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Revisiting the histological patterns of storage tissues: beyond the limits of gall-inducing taxa

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Revisiting the histological patterns of storage tissues: beyond the limits

of gall-inducing taxa

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Abstract

Gall-inducing Aphididae may feed directly on phloem, while Eriophyidae and Nematoda feed on cells lining the gall chambers. We assume that a variation in structural complexity will occur within galls induced by each *taxon*, and that the complexity of the galls could be related with the types of storage tissue they have. Histological, histometric and histochemical analyses were used to compare six gall systems, which have different levels of complexity. Such levels are not *taxon*-related, even though eriophyid galls are usually simpler than nematode and aphid galls. The histological features of galls allowed the classification of storage tissues in three types: 'typical nutritive tissues' (TNT), 'common storage tissues' (CST), and 'nutritive-like tissues' (NLT). The TNT and NLT have cells with dense cytoplasm and a prominent nucleus. The CST cells are vacuolated, and may store starch and other energy-rich molecules, as do the NLT cells. In contrast to NLT or CST, the TNT serves as direct food source for gall inducers, and it is present in nematode and some eriophyid galls. NLTs may be present in some aphid galls, but are not the direct feeding site. The CST occurs on galls of all three inducing *taxa*.

Key words: Aphid galls; Eriophyid galls; Histology; Nematode galls; Nutritive tissue; Storage cell

Introduction

Galls are products of an intrinsic relationship between specific parasites and their host plants (Mani 1964), where the gall inducers govern plant developmental processes such as cell hypertrophy and redifferentiation, hyperplasia, and/or alteration of meristematic activity (Mani 1964; Bronner 1992; Álvarez et al. 2009, 2013; Oliveira and Isaias 2010; Ferreira and Isaias 2013; Fleury et al. 2015). The alterations in plant histogenesis are linked to the sink strength in galls (Larson and Whitham 1991; Inbar et al. 1995; Castro et al. 2012; Fleury et al. 2015), and provide essential energy-rich molecules for gall growth and maintenance, and for the inducer's nutrition. Even though the galling habit evolved independently in non-related groups of Animalia (Acari, Nematoda, and Insecta) (Meyer 1987), the galls show convergent structural arrangements related to the similar feeding habits of the inducers (Larew 1981; Bronner 1992; Rohfritsch 1992; Oliveira and Isaias 2010; Oliveira et al. 2011; Ferreira and Isaias 2013). In addition to providing nutrition, gall structure may offer protection against natural enemies, such as parasitoids, pathogens and inquilines, as well as an adequate microenvironment for the development of the offspring (Price et al. 1987; Stone and Schonrogge 2003). Moreover, some conservative features such as storage, cell and trichome types, secretory structures, arrangement of vascular bundles, and some cytological features may represent constraints imposed by the host plant, and consequently determine gall histology (Moura et al. 2009; Oliveira et al. 2010; Ferreira and Isaias 2014).

Galls induced by insects can be histologically divided into two groups: (1) those induced by chewing (Lepidoptera and Hymenoptera) and scraping-

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sucking insects (Diptera: Cecidomyiidae); and (2) those induced by sucking

insects (Hemiptera) (Weidner 1961 *apud* Larew 1981). The galls of chewing

and scraping-sucking insects have 'typical nutritive tissues' (TNT) surrounding

the gall chamber. The TNT is a storage tissue used as the food source for the

inducer. Its cells are usually cytoplasm-rich, with a prominent nucleus and

nucleoli (Kostoff and Kendall 1929; Bronner 1992; Ferreira et al. 2015; Oliveira

et al. 2011, 2016). TNT cells usually accumulate proteins, lipids or reducing

sugars, depending on the gall-inducing species (Bronner 1992; Oliveira and

Isaias 2010; Vecchi et al. 2013; Ferreira and Isaias 2013, 2014).

Galls induced by sucking insects are not thought to have nutritive tissues,

because their inducers suck directly on vascular bundles (Larew 1981; Bronner

1992). However, storage tissues with cells that accumulate starch, here termed

'common storage tissues' (CST), surround the vascular bundles (Álvarez et al.

2009, 2013; Oliveira and Isaias 2010; Isaias et al. 2011; Muñoz-Viveros et al.

2014). In specific cases, when the cells store starch and reducing sugars and

have an active nucleus and dense cytoplasm (Carneiro and Isaias 2014, 2015;

Carneiro et al. 2014; Richardson et al. 2016), the CST are termed 'nutritive-like

tissues' (NLT) (*sensu* Richardson et al. 2016). NLT and CST are histologically

distinguished by the vacuoles (large in CST; fragmented in NLT), nucleus (non-

prominent in CST; prominent in NLT), and cytoplasm (peripheral and

inconspicuous in CST; dense in NLT). Sucking gall-inducing animals such as

Nematoda and Acari induce colonial galls with TNT (Kendall 1930; Goodey

1939, 1948; Watson and Shorthouse 1979; Skinner et al. 1980; Larew 1981;

Westphal et al. 1981; Moura et al. 2008, 2009; Ferreira et al. submitted). The

stylets of these inducers are shorter than the stylets of the hemipterans, and

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they are accordingly capable of feeding only on the cells immediately adjacent

to the chamber (Dropkin 1969; Westphal et al. 1981).

The different types of storage parenchyma may be present in galls

induced by different animal groups, and these types vary cytologically according

to the functions of the cells in each galling system. Different authors distinguish

storage tissues in galls with an array of terms, such as: 'parenchymatic nutritive

tissue' and 'nutritive tissue' (Sliva and Shorthouse 2006), 'nutritive-like

parenchyma' (Oliveira and Isaias 2010), 'storage nutritive tissue' and 'typical

nutritive tissue' (Ferreira and Isaias 2013; Ferreira et al. 2015), 'true nutritive

tissue' not eaten by the inducer (Carneiro and Isaias 2015) and 'nutritive-like

tissue' (Richardson et al. 2016). Detailed analyses of these types of tissues

revealed some convergent and divergent structural and functional features,

which led us to propose a standard classification for these storage tissues. We

believe that the use of a standard classification will be an important step for

future investigations of cytological, histochemical, and functional patterns in gall

storage tissues.

Presuming that the feeding habits of the gall inducers would predict the

histological patterns of the galls, colonial galls should have the same

histological patterns as solitary galls. We aimed to compare features of galls

induced by three groups: aphids, nematodes, and mites, using an array of

morphological tools and data from the literature. The level of structural

complexity of the gall is as high as the range of developmental alterations they

have. We considered the occurrence of hyperplasia, cell hypertrophy,

hypertrophy of vascular bundles, cell redifferentiation, histochemical changes,

and manipulation of meristematic activity as the wider amplitude of gall

complexity. Assuming the range of developmental alterations within each gall group, we test if the complexity of the galls is related to the types of storage tissues they have. Finally, we propose to standardize the nomenclature for storage tissues in galls, based on galls induced by three phylogenetically distant groups of gall inducers.

Material and methods

Collection and histological processing

Non-galled leaves (controls) and galls (Table 1) were collected (n = 5 for each category), fixed in Karnovsky's solution (Karnovsky 1965) or in FAA (Johansen 1940), dehydrated in a butyl or ethyl series (Kraus and Arduin 1997), embedded in Paraplast®, and sectioned using a rotary microtome (12 µm). The slides were deparaffinized, stained with 0.5% astra blue and 0.5% safranin (9:1) (Kraus and Arduin 1997), or with 1% safranin and 1% fast green (Johansen 1940) in order to distinguish cell walls with lignin or suberin, vacuoles with phenolics, and nucleus (red stained) from pectocellulosic cell walls and cytoplasm (blue stained). After staining, the slides were dehydrated and mounted in clear varnish (Paiva et al. 2006) or Entellan®.

Histochemistry

The sections were treated with Lugol's reagent to detect starch (Johansen 1940), Fehling's reagent for reducing sugars (Sass 1951), Ponceau 2R for proteins (Ventrella et al. 2013), or Sudan red B for lipids (Jensen 1962).

