UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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Phabliny Martins Silva Bomfim

Uberlândia – MG
Fevereiro/2020

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Orientador
Prof. Dr. Denis Coelho de Oliveira

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Resumo

Bomfim, Phabliny M. S. 2020. Ovule gall stimulating a big fake fruit on *Miconia chamissois* Naudin (Melastomataceae): a structural overview. Dissertação de Mestrado em Ecologia e Conservação de Recursos Naturais. UFU. Uberlândia-MG. 36p.

Galhas são uma relação extremamente íntima entre plantas e um outro organismo, o galhador. Esse organismo é capaz de fazer diversas alterações morfológicas no órgão induzido. O galhador pode prejudicar a planta da forma mais comum que é realocando substâncias para seu próprio benefício ou pode também impactar a reprodução da planta. Para afetar diretamente a reprodução da planta hospedeira é necessário que a galha ocorra em um órgão reprodutivo e acabe evitando ou reduzindo a dispersão de sementes. Nosso principal objetivo foi avaliar se o galhador *Allorhogas uberlandiensis* afeta negativamente as taxas reprodutivas em botões florais galhados na planta *Miconia chamissois*. Os novos tecidos formados na galha também foram observados e comparados com seus tecidos originais, além de verificar quais compostos foram acumulados neles. O volume do "fruit like gall" (toda a estrutura induzida pelo galhador) é em média 5,4 vezes maior que os frutos maduros (sendo a maior estrutura reprodutiva da planta hospedeira) e galhas são em média 20 vezes maiores que os óvulos. Na galha são formados um tecido nutritivo típico e um tecido reserva. Ocorre o acúmulo de substâncias como lipídeos, proteínas e pectinas. Com a análise anatômica é possível observar que a indução da galha acaba criando uma barreira física que impede que as estruturas reprodutivas se conectem ao exterior, o que é essencial para a fertilização. Assim, é possível inferir que 100% dos óvulos presentes em um botão floral galhado se tornam inviáveis. Como o galhador tem um impacto significativo nos botões florais, reduzindo a taxa de reprodução da planta hospedeira, nesse sistema o galhador pode ser considerado uma praga ou um agente de controle biológico, de acordo com o status da *Miconia chamissois* no local (como planta nativa ou invasora).

Keywords: Himenóptero, Planta anatomia, Histoquímica, Tecido nutritivo

Abstract

Bomfim, Phabliny M. S. 2020. Ovule gall stimulating a big fake fruit on *Miconia chamissois* Naudin (Melastomataceae): a structural overview. Dissertação de Mestrado em Ecologia e Conservação de Recursos Naturais. UFU. Uberlândia-MG. 36p.

Galls are an extremely close relationship between plants and some other organism, the galler. This organism is capable of induced a lot of morphological changes in the induced organ. The galler can bring harm to the plant in the common way that is to relocating substances of the plant for their benefit or may impact the reproduction of the plant too. For to directly affect the reproduction of the host plant it is necessary for the gall to occur in a reproductive organ and end up preventing or reducing the seed dispersal. Our main objective was to evaluate if the galler *Allorhogas uberlandiensis* negatively affects reproductive rate in a floral bud galled in the plant *Miconia chamissois*. The news tissues formed by gall induction were also observed in comparison with their original tissues, in addition to the compounds accumulated in them. The "fruit-like gall" (whole structure induced by galler) volume is on average 5.4 times larger than mature fruits (these being the largest reproductive structure of the host plant) and galls are on average 20 times larger than the ovules. In the gall a typical nutritive tissue and a reserve tissue are formed. Occurs the accumulation of lipids, proteins and pectins. With the anatomical analysis it is possible to observe that the induction of gall ends up creating a physical barrier that obstructs the reproductive structures from connecting to the outside, which is essential for fertilization to occur. Thus, it is possible to infer that 100% of the ovule present in a galled floral bud become unviable. As the galler has a significant impact on floral buds reducing the reproduction rate of the host plant, in this system the galler can be considered a pest or a biological control agent according with the status of *Miconia chamissois* in the location (as a native or invasive plant).

Keywords: Hymenoptera, Plant anatomy, Histochemistry, Nutritive tissue

INTRODUÇÃO GERAL

Dentre as interações mais específicas entre insetos e plantas destacam-se aquelas que levam a formação de galhas (Mani 1964; Shorthouse & Rohfritsch 1992). Estes insetos são capazes de manipular os tecidos vivos da planta hospedeira e construir seu próprio habitat, as galhas (Oliveira *et al*. 2016). As galhas oferecem aos galhadores enormes vantagens em relação aos seus ancestrais de vida livre como abrigo, proteção contra as intempéries do ambiente e inimigos naturais, além de recursos alimentares (Price 1992). Esses insetos apresentam aparelho bucal associado aos seus hábitos alimentares específicos, que se enquadram primordialmente em guildas de mastigadores, raspadores e sugadores (Oliveira *et al*. 2016). Independente do hábito alimentar, organismos galhadores promovem alterações estruturais e metabólicas nos tecidos da hospedeira (Oliveira *et al*. 2006; Moura *et al*. 2008; Oliveira *et al*. 2017) relacionadas diretamente ao estabelecimento de um estresse biótico (Oliveira *et al*. 2016). Estas alterações estruturais e metabólicas interagem não só com o organismo galhador, acabam interagindo também com a comunidade de organismos ao redor da galha (Barônio & Oliveira 2019), especialmente com inimigos naturais associados ao sistema galhador-planta hospedeira (Rezende *et al*. 2019). Raízes, caules, folhas e flores podem ser usados como órgãos hospedeiros para desenvolvimento de galhas. Quando a oviposição e desenvolvimento de galhas ocorre em estruturas reprodutivas (botão floral, flor e fruto) ocorre uma perda imediata do investimento da hospedeira em reprodução, uma vez que a galha em geral pode impedir a fecundação e/ou a formação de frutos e sementes viáveis (Mani 1964; Badenes-Perez & Johnson 2007; Chavarría *et al*. 2009).

Galhas em um contexto de controle biológico

Em interações inseto-planta em que o inseto é fitófago, estes muitas vezes são vistos apenas como pragas de plantas, principalmente quando a planta em questão apresenta um interesse econômico. Deste modo ocorre um direcionamento dos estudos para que esses insetos sejam combatidos de forma a se obter uma produção vegetal mais rentável. Um bom exemplo dos impactos que um inseto pode causar em uma espécie vegetal é o caso da vespa-da-madeira *Sirex noctilio* (Hymenoptera: Siricidae), considerada uma praga por causar um grande prejuízo econômico em plantios de *Pinus* spp. (Pinaceae). Com o intuito de monitorar e controlar a espécie *S. noctilio* foi criado no Brasil o Programa Nacional de Controle à Vespa- da- Madeira, assim foram tomadas medidas para controle biológico usando o nematóide *Deladenus siricidicola* que atua

deixando fêmeas da vespa-da-madeira estéreis e ovos do parasitóide *Ibalia leucospoides* (Hymenoptera: Ilbaliidae), além do uso dos ectoparasitas de larvas maduras *Megarhyssa nortoni* e *Rhyssa persuasoria* (Hymenoptera, Ichneumonidae) (Iede *et al*. 1998; Iede & Zanetti 2007). É possível notar que diferentes espécies de himenópteros possuem papéis discrepantes quanto aos seus efeitos em cultivos, pois podem ser considerados pragas de plantas ou atuam no controle ambiental reduzindo populações de insetos considerados pragas.

Dentro do gênero *Allorhogas* (Hymenoptera: Braconidae) existem espécies que são consideradas como agentes de controle biológico, como no caso das espécies fitófagas que se alimentam frutos/sementes. Quando os frutos de *Conostegia xalapensis* (Melastomataceae) estão infestados por *Allorhogas conostegia* as sementes passam a ser 75% menores e com aparência menos saudável do que as presentes em fruto não infestados (Chavarría *et al*. 2009). *Miconia calvescens* (Melastomataceae), quando tem os frutos infestados por *Allorhogas granivorus* apresentam frutos maiores (20%) e com menos sementes (79%) do que quando os frutos não são infestados, com consumo das sementes consequentemente o inseto acaba reduzindo a propagação desta espécie de planta (Badenes-Perez & Johnson 2007; Zaldívar-Riverón *et al*. 2018) . Neste último caso o inseto é considerado pelos autores como tendo uma importância maior regulando as populações de *M. calvescens*, uma vez que esta espécie tem um potencial como invasora maior do que a *C. xalapensis* (Chavarría *et al*. 2009).

