fitness reduction, and ultimately, the loss of productivity reported by the producers.

Author's Contributions

AMAS collected and prepared the samples and wrote the first draft; RMSI designed and coordinated the experiment, analyzed the data, and revised the manuscript; EATP assisted in sample collection, data analysis, and revision of the manuscript.

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Conflict of Interest

The authors had no conflicts of interest during the production of this paper.

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Table and Figure Captions

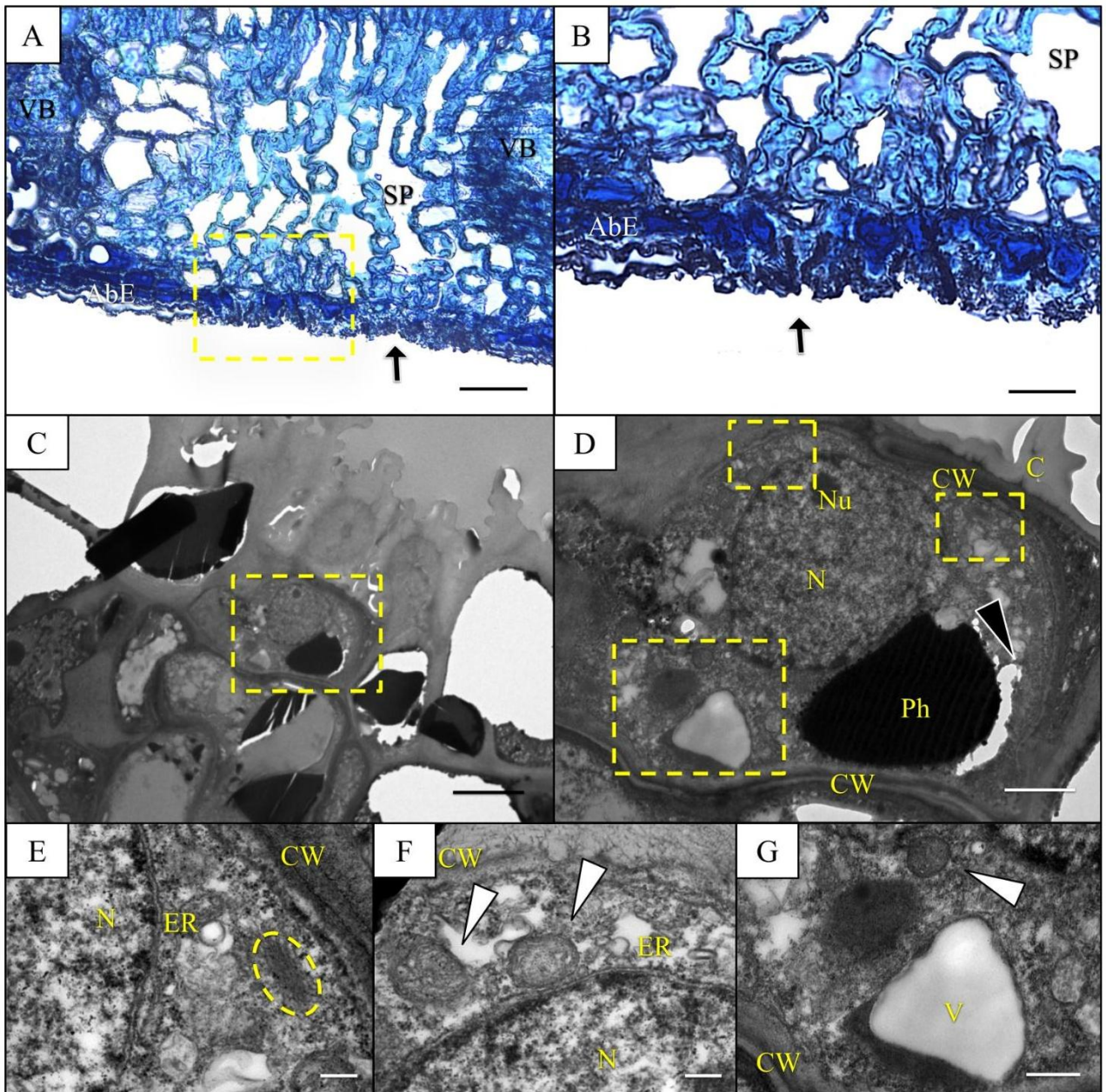
Figure 1: Anatomy (A, B) and cytology (C - G) of the abaxial epidermal cells of non-galled *Litchi chinensis* Sonn. leaves. SP = spongy parenchyma. AbE = abaxial epidermis. VB = vascular bundles. Black arrows = stomata. CW = healthy and smooth cell wall. C = thick cuticle. V = vacuole. Ph = Phenolics. ER = endoplasmic reticule. N = healthy nucleus. Nu = evident nucleolus. White arrowheads = healthy mitochondria. Black arrowhead = healthy chloroplast. Dotted circle = Golgi apparatus. Scale: 200 μm (A), 50 μm (B), 5 μm (C), 1 μm (D), 200 nm (E, F), and 500 nm (G).