Histometry

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In order to evaluate the occurrence of hyperplasia and hypertrophy, 3 randomly selected regions of median sections of the galls and controls (n = 5) were photographed, and the number of cell layers was counted. The area of 5 cells of each tissue was measured in each section. In aphid galls and their controls, the height (anticlinal diameter) of the xylem and phloem (transverse sections) of secondary veins was measured. In midrib galls induced by *Geoica utricularia* on *Pistacia terebinthus*, the tissues of the non-galled midrib were considered as control tissues, and the two rows of vascular bundles (adaxial supernumerary bundles and main central bundles) were measured, separately.

The measurements were compared using the software SigmaStat® by means of a t-test (when raw or transformed data showed a normal distribution and were homoscedastic) or a Mann-Whitney test (when the data, even after transformations, were not normal and/or homoscedastic). In some cases, two kinds of leaf parenchyma were compared with gall parenchyma(s), and the data were compared with one-way analysis of variance (ANOVA) followed by Tukey's post-test (normal and homoscedastic data) or Kruskal-Wallis test followed by Dunn's post-test (non-normal and/or non-homoscedastic data even after transformations). We considered the differences to be statistically significant when $p < 0.05$.

Results

Aphid galls

<u>*Eriosoma ulmi*</u>. The galls are formed by rolling one leaf margin toward its abaxial surface (Fig. 1A), and have three kinds of tissue regions. The first is similar to the non-galled mesophyll (Fig. 1B); the second is almost unaffected

but with some tissue homogenization (Fig. 1C); and the third is the folding

region, with noticeable hypertrophy of the parenchyma cells and hyperplasia

(Fig. 1D). The sites of parenchyma hyperplasia have 2-fold thicker mesophyll

than that of the non-galled leaf lamina (Table 2). The phloem faces the gall

chamber (Fig. 1D). The epidermal cells on both surfaces and the parenchyma

cells are hypertrophied (Table 2). Xylem and phloem areas increase in gall sites

compared with the controls (Table 2). Gall parenchymatic cells are highly

vacuolated, with thin cytoplasm. Reducing sugars accumulate in the gall

vascular bundles and endodermis (Fig. 1E), as well as in the controls. Small

amounts of accumulated proteins (Fig. 1F-G) but no starch was detected in the

gall tissues (Table 2). No storage tissues differentiate (Table 3).

Geoica utricularia: The galls are globoid (Fig. 2A), induced on the midrib (Fig.

2B) of young leaflets. They are formed by the growth of a pouch on the midrib

toward the abaxial surface. Unicellular trichomes close the small aperture. The

inner (adaxial) epidermis is multiseriate, with small holes linking the gall

chamber and gall parenchyma (Fig. 2C). The gall wall can be divided into inner

and outer homogeneous parenchyma (Fig. 2C). The parenchyma shows

marked hyperplasia and cell hypertrophy (Table 2). The inner parenchyma

layers accumulate proteins, starch, and reducing sugars (Fig. 2D; Table 2), with

cytoplasm-rich cells that have prominent nucleus (Fig. 2E; Table 3). The midribs

of the control (Fig. 2B) and of the galls (Fig. 2C) have two rows of vascular

bundles: the adaxial supernumerary and the abaxial main bundles. The phloem

portion of the supernumerary bundles faces the adaxial surface (Fig. 2C, E),

and is significantly hypertrophied in galls (Table 2). Both phloem bundles of the

galls have secretory ducts (SD) (Fig. 2C, E), whereas the supernumerary

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bundles of the controls lack the secretory duct associated with the phloem (Fig. 2B).

Nematode galls

<u>*Ditylenchus gallaeformans* on *Miconia albicans.*</u> The globoid verrucous galls are induced on leaves (Fig. 3A-B), stems and inflorescences of *M. albicans* (Melastomataceae). Colonies of nematodes are surrounded by gall emergences (Fig. 3C-E). The outer epidermis is not in contact with the colonies, and is densely covered with arachnoid trichomes (Figs. 3C-E). These trichomes are lignified and form a denser layer on the galls than on the controls (Fig. 3C; Table 4). The subjacent outer homogeneous parenchyma develops under the outer epidermis (Fig. 3C-G), and has polyhedral cells and idioblasts with druses (Fig. 3E), better seen under polarized light (Fig. 4A). Discrete collateral vascular bundles are embedded in the outer parenchyma (Fig. 3E-F). The cells in contact with the larvae are meristematic, and originate new emergences that grow to surround the nematode colonies (Fig. 3D-E). The cells of the inner parenchyma and epidermis of the nematode chamber constitute the nutritive tissue (Fig. 3F-G). These cells are smaller than the cells of the non-galled leaf portions (Fig. 3E-F; Table 4), and have a dense granulose cytoplasm, a prominent nucleus, and thin cell walls (Figs. 3F-G). Adventitious leaves may also appear in the lateral regions. Starch grains occur in the outer parenchyma cells (Fig. 4B), while proteins and reducing sugars (Fig. 4C) occur in the nutritive cells (Table 3). Parenchyma hyperplasia is the main process that forms these galls, while cell hypertrophy is not observed (Table 4). The inner (nutritive) cells are smaller than the cells of the non-galled leaves (Table 4).

Ditylenchus gallaeformans on *Miconia ibaguensis.* The globoid verrucous galls are induced on leaves of *M. ibaguensis* (Fig. 5A-B). Emergences surround the colonies of nematodes (Fig. 5B-C). The outer epidermis, not in contact with the nematodes, is densely covered with stellate trichomes (Fig. 5C). An outer parenchyma with polyhedral cells and idioblasts with druses develops under the outer epidermis (Fig. 5B-C). Discrete collateral vascular bundles are embedded in the outer parenchyma (Fig. 5D). The cells in contact with the larvae are meristematic, and originate new emergences that grow to surround the nematode colonies (Fig. 5C-D). The cells of the inner parenchyma and epidermis of the nematode chamber constitute the nutritive tissue (Fig. 5D). These cells are smaller than the cells of the non-galled leaf portions (Table 4), and have a dense granulose cytoplasm, a prominent nucleus, and thin cell walls (Fig. 5D). Adventitious leaves usually appear in the lateral regions (Fig. 5A-B). Starch grains occur in the outer parenchyma cells, while proteins (Fig. 5E) and reducing sugars (Fig. 5F) occur in the nutritive cells (Table 3). Parenchyma hyperplasia is the main process that forms these galls, while cell hypertrophy is not observed (Table 4). The inner (nutritive) cells are smaller than the cells of the non-galled leaves (Table 4).

Eriophyid galls

Galls of unidentified eriophyid: The galls are formed by a bulging of the leaf lamina of *M. ibaguensis*, usually forming blisters directed toward the adaxial surface (Fig. 6A-B), with a dense indumentum on the abaxial surface (Fig. 6C-D), where the mites live and feed. Sometimes a dense indumentum may also develop on the adaxial surface. The galls have homogeneous parenchyma (Fig. 6D-E), with no alterations in the number of cell layers or in the thickness of the

mesophyll compared to the control leaves (Table 5). Cell hypertrophy does not occur (Table 5). The indumentum is formed by long emergences with one layer of epidermis and 1-3 layers of parenchyma (Fig. 6E-G). The emergences are considerably larger and denser than in the controls (Fig. 6E-D) (Table 5). The mites feed on vacuolated cells of the epidermis located among and on the bases of the emergences (Fig. 6E-F; Table 3). Reducing sugars (Fig. 6H) occur in the epidermis of the chamber (Table 3). Proteins, starch (Fig. 6I), and lipids accumulate equally in galled and non-galled parenchyma (Table 5).

Aceria tristriata: The galls are small and lenticular (Fig. 7A-B). An aperture is usually present on the abaxial surface. In galls, the leaf thickness increases considerably (Fig. 7A) through hyperplasia and cell hypertrophy (Table 5). The epidermis is simple, and the gall mesophyll has homogeneous parenchyma, sometimes with a small bundle embedded in the median region (Fig. 7C). The surface of the nymphal chamber consists of nutritive cells, sometimes disrupted by the eriophyid feeding (Fig. 7D). The nutritive cells have a large prominent nucleus and granulose cytoplasm (Fig. 7D-F), and accumulate a considerable amount of proteins (Fig. 7E) and some oil drops (Table 3). The non-nutritive parenchyma cells are not in contact with the mites, and have large vacuoles and a peripheral cytoplasm (Fig. 7D, 7G). Starch and reducing sugars accumulate in the outer parenchyma cells (Tables 3, 5), but in smaller amounts than in the non-galled leaves.

