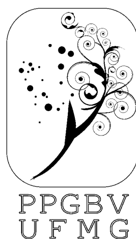

UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

BRUNO GARCIA FERREIRA

PROTAGONISTAS DA INTERAÇÃO E SEU PAPEL NA DETERMINAÇÃO DOS PERFIS ANATÔMICOS E FISIOLÓGICOS EM GALHAS

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Biologia Vegetal.

Área de Concentração: Morfologia, Sistemática e Diversidade Vegetal

BELO HORIZONTE – MG

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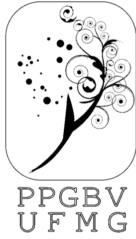

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Orientadora: Profa. Dra. Rosy Mary dos Santos Isaias
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"É de sonho e de pó

O destino de um só

Feito eu perdido em pensamentos

Sobre o meu cavalo

É de laço e de nó

De gibeira o jiló

Dessa vida cumprida a Sol"

(*Romaria*. Renato Teixeira)

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RESUMO

Esta tese apresenta padrões histológicos, histoquímicos e fisiológicos de galhas induzidas por nematódeos, ácaros e insetos, e sua relação com modo de alimentação, com o táxon indutor e com as capacidades fisiológicas da espécie hospedeira. Desta forma, avalia-se se o indutor, a planta hospedeira, ou ambos atuam como protagonistas da interação, ou seja, se são os principais determinantes do fenótipo da galha. A hipótese central foi que "a diversidade de possíveis respostas ontogenéticas e metabólicas das plantas aos fatores exógenos determina a diversidade anatômica e fisiológica das suas galhas". Seguindo esta linha, esta tese foi dividida em quatro capítulos, discutindo (1) os padrões anatômicos comparativos em galhas de nematódeos, ácaros e insetos; (2) a influência de taxa distintos de galhadores coloniais sobre os meristemas vegetativos de hospedeiras distintas; (3) a influência de uma mesma espécie de nematódeo galhador, *Ditylenchus gallaeformans*, sobre os meristemas reprodutivos de *Miconia albicans* e *M. ibaguensis* (Melastomataceae); e (4) alterações de parâmetros de fluorescência da clorofila, do teor de polifenóis, carotenoides e fosfolipídios peroxidados em galhas de nematódeos e ácaros em *M. albicans* e *M. ibaguensis*. Detectou-se que os galhadores de diversos grupos induzem padrões histológicos e histoquímicos diferentes de acordo com seu modo de alimentação e sua espécie, tendo como base o programa de desenvolvimento contido nas células do órgão hospedeiro. Ambos, galhadores e hospedeiras, apresentaram papéis importantes na determinação de respostas fisiológicas nas galhas, com estratégias antioxidantes divergentes, mas convergindo à função de manutenção da homeostase oxidativa em suas células. Os nematódeos induzem galhas em diversas espécies de plantas. Tais galhas não são apenas fenótipos estendidos dos galhadores, mas seu desenvolvimento e metabolismo são também determinadas pelas hospedeiras. Estes parasitas são, portanto, capazes de se adaptar a reações peculiares de cada hospedeira. Com as discussões reunidas, pôde-se vislumbrar que tanto os galhadores quanto as plantas hospedeiras são protagonistas imprescindíveis na determinação fenotípica das galhas, e por isso a variação das espécies hospedeira e indutora determina a distinção estrutural e metabólica das galhas.

PALAVRAS-CHAVE: fotossíntese, galhas, meristemas, Nematoda, sistema antioxidante, tecidos de reserva.

RESUMEN

La presente tesis doctoral presenta los patrones histológicos, histoquímicos y fisiológicos de agallas inducidas por nematodos, ácaros e insectos, y su relación con el hábito de alimentación, con el taxón inductor y con las características fisiológicas de la especie hospedadora. Se evalúa si el inductor, la planta hospedadora, o ambos, actúan como protagonistas de la interacción, es decir, si son los principales determinantes del fenotipo de la agalla. La hipótesis central fue que "la diversidad de posibles respuestas ontogenéticas y metabólicas de las plantas a los factores exógenos determina la diversidad anatómica y fisiológica de sus agallas". Siguiendo esta línea, la presente tesis doctoral fue dividida en cuatro capítulos, en los que se estudian (1) los patrones anatómicos comparativos de agallas inducidas por nematodos, ácaros e insectos; (2) la influencia de distintos tipos de agallas coloniales sobre los meristemos vegetativos de hospedadores distintos; (3) la influencia de una misma especie de nematodo gallícola, *Ditylenchus gallaeformans,* sobre meristemos reproductivos de *Miconia albicans* y *M. ibaguensis* (Melastomataceae); y (4) las alteraciones en la fluorescencia de clorofila, y en el contenido de polifenoles, carotenoides y fosfolípidos peroxidados en agallas de nematodos y ácaros en *M. albicans* y *M. ibaguensis*. Se observó agallas de grupos distintos inducen patrones histológicos e histoquímicos diferentes, de acuerdo con el hábito de alimentación y la especie del inductor, teniendo como base el programa de desarrollo contenido en las células del órgano hospedador. Ambos, los indivíduos gallícolas y lasplantas hospedadoras, presentaron papeles importantes en las respuestas fisiológicas y en la formación de agallas, con estrategias antioxidantes divergentes, pero que convergen en el mantenimiento de la homeostasis oxidativa en sus células. Los nematodos inducen agallas en diversas especies de plantas. Como las agallas no son solamente fenotipos extendidos de los inductores, estos parásitos son capaces de adaptarse a reacciones peculiares de cada hospedera. Se puede vislumbrar, reuniendo los datos aquí discutidos, que tanto las especies gallícolas como las plantas hospedadoras son protagonistas imprescindibles en la determinación fenotípica de las agallas, y por eso la variación de las especies hospedadoras e inductoras determina las características estructurales y metabólicas de las agallas.

PALABRAS CLAVE: agallas, fotosíntesis, meristemos, Nematoda, sistema antioxidante, tejidos de reserva.

ABSTRACT

This thesis presents histological, histochemical and physiological patterns of galls induced by nematodes, mites and insects, and their relationship with the feeding habit, the gall-inducing taxon, and the physiological capabilities of their host plants. Thus, it was evaluated if the gall-inducing organisms, the host plants, or both of them act as the main determiners of gall phenotypes. The central hypothesis proposes that "the diversity of possible plant ontogenetic and metabolic responses to exogenous factors determines the anatomical and physiological diversity of galls". Following this line of thinking, this thesis was divided into four chapters discussing: (1) comparative patterns of galls of nematodes, mites and insects; (2) the influence of distinct groups of colonial gall inducers on vegetative meristems of distinct host plants; (3) the influence of a single species of a gall-inducing nematode, *Ditylenchus gallaeformans*, on the reproductive meristems of *Miconia albicans* and *M. ibaguensis* (Melastomataceae); and (4) the alterations of chlorophyll fluorescence parameters, and of polyphenol, carotenoid and peroxided lipid contents in galls of nematodes and mites on *M. albicans* and *M. ibaguensis*. Gall inducers of diverse taxonomic groups induce different histological and histochemical patterns on their host plants according to their feeding habits and species, but based on the developmental programs of the host organ cells. Both gall inducers and host plants had important roles in the determination of gall physiological responses, with distinct antioxidant strategies, but converging to the oxidative homeostasis maintenance in gall cells. A single species of Nematode may induce galls on diverse host plant species, and their galls could not be considered strict extended phenotypes of the gall inducers. Peculiarly, these parasites are able to be adapted to peculiar reactions of each host plant. With current approaches, it was possible to foresee that both gall inducers and host plants are essential protagonists in the phenotypical determination of galls, and therefore the species of host plants and of gall-inducing organisms determine the structural and metabolic distinctions in galls.