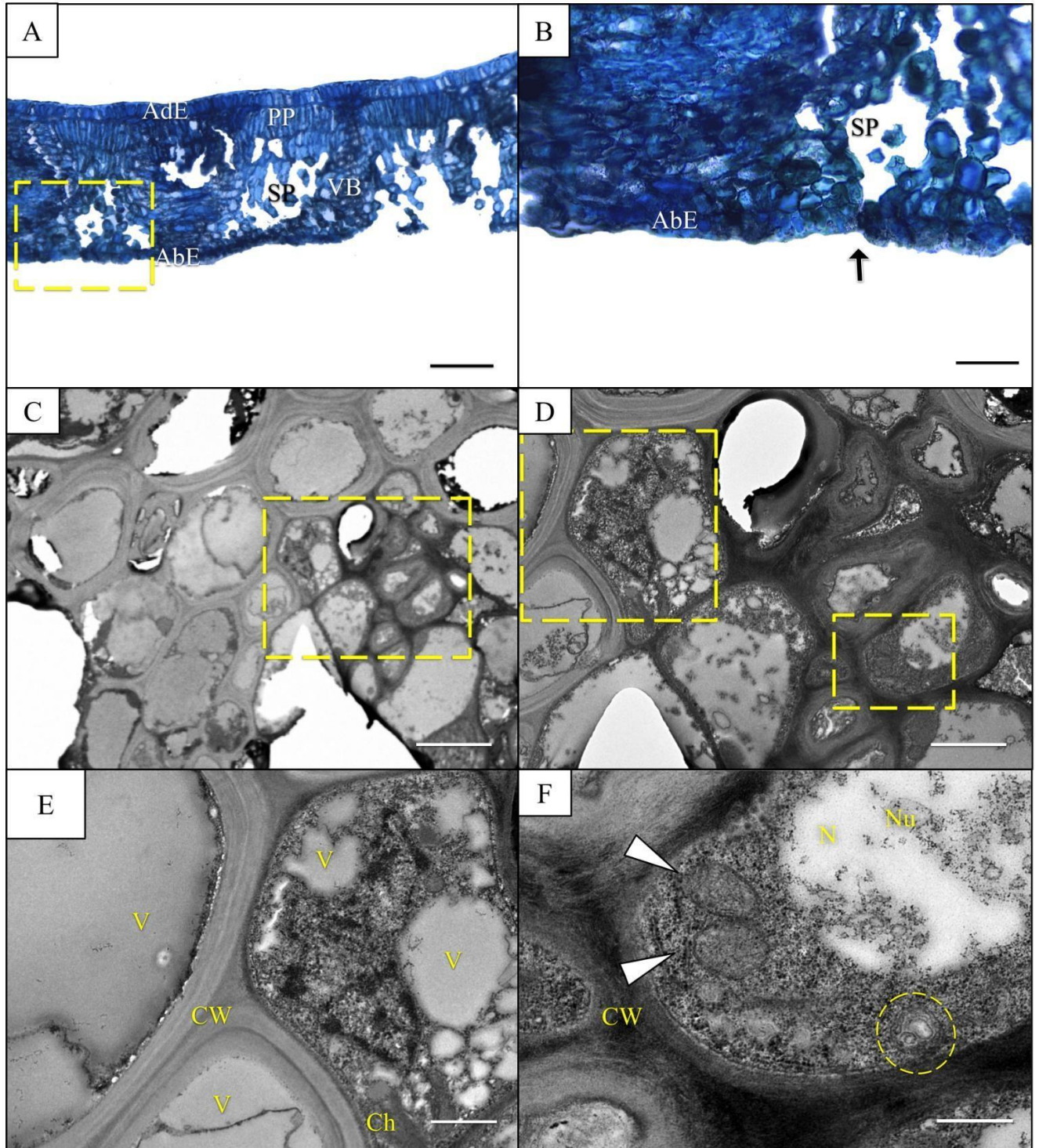
Figure 2: Anatomy (A, B) and cytology (C - F) of the abaxial epidermal cells of young *Aceria litchii* (Keifer, 1943) leaf galls. AdE = adaxial epidermis. AbE = abaxial epidermis. VB = vascular bundles. Black arrow = stomata. CW = altered cell wall. C = thinner cuticle. V = enlarged vacuole. N = altered nucleus. Nu = evident nucleolus. M = altered mitochondria. Ch = altered chloroplast. Dotted circle = enlarged Golgi apparatus. Scale: 200 μm (A), 50 μm (B), 2 μm (C, D), 1 μm (E) and 500 nm (F).