Em uma das estratégias mais eficientes de utilização dos vegetais pelos herbívoros, ocorre a formação de galhas (Roskam 1992; Shorthouse & Rohfritsch 1992; Shorthouse *et al*. 2005) e assim, os insetos capazes de induzir estas estruturas poderiam controlar a população de suas hospedeiras. Neste contexto, galhas que são induzidas em estruturas reprodutivas em plantas poderiam impactar o fitness destas hospedeiras e controlar populações de espécies vegetais, como é o caso da *Miconia chamissois* (Melastomataceae). Esta espécie apresenta galhas induzidas por *Allorhogas uberlanidiensis*, sendo as galhas induzidas em óvulos dentro de botões florais. A indução destas galhas simularia o desenvolvimento de uma semente e a formação de uma estrutura semelhante a um fruto, chamada de "fruit-like gall" (Joele *et al*. 2019). Com a indução da galha cria-se uma barreira que evita a fecundação dos os óvulos o que poderia impedir a formação de sementes, e assim este galhador apresentaria um grande potencial como agente de controle biológico ou como uma praga dependendo do potencial invasor da planta hospedeira no local. Neste sistema a galha propriamente dita

é apenas uma parte de toda uma estrutura formada pelo estímulo do galhador, sendo que cada óvulo que recebe esse estímulo se torna uma galha e o ovário em decorrência do mesmo estímulo acaba se desenvolve formando o "fruit-like gall". Sendo daqui para frente chamados de fruit-like gall toda a estrutura que é induzida pelo galhador, já a galha passa a ser a parte específica que vai do galhador que se encontra na câmara larval e todos os tecidos ao seu redor que induzidos com o propósito de alimentação (tecido nutritivo e de reserva).

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Ovule gall stimulating a big fake fruit on *Miconia chamissois* Naudin (Melastomataceae): a structural overview

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Abstract

The galling insects manipulates the host plant tissues to get access to shelter and protection directly or indirectly against natural enemies and abiotic factors, as well as improve your diet. When the galls are induced in the reproductive host organs may compromise the host plant reproduction. *Allorhogas uberlandiensis* (Hymenoptera) induce galls on reproductive organ of *Miconia chamissois* (Melastomataceae). The galls are induced in the ovules before the fertilization, leading to ovary growth and developing a "fruit-like gall". Herein, we look for evaluate the morphological, histological and cytological changes caused by *A. uberlandensis* on the reproductive host tissues of *M. chamissois*. The "fruit-like gall" (whole structure induced by galling organism) volume is on average 5.4 times larger than mature fruits (these being the largest reproductive structure of the host plant) and ovule-galls are on average 20 times larger than the ovules. There is a formation of a typical nutritive tissue in the ovule-gall that accumulates lipids, proteins and pectins. It is possible to observe that the induction of gall ends up creating a physical barrier that obstructs the reproductive structures from connecting to the outside, which is essential to fertilization processes. Thus, it is possible to infer that 100% of the ovule present in a galled floral bud become unviable. As the galling organism has a significant impact on floral buds, there are a reducing of the reproduction rate of the host plant. Thus, *A. uberlandensis* can be considered a pest or a biological control agent according with the status of *Miconia chamissois* in the location (as a native or invasive plant).

Keywords: Hymenoptera, Plant anatomy, Histochemistry, Nutritive tissue

Introduction

Galls are a very intimate relationship between the host plant and the parasite organism. These structures develop through specific stimulus of the galling organism, leading to host tissue modification and gall structure formation on both vegetative and reproductive plant organs (Shorthouse & Rohfritsch 1992; Shorthouse *et al*. 2005, Oliveira *et al*. 2016). The most representative taxa of galling organism in nature are the insects (Espirito-Santo & Fernandes 2007). It can manipulate the host plant phenotype to build the gall (Martini *et al*. 2019) in a most efficient strategy for the plant tissues utilization by herbivores (Roskam 1992). So, the galling insects manipulates the host plant tissues (Oliveira *et al.* 2016) to get access to shelter and protection directly or indirectly against natural enemies and abiotic factors (Price *et al*. 1986; Rohfritsch & Anthony 1992). The success of the interaction and gall structure development is dependent on the continuous chemical or mechanical stimulus of the galling organism (Mani 1964; Meyer & Maresquelle 1983; Bronner 1992; Rezende *et al*. 2019). In addition to shelter and protection, the gall structure provides resources for galling insect feed (Stone & Schonrogge 2003). These nutritional sources are produced in a redifferentiated nutritive tissue induced by different taxa of galling organisms (Bronner 1992; Ferreira *et al*. 2017). Thus, the induction and the cytological and histochemical profile of the nutritive tissue seems to depend, firstly, on the taxa of the galling organism (Bronner 1992).

According with the feeding behavior, the genus *Allorhogas* (Hymenoptera) can be diversified once seems to have different feeding habits including phytophagous predating (Zaldiver-Riveron *et al*. 2018), gall inducers (Marsh *et al.* 2000, Macêdo *et al.* 1998), as well as inquilines of other galls (Zaldiver-Riveron *et al*. 2018) and parasitoids (Martínez & Zaldívar-Riverón 2013). Some *Allorhogas* sp. induce galls in *Miconia* spp. (Melastomataceae) (Macedo *et al*. 1998; Chavarría *et al*. 2009; Centrella & Shaw 2010; Zaldívar-Riverón *et al*. 2018), while other species of *Allorhogas* are described like a possible galler in seeds of Fabaceae (Marsh *et al*. 2000; Marsh 2002) and Melastomataceae fruits (Chavarría *et al*. 2009; Zaldívar-Riverón *et al*. 2018). The galls induced by *Allorhogas* can reduce the fruit and/or seed viability, as has been observed in Melastomataceae. In this context, galls induced in reproductive organs of plants could to impact the host's fitness and control the plant population. On this way the galling organism could act like an agent of biological control when the plant is considered as

invasive (Macêdo & Monteiro 1989; Marsh *et al*. 2000; Badenes-Perez & Johnson 2007; Chavarría *et al*. 2009; Zaldívar-Riverón *et al*. 2018) or a pest in native or with economic interest plants.

Miconia chamissois (Melastomataceae galls are induced by *Allorhogas uberlandiensis*. The galls are induced in ovules of *M. chamissois* before the fertilization, leading to ovary growth and developing a "fruit-like gall" with shape and color distinct from the fruit (Joele *et al*. 2019). Herein, we look for evaluate the morphological, histological and cytological changes caused by *A. uberlandensis* on the reproductive host tissues of *M. chamissois*. Thereby we ask (I) how different is the morphology of fruit-like galls and the fruit, (II) how much the fruit-like structure and ovule-gall are different in size from fruit and ovule of *M. chamissois*, (III) if there is a formation of a true nutritive tissue in the ovule-gall and (IV) what kind of chemical compounds are allocated in each gall compartment.

Material and Methods

Study site and gall system

The present study was carried in two places. Clube Caça e Pesca Itororó (18º30' – 19º30' S a 47º50' – 48º50' W) localized at 8 km west from the center of Uberlândia municipality (Lima *et al*. 1989) and in the Parque do Sabiá, a urban park in Uberlândia (18°54'52"S and 48°14'02" W). *Miconia chamissois* (Melastomataceae) is a type of bush reaching up to 3 meters in height, has small and round fruits like berry arranged in infructescence, which invests more in number of seeds than in nutritional reserves (Snow 1965). *Allorhogas uberlandiensis* (Hymenoptera) induce galls on reproductive organ of *M. chamissois* (Joele *et al*. 2019). This gall is induced on the ovule of floral buds and its development stimulates the formation of a structure similar to the fruit, the fruit-like gall (Joele *et al*. 2019).