Discussion

Variations in histological complexity occur within each group of galls induced by the same *taxon*. Simple galls, such as those induced by *E. ulmi* on

leaves of *U. minor*, and those induced by an unidentified Eriophyidae on *M. ibaguensis*, have fewer histological alterations. Hyperplasia occurs both in simple and complex galls, being the unique histological process common to all studied systems. Storage tissues are absent in less complex galls. Therefore, the presence and types of storage tissues may indicate the complexity level of a gall.

Different colonial taxa *manipulate host-plant tissues differently*

Current results have shown that histological patterns determined by each galling *taxon* also occur in colonial galls. However, the feeding habit of gall inducers alone does not explain these patterns. In fact, distinct groups of superficial-sucking gall inducers, i.e., nematodes and eriophyids, have different structural peculiarities.

The unprecedented comparisons among the three phylogenetically distant groups of gall-inducers, using histological, histometric, and histochemical tools, have shown that aphid galls have the most extensive range of histological alterations when compared to nematode and eriophyid galls. Aphid galls may have different levels of complexity (Álvarez et al. 2013), but all usually show hyperplasia, hypertrophied parenchyma cells and phloem bundles, and accumulate reducing sugars. Hypertrophy of the vascular bundles is expected in aphid galls, because the phloem is their food source (Wool et al. 1999), and directly receives the stimuli of gall induction. However, the orientation of the vascular bundles is maintained, and the stylets of the inducers may cross the xylem to suck the phloem sap (Álvarez et al. 2009). In addition, the two rows of vascular bundles observed in galls of *G. utricularia* have the

same overall vascular organization as the control midrib (Álvarez 2011, 2012),

as occurs in other aphid galls (Wool et al. 1999; Álvarez et al. 2014; Liu et al.

2014). Therefore, the complexity of aphid galls also depends on the complexity

of the structure of the host organ. The presence of storage tissues, holes in the

inner epidermis (Álvarez 2011, 2012; Álvarez et al. 2014), and mechanical

tissues (Álvarez et al. 2009, 2016; Kurzfeld-Zexer et al. 2015) are other

indicators of the complexity of aphid galls. The colonial galls induced by aphids

are histologically similar to galls induced by solitary phloem-sucking gall

inducers such as members of Psylloidea (Larew 1981; Bronner 1992; Dias et al.

2013; Carneiro and Isaias 2014, 2015; Carneiro et al. 2014), but the presence

of several gall inducers per chamber increases the degree of hyperplasia and

cell hypertrophy.

The nematode galls have shown a wide range of histological alterations,

including the formation of a nutritive tissue with promeristematic features –

namely, the capability of differentiating several cell types – a novelty in

comparison to the other gall systems. All nematode galls are formed by

hyperplasia and homogenization of the parenchyma (Goodey 1939, 1948;

Watson and Shorthouse 1979; Skinner et al. 1980), but these changes show no

clear relationship to the cell size in nematode galls. The redifferentiation of cells

in nematode gall developmental sites may result in larger (Goodey 1948; Bird

1961; Watson and Shorthouse 1979; Yousif 1979; Skinner et al. 1980), similar-

sized (Goodey 1939; Watson and Shorthouse 1979; Skinner et al. 1980) or

smaller cells (Goodey 1948) in relation to the controls. The study of galls

induced by *D. gallaeformans* reinforced the ability of gall-inducing nematodes to

manipulate feeding cells in several ways, forming several types of nutritive

tissues. Nutritive cells of galls of *D. gallaeformans* are smaller than the control

cells, in concordance with their redifferentiation toward a meristematic status.

The accumulation of reducing sugars and proteins, and the cytological features

of these cells configure them as a typical nutritive tissue (TNT) (*sensu* Kostoff

and Kendall 1929), as they are used directly for nematode feeding. The

diversity of nematode galls is observed mainly in their nutritive structures, which

may be giant polynucleated cells, syncytia, tissues with uninucleate or

polynucleate cells (Dropkin 1969; Weischer and Wyss 1976; Meyer 1987; Wyss

1997), or a meristematic nutritive tissue (Goodey 1948; Ferreira et al.

submitted; this work). Starch may sometimes accumulate in the outer

parenchyma, and form a 'common storage tissue' (CST). The differentiation of

mechanical and other specialized tissues is rare in nematode galls. However, a

neoformed collenchyma was reported in galls of *Anguina balsamophila* on

Wyethia amplexicaulis (Goodey 1948).

Galls of Eriophyidae members are less histologically complex than the

nematode and aphid galls. In addition, current results demonstrated that

eriophyid galls may have distinct complexity levels, as seen by the diversity of

storage tissues they have. The galls induced on *M. ibaguensis* may be called

'filzgalls' (*sensu* Mani 1964), for they have homogeneous cells, with little

modification from the non-galled mesophyll, and are covered with a denser

indumentum (Larew 1981). Even though an outer CST with starch is present in

both eriophyid galls studied here, a TNT with proteins occurs only in *A. tristriata*

- *Juglans regia* galls. The vacuolated epidermal cells with reducing sugars are

the feeding sites for eriophyids in *M. ibaguensis,* but they are not considered a

TNT because their cells have a non-granulose cytoplasm and the nucleus is not

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prominent. Finally, the simultaneous ontogenetic processes (homogenization, hypertrophy, hyperplasia, and differentiation of TNT) of *A. tristriata* galls characterize these galls as more complex structures than the galls of *M. ibaguensis* (with only homogenization and neoformation of emergences). Neoformation of specialized tissues, other than the CST or the TNT, is not commonly observed in eriophyid galls (*see* Kendall 1930; Larew 1981; Westphal et al. 1981; Moura et al. 2008, 2009). An exception is the galls of *Eriophyes ulmicola* on *Ulmus campestris*, the only eriophyid gall with mechanical tissue that has been studied. These galls represent the "maximum histocytological differentiation" of the group (Meyer 1987).

Storage tissues are present especially in complex galls

Storage cells with lipids, proteins, starch or reducing sugars occur widely in plants (Evert 2006), as well as in galls (Bird 1961; Bronner 1992; Rohfritsch 1992; Oliveira and Isaias 2010; Ferreira et al. 2013; Vecchi et al. 2013). Considering the accumulated metabolites, the location of the storage cells, and whether or not they are the direct feeding site of the inducers, we defined three types of storage tissues, present in galls induced by eriophyids, aphids and nematodes: the TNT, typical nutritive tissues; the CST, common storage tissues; and the NLT, nutritive-like tissues, with different levels of activity and functions (Table 3). Current proposal on terminological standardization will facilitate the direct and precise reference of storage cells in future developmental studies.

TNT are commonly induced by chewing and scraping galling insects (Kostoff and Kendall 1929; Larew 1981; Bronner 1992; Ferreira and Isaias

2013), and their cells are the direct feeding sites of the galling herbivores. These cells are adjacent to the gall chamber, and may store lipids, proteins and/or reducing sugars. The TNT cells are usually recognized by their granulose cytoplasm and prominent nucleus, and also occur in galls induced by nematodes (Goodey 1948; Bird 1961; Dropkin 1969; Ferreira et al. submitted), such as those of *D. gallaeformans*, and in complex galls induced by eriophyids (Kendall 1930; Westphal et al. 1981; Moura et al. 2009), such as those of *A. tristriata*. Eriophyid galls in *M. ibaguensis* do not differentiate a TNT, an aspect of the relative simplicity of these filzgalls.