KEYWORDS: antioxidant system, galls, meristems, Nematoda, photosynthesis, storage tissues.

INTRODUÇÃO GERAL

Diversos seres vivos podem manipular a morfogênese das plantas, induzindo neoformações que são conhecidas como galhas (Mani 1964). Quando induzidas por microrganismos, tais neoformações podem envolver recombinação gênica, como ocorre durante a infecção por *Agrobacterium tumefasciens* em diferentes plantas hospedeiras (Tooker & Helms 2014; Taiz et al. 2015). Animais galhadores, também chamados de cecidozoários – do grego, *kekis,* galha + *zoion*, animal (Meyer & Maresquelle 1983) – induzem galhas por meio de sinalização química ou mecânica sobre os tecidos da planta hospedeira, quando de sua atividade alimentar ou, em alguns casos, no momento da oviposição (Hough 1953; McCalla et al. 1962; Westphal et al. 1981; Meyer 1987; Mapes & Davies 2001; Oldfield 2005; Raman et al. 2005; Favery et al. 2016).

As galhas induzidas por animais, ou zoocecídias, se desenvolvem por processos como hipertrofia celular, hiperplasia e, ou, homogeneização de parênquima (Mani 1964). Tais processos são observados durante o desenvolvimento das galhas induzidas por nematódeos, ácaros e insetos (Goodey 1948; Mani 1964; Meyer & Maresquelle 1983; Meyer 1987; Rohfritsch 1992; Moura et al. 2008; Ferreira & Isaias 2013; Magalhães et al. 2014; Ferreira et al. 2017a, 2017b), ainda que fenômenos adicionais como rediferenciação celular, neoformação vascular, rediferenciação de conjuntos de esclereides, e metaplasia[1] possam fazer parte do desenvolvimento e maturação de tais estruturas (Meyer 1987; Arduin et al. 2005; Oliveira & Isaias 2010b; Ferreira & Isaias 2013; Oliveira et al. 2011, 2016; Fleury et al. 2015; Ferreira et al. 2017a).

As galhas induzidas por ácaros e insetos são em geral espécie-específicas (Meyer 1987; Dreger-Jauffret & Shorthouse 1992), com uma espécie indutora usualmente parasitando uma única espécie hospedeira (Mani 1964; Meyer 1987; Raman 2011). Nematódeos, por outro lado, podem induzir galhas em um número maior de espécies hospedeiras (Goodey 1948; Mani 1964; Oliveira et al. 2013). Este fato coloca em evidência o conceito de galhas como fenótipo estendido dos indutores, que considera as peculiaridades estruturais e fisiológicas das galhas como uma extensão do fenótipo do parasita sobre o desenvolvimento da planta hospedeira (Dawkins 1982; Carneiro et al.

[1] Este termo, frequentemente utilizado em histologia animal, foi discutido por Meyer & Maresquelle (1983, p. 18) no contexto da ontogênese das galhas. Metaplasia foi definida como a rediferenciação de uma célula sem que a mesma se prolifere, como ocorre no momento da indução de algumas galhas de Cecidomyiidae ou Eriophyidae, em que há formação de células nutritivas a partir das células da epiderme, sem que haja prévia desdiferenciação e proliferação das mesmas.

2015). Deste modo, seria esperado que as galhas de um mesmo nematódeo sobre diferentes espécies hospedeiras demonstrassem mesmos padrões fisiológicos e estruturais, porém algumas distinções ocorrem de acordo com a planta hospedeira (Goodey 1939; Ferreira & Isaias 2017b, 2017c, 2017d). Sem dúvidas, a conformação morfológica e anatômica das galhas é um bom indicador da espécie que as induz, já que em uma superhospedeira cada sistema galhador-planta hospedeira possui uma estrutura peculiar própria (Isaias et al. 2013). O conceito de fenótipo estendido é fundamental e usualmente usado como base para determinar a diversidade e riqueza de galhas em inventários em diferentes ambientes. Independente das pressões ambientais de cada ecossistema, as galhas têm valor adaptativo para os galhadores seguindo três principais hipóteses: (1) provêm nutrição especializada; (2) garantem um microambiente que protege contra limitações ambientais distintas; e (3) protegem contra os seus inimigos naturais, como predadores, parasitas e parasitoides (Stone & Schonrögge 2003; Price et al. 1987; Fernandes & Price 1992).

Os estudos de anatomia, histoquímica e citologia têm corroborado o fato de as zoocecídias proverem aos galhadores alimentação enriquecida, por meio do acúmulo de substâncias nutricionalmente energéticas armazenadas nos tecidos utilizados como fonte alimentar (Kendall 1930; Bird 1961; Dropkin 1969; Larew 1981; Westphal et al. 1981; Bronner 1992; Ferreira & Isaias 2013; Vecchi et al. 2013; Carneiro et al. 2014; Ferreira et al. 2015; Bragança et al. 2017). O modo alimentar do indutor, portanto, parece determinar algumas peculiaridades histológicas das galhas (Mani 1964; Meyer & Maresquelle 1983; Bronner 1992; Rohfritsch 1992). Células com citoplasma mais denso, contendo muitos ribossomos, polissomos, um sistema de endomembranas conspícuo, e núcleos e nucléolos volumosos são comumente observadas em contato direto com os habitantes das câmaras larvais. Quando tais células são utilizadas diretamente para a alimentação dos galhadores, estas constituem o assim denominado tecido nutritivo típico (TNT), podendo acumular lipídios, proteínas, e, ou, açúcares redutores (Kostoff & Kendall 1929; Bird 1961; Mani 1964; Meyer 1987; Bronner 1992; Kraus et al. 1993; Arduin & Kraus 1995; Moura et al. 2009; Oliveira et al. 2011; Ferreira & Isaias 2013; Vecchi et al. 2013; Ferreira et al. 2015, 2017a, 2017b, 2017c).

Galhas induzidas por cecidozoários sugadores com estiletes curtos, que se alimentam de células superficiais, como nematódeos da ordem Tylenchida (classe Secernentea) (Goodey 1948; Krusberg 1963; Dropkin 1969; Wyss 1997; Ferreira et al.