Morphometric analysis

In the field were randomly selected 30 samples of each, being each taken from a different host plant. The height and width of fruit-like galls, fruits, ovule-galls and seeds. Were measure using a digital caliper (Digimess®). The volume was estimated using the formula who has a better adequation with the samples' shape. For both categories was used the ellipsoid formula volume (4/3)* × pi × r1 × r2 × r3, where r1 is the height, r2 is the width and r2 = r3.

To compare the data was used a t-test between the two categories, big structures (fruit-like gall and fruit) and small structures (ovule-gall and seed). The normality distribution and homogeneity of variance were made using Shapiro test and graphics observation. Based that, all the data were transformed to logarithm, in agreement with the premises. The statistical analysis was made in the R environment version 3.4.1 (R Core Team 2018).

Anatomic analysis

Young and mature fruit-like gall, ovule-gall, floral bud, fruit and seed were fixed in FAA (formalin, acetic acid, 50% ethanol, 1:1:18 v/v/v) (Johansen 1940), dehydrated in an ethanol graded series (Kraus & Arduin 1997), and embedded in 2-hydroxyethylmethacrylate (Historesin, Leica®). Sections were obtained using a rotatory microtome (Ancap®, YD-315) at 5µm of thickness, stained with 0.05% toluidine blue - pH 4.7 (O'Brien *et al*. 1964) and mounted in Entelan®. The samples were observed under a light microscope (Leica® DM500) and photographed with a coupled U-photo system (Leica® ICC50HD).

Histochemical analysis

Fresh samples of fruit-like gall, ovule-gall, fruit, and seeds were prepared by free-hand cuts (Johansen 1940). The samples were submitted to histochemical tests for the detection of total lipids with Sudan Red B (Brundett *et al*. 1991), total proteins with bromophenol blue (Baker 1958), starch with Lugol's reagent (Johansen 1940), and reducing sugars with Fehling reagent (Sass 1951). As control tests the samples were compared with blank samples. All samples were visualized and photographed in a Microscope (Leica® DM500) coupled with a U-photo system (Leica® ICC50HD).

Cytological analysis

Samples of young and mature ovule-galls were fixed in Karnovsky's solution (4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer – 0.1M, pH 7.2) (Karnovsky 1965). The samples were washed twice in 0.1 M sodium cacodylate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide prepared in 0.1M sodium cacodylate buffer (pH 7.2) for 2 hours at 48° C. Following post fixation, the material was washed twice with distilled water for 5 minutes each and subjected to dehydration with a crescent acetone series. The dehydrated materials were embedded in Epon 812 resin (electron

microscopy sciences/EMS). After the samples were cut into ultrathin sections (50-70nm) and contrasted with 2% uranyl acetate and 0.2% lead citrate. Copper grids were examined in a JEM-2100 transmission electron microscopy (Jeol) equipped with energy dispersive spectroscopy (EDS, Thermo Scientific) in the Laboratório Multiusuário de Microscopia de Alta Resolução of Universidade Federal de Goiás (LabMic, UFG).

Results

Description of the gall morphology

The gall induced by *Allorhogas ubernandiensis* in *Miconia chamissois* has a globoid shaped (Fig. 1 A-B) and are induced only in inflorescences, more specifically in floral bud and rarely in flowers. The external morphology of the fruit and fruit-like gall are similar. The fruit-like gall is green or with some parts tending to red, while the fruit is green when are unripe and dark purple when are ripe. The fruit-like gall present hypertrophied locules (Fig. 1 A-B) different from fruit in which the locules are filled by the mesocarp (Fig. 1 C-D) and from the floral bud which has very small locules (Fig. 1 E-F). The true gall is induced in the ovule and looks like a cocoon shaped; with texture it looks like an egg shell when cut (Fig. 1 A-B).

The fruit-like gall may have 2 to 5 locules according to the characteristics of the host plant. Each locule in the fruit-like gall is occupied by just one gall but, rare observations, can occur two galls in the same locule. The ovule-gall shelter just one galling insect in the larval chamber. The floral bud attacked by the galling insect do not open and the stamens and style are retained in the apical portion of fruit like gall, making the ovule fertilization impossible (Fig. 2 A-B). At the beginning of the gall development, these floral structures seen to be viable (anthers and ovules) but, along the gall development these structures come to deteriorate (Fig 2. C-D).

The fruit-like galls has the volume 5.4 times bigger (height average = 8.40 mm, S = 1.18/ width average = 9.45 mm, S = 1.41/ volume average = 3330.04 mm3, S= 1461,24) than the mature fruits (height average = 4.57 mm, S = 0.62/ width average = 5.59 mm, S = 0.63/ volume average = 614.3 mm^3, S= 200.2) ($t = -17.03$, $df = 58$, $p<0.001$), besides the width being greater than the height in both cases. While the ovule-galls (height average = 2.46 mm, S = 0.14/ width average = 1.47 mm, S = 0.13/ volume average = 2.82 mm^3, S= 0.63) has the volume 20 times bigger than seeds (height average = 0,48 mm, S = 0,05/ width average = 0,28 mm, S = 0,04/ volume average = 0,14 mm3, S= 0,02) ($t=64,196$, $df=58$, $p<0,001$), in this case the height are greater than the height.

Anatomy

Miconia chamissois exhibits perfect flowers in which sepals, petals, and stamens are inserted at the distal region of hypanthium (Fig. 3A). In flower buds, the stamens are curved inwards and positioned around the pistil (Fig. 3A). The gynoecium presents a 2-5 locular ovary and a long linear style with an apical stigma (Fig. 3A-C). The ovary is 1/3 to 1/2 adhered to the hypanthium, with a free apex (Fig. 3 A-B). Each ovary locule exhibits some ovules (Fig. 3A) with axillary placentation.

Anatomically, the region where ovary and hypanthium are fused is quite similar to the ovary wall. It is formed by an external and internal uniseriated epidermis and a mesophyll with a chlorophyll-bearing spongy parenchyma, where vascular bundles are distributed (Fig. 3C). The ovules are anatropous and bitegmic, with the outer and inner integuments formed by three and two cell layers, respectively. In the raphe, phenolic compounds are observed in cells around the vascular bundle (Fig. 3D).

The fruit is formed mainly by the inferior portion of the ovary. The exocarp originates from the outer hypanthium epidermis. It is followed by a vascularized mesocarp, which consists of a parenchyma and idioblast cells containing druses and phenolic compounds. Few intercellular spaces occur in the most external zone of the mesocarp, which includes some/ (3-4) subepidermal layers. In contrast, more intercellular spaces emerge from the internal zone. The seeds of *M. chamissois* are testal and exhibit non-multiplicative integuments (Fig. 3 E). The testa is formed by two layers: the exotesta, which shows anticlinal and outer periclinal cells walls lignified at the antiraphal side; and the endotesta, composed by lignified cuboidal cells, which may exhibit druses (Fig. 3E). In the tegmen occurs a cell layer with phenolic compound, surrounding the embryo cavity.

The fruit-like gall has cells hypertrophied and varying in sizes equal to those present in the mesocarp of the fruits (Fig 3 F-4D). In the fruit-like gall the vascular bundles are more concentrated in the insertion of the gall in the ovary wall (Fig 4F), while in the flower bud and fruit they are well distributed.

At the beginning of the gall formation, half of its surface is adhered to the ovary (fig. 4A-B). During the development of the gall it becomes better defined and its connection with the ovary decreases a lot (Figure 4C). The galls are formed by a uniseriated epidermis and other two compartments, the storage tissue and a nutritive one. The storage tissue has elongated and flat cells, being this tissue compact without intracellular space. While the nutritive tissue has cells very hypertrophied (Fig. E). When

the galls are mature most of the nutritive tissue of the galls are absent due to its consumption by the galling.