The CST is present in the outer layers of the eriophyid and nematode galls, and its cells have peripheral cytoplasm and should mobilize starch to reducing sugars for gall structural maintenance, as is commonly true for insect galls (Oliveira et al. 2010; Ferreira and Isaias 2013, 2014). The CST is also present in galls induced by phloem-sucking insects (Álvarez et al. 2009, 2013; Oliveira and Isaias 2010; Isaias et al. 2011; Álvarez 2011; Carneiro et al. 2014).

Sometimes, the storage cells have evident cytoplasmic activity, with cytoplasm granulation and a prominent nucleus, similar to those of the TNT, as observed in hemipteran galls (Carneiro and Isaias 2014, 2015; Richardson et al. 2016). However, they are here termed the NLT (Table 3), as they are not the direct feeding sites of the gall inducers (Richardson et al. 2016). Based on current results and literature on Psylloidea (Oliveira and Isaias 2010; Oliveira et al. 2010; Carneiro and Isaias 2014, 2015) and Aphididae galls (Alvarez et al. 2009, 2013; Richardson et al. 2016), the arrangement of storage tissues in galls induced by phloem-sucking insects should be similar. The most complex galls should have a NLT near the gall chamber and surrounding vascular bundles,

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and an outer CST, as observed in *G. utricularia* galls. The simpler galls should have no storage tissues, as observed in *E. ulmi* galls, or only a CST. The differences in hyperplasia and hypertrophy rates occurring on colonial (aphid) and solitary (psylloid) galls should reflect direct responses to the number of inducers and their influence on gall growth rates, which is yet to be evaluated.

As galls are usually sinks of photoassimilates (Larson and Whitham 1991; Inbar et al. 1995; Castro et al. 2012; Fleury et al. 2015), storage cells will differentiate in galls capable of draining a large amount of photoassimilates. The degree of starch accumulation in cortical tissues of galls is related to the strength of the sinks (Jones and Payne 1977; Kellow et al. 2004), which is directly influenced by the size of the colony. We have shown here that the histologically simplest galls may lack storage tissues, such as the galls of *E. ulmi*; they are presumed to be weaker sinks, draining fewer photoassimilates than the complex galls.

Conclusions

We have demonstrated that more complex galls, namely, those with more histological, histochemical and histometric alterations, have more specialized storage tissues than simpler galls. In addition, the distinct levels of complexity are not *taxa*-related, even though eriophyid galls are usually simpler than nematode and aphid galls. Even though a wider range of histological processes is usually required during the development of aphid galls, the nematodes have a broad capability for manipulating meristematic sites. This capability culminates in more-specialized typical nutritive tissues (TNT) in galls induced by nematodes, where meristematic nutritive sites, syncytial areas, or

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giant polynucleate cells may occur, depending on the inducing species.

Independently of the galling *taxa*, the data from the literature refer to similar

storage tissues with different names, and to different storage tissues with similar

names. We revisited this nomenclature and proposed the current

standardization. Accordingly, the storage tissues in galls may be divided into

typical nutritive tissues (TNT), common storage tissues (CST), and nutritive-like

tissues (NLT). Galls with TNT and NLT are more complex than those where

only CST or no storage tissues occur.

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Tables

Table 1. Galls studied.

Gall inducer	Group	Host plant	Sampling area
Eriosoma ulmi	Aphididae	*Ulmus minor*	León, Spain
Geoica utricularia	Aphididae	*Pistacia terebinthus*	León, Spain
Ditylenchus gallaeformans	Nematoda	*Miconia albicans*	Belo Horizonte, Brazil
Ditylenchus gallaeformans	Nematoda	*Miconia ibaguensis*	Belo Horizonte, Brazil
Unidentified	Eriophyidae	*Miconia ibaguensis*	Belo Horizonte, Brazil
Aceria tristriata	Eriophyidae	*Juglans regia*	León, Spain

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Table 2. Histometry and histochemistry in controls and aphid galls.

	E. ulmi - U. minor		G. utricularia - P. terebinthus	
	Control	Gall	Control	Gall
Wall thickness	196.74 b	447.33 a	414.29 b	660.10 a
Adaxial epidermis				
Cell area	408.45 b	1086.43 a	207.99 b	330.16 a
Parenchyma				
Cell layers	5.84 b	7.35 a	14.20 b	26.28 a
Cell area				
Adaxial	441.99 b	3223.23 a	288.68 b	485.99 a
Abaxial	223.71 b		234.99 b	659.40 a
Starch	+	-	+	++
Reducing sugars	+	+	+	++
Proteins	+++	+	+	+
Oil	-	-	+	-
Phloem				
Main bundle	49.10 b	86.29 a	169.44 a	97.76 b
Supernumerary bundle	NA	NA	32.09 b	77.64 a
Xylem				
Main bundle	27.70 b	59.09 a	92.22 a	33.11 b
Supernumerary bundle	NA	NA	20.11 a	30.60 a
Abaxial Epidermis				
Cell area	260.79 b	1377.98 a	127.64 a	156.12 a

The comparisons were performed between the control and the respective gall.

Data followed by different letters are statistically different. Units: wall thickness

(μm); cell area (μm^2); cell layers (number). Histochemistry results: (-) absence;

(+), (++), (+++): semi-quantitative levels of presence. NA = not applicable.

Table 3. Classification of storage tissues in the galls studied, based on reserves, cytological features and whether it is a feeding tissue.

| | Aphididae | | | | *Ditylenchus gallaeformans* | | | | Eriophyidae | | | |
| | *E. ulmi* | | *G. utricularia* | | on *M. albicans* | | on *M. ibaguensis* | | on *M. ibaguensis* | | *Aceria tristriata* | |
	End	Mph	OP	IP	OP	IP	OP	IP	Mph	AbE	OP	IP
Reserves												
Reducing sugars	++	-	+	++	-	+++	-	+++	-	++	+	+
Starch	-	-	++	++	++	-	++	-	+	-	+	-
Proteins	-	-	-	+	-	++	-	++	+	+	-	+++
Oil	-	-	-	-	-	-	-	-	+	-	-	++
Cytological features												
Cytoplasm granulation	-	-	-	+	-	+	-	+	-	-	-	+
Prominent nucleus	-	-	-	+	-	+	-	+	-	-	-	+
Feeding tissue	No	No	No	No	No	Yes	No	Yes	No	Yes	No	Yes
Storage tissue type	-	-	CST	NLT	CST	TNT	CST	TNT	CST	-	CST	TNT

AbE: abaxial epidermis; CST: common storage tissue; End: endodermis; IP: inner parenchyma; Mph: mesophyll; NLT: nutritive-like tissue; OP: outer parenchyma; TNT: typical nutritive tissue. Histochemistry results: (-) absent; (+), (++); (+++): semi-quantitative levels of presence. Cytological features: (-) absent; (+) present.

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Table 4. Histometry and histochemistry in controls and nematode galls.

	D. gallaeformans - M. albicans		D. gallaeformans - M. ibaguensis	
	Control	Gall	Control	Gall
Wall thickness	143.25 b	302.12 a	174.09 b	409.65 a
Adaxial/outer epidermis				
Cell area	604.65 a	148.54 b	237.82 a	168.11 b
Trichomes	-	+++	-	+++
Parenchyma				
Cell layers	5.67 b	15.39 a	8.20 b	20.2 a
Cell area				
Adaxial	701.50 a	528.67 a	960.71 a	883.92 a
Abaxial	139.61 b	84.78 b	312.81 b	128.13 c
Starch	++	+	++	+
Reducing sugars	+	+++	+	+++
Proteins	+	+	+	+
Oil	+	-	+	-
Abaxial/nutritive epidermis				
Cell area	92.37 a	63.92 b	151.77 a	113.83 b
Trichomes	++	-	++	-

The comparisons were performed between the control and the respective gall.

Data followed by different letters are statistically different. Units: wall thickness

(μm); cell area (μm^2); cell layers (number). Histochemistry results: (-) absence;

(+), (++); (+++): semi-quantitative levels of presence.