2017a, 2017b, 2017c), ácaros da família Eriophyidae (ordem Trombidiformes) (Kendall 1930; Westphal et al. 1981; Ferreira et al. 2017c), e tripes (classe Insecta, ordem Thysanoptera) (Mound & Morris 2005; Raman 2012) apresentam células nutritivas típicas. Galhas induzidas por insetos cujas larvas são capazes de mastigar as células vegetais, como insetos lepidópteros (ordem Lepidoptera) (Mani 1964; Meyer 1987; Ferreira & Isaias 2013; Vecchi et al. 2013), himenópteros (Hymenoptera) (Mani 1964; Meyer 1987; Bronner 1992) e coleópteros (Coleoptera) (Barnewall & De Clerck-Floate 2012), bem como as larvas especializadas de dípteros (Diptera), que podem romper as células superficiais, realizar digestão extracorpórea e sorver o conteúdo desta digestão (Meyer & Maresquelle 1983; Meyer 1987; Rohfritsch 1992; Harris 1994) também apresentam células nutritivas típicas. Algumas especializações podem ser observadas em galhas induzidas por nematódeos, como células gigantes polinucleadas (Bird 1961; Rodiuc et al. 2014), células nutritivas totipotentes (Ferreira et al. 2017b, 2017c) e tecidos nutritivos sinciciais[2] (Jones & Northcote 1972), demonstrando a especificidade na indução destes tipos celulares nutritivos.

Galhas induzidas por hemípteros (ordem Hemiptera), que possuem estiletes longos, podendo sugar em regiões mais profundas dos órgãos hospedeiros, em geral se alimentam sugando o conteúdo floemático de feixes vasculares hipertrofiados (Wool et al. 1999; Arduin et al. 2005; Ferreira et al. 2017a, 2017b), e, em alguns casos, também do conteúdo de células parenquimáticas (Meyer 1987). Neste tipo de galhas, também pode ocorrer o enriquecimento citoplasmático das células parenquimáticas próximas à câmara da galha (Carneiro & Isaias 2015; Richardson et al. 2017; Ferreira et al. 2017b). Estas células, não associadas diretamente à alimentação dos galhadores, armazenam amido, diferentemente daquelas do TNT, mas possuem similaridades ultraestruturais com as mesmas, formando um tecido que pode ser denominado como tecido similar ao nutritivo (TSN) (Richardson et al. 2017; Ferreira et al. 2017b). De qualquer maneira, o acúmulo de reservas em galhas é essencial para o suprimento energético demandado pelo constante dreno alimentar causado pelos galhadores e pelo crescimento, divisão e diferenciação de células (Motta et al. 2005; Oliveira & Isaias 2010a; Carneiro & Isaias 2015; Richardson et al. 2017; Ferreira et al. 2017b). Comumente, portanto, os compartimentos teciduais

[2] Considerou-se, neste texto, que uma *célula gigante polinucleada* é originária de múltiplas cariocineses sem citocineses, enquanto aqueles *tecidos nutritivos sinciciais* foram considerados sinônimos às células gigantes sinciciais descritas por Meyer & Maresquelle (1983, p. 327), e são, portanto, tecidos sem delimitação de protoplastos, multinucleados, resultantes não só de fusões de células por digestão de suas paredes, mas também de divisões cariocinéticas sem simultânea citocinese.

externos nas galhas são formados por parênquima de reserva homogêneo, com células em geral hipertrofiadas, contendo plastídios armazenadores de grãos de amido, sendo então denominadas como tecido comum de reserva (TCR) (Ferreira et al. 2017a, 2017b, 2017c). Os tipos de reserva primária, os tipos celulares diferenciados e a maquinaria enzimática responsável pelo desenvolvimento e metabolismo das células nas galhas, depende primariamente do potencial genético das plantas hospedeiras, que é manipulado sob os próprios limites destas plantas (Moura et al. 2008; Oliveira & Isaias 2010a; Ferreira & Isaias 2014; Ferreira et al. 2017a, 2017b, 2017c).

O acúmulo de derivados fenólicos é comum em muitas galhas de insetos (Abrahamson et al. 1991; Hartley 1998, 1999; Nyman & Julkunen-Tiitto 2000; Motta et al. 2005; Formiga et al. 2009; Guedes et al. 2016), sendo este acúmulo detectado histoquimicamente nos compartimentos teciduais externos, onde também podem ocorrer outros metabólitos secundários como alcaloides ou terpenoides (Ferreira & Isaias 2013; Bedetti et al. 2014, 2017; Carneiro et al. 2014; Suzuki et al. 2015; Bragança et al. 2017). Não é incomum, entretanto, a presença de tais metabólitos nos compartimentos teciduais internos das galhas (Ferreira et al. 2014, 2017d; Amorim et al. 2017). Os metabólitos secundários são tradicionalmente considerados defesas contra herbivoria e patógenos em plantas. Contudo, sua importância no metabolismo e no desenvolvimento das plantas tem sido desvendada nas últimas décadas. Alguns trabalhos têm sugerido que estes metabólitos estão envolvidos na dissipação do excesso de luz e do estresse oxidativo (Larson 1988; Close & McArthur 2002; Møller et al. 2007; Apel & Hirt 2004), destacando-se o *boom* oxidativo gerado em galhas de insetos (Gopinathan & Suresh 1985; Isaias et al. 2015; Oliveira et al. 2016, 2017). Nestas, os fenólicos podem também estar envolvidos com o crescimento celular, já que eles podem inibir a ação de enzimas responsáveis pela degradação de auxinas, ocasionando seu acúmulo (Abrahamson et al. 1991; Hori 1992; Hartley 1999; Bedetti et al. 2014, 2017; Tooker & Helms 2014; Suzuki et al. 2015). O papel dos fenólicos como defensores contra herbívoros parece ser secundário e muito mais relacionado àqueles generalistas, não adaptados às concentrações de polifenóis de uma planta específica (Carmona et al. 2011). De qualquer modo, o aumento da concentração de polifenóis que usualmente ocorre em galhas de insetos em comparação aos órgãos não galhados também deve depender do potencial metabólico das plantas hospedeiras, refletindo a influência do galhador sobre as potencialidades da planta.