Hystochemistry

Using histochemical assays was possible to two distinct tissues in the mature galls, the typical nutritive tissue (inner cortex) and the storage tissue (outer cortex) (Fig 5 B, C, E, F, H and I). Lipids (Fig 5 A-C) and proteins (Fig 5 D-E) were detected in seeds and ovule-gall tissues. Starch and reducing sugars were detected in the seeds but were not detected in the fruit's mesocarp and fruit-like gall. The pectin assessed by ruthenium red was detected especially in the nutritive tissue of the ovule ovule-gall (Fig 5 G-I).

Cytological profiles of the ovule gall

The young ovule gall presents a clear distinction between the outer tissue (which should develop into a seed tegument) and an inner tissue, the nutritive one. An ultrastructural analysis showed cells in different size and shapes, different degrees of cell wall thickness and conspicuous nuclei (Figure 6A-C). We can note a thin and sinuous cell wall (Figure 6A, B) in both outer and inner tissues of the young gall. The inner tissue develops into a nutritive tissue that present conspicuous nuclei and nucleolus as well as mitochondria and plastids associated (Figure 6C). However, despite of young gall, symptom of oxidative stress as multivesicular bodies were observed in both outer and inner tissues (figure 6D). In the mature galls, when the galling insect is on the larval developmental stage, the outer tissue presents a thick cell wall and accumulate substances in the vacuole (phenolics probably) (Figure 7A). Surprisingly, some chloroplast with well development membrane system and starch were observed in the cells of outer tissue (Figure 7B). The nutritive cells in the mature galls have conspicuous nuclei, thin cell wall, high numbers of mitochondria and lipid droplets associated with endoplasmic reticulum and Golgi apparatus (Figure 7C – E). In the nutritive tissue, we can note cells with symptoms of oxidative stress as the multivesicular bodies associated with lipid droplets and membrane system degeneration.

Discussion

As well as the seeds are structures that provide protection and specialized food (endosperm) for the embryo, the gall tissues through the nutritive cells provide rich

nutritional resources for galling development. *Allorhogas uberlandiensis* induce galls on the ovules of reproductive structures of *Miconia chamissois*. The gall induction changes the ovule cell fates for a news tissues in the ovule-gall, blocking the fertilization and seed development. However, the development of ovule-gall works as a physiological seed, stimulating the ovary wall growth and thus developing a big fake fruit, the fruit-like gall. The ovule-gall, inside the fruit-like gall, present a typical nutritive tissue with cells rich in proteins and lipids substances as well as cytological and histochemical profiles whose found in other galls induced by hymenopterans (Bronner 1992; Ferreira *et al*. 2017).

The big fake fruit can compromise the host reproductive effort

The fruit development in Angiosperms depends on the successful competition of pollination, double fertilization and nuclei fusion to embryo formation (Raghavan 2003). These events lead to seed formation which control the cell division and elongation during ovary wall development and fruit formation. In addition, consistent evidence supports that combined action of phytohormones as auxin, gibberelins and cytokinin regulate the fruit growth (Kumar et al 2014). Herein, *A. uberlandiensis* induce gall on ovules of *M. chamissois* and this ovule-gall works as a physiological seed/embryo stimulating the ovary growth developing into a fruit-like gall. This fruit-like gall is bigger than the health fruit of the host plant, as well as the ovule-gall is bigger than the seed. For fruit-like galls and ovule-gall the size patterns are similar to the most correlated structure (fruit and ovule/seed respectively), in terms of being larger in height or width. The ovary enlargement has already been detected in galls induced by *Asphondylia sesami* on *Sesamum indicum* (Pedaliaceae), specially by the increase of cell divisions and hypertrophy (Mehalingam 2015). As well as the gall induced by *A. uberlandensis* on the flowers bud of *M. chamissois*, galls induced by *Adelges cooleyi* (Hemiptera) on *Piceae* sp. and *Diplolepis rosae* (Hymenoptera) on *Rosa* sp. (Rosaceae) develops a fruit-like gall structure (Kohnen *et al*. 2012; Schultz *et al*. 2019; Gumming 1959). The ovule-gall formation and consequent fruit-like gall development compromise the reproductive effort of the *M. chamissois*, as discussed by Guimarães *et al*. (2014) for gall induced by *Brugmanniella byrsonimae* (Cecidomyiideae) on reproductive organ of *Byrsonima sericea* (Malpighiaceae).

The negative effects of galls on the reproductive effort of the *M. chamissois* put the galling species *A. uberlandensis* as a potential biological agent, as occur in the phytophagous species of *Allorhogas* who feed on fruits/seeds. As an example, *A.*

conostegia consume fruits of *Conostegia xalapensis* (Melastomataceae) decreasing the seed viability (Chavarría *et al*. 2009). In other hand, although *A. granivorus* infest *M. calvescens* (Melastomataceae), the fruits increase in 20% size and reduce the number of seeds in 79% in relation to fruits without insect infestation (Badenes-Perez & Johnson 2007; Zaldívar-Riverón *et al*. 2018). Here, *A. uberlandiensis* decrease the reproductive effort of *M. chamissois* in a different way. When the galling insect induce the ovule-gall all the other ovules in the carpel degenerate and comes unable to be fertilized, consequently there is no seed formation. Sometimes, in rare cases, the galls are induced by *A. uberlandensis* after the anthesis. However, in all these cases there are no fertilization. When galls are induced interrupt any chance of this floral bud reproduction, it occurs in consequence of the gall grow. This way the stigma and style cease to have a direct connection with the ovaries and consequently ovules, forming a physical barrier preventing the fertilization. When the plant is more invasive the importance of the insect as biological agent are bigger.

Anatomical and histochemical profile of ovule-gall

The galling feeding activity and the establishment of the ovule gall works as a true physiological seed, stimulating the growth of the ovary wall and the formation of a fruit-like galls. Thus, the ovule-galls are bigger than ovules and seeds and consequently, the fruit-like galls are bigger than fruits. The main anatomical features associated to this growth is the cell hypertrophy. The galling insect *A. uberlandiensis* changes the morphogenetical patterns of the ovule in the host reproductive organ leading to develop an ovule gall. The outer and inner integuments of the ovule differentiate into an outer and inner tissue of the gall, especially by the cell hypertrophy and tissue hyperplasia, common events in gall development (Isaias *et al*. 2014; Magalhães *et al*. 2014; Oliveira & Isaias 2010; Oliveira *et al*. 2016). The outer cortex showed hypertrophied and vacuolated cells, with a probable presence phenolic compounds detected in electronic microscopic. However, no reserve substances were detected in the outer cortex and thus, there is no formation of a storage tissue. The inner cortex is rich in proteins and lipids substances with small cells with dense cytoplasm and thus, can be characterized as a nutritive tissue where the *A. uberlandiensis* feed. As expected, gall induced by hymenopterans induce the development a feeding site, the nutritive tissue, as previously discussed by Bronner (1992) and revisited by Ferreira *et al*. (2017).

For instance, the nutritive tissue showed typical cytological features as cytoplasm-rich, noticeable nucleus and nucleoli, develop membrane systems, mitochondria-rich (Bronner 1992; Oliveira *et al*. 2011, 2016; Ferreira *et al*. 2017; Rezende *et al*. 2019) and, in the ovule gall induced by *A. uberlandiensis*, large amounts of rough and smooth endoplasmic reticulum. In the nutritive cells of ovule-galls there are large amount of elaioplasts that can be used for galling feed. These cytological features can be associated with the histochemical detection of proteins and lipids in the nutritive tissue, commonly observed in galls induced by hymenopterans (e.g. Bronner 1992). Lipids are currently accumulated as energetic reserve of seeds in *M. chamissois*, thus, the galling insect use this class of substance intrinsically produced in the host tissue a food for its growth and development. Once the lipids seem to be an excellent energetic substance typical in galls and plants (Bronner 1992; Buchanan *et al*. 2000). The intense feeding activity of the galling insect increase the oxidative stress and produce clear cytological symptoms in the nutritive tissue as pyknotic nuclei, membrane system packaging and presence of multivesicular bodies (Oliveira & Isaias 2010; Ferreira *et al*. 2015; Rezende *et al*. 2019).