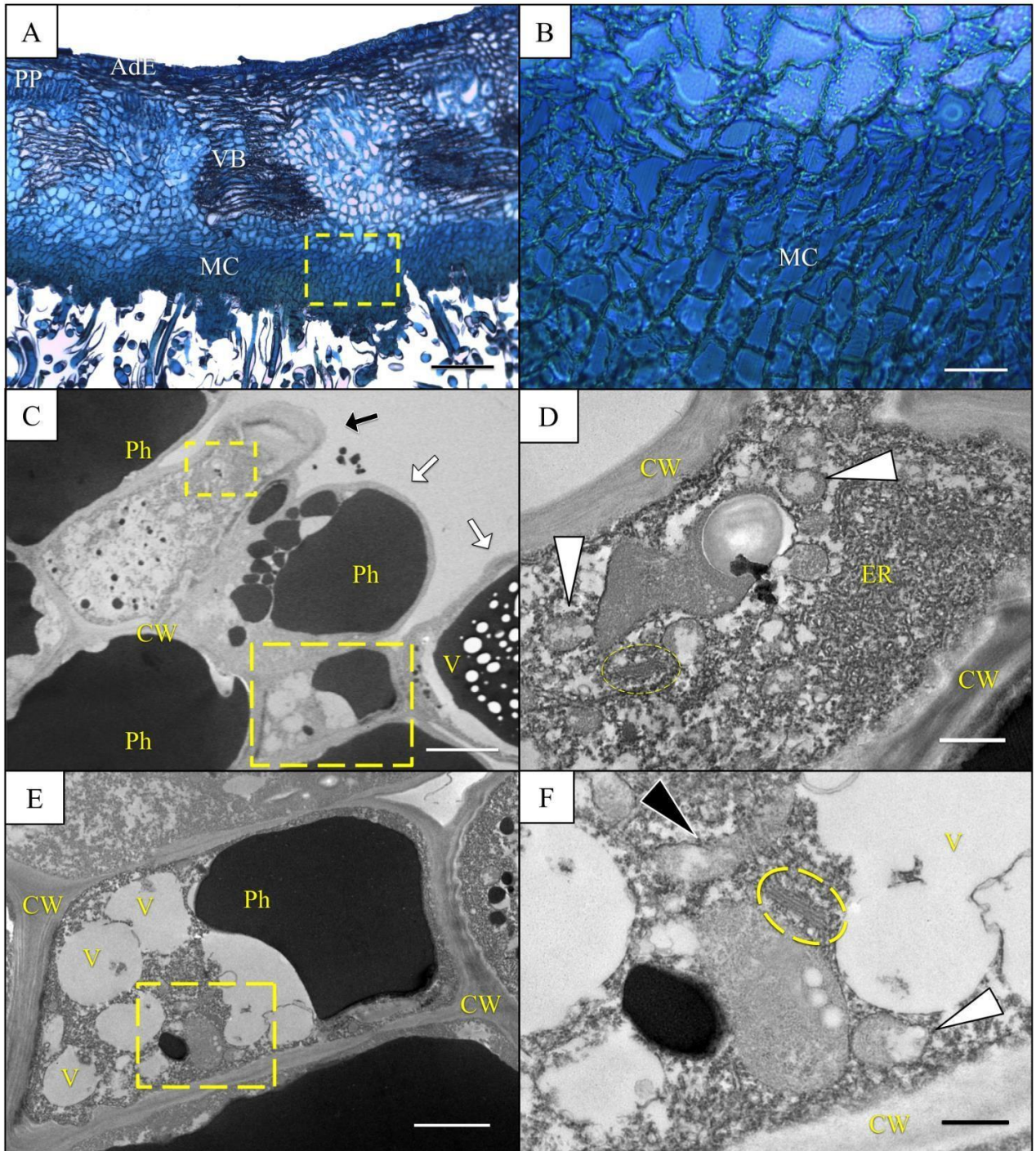
Figure 3: Anatomy (A, B) and cytology (C - F) of mature *Aceria litchii* (Keifer, 1943) leaf galls. AdE = adaxial epidermis. VB = vascular bundles. MC = meristem-like cells. CW = thin cell wall. C = thin cuticle. V = enlarged vacuoles. Ph = Phenolics. ER = enlarged endoplasmic