A partir do estudo de galhas induzidas por nematódeos, ácaros e insetos, o objetivo central desta tese é detectar os padrões histológicos, histoquímicos e fisiológicos em tipos distintos de galhas, relacioná-los ao modo de alimentação, ao táxon do indutor, ao hábito colonial e às respostas estruturais e fisiológicas da espécie hospedeira, avaliando quais são os principais determinantes na estrutura dessas neoformações, e qual o papel dos protagonistas destas interações. Foram avaliadas as especificidades estruturais e fisiológicas de galhas induzidas por nematódeos em partes aéreas, raramente estudadas, principalmente nos trópicos (Ferreira et al. 2017b, 2017c). As galhas de nematódeos foram então avaliadas comparativamente às galhas de ácaros e insetos. A diversidade de respostas ontogenéticas e metabólicas das plantas se reflete em diferenças anatômicas e fisiológicas nas galhas que hospedam. Deste modo, sendo os nematódeos capazes de induzir galhas em diversas espécies de plantas, suas galhas não seriam apenas fenótipo estendido dos mesmos, refletindo também os padrões determinados pelas vias de desenvolvimento da hospedeira, bem como a capacidade destes parasitas se adaptarem a tipos distintos de respostas. Procurando testar tal hipótese, estudou-se comparativamente (1) os padrões anatômicos em galhas de nematódeos, ácaros e insetos (Ferreira et al. 2017a); (2) a influência de grupos distintos de galhadores coloniais sobre os meristemas vegetativos de hospedeiras distintas (Ferreira et al. 2017b); (3) influência de uma mesma espécie de nematódeo galhador, *Ditylenchus gallaeformans* (Tylenchida: Anguinidae), sobre os meristemas reprodutivos de espécies de *Miconia albicans* e *M. ibaguensis* (Myrtales: Melastomataceae) (Ferreira et al. 2017c); e (4) a influência de ácaros e nematódeos sobre os sistemas fotossintéticos e antioxidantes em *Miconia albicans* e *M. ibaguensis* (Ferreira et al. 2017d). Este trabalho, portanto, foi dividido em quatro capítulos, cada um deles focando em um dos aspectos relatados acima, buscando entender qual o papel de cada organismo associado como protagonista nestas interações.

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Capítulo 1

Feeding and other gall facets: patterns and determinants in gall structure

Manuscrito submetido ao periódico científico: **The Botanical Review**

Feeding and other gall facets: patterns and determinants in gall structure

Running head: **Patterns and determinants in gall structure**

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VIII. References

I. Abstract

Anatomical traits observed in animal-induced galls involve manipulation of plant morphogenesis in convergent ways. Nematode, mite and insect galls usually contain homogeneous storage parenchyma and develop due to hyperplasia and cell hypertrophy. The development of typical nutritive tissues, giant cells, or hypertrophied vascular bundles may occur. Some other anatomical features may be usually restricted to galls induced by specific taxa, but they may eventually be related to the developmental potentialities of the host plants. The combination of distinct morphogenetic peculiarities in each gall system culminates in extant gall structural diversity. Convergent anatomical traits are observed according the feeding mode of the gall inducers, representing potentiation or inhibition of similar events of host plant morphogenesis and differentiation, independent of gall-inducing taxa.

Keywords: cell redifferentiation; Eriophyidae galls; insect galls; nematode galls; nutritive tissue; plant anatomy

II. Introduction

Animal-induced galls, the zoocecidia, may develop on all plant organs, under the stimuli of gall-inducing organisms (the cecidozoa) (Mani, 1964; Meyer & Maresquelle, 1983; Roskam, 1992; Stone & Schonrögge, 2003; Oliveira et al., 2016). Gall anatomical structure may protect the gall inducers against natural enemies and unfavorable environmental conditions (Price et al., 1987; Fernandes & Price, 1992; Stone & Schonrögge, 2003). Moreover, gall tissue organization has been usually related to the taxa of insects and their feeding habit (Larew, 1981; Meyer & Maresquelle, 1983; Bronner, 1992; Rohfritsch; 1992; Ferreira et al., 2017a). Even though nematode and mite galls also have their own patterns and histological peculiarities (Mani, 1964; Larew, 1981), a thorough comparison among galls induced by Nematoda, Acarina and Insecta has been rarely performed (Larew, 1981; Meyer & Maresquelle, 1983; Ferreira et al., 2017a), and few of their systems have been well-studied (Table 1).

The histological profiles of the zoocecidia may be simple, i.e., similar to those of their host organs, or complex, with several neo-formed tissues or structures, which provide a better microenvironment for the development of the gall inducers (Wells, 1920; Mani, 1964; Larew, 1981; Meyer & Maresquelle, 1983; Ferreira et al., 2017a). Regarding the histological profiles, galls may be classified as organoids or histioids. The organoid galls do not histologically differ from their host organs, but macroscopic alterations may be observed (Sinnott, 1960). They are generally induced by fungi, viruses or bacteria, and may be simply shortened branched shoots, or sometimes with leaves with reduced surface area (Meyer, 1987). Witches' brooms and fasciations are typical organoid galls. Fasciations modify host plant meristems, and originate ribbon-like, elongated, or crisped structures instead of polygonal or rounded axis (White, 1948). The histioid galls comprise alterations in histological structure and are usually induced by animals (Mani, 1964). They are divided into kataplasmatic galls (without a constant external shape and size), and prosoplasmatic galls (with a typical, constant and definitive shape and size) (Küster, 1911; Wells, 1920; Mani, 1964). Herein, we considered the number of events of structural modification (Ferreira et al., 2017a) to evaluate the level of gall complexity. Tissue reorganizing events, such as: (a) parenchyma homogenization; (b) occurrence of hyperplasia; (c) occurrence of cell hypertrophy or hypotrophy; (d) increment in the density of trichomes/emergences; (e) redifferentiation of a nutritive tissue and/or a reserve tissue; (f) redifferentiation of a mechanical layer; (g) hypertrophy of vascular bundles; and (h) neo-formation (or redifferentiation) of specialized structures are discussed in the following sections. Simple and complex zoocecidia induced by Nematoda, Acarina and Insecta will be comparatively categorized.

In gall structural studies, plant developmental machinery is supposed to be similarly stimulated by the feeding habit of distinct gall-inducing taxa. In this case, the colonial habit is responsible only for potentiating the cecidogenetic factors, and therefore gall histological pattern

do not alter according to the colonial or solitary habits of the cecidozoa. Recently discovered novelties on Neotropical galls, which have shown more diverse histological and cytological patterns from those studied in the Temperate regions, guided current discussion. Reuniting a vast literature data from 20th and 21th centuries, we expect to demonstrate that galls are results of a combination of distinct morphogenetical potentialities of the host plants, which results in the extant gall structural diversity. Therefore, the host plants should also be considered "protagonists" in gall structural determination, as the gall-inducing organisms are.