Table 5. Histometry and histochemistry in controls and eriophyid galls.

| | Eriophyidae - *M. ibaguensis* | | *A. tristriata - J. regia* | |
	Control	Gall	Control	Gall
Wall thickness	174.09 a	154.63 a	127.42 b	782.06 a
Adaxial epidermis				
Cell area	237.82 a	328.17 a	241.07 b	393.90 a
Parenchyma				
Cell layers	8.20 a	7.60 a	5.80 b	18.70 a
Cell area				
Adaxial	960.71 a	319.02 b	197.75 b	1836.16 a
Abaxial	312.81 a	351.48 a	136.56 b	1720.31 a
Starch	++	+	++	+
Reducing sugars	+	++	+	+
Proteins	+	+	+	++
Oil	+	+	+	+
Abaxial epidermis				
Cell area	151.77 a	211.53 a	162.09 b	348.53 a
Emergences	+	+++	-	-

The comparisons were performed between the control and the respective gall.

Data followed by different letters are statistically different. Units: wall thickness

(μm); cell area ($μm^2$); cell layers (number). Histochemistry results: (-) absence;

(+), (++); (+++): semi-quantitative levels of presence.

Figure captions

Figure 1. Non-galled leaf and leaf-rolling gall of *Eriosoma ulmi* on *Ulmus minor.*
(A) Leaf-rolling gall, macroscopic view. (B) Transverse section of non-galled
leaf. (C-E) Leaf-rolling gall, transverse sections. (C) Region similar to non-galled
mesophyll. (D) Folding region, with homogenization of parenchyma,
hyperplasia, cell hypertrophy, and hypertrophy of xylem and phloem. (E)
Reducing sugars accumulated mainly in the area of vascular tissues (dotted
line, brown precipitates) and endodermis (arrow). (F) Low accumulation of
proteins in galls. (G) Accumulation of proteins in controls. Abbreviations: Ar:
aerenchyma. En: endodermis. GC: gall chamber. HP: homogeneous
parenchyma. NGL: non-galled leaf. Ph: phloem. PP: palisade parenchyma. Tr:
trichome. VB: vascular bundle. Xy: xylem. Bars: (A) 5 mm. (B-G) 100 µm.
Staining: safranin and fast green (B-D); Fehling's reagent (E); Ponceau 2R (F-
G).

Figure 2. Non-galled leaflet midrib and gall of *Geoica utricularia* on *Pistacia
terebinthus.* (A, C-D) Globoid gall associated with midrib. (B-D) Transverse
sections. (B) Midrib of non-galled leaflet (NGL), with main and supernumerary
(arrow) vascular bundles. (C) Gall with main (MB) and supernumerary vascular
bundles (SB), homogeneous parenchyma differentiated into inner (IP) and outer
(OP) regions. Small holes (arrows) in the inner epidermis. Details of the main
vascular bundles is shown in the box. (D) Reducing sugars accumulate
abundantly in storage tissues (arrows). (E) Inner region of gall, with Nutritive-
Like (NL) cells, with starch accumulation. Small holes in inner epidermis
(arrow). Detail of the supernumerary vascular bundle (SB). Abbreviations: GC:
gall chamber. Ph: phloem. SD: secretory duct. Xy: xylem. Bars: (A) 1 cm. (B-C)

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100 μm. (D) 200 μm. (E) 50 μm. Staining: safranin-fast green (B-C, E); Fehling's reagent (D).

Figure 3. Galls of *Ditylenchus gallaeformans* on *Miconia albicans.* (A-B) Globoid galls induced on abaxial surface of *M. albicans.* (A) Older verrucous gall (arrow). (B) Younger gall. (C) Gall with overlapped curved emergences (arrow). (D) Transition zone between non-galled leaf with palisade and spongy parenchyma, and gall with homogeneous parenchyma and inner regions with nutritive tissue and young emergences. (E) Details of emergences and nutritive tissue of galls. The outer parenchyma has polyhedral cells and some idioblasts with druses (arrows). (F-G) Differences among cells of nutritive tissue, with denser cytoplasm and prominent nucleus, and outer parenchyma, with larger and vacuolated cells. Abbreviations: Cy: cytoplasm. HP: homogeneous parenchyma. LN: leafy neoformation. NGL: non-galled portion of leaf. Nm: nematodes. NT: nutritive tissue. Nu: nucleus. OP: outer parenchyma. PP: palisade parenchyma. SP: spongy parenchyma. Tr: arachnoid trichomes. Vc: vacuole. VT: vascular tissue. YE: young emergences. Bars: (A) 5 cm. (B) 5 mm. (C) 500 μm. (D) 100 μm. (E) 50 μm. (F) 20 μm. (G) 10 μm. Staining: safranin-astra blue (C-E); toluidine blue (F-G).

Figure 4. Galls of *Ditylenchus gallaeformans* on *Miconia albicans.* Transverse sections*.* (A) Polarized light image, showing lignified trichomes and idioblasts with druses (arrow) in gall parenchyma*.* (B) Starch grains in outer parenchyma (arrows), but absent in the adjacent nutritive tissue. (C) Greater accumulation of reducing sugars in nutritive tissue, vascular bundles and young emergences of galls in comparison with the non-galled region of the leaf. Abbreviations: GC: gall chamber. NGL: non-galled region of leaf. Nm: nematodes. NT: nutritive

tissue. OP: outer parenchyma. Tr: arachnoid trichomes. VB: vascular bundle.

YE: young emergences. Bars: (A) 200 µm. (B) 50 µm. (C) 500 µm. Staining:

safranin-astra blue (A); Lugol (B); Fehling's reagent (C).

Figure 5. Galls of *Ditylenchus gallaeformans* on *Miconia ibaguensis.* (A)

Globoid verrucous galls (white arrow) induced on adaxial surface*,* with leafy

neoformations (black arrow). (B-F) Transverse sections. (B) Gall with curved

vascularized emergences (arrows) and leafy neoformation. (C) Details of

mature and young emergences in a gall. (D) Detail of a mature emergence, with

meristematic nutritive tissue in contact with nematodes, and collateral vascular

bundle. (E) Proteins in nutritive tissues (arrows) and vascular bundles. (F)

Greater accumulation of reducing sugars in nutritive tissue, vascular bundles

and young emergences of galls. Abbreviations: GC: gall chamber. NGL: non-

galled portion of leaf. NL: neoformed leaf. Nm: nematodes. NT: nutritive tissue.

OP: outer parenchyma. Ph: phloem. PP: palisade parenchyma. SP: spongy

parenchyma. Tr: stellate trichomes. VB: vascular bundle. YE: young

emergences. Xy: xylem. Bars: (A) 5 mm. (B) 500 µm. (C, E, F) 200 µm. (D) 50

µm. Staining: safranin-astra blue (B-D); Ponceau 2R (E), and Fehling's reagent

(F).

Figure 6. Gall of Eriophyidae on *Miconia ibaguensis.* (A-C) Blister-shaped galls,

with overdifferentiated indumentum in abaxial region (C). (D-I) Transverse

sections of galls. (D) Blister gall in continuity with non-galled leaf region (NGL).

Several neoformed emergences are visible in abaxial region. (E-F) Gall with

homogeneous parenchyma, neoformed emergences, and epidermis, where the

eriophyids feed. (G) Polarized light image showing druses (arrows), but no

lignified tissue. (H) Reducing sugars in epidermis and emergences. (I) Starch

(arrows) in homogeneous gall parenchyma. Abbreviations: Em: emergences.

Er: eriophyid. Ep: epidermis. GC: gall chamber. HP: homogeneous

parenchyma. NGL: leaf non-galled region. NT: nutritive tissue. OP: outer

parenchyma. Bars: (A) 1 cm. (B) 4 mm. (C) 2 mm. (D) 500 μm. (E, H, I) 50 μm.

(F) 25 μm. (G) 200 μm. Staining: astra blue-safranin (D, G); toluidine blue (E-F);

Fehling's reaction (H); Lugol (I).

Figure 7. Gall of *Aceria tristriata* (Eriophyidae) on *Juglans regia.* (A, C-G)

Transverse sections of lenticular gall. (A) Gall with hypertrophied homogeneous

parenchyma compared with non-galled leaf regions. The mites are present in

the cavities (arrows). (B) Lenticular galls (arrow) on abaxial surface. (C)

Chamber (dotted line) of the eriophyids, surrounded by a nutritive tissue. (D)

Nutritive cells are perforated by eriophyids and appear disrupted (black arrow).