Conclusion

In our study, we have shown that galling insect stimulates the development of a big fake fruit, the fruit-like gall, where the ovule-gall develop in. During the ovule-gall growth the galling organism stimulate the accumulation of primary substances in the nutritive tissue of gall, mainly lipids and proteins. In the seed were found a lot of lipids just like in the gall, this way is possible to suppose that the galling takes advantage of this location that already has great potential to store nutrients for its own benefit. The fruit-like gall and ovule-gall are considerable bigger than the corresponding plant organs, indicating that the gall take an advantage from the innate potential and patterns of the reproductive structures to grow just as it does with the accumulation of substances. Because this fruit-like gall is different from the fruit may have any effect on seed dispersal by birds, besides galling has a potential as agent of biological control due to the fact that all the floral bud galled becomes unviable.

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TABLE AND FIGURES

Table 1. Histochemistry results separated in gall and other parts of the fruit-like gall and fruit separated in seed and mesocarp. The accumulation of each substance is represented by the + and the absence for the -, for the starch, lipids, proteins, pectin and reducing sugars.

	Fruit-like gall		Fruit	
	Ovule-gall	**Other parts**	**Seed**	**Mesocarp**
Starch	-	-	+	-
Lipids	+	-	+	-
Proteins	+	-	+	-
Pectin	+	+	-	-
Reducing sugars	-	-	+	-

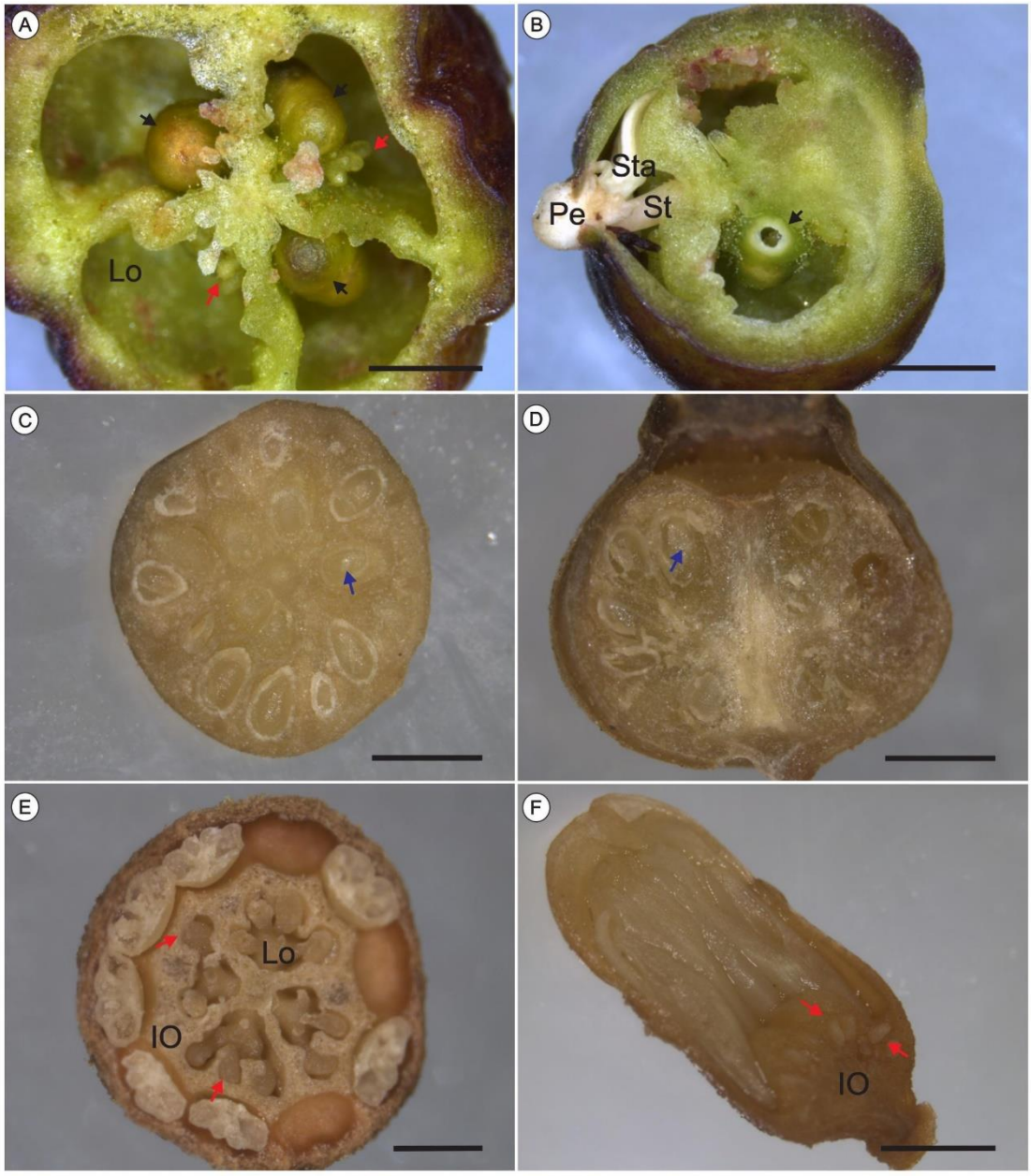


Figure 1. (A) Transverse section (TS) of the fresh Fruit like gall; (B) Longitudinal section (LS) of the fresh Fruit like gall; (C) TS of the fruit preserved in 70% etanol; (D) LS of the fruit preserved in 70% etanol; (E) TS of the floral bud preserved in 70% etanol; (F) LS of a floral bud preserved in 70% etanol. Black arrow – Gall; Blue arrow – Seed; Red arrow – Ovule. Abbreviations: LO – Locule; OI - Ovary inferior portion; Pe – Petal; St – Style; Sta – Stamens. Bars: (A, D) - 2 mm. (E) - 0,5 mm. (F) - 1 mm.

Figure 2. Longitudinal section in the fruit-like gall (A) Upper portion with the retained floral parts viable; (B) Upper portion with the retained floral parts unviable; (C) Middle portion with the ovules viable; (D) Middle portion with the ovules unviable. Detail –

Showing when a flower open even with the gall being induced. Abbreviations: LO – Locule; OI - Ovary inferior portion; Pe – Petal; St – Style; Sta – Stamens. Bars: (A, A Detail, B) - 1mm. (C, D) - 0,5 mm.