III. Gall types

A. Some simple galls may be called pseudogalls. Phylogenetic studies suggested that the galling habit, at least in some groups, evolved from leaf rolling and leaf folding galls (Inbar et al., 1995; Crespi & Worobey, 1998; Nyman et al., 2000). Therefore, the simplest galls should be the leaf rolling/folding galls, which may be induced by mites, thrips, aphids, psylloids, lepidopterans, hymenopterans, and cecidomyiids (Raman & Ananthakrishnan, 1983; Inbar et al., 1995; Nyman et al., 2000; Souza et al., 2000; Arduin et al., 2005; Rancic et al., 2006; Álvarez et al., 2009, 2016; Magalhães et al., 2014; Portugal-Santana & Isaias, 2014; Bedetti et al., 2015). These galls may be slight modifications of host tissues, as the eriophyid galls on *Cirsium arvense* (Rancic et al., 2006), or may also be formed by hyperplasia, cell hypertrophy and/or parenchyma homogenization, as demonstrated for thrips-induced galls on several host plants (Raman & Ananthakrishnan, 1983; Gopinathan & Ananthakrishnan, 1985; Jorge et al., 2016), aphid galls induced by *Eriosoma ulmi* on *Ulmus minor* (Sano & Akimoto, 2011; Álvarez et al., 2013; Ferreira et al., 2017a), psyllid galls on *Baccharis reticularia* (Formiga et al., 2015), cecidomyiid galls induced by *Dasineura affinis* on *Viola odorata* (Meyer, 1987), and sawfly (hymenopteran) galls on *Salix* spp. (Nyman et al., 2000).

Some authors consider leaf rolling galls as pseudogalls (Wool, 2004), due to the fact that their development depends on the feeding activity and death of epidermal cells, which causes host leaf deformation. However, several leaf rolling galls are considered true galls, for they result from hypertrophy of vascular bundles, parenchyma and epidermal cells, overdifferentiation of trichomes, hyperplasia and parenchyma homogenization (Mani, 1964; Álvarez et al., 2013; Formiga et al., 2015; Jorge et al., 2016; Ferreira et al., 2017a). More complex leaf folding galls may have mechanical tissues (sclerenchyma layer surrounding gall chamber), multiseriate epidermis, and nutritive-like tissues or common storage tissues, as observed for some aphid galls (Álvarez et al., 2009, 2016; Kurzfeld-Zexer et al., 2015).

The concept of galls as new organs may be applied to galls containing redifferentiated tissues with new specialized functions (Oliveira & Isaias, 2010a, 2010b; Ferreira & Isaias, 2013, 2014; Ferreira et al., 2015; Richardson et al., 2017). Due to the variety of tissue reorganization, it is controversial to consider the leaf rolling/folding galls as new organs. Generally, the concept of galls as new organs will depend on the occurrence of tissue neo-formation and cell redifferentiation, and also on altered physiological parameters. Independent of the galls being new organs or not, Mani (1992, p. 3-4) amplified the concept of galls to "*a bewildering variety of plant structures and growth forms, ranging from the nearly normal plant organs to extreme bizarre and highly complex growth abnormalities*", recognizing all types of tissue manipulation as galls.

B. Colonial, solitary and coalescent galls

Colonial galls shelter several individuals in a single chamber, and are commonly induced by nematodes and mites, but rarely by insects (just some Thysanoptera, Hemiptera: Aphididae and Hemiptera: Adelgidae) (Table 1). Indeed, most insects induce solitary galls, with one insect per chamber. Several Hymenoptera may induce galls by ovipositing in very close sites of the host organs (Table 2). In such cases, the coalescent galls share common tissues, but each larva is solitary and occupies an exclusive chamber (Mani, 1964; Meyer, 1987) (Table 1). The advantages of these multichambered (multilocular) galls should be the relative protection of some individuals

from predation or parasitism, considering that the superficial individuals are more susceptible to the attack of parasitoids (Stone & Schonrögge, 2003).

1) Influence of induction modes on solitary, coalescent and colonial habits

The induction habit of the gall-inducing species is determinant for the colonial or solitary habit (Table 2). When a female induces galls by its feeding activity, and oviposits or generates its offspring parthenogenetically, these galls are usually colonial, containing the mother and its offspring. When the feeding activity (chewing or sucking plant tissues) of the larva or the nympha is the stimuli for gall induction, galls are usually solitary. In a few groups, the oviposition is responsible for the first steps of gall induction, and galls may be solitary or coalescent. Independent of the solitary or colonial feature, gall induction usually occurs in meristematic tissues (Mani, 1964; Álvarez, 2011; Dias et al., 2013a; Ferreira & Isaias, 2014; Fleury et al., 2015), but there are some cases in which parenchymatic or epidermal tissues are the oviposition sites (Oliveira & Isaias, 2010b; Ferreira & Isaias, 2013). These cases demonstrate that most living plant cells are truly totipotent cells, being capable of redifferentiate into any other plant cell type (see Lev-Yadun, 2003).

Both colonial and solitary galling habits are strategies that have arisen independently among Arthropoda. Colonial insect galls are induced by Thysanoptera, and Hemiptera such as Aphididae, Phylloxeridae and Adelgidae. The Thysanoptera females induce galls by feeding stimuli prior to oviposition, and the nymphs may intensify plant tissue responses. Similarly, aphid and phylloxerid galls are induced by a female, the fundatrix, and its offspring maintain the galling stimuli (see below). The solitary galls independently arose in distinct taxa of insects, and usually have one gall-inducing individual per chamber (Table 1, Table 2). The galls with chewing and rasping-sucking larvae are predominantly solitary (Table 2). It is important to notice that colonial galls are usually induced by hemimetabolous species (such as Thysanoptera and Hemiptera) (Kjer, 2006), cases in which adults and offspring have the same feeding habits. The solitary galling habit is of higher occurrence in taxa of Endopterygota (the holometabolous, such as Hymenoptera, Coleoptera, Lepidoptera and Diptera), with chewing or rasping larvae as inducing individuals.

Among the hemimetabolous, some Hemiptera (Psylloidea and Coccoidea) may induce solitary galls, where the nymphs, and not the adult females, are responsible for gall induction. The distinct stages of an individual, i.e., larva, pupa and adult of the holometabolous organisms usually inhabit solitary galls, while for the hemimetabolous, distinct generations may cohabit a colonial gall. Among the holometabolous, the Diptera: Cecidomyiidae and Hymenoptera: Cynipidae induce the most diverse galls (Espírito-Santo & Fernandes, 2007), which are histologically and histochemically peculiar (Wells, 1920; Rohfritsch, 1992; Harris, 1994; Stone & Schonrögge, 2003; Ferreira & Isaias, 2014). Such diversity may be consequence of the capability of these solitary gall-inducing organisms to both manipulate plant tissues and tolerate chemical constraints imposed by the host plants (Amorim et al., 2017).

2) Influence of number of individuals in gall growth and development

The solitary and colonial habit may influence the size of the gall and the rates of hypertrophy and hyperplasia, but the tissue arrangement tends to be similar. In mite galls, the number of inducers and gall size are positively correlated, even though additional tissue layers and larger cells are not observed (Moura et al., 2009b). In galls induced by several species of thrips on *Acacia* spp., the more generations inside a gall, the greater is the gall inner surface area related to gall volume (Crespi & Worobey, 1998). The histological patterns of galls induced by colonial (aphids) (Fig. 2a, Fig. 3b) and solitary phloem-sucking insects (psylloids) are similar (Fig. 1). The size of aphid-induced galls is also directly related to the number of individuals inside gall chamber (Zha et al., 2016). Therefore, only lateral growth – in an action of "plate meristem" (see Evert, 2006), with anticlinal cell divisions in the gall walls – seems to be potentiated. Coalescent galls also have a potentiation of growth directly related to number of individuals in their structure, as observed by Arduin et al. (1989) for hymenopteran galls induced on

Struthanthus vulgares leaves. These data suggest that the number of inducers does not affect gall histological patterns, but only gall size. It seems to be merely a response to increased concentrations of growth signals. Therefore, colonial and coalescent galls should be structurally similar to solitary galls induced by closely-related gall-inducing species with same feeding habit, but greater dimensions than those of solitary galls are expected.