Adjacent cells become nutritive (white arrows), with prominent nucleus and

granulose cytoplasm. (E) Protein granules (arrowheads) detected in TNT, near

the eriophyids. (F) Typical nutritive tissue. (G) Hypertrophied and vacuolated

outer cell, with smaller nucleus. Abbreviations: Cy: cytoplasm. Er: eriophyids.

HP: homogeneous parenchyma. NGL: leaf non-galled region. Nu: nucleus. PP:

palisade parenchyma. SP: spongy parenchyma. TNT: typical nutritive tissue.

Vc: vacuole. VB: vascular bundle. Bars: (A) 200 μm. (B) 5 mm. (C, E) 100 μm.

(D) 50 μm. (F, G) 10 μm. Staining: safranin-fast green (A, C-D, F-G); Ponceau
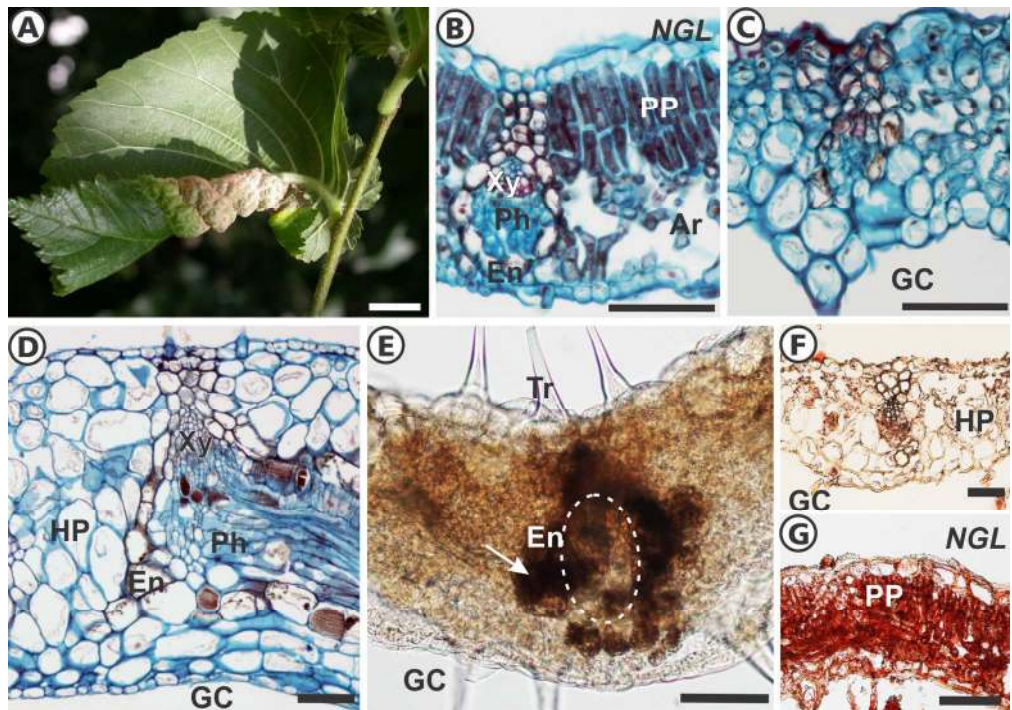
2R (E).
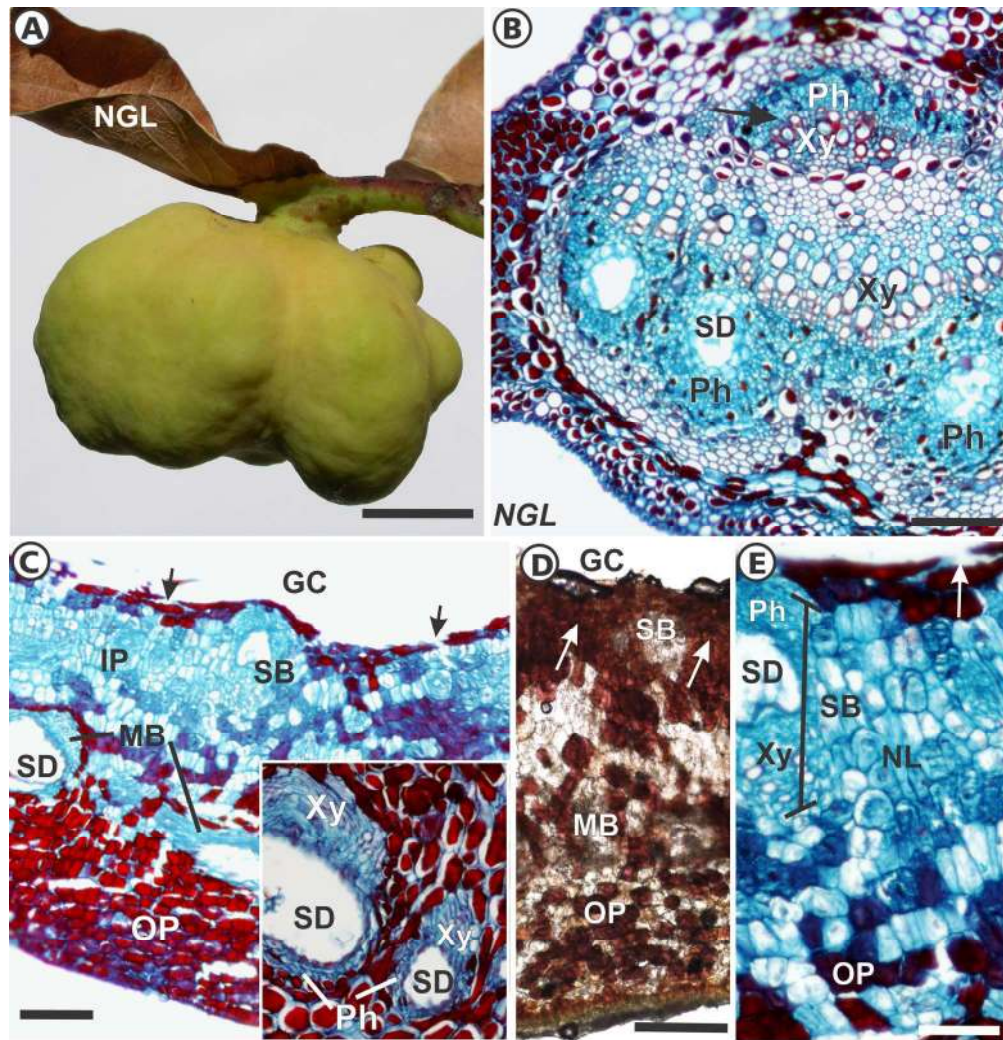
Fig. 1
Fig. 1
170x119mm (300 x 300 DPI)
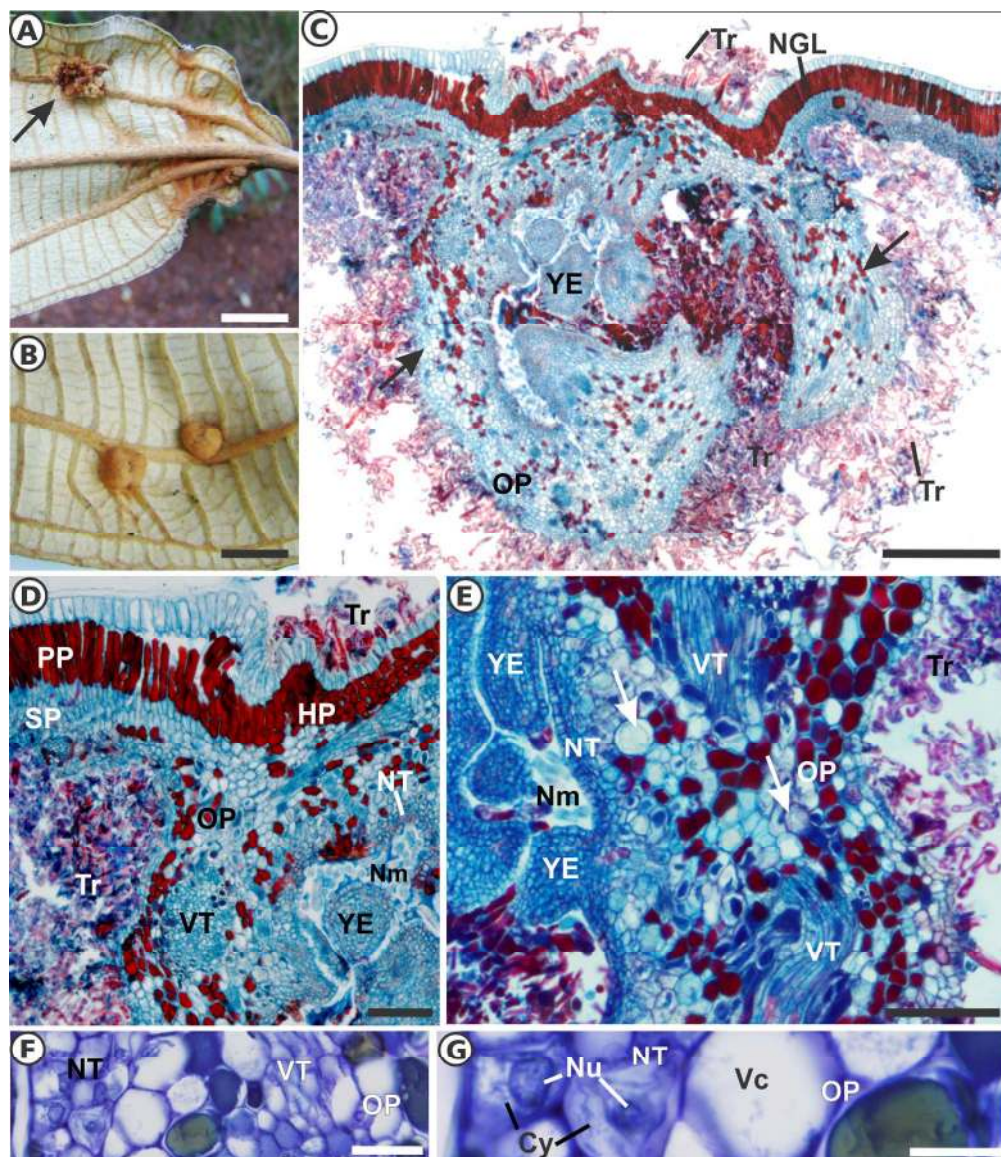
Fig. 2
Fig. 2
163x168mm (300 x 300 DPI)