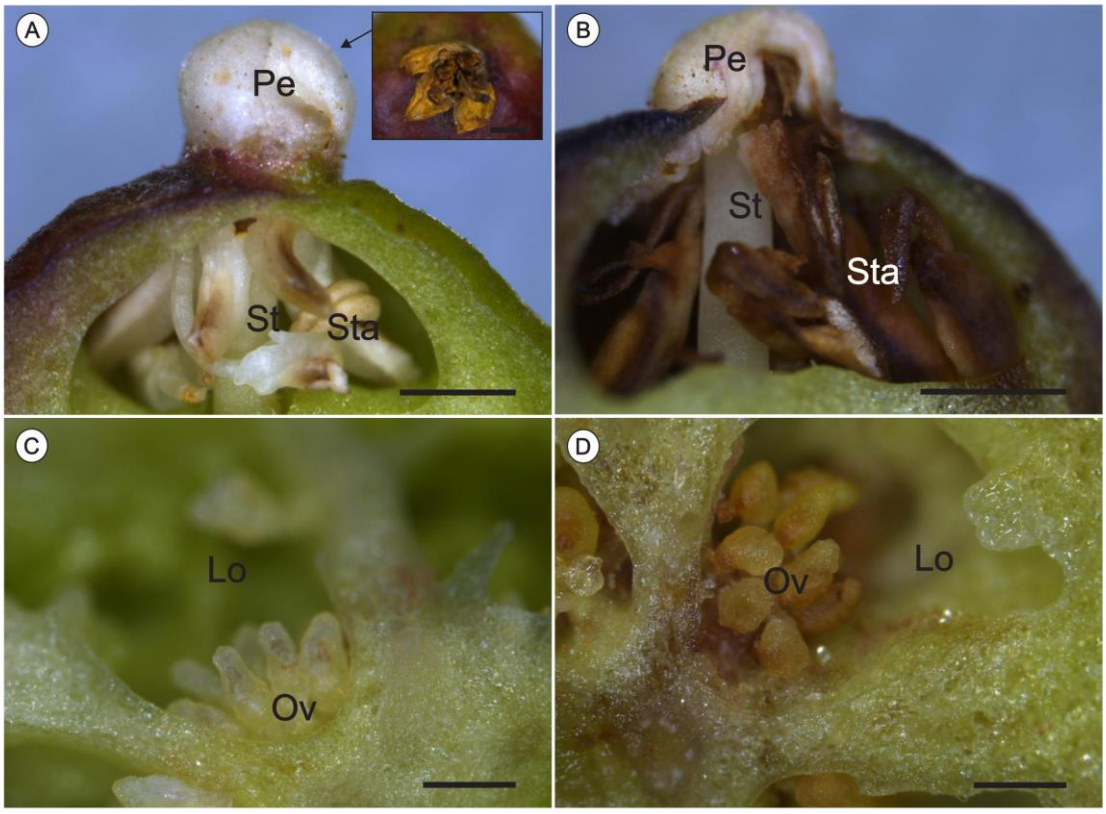
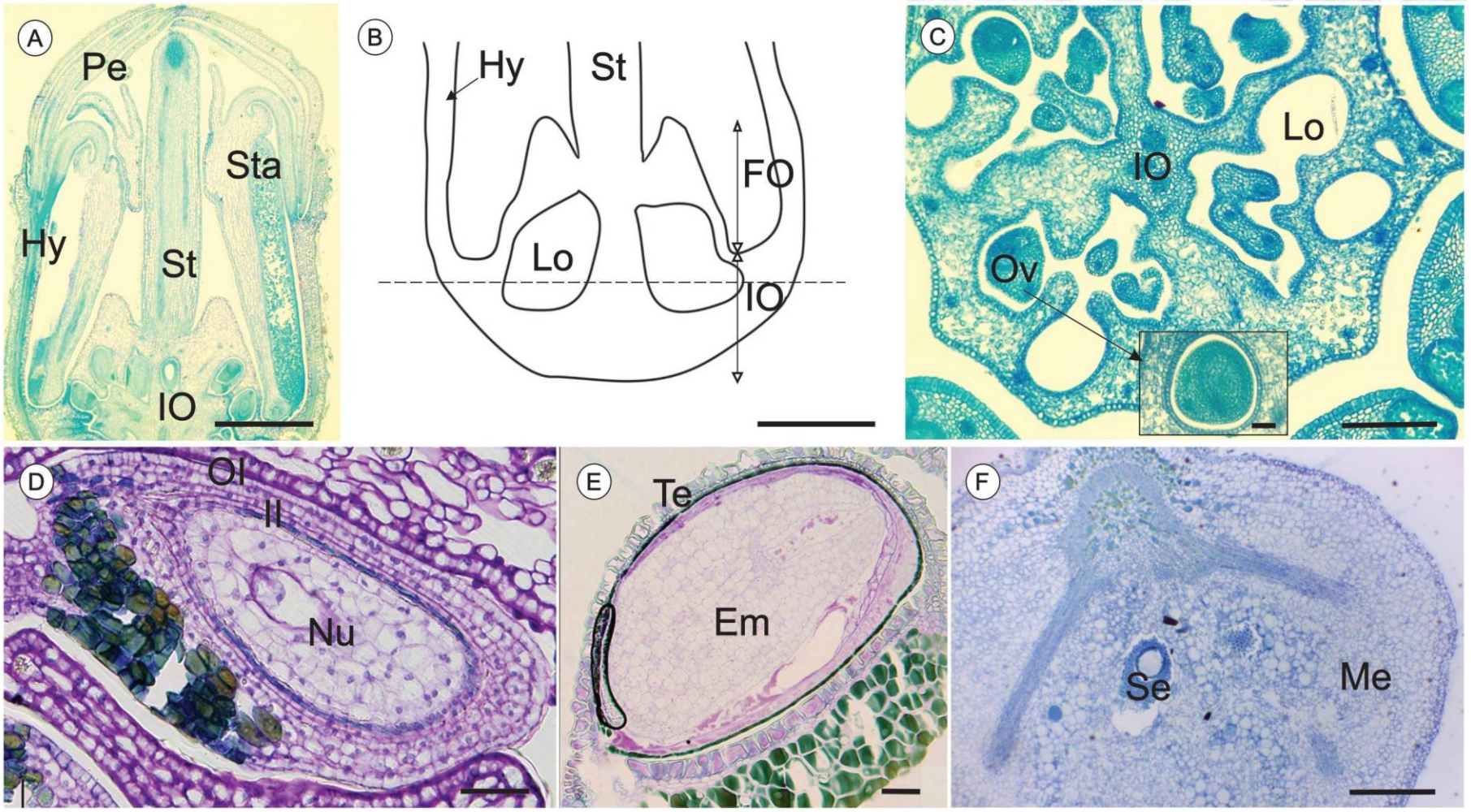


Figure 2. Longitudinal section in the fruit-like gall (A) Upper portion with the retained floral parts viable; (B) Upper portion with the retained floral parts unviable; (C) Middle portion with the ovules viable; (D) Middle portion with the ovules unviable. Detail – Showing when a flower open even with the gall being induced. Abbreviations: LO – Locule; OI - Ovary inferior portion; Pe – Petal; St – Style; Sta – Stamens. Bars: (A, A Detail, B) - 1mm. (C, D) - 0,5 mm.


A
Pe
Sta
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Em
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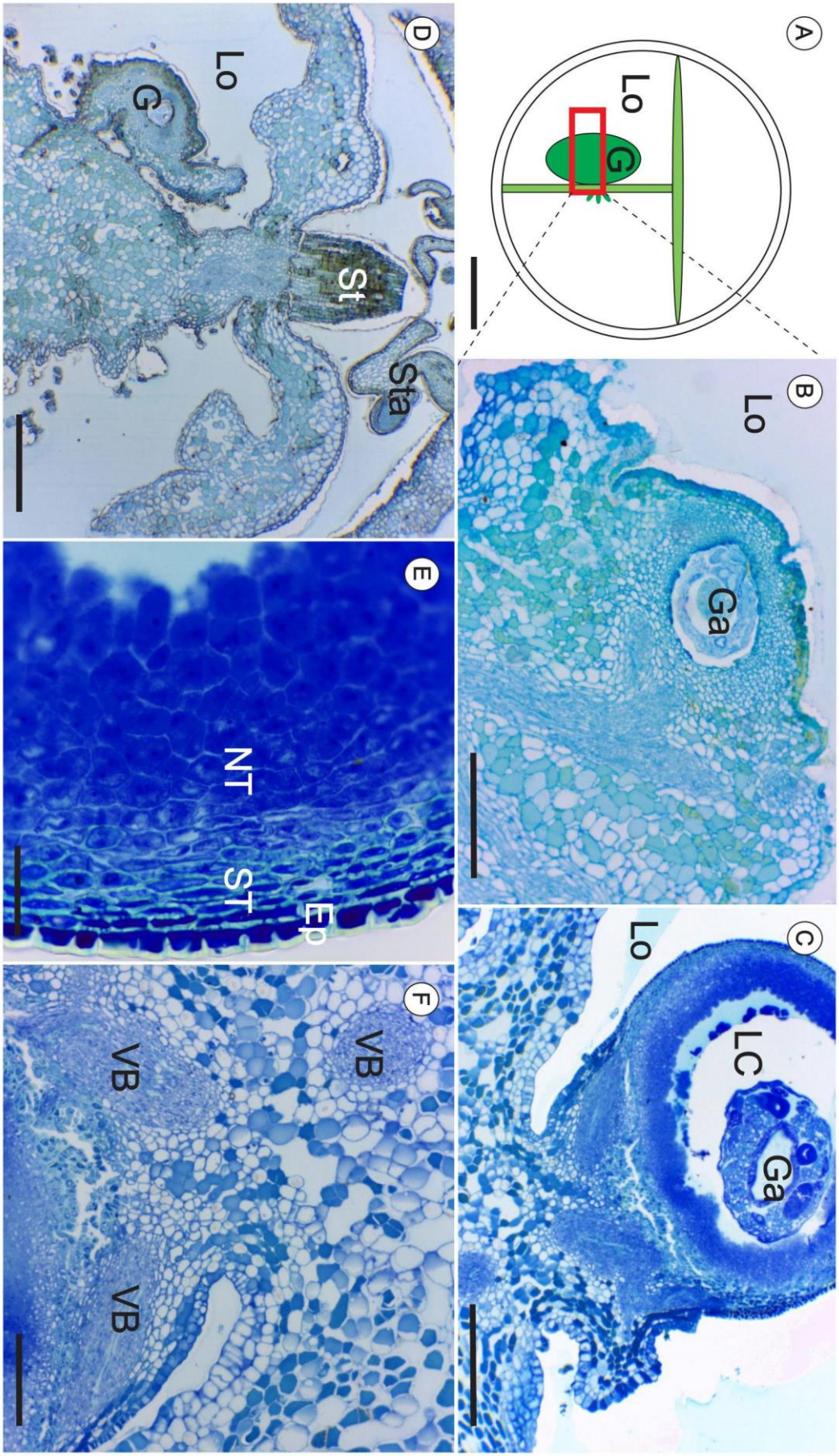
Figure 3. (A) Longitudinal section (LS) of the floral bud; (B) Schematic drawing of a floral bud without the stamens, showing with the sectioned line when was made the C section; (C) Transverse section of the ovary in a floral bud (D) Ovule (E) Seed (F) Fruit. Abbreviations: Em – Embryo; FO - Ovary free portion; Hy - Hypanthium; II – Inner integument; IO - Ovary inferior portion; Lo – Locule; Me – Mesocarp; Nu - Nucellus; OI – Outer integument Ov – Ovule; Pe – Petal; Se – Seed; St – Style; Sta – Stamens; Te – Testa. Bars: (A, B) - 500 μm. (C) 200 μm. (C detail) - 50 μm. (D) 50 μm. (E) 20 μm. (F) 500 μm.