IV. Multiple evolution of galling taxa

A. Taxonomical distribution of gall inducers

Insecta is the richest group of independently-evolved families with gall-inducing species (Mani, 1964; Meyer, 1987; Roskam, 1992) (Table 1). Considering that only the most representative clades of gall-inducing species have been listed in Table 1, the galling habit appeared several independent times in metazoan evolution – representing more than 52 insect families (133,000 estimated species) (Fernandes et al., 2012), 4 mite families (3,600 described species) (Raman et al., 2005), and at least 4 nematode families (without estimations on the number of galling species) (Meyer, 1987). The galling habit evolved in parallel in several distinct families of insects, from ancestral guilds with distinct life habits (Roskam, 1992). The gall-inducing Cecidomyiidae seems to have evolved directly from detritivorous/mycetophagous ancestors, while other insect groups have evolved from: spores/pollen feeders (such as Thysanoptera: Phloeothripidae); plant sap feeders (as Hemiptera: Adelgidae, Eriosomatidae, Psyllidae, and Coccidae); foliage feeders (as Coleoptera: Curculionidae and Hymenoptera: Tenthredinidae); stem borers (as Hymenoptera: Cephidae); leaf miners (as Lepidoptera: Gelechiidae, and Diptera: Tephritidae and Agromyzidae); and from zoophage parasitoids (as Hymenoptera: Cynipidae) (Roskam, 1992).

B. Gall induction mechanisms

1) Induction of Nematoda galls. The most common nematode galls are induced in roots, where the adult females (*Nacobbus, Rotylenchulus, Tylenchulus*) or second-stage juveniles (*Globodera*, *Heterodera*, *Meloidogyne*) usually penetrate into plant tissues (Krusberg, 1963; Dropkin, 1969; Meyer, 1987; Wyss, 1997). The nematodes perforate cell walls and introduce salivary secretions, which immediately induce cytoplasm aggregation around the stylets (Krusberg, 1963; Dropkin, 1969; Wyss, 1997). Some proteins trigger cytoplasm changings, and influence the genetic expression of plant cells (Favery et al., 2016), activating the differentiation of meristematic cells into nutritive cells, or the redifferentiation of parenchymatic cells into new cell types (Goodey, 1948; Bird, 1961; Ferreira et al., 2017b).

Usually, the first feeding sites are meristematic cells, which respond to the galling stimuli of nematodes. The feeding of the nematodes causes the surrounding cells and nuclei to become larger, and the cytoplasm to be denser (Krusberg, 1963; Dropkin, 1969). There is an increase in ribosomes, polyribosomes and cell organelles in cortical parenchyma (as in *Xiphinema* galls) (Weischer & Wyss, 1976; Wyss, 1997, 2002) or in xylem parenchyma cells (as in *Meloidogyne* galls) (Bird, 1961; Yousif, 1979; Finley, 1981; Wyss, 1997, 2002; Di Vito et al., 2004) due to the nematodes feeding activity (Wyss, 1997). In general, the feeding activity of the nematodes induces the redifferentiation of meristematic or parenchymatic cells, and specialized nutritive tissues develop. The nutritive cells in root nematode galls may be multinucleate giant cells, non-hypertrophied uninucleate nutritive cells, or syncytia, depending on the gall-inducing species (Dropkin, 1969; Meyer, 1987; Wyss, 1997). The multinucleate cells are usually formed by nuclear divisions without cytokinesis, while syncytia are formed by fused protoplast of cells after partial wall dissolution (Wyss, 1997). In fact, considering the distinct types of induced nutritive cells, each nematode species should be responsible for activating specific cell cycle genes of the host plant (Favery et al., 2016). Besides the formation of nutritive cells or tissues, the root parenchymatic tissues undergo hyperplasia, forming cysts or root-knots (Wyss, 1997).

In aerial parts, galling Nematoda usually penetrate young leaves or stems, inducing the redifferentiation of meristematic cells, and necrotic regions are rarely observed (Goodey, 1939, 1948; Dropkin, 1969; Watson & Shorthouse, 1979; Skinner et al., 1980). In 2 or few days, a typical nutritive tissue may be observed surrounding the colonies (Goodey, 1939, 1948; Skinner et al., 1980). The nutritive cells may be hypertrophied or not, and may have one or more nuclei and nucleoli (Watson & Shorthouse, 1979; Skinner et al., 1980). Even though most galling nematodes penetrate plant meristems, there are some exceptions. *Ditylenchus gallaeformans* remains external to plant tissues, and induces galls by feeding on the meristem surfaces of *Miconia* spp. aerial parts (Ferreira et al., 2017a, 2017b).

2) Induction of Acarina galls. Eriophyidae galls are also induced by adult females (Table 2) (Meyer, 1987), which feed on young leaf surfaces. After 15-20min, it is possible to observe a cone-shaped callose thickening surrounding the perforation region of the outer periclinal wall of the epidermal cell. In one-hour time, the first nutritive cells differentiate next to the perforation, with dispersion of chromatin, nucleoli enlargement, vacuome formation, and cytoplasm enrichment (Westphal et al., 1981). Plant tissue invagination may occur after some hours to two days of motionless position of the female (Oldfield, 2005). The mites may feed only on epidermis, inducing the differentiation of superficial nutritive cells. Meyer and Maresquelle (1983) considerate the direct differentiation of epidermal cells into nutritive cells in these galls as metaplasia, since they not dedifferentiate and proliferate before redifferentiation. In other cases, the feeding activity may cause cell degradation of the feeding tissues, and the mites may gradually penetrate plant tissues forming a gall chamber. Nutritive cells redifferentiate from common parenchyma cells, around the feeding route of the mites, which seem to be the main stimuli for this redifferentiation (Kendall, 1930; Westphal et al., 1981; Ferreira et al., 2017a). After a variable time of feeding, the females oviposit (the eggs are often produced by parthenogenesis), and quit the gall (Oldfield, 2005).