Fig. 3
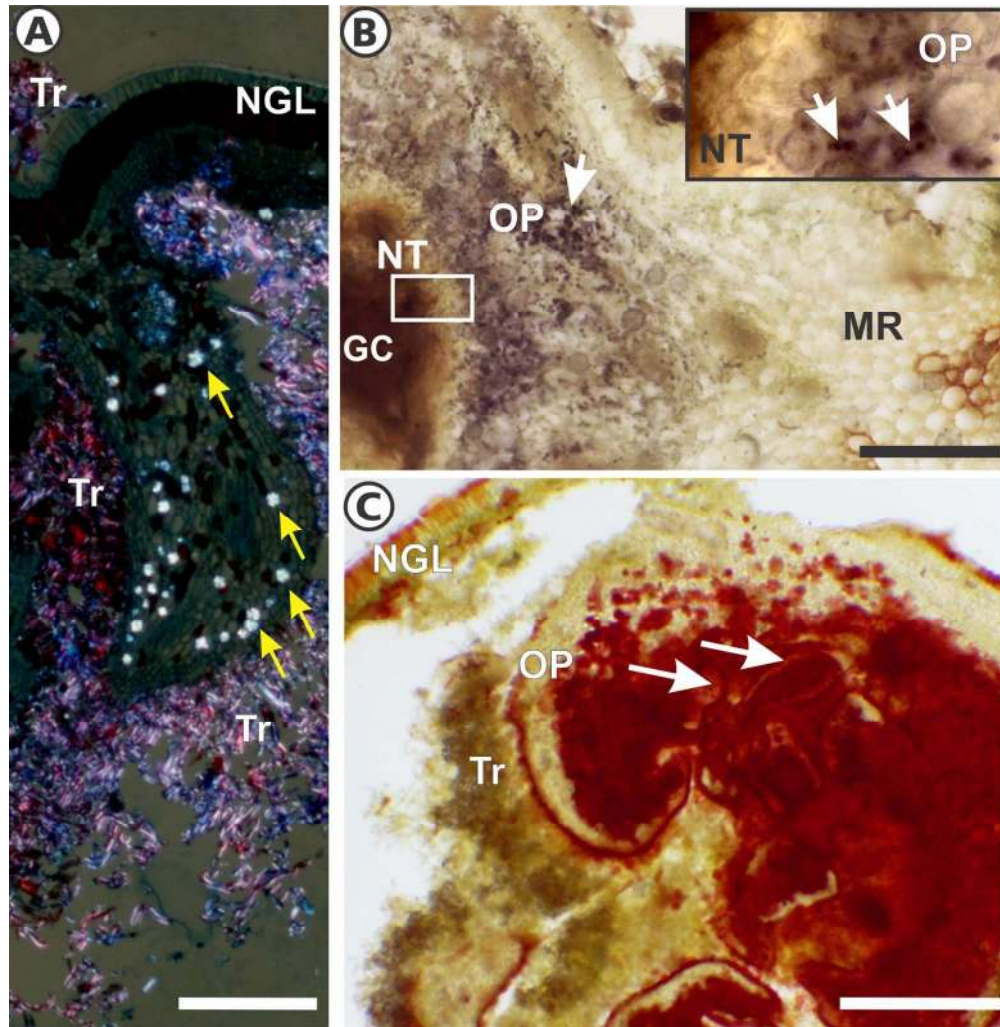Fig. 3
178x205mm (300 x 300 DPI)
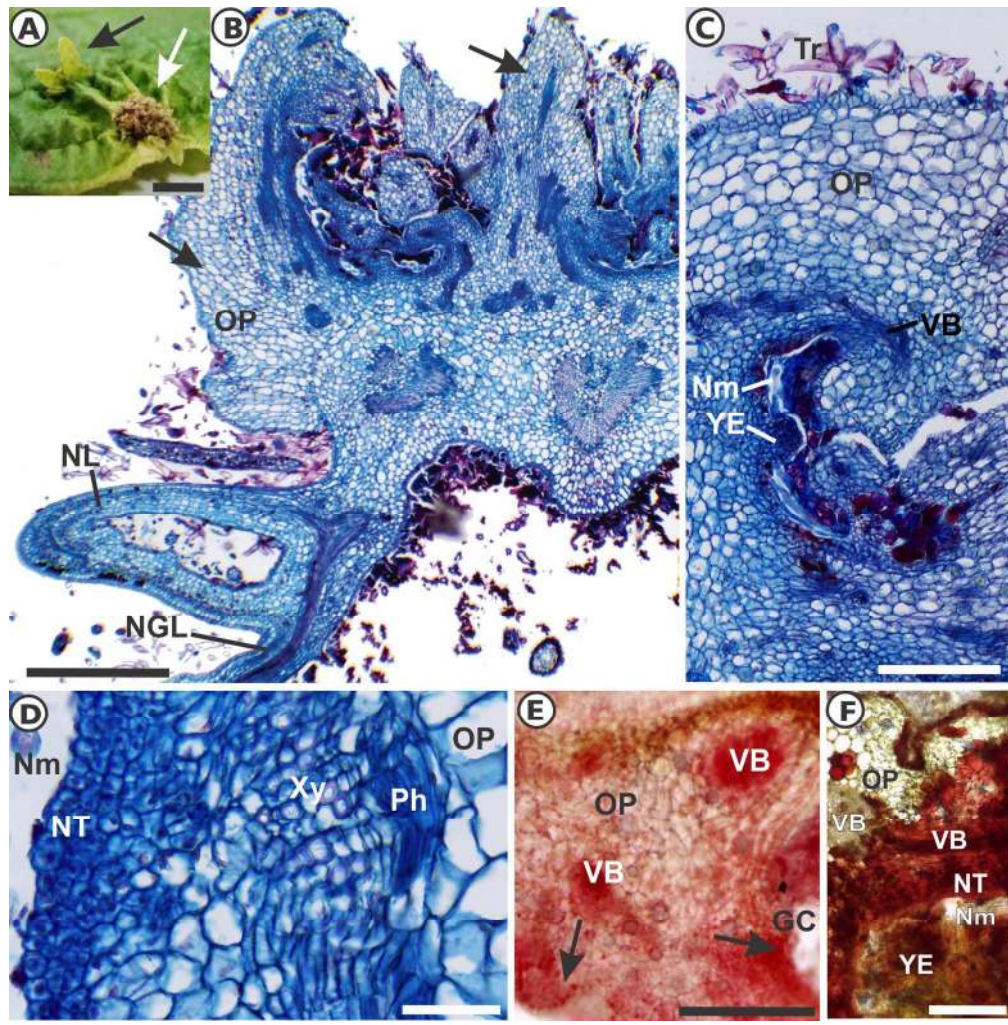
Fig. 4
Fig. 4
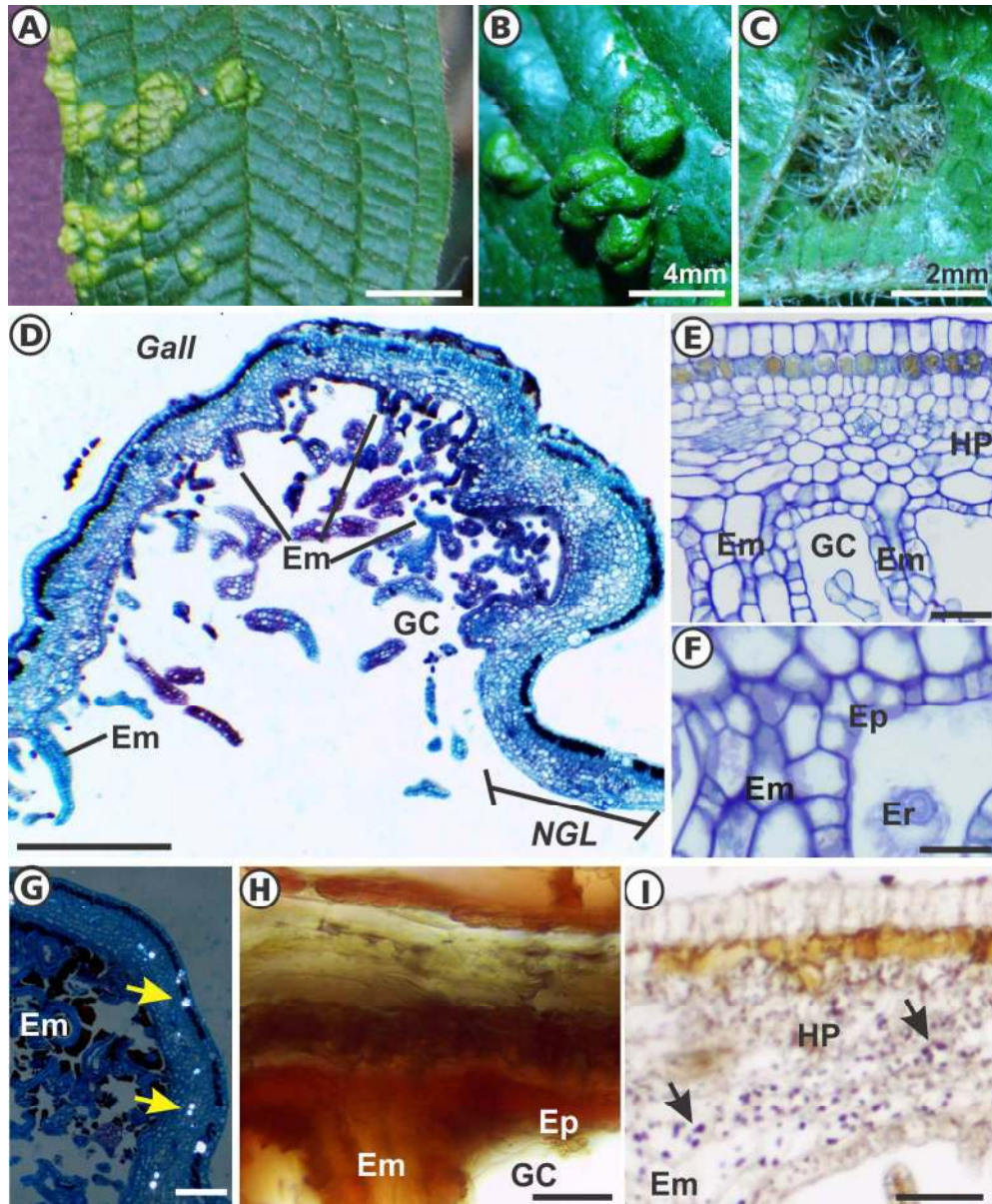126x129mm (300 x 300 DPI)