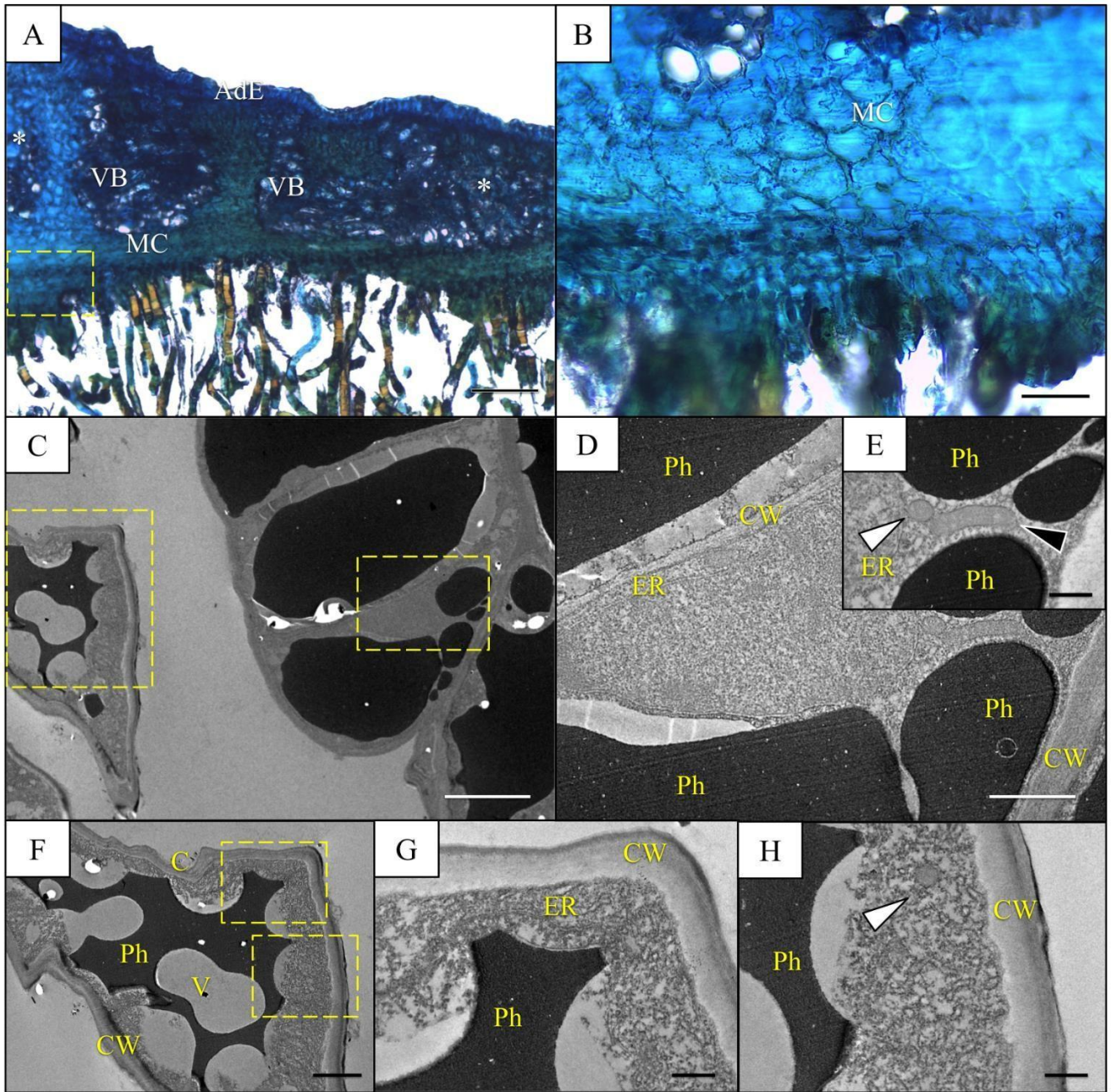
reticule. Dotted circle = enlarged Golgi apparatus. Black arrow = meristem-like cell. White arrows = erineae basal cells. White arrowhead = ruptured mitochondria. Black arrowhead = ruptured chloroplast. Scale: 200 μm (A), 50 μm (B), 5 μm (C), 1 μm (D) and 500 nm (E).