3) Induction of Insecta galls. The real causes of the insect galls were firstly unveiled by Marcello Malpighi in the 17th century, with his book "De Gallis" published in 1679 (Fagan, 1918). However, the mechanisms of gall induction were actually experimented and discussed along the late 19th and 20th centuries, by studies applying mechanical stimuli, insects' salivary mixtures, and insects' entire extracts to plant tissues (Felt, 1936; Hough, 1953). However, the physiological mechanisms of insect gall induction remain obscure (Wool, 2004; Oliveira et al., 2016). Even though some experiments may stimulate hyperplasia in host plant tissues, the growth and development is not complete as they are in galls naturally induced by the insects (Hough, 1953; McCalla et al., 1962; Mapes & Davies, 2001). However, the role of the mechanical wounding (by perforation or chewing), and mainly the insect's saliva or egg's secretions are essential for gall induction (Felt, 1936; Hough, 1953; Raman et al., 2005). Additionally, plant growth regulators may be found in the gall-inducing insect extracts, reinforcing the chemical hypothesis (Byers, 1976; Meyer & Maresquelle, 1983; Hori, 1992; Mapes & Davies, 2001). It is also assumed that the constant feeding of the inducing animal on gall tissues is essential for gall development, maturation, and dehiscence (Felt, 1936; Hough, 1953; Oldfield, 2005). However, the initial stimuli are not necessarily related to the feeding activity, as some insect galls may be induced by oviposition injury and associated secretions (Felt, 1936; Hough, 1953; Raman et al., 2005).

a) Feeding activity. The inseminated female and offspring of the thrips (Thysanoptera) induce galls only by sucking on individual leaf epidermal cells (Mound & Morris, 2005; Raman, 2012), which end up dying, causing leaf distortion. The surrounding mesophyll cells are responsible for repairing this damage, originating outgrowths into the tissues often causing leaf folding (Mound, 1994; Mound & Morris, 2005; Raman, 2012).

Some gall-inducing species of Hemiptera induce either solitary or colonial galls. Aphididae and Phylloxeridae induce colonial galls, by the feeding activity of a single female, usually the fundatrix, which reproduces parthenogenetically (Wool, 2004). Some genera of Aphididae: Fordini (*Forda*, *Smynthurodes*) may induce two distinct galls on a single plant species: the fundatrix (F1) induces a temporary solitary gall; in a few weeks, the fundatrix offspring, the

fundatrigeniae (F2) induce a definitive gall. The parthenogenetic fundatrigenia oviposits inside the chamber, and therefore the definitive galls are colonial (Wool, 2004; Álvarez et al., 2014; Kurzfeld-Zexer et al., 2015). Solitary galls of Psylloidea (Hemiptera) are induced by the feeding activity of first-instar nymphs (1-3 individuals), and covering or pouch galls develop (Burckhardt, 2005), as is true for *Nothotrioza* spp. (Triozidae) on leaves of *Psidium* spp. (Myrtaceae) (Carneiro et al., 2014b; Carneiro & Isaias, 2015). Nevertheless, some species such as *Baccharopelma dracunculifoliae* (Psyllidae) nymphs may induce galls with one to 21 individuals inside the chamber. The sucking activity of *B. dracunculifoliae* nymphs is the stimulus responsible for the hyperplasia and cell hypertrophy, which culminate in the entire folding of *Baccharis dracunculifolia* (Asteraceae) leaves (Arduin et al., 2005).

In Coccoidea (Hemiptera), the first nymphs (crawlers) were known to induce solitary galls by sucking on phloem or on parenchymatic tissues (Meyer, 1987). In the Neotropics, second-instar Eriococcidae nymphae are responsible for gall induction in three systems: *Pseudotectococcus rolliniae – Rollinia laurifolia* (Annonaceae), *Eriogalococcus isaias – Pseudobombax grandiflorum* (Malvaceae), and *Bystracoccus mataybae – Matayba guianensis* (Sapindaceae). The first-instar nymphs of *P. rolliniae* induce dormancy stem galls on *R. laurifolia*, and sexually dimorphic leaf galls are induced by the second-instar nymphs (Gonçalves et al., 2005). In the other two studied systems, the fist-instar nymphae use the bark of *M. guianensis* and *P. grandiflorum* as the sites for dormancy (diapause), but no tissue alteration is observed. After molting, they move to leaves and induce dimorphic galls, i.e., the female galls are distinct from those induced by the males (Hodgson et al., 2013; Magalhães et al., 2015).

In the case of Cecidomyiidae (Diptera), the main gall-inducing factor is the cell disruption caused by the larva, in most cases using the prothoracic spatula for puncturing surficial cells (Rohfritsch, 1992; Harris, 1994). The released cellular fluids are extracorporeally digested and then sucked by the larvae. Probably the cell disruption and salivary fluids triggers the development of a pouch, covering growth, or distinct growth of buds (Rohfritsch, 1992; Harris, 1994; Ferreira & Isaias, 2014; Fleury et al., 2015). The larva may induce galls on the epidermal surface (and metaplasia may occur) or may penetrate into plant tissues, depending on the oviposition site and its behavior (Meyer, 1987).

The Lepidoptera induce galls through the voracious feeding of their larva, leading to the redifferentiation of host plant cells into typical nutritive cells. The oviposition occurs on superficial tissues, and when the eggs hatch (Meyer, 1987; Miller, 2005), the larvae chew plant cells, and enter the host plant organ. The nutritive cells accumulate lipids and are capable of self-regenerating (Meyer, 1987; Ferreira & Isaias, 2013; Vecchi et al., 2013; Ferreira et al., 2015). Even though Hymenoptera and Coleoptera also have chewing mouthparts, and their feeding activity is important to maintenance of gall development, their galls are initially induced by oviposition, and therefore they were discussed below.

b) Oviposition. The oviposition is an important induction factor in Hymenoptera and Coleoptera galls (Table 2). Cynipidae (Hymenoptera) oviposition into plant tissues stimulates cell lysis and the formation of a gall chamber (Meyer, 1987; Csóka et al., 2005). The cells surrounding the oviposition site go through metaplasic reaction, and the cytoplasm becomes granulose. Gall development proceeds by cell hypertrophy and hyperplasia of surrounding tissues. Several Cynipidae galls, however, are induced only after the eclosion of the larvae (McCalla et al., 1962), which feeds, and induces the redifferentiation of additional nutritive cells in the outer layers (Meyer, 1987; Bronner, 1992; Ferreira et al., 2017a).

The oviposition is also the determinant factor for tissue modification in Coleoptera galls (Korotyaev et al., 2005). The adult females of Curculionidae excavate the host stems, oviposit and immediately leave plant tissues. Controlled experiments have shown that the females digging without oviposition is followed by fast reintegration of stem tissues. Accordingly, the oviposition fluids and the egg secretions were essential for the formation of hyperplasic and nutritive tissues in these galls (McCalla et al., 1962; Barnewall & De Clerck-Floate, 2012). However, the complete development of Coleoptera- and Hymenoptera-induced galls requires the larval feeding by

chewing the typical nutritive cells (Meyer, 1987; Rohfritsch, 1992; Barnewall & De Clerck-Floate, 2012).

V. Histological and histochemical convergences in galls

During gall development, the most the morphogenetic changes are, the most complex the gall is (Formiga et al., 2015; Ferreira et al., 2017a). Therefore, histochemical, histological, and histometric evaluations must be considered in order to determine the comparative complexity levels of galls (Ferreira et al., 2017a), considering distinct types of developmental alterations (Table 3).