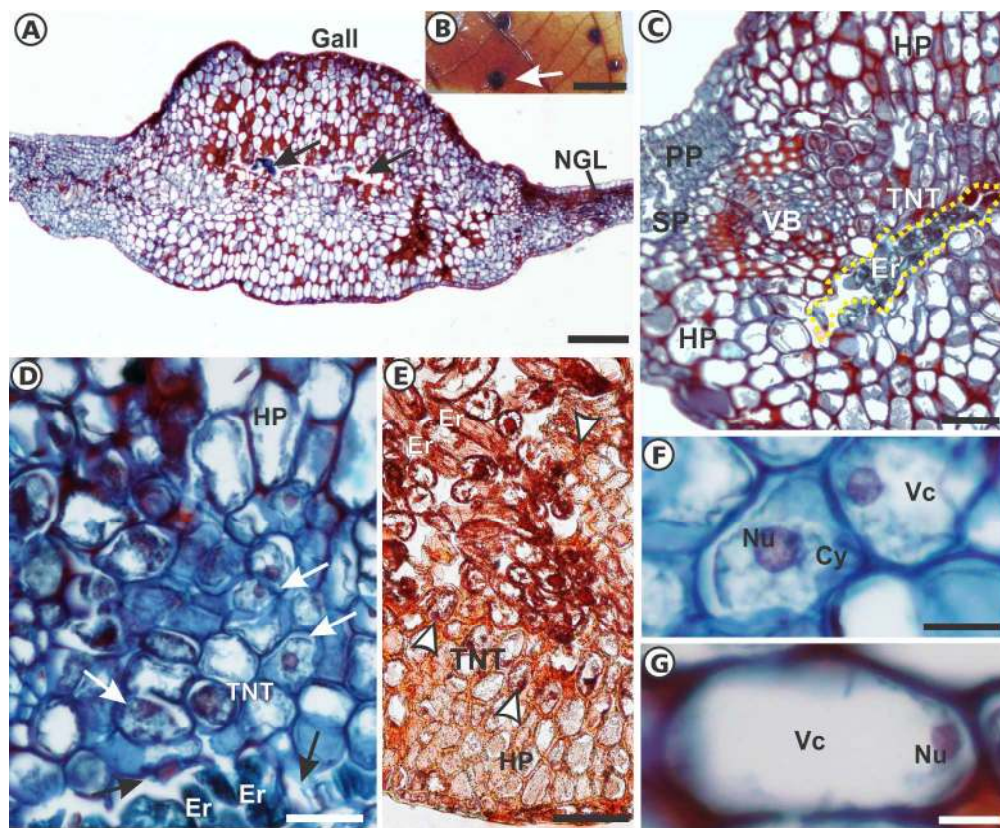
Fig. 5
Fig. 5
166x168mm (300 x 300 DPI)

Fig. 6
Fig. 6
154x187mm (300 x 300 DPI)

Fig. 7
Fig. 7
197x161mm (300 x 300 DPI)