A
Lo
G
B
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Ga
C
Lo
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Ga
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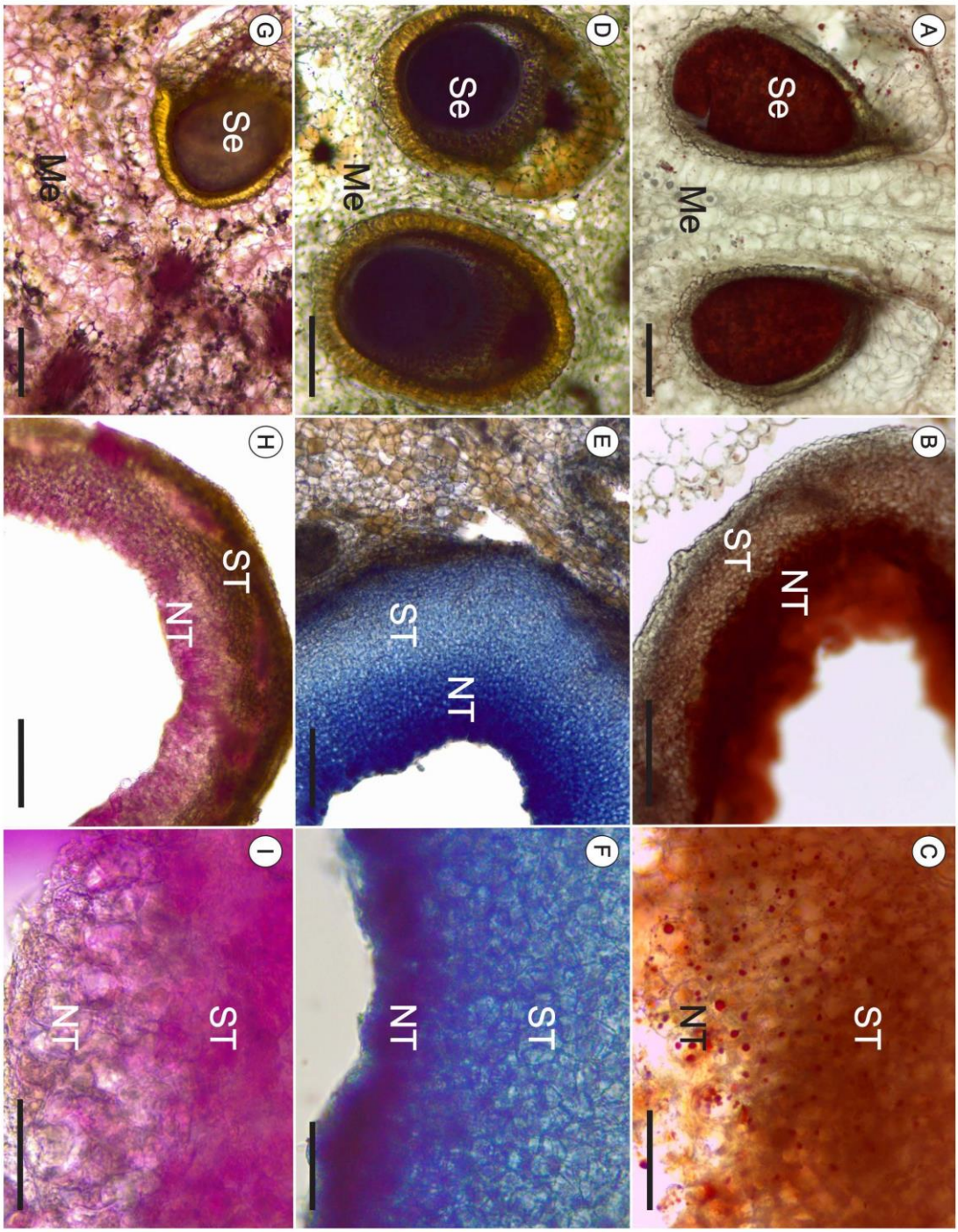
Lo
G
St
Sta
E
NT
ST
Ep
F
VB
VB
VB

Figure 4. (A) Longitudinal schematic drawing of a fruit-like gall, showing with the sectioned line when was made the B and C section; (B) Young gall; (C) Mature gall; (D) LS of the young gall; (E) Show in detail the nutritive and storage tissue in a mature gall; (F) Show the connection between the central portion of the fruit-like gall mature and the gall. Abbreviations: EP – Epidermis; G – Gall; Ga – Galler; LC – Larval chamber; Lo – Locule; NT – Nutritive tissue; ST – Storage tissue; St – Style; Sta – Stamens; VB –



Vascular bundles. Bars: (A) 2 mm. (B) 200 µm. (C, D) 500 µm. (E) 50 µm. (F) 200 µm.

Figure 5. Results of the histochemistry tests (A) Accumulation of lipids in seeds; (B) Accumulation of lipids in galls; (C) Accumulation of lipids in galls showing the typical nutritive and storage tissues; (D) Accumulation of proteins in seeds (E) Accumulation of proteins in galls; (F) Accumulation of proteins in galls showing the typical nutritive and storage tissues; (G) Absence of accumulation of pectin in fruit seed (H) Accumulation of pectin in cell walls in the gall; (I) Accumulation of pectin the typical nutritive and storage tissues in the gall. Abbreviations: Me – Mesocarp; NT – Nutritive tissue; Se – Seed; ST – Storage tissue. Bars: (A, B, D, E, G, H) 200 µm. (C, F, I) 50 µm.