With the comparison of galls induced by mites, nematodes and insects, not only the feeding habit, but also gall-inducing taxa, also determinate patterns in gall development. For example, nematodes and mites have similar feeding habits, and induce galls with several cytohistological distinctions, mainly their nutritive tissue types (Fig. 1). Mite-induced galls are usually simple, and a typical nutritive tissue is not always essential, while nematode-induced galls may have different levels of complexity. Nematode galls may have diverse nutritive tissues, whose patterns are related to the inducing species. Sucking animals, as eriophyids, nematodes, hemipterans, and thrips, induce distinct galls, according to their feeding sites on plant organs (Fig. 2a-e, Fig. 3a-b). Phloem-sucking insects, with long stylets, induce phloem hypertrophy, while superficial sucking animals, as thrips, mites, nematodes, and rasping and chewing insects may induce galls with typical nutritive tissues around the larval chamber. Usually, the gall-inducing individuals induce the growth and enrichment of those cells that are sources of their feeding. Simple galls of distinct groups result at least from one of these three basic processes: parenchyma homogenization, cell hypertrophy or hyperplasia (Fig. 1). Complex galls result from these processes and additional ontogenetic alterations.

A. Hyperplasia. The increasing cell divisions, hyperplasia, is observed in almost all galls (Fig. 1), and must be diagnosed by the increment of cell layers or number of cells per tissue area (Goodey, 1948; Mani, 1964; Meyer, 1987; Moura et al., 2008; Ferreira & Isaias, 2013; Ferreira et al., 2017a). In some cases, the intumescence of host organs results only from parenchyma hyperplasia (Goodey, 1948; Ferreira et al., 2017a).

Hyperplasia occurs mainly in the beginning of gall development, being persistent in most galls until the end of gall growth and development phase (Rohfritsch, 1992; Arduin & Kraus, 1995). The increment of cell layers occurs mainly before cell hypertrophy and differentiation, and contributes to the final shape of the galls, depending on its topographical concentration. The intumescence of a stem gall is due to the hyperplasia in the cortical cells, where periclinal divisions occur during growth and development phase (Goodey, 1948; Ferreira & Isaias, 2013). Similarly, the formation of pouch galls (Moura et al., 2009b; Oliveira & Isaias, 2010a; Álvarez, 2011; Dias et al., 2013a; Carneiro & Isaias, 2015), leaf rolling and folding galls (Souza et al., 2000; Arduin et al., 2005; Álvarez et al., 2009; Ferreira et al., 2017b) occurs by unequal hyperplasia and hypertrophy rates between the adaxial and abaxial ground meristems. In the formation of a covering gall (Arduin & Kraus, 1995), periclinal and oblique divisions originate emergences, which cover the gall-inducing egg, larva or nymph. In some galls, the hyperplasia rates are high until the end of the maturation phase, due to the constant feeding activity of the larva (Ferreira & Isaias, 2013).

B. Cell hypertrophy. Cell hypertrophy may occur either in outer or inner gall tissue compartments (*sensu* Bragança et al., 2017). In the most complex galls induced by nematodes, cell hypertrophy is limited to the inner compartment, with redifferentiation of giant nutritive cells (Bird, 1961; Watson & Shorthouse, 1979). Such cells are polynucleated, which may explain their great hypertrophy rates when compared to other galls (Jones & Northcote, 1972; Rodiuc et al., 2014). In mite-induced galls the hypertrophy may occur in the outer parenchyma cells, even though it is not a strict pattern (Moura et al., 2009b; Ferreira et al., 2017a). Nevertheless, insect-

induced galls usually have hypertrophic outer compartments with a large rate of cell hypertrophy (Kostoff & Kendall, 1929; Mani, 1964; Kraus et al., 2002; Álvarez et al., 2009; Isaias et al., 2011; Ferreira & Isaias, 2013, 2014; Suzuki et al., 2015), which develop at the end of growth and development phase or in the maturation phase. Outer parenchyma cells are usually vacuolated and store starch, being named the 'common storage tissue' (see next subsection), for they are not directly involved in the nutrition of the galling insect (Oliveira et al., 2011; Bragança et al., 2017; Ferreira et al., 2017a). Outer parenchyma cells may also accumulate secondary metabolites, being related to UV protection, defense against enemies, or to conferring distinct colors to the galls (Dias et al., 2013b; Bragança et al., 2017; Ferreira et al., 2017c). Phenolics and auxins have been co-localized in these tissue compartments, which have been associated with cell hypertrophy (Hori, 1992; Bedetti et al., 2014; Suzuki et al., 2015).

C. Hypertrophy of internal glands. The internal glands are common on some host plant families and the activity of the gall inducing agents causes their hypertrophy. Hypertrophied internal glands immersed on phloem commonly occur in galls induced by phloem-sucking hemipterans on Anacardiaceae (Álvarez, 2011; Dias et al., 2013a; Álvarez et al., 2014, 2016; Muñoz-Viveros et al., 2014). In this case, the hypertrophy of the internal glands accompanies the hypertrophy of the phloem, the primary feeding source of the inducing aphids, and therefore the responsive tissue in these galls. Other phloem-sucking insects induce the hypertrophy of secretory cavities non-associated to vascular bundles in host leaves (Arduin et al., 2005). The hypertrophy of secretory structures seems to accompany the common processes of hypertrophy and hyperplasia of the outer parenchyma cells (Arduin et al., 2005; Magalhães et al., 2014), and not necessarily the direct stimuli of the feeding activity. It may be an indirect response to a hormonal synergism (e.g., auxins and cytokinins) caused by translocation and consequent alteration of cell expansion and proliferation.

D. Parenchyma homogenization. Independent on the gall ontogenesis, the occurrence of parenchyma homogenization in galls induced by nematodes, mites, and insects is common (Fig. 1). It is observed in simple leaf rolling galls (Álvarez et al., 2013; Ferreira et al., 2017a), *filzgalls* (Ferreira et al., 2017a), leaf folding galls (Souza et al., 2000; Álvarez et al., 2009; Bedetti et al., 2013; Magalhães et al., 2014; Formiga et al., 2015), pouch galls (Moura et al., 2009b; Carneiro et al., 2014b; Carneiro & Isaias, 2015), covering galls (Ferreira et al., 2017a), and stem/root swelling galls (Kraus & Tanoe, 1999; Ferreira & Isaias, 2013). Parenchyma homogenization may be related to some developmental factors: (1) the overwhelming of gall-inducing stimuli upon the environmental and endogenous signals for cell elongation in the host plant organ; (2) the maintenance of some meristematic sites in gall tissues; and (3) the combination of high hypertrophic and hyperplasic rates all over the gall structure. These developmental factors isolated or grouped are commonly diagnosed in gall developmental sites.

Regarding gall development, some histological features reflect cytological aspects, such as the