Figure 4: Anatomy (A, B) and cytology (C - H) of senescent *Aceria litchii* (Keifer, 1943) leaf galls. AdE = adaxial epidermis. VB = vascular bundles. MC = meristem-like cells. Asterisk = necrotic tissue. CW = thin cell wall. C = thin cuticle. V = enlarged vacuole. M = ruptured mitochondria. ER = enlarged endoplasmic reticule. N = faded nucleus. Nu = altered nucleolus. White arrowhead = ruptured mitochondria. Black arrowhead = ruptured chloroplast. Scale: 200 μm (A), 50 μm (B), 5 μm (C), 2 μm (D, F), and 500 nm (E, G, H).









CONSIDERAÇÕES FINAIS

As galhas foliares de *A. litchii* apresentam processos de hipertrofia e hiperplasia iniciando na epiderme abaxial, com formação de um campo cecidogenético em direção à epiderme da face adaxial da folha. Os processos de intumescimento e enrolamento da lâmina foliar são acompanhados pela neoformação de tecidos com processos de dediferenciação e rediferenciação celular, originando conjuntos de células meristemáticas e eríneas.

O perfil histoquímico revela o acúmulo de recursos no sítio de desenvolvimento da galha, em especial de metabólitos primários como açúcares redutores e proteínas nas eríneas, das quais as ninfas se alimentam, e de compostos fenólicos nas células basais da camada meristemática, onde os processos de divisão e rediferenciação celular são mais intensos. Em virtude da posição e densidade das eríneas, a lâmina foliar se torna incapaz de produzir fotoassimilados *in situ*, se tornando, portanto, um dreno permanente para a planta.

Além disso, as constantes divisões celulares e a atividade alimentar da colônia de ácaros induzem a formação constante de eríneas, o que expõe a maquinaria da célula ao estresse oxidativo. O acúmulo de fenólicos nas células meristemáticas neoformadas parece não ser capaz de capturar o excesso de radicais livres, pois há degradação das organelas citoplasmáticas. A diagnose anatômica, histoquímica e ultraestrutural se relaciona à queda acentuada de produtividade relatada em pomares ao redor do mundo.

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