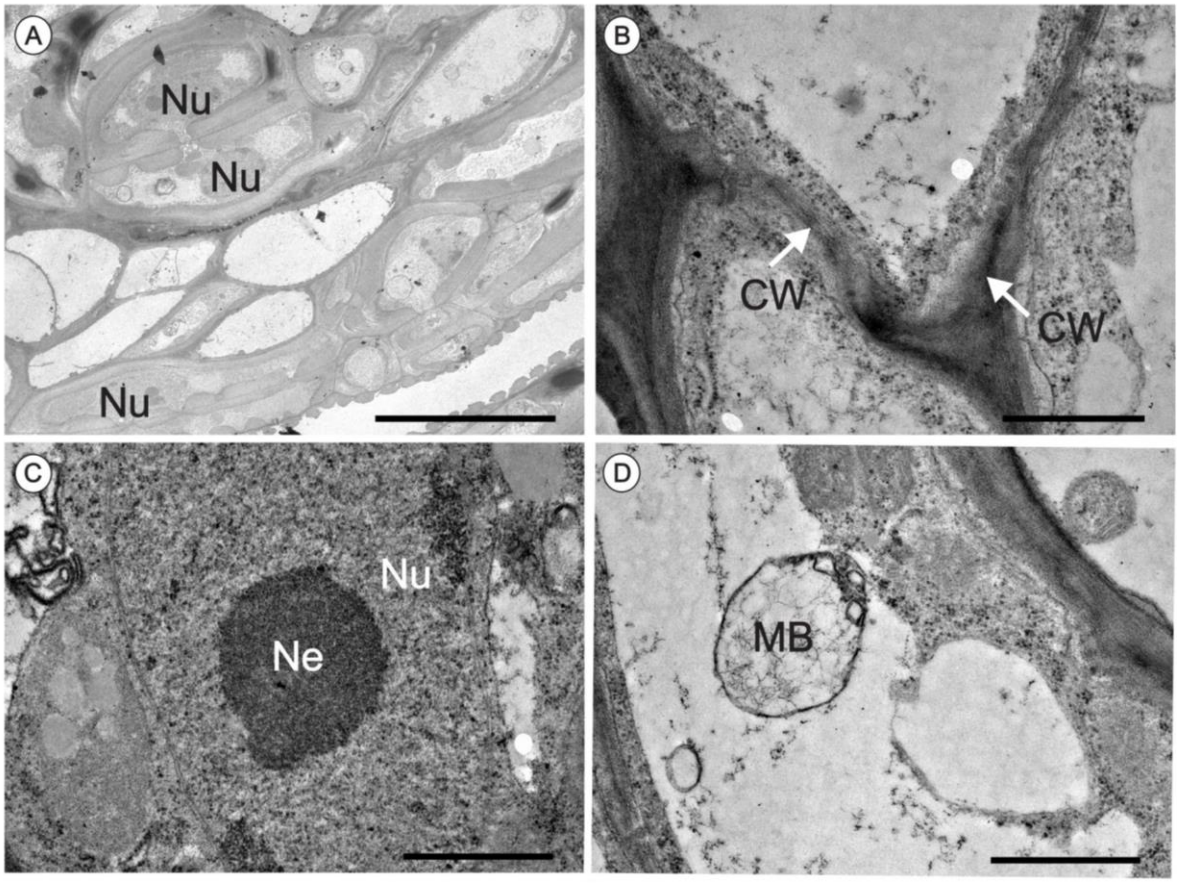


Figure 6. Cells of young galls of the System *M. chamissois-A. uberlandiensis* in transmission electron microscopy. (A-B) Cells with a thin and sinuous wall; (C) Conspicuous nuclei and nucleolus; (D) Multivesicular bodies. Abbreviations: CW – cell wall; MB – multivesicular body; Ne – nucleolus; Nu – nucleus. Bars: (A) 100 μm. (B, C, D) 1 μm.

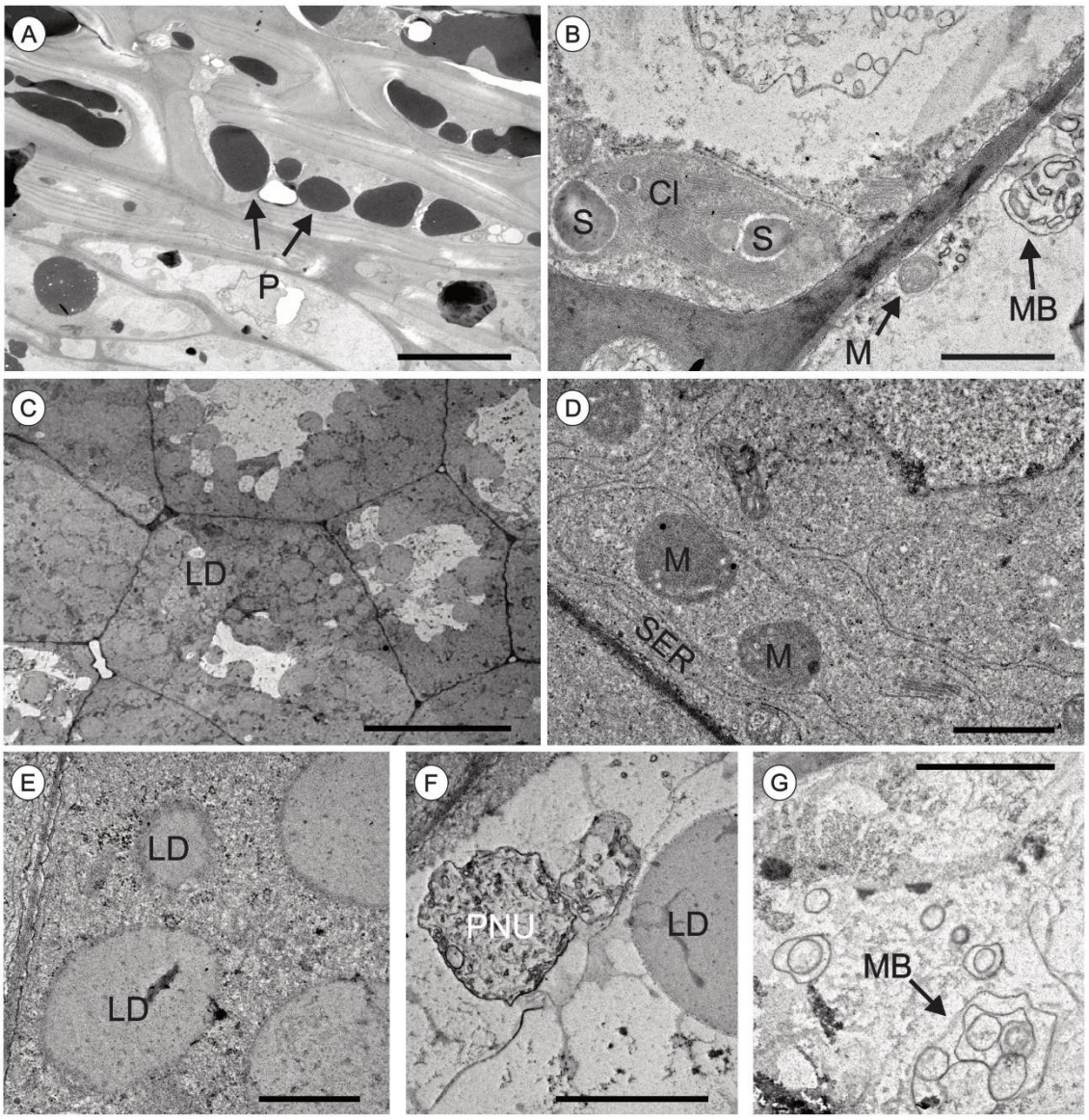


Figure 7. Cells of mature galls of the System *M. chamissois-A. uberlandiensis* in transmission electron microscopy. (A) Cells with a thick wall and accumulate substances in the vacuole (possible phenol); (B) The nutritive cells in the mature galls; (C) Cells with a lot of lipids droplets; (D) Smooth endoplasmic reticulum and mitochondria; (E) Detail of the lipids droplets (F) Pyknotic nuclei; (G) Multivesicular bodies. Abbreviations: Cl – Chloroplast; CW – Cell wall; LD – lipid droplets; MB – Multivesicular body; Mi – Mitochondria; Ne – nucleolus; Nu – nucleus; P – Phenol; PNU – Pyknotic nuclei; SER – Smooth endoplasmic reticulum; St – starch; Va – Vacuole. Bars: (A) 5 µm. (B, D, E, F, G) 1 µm. (C) 10 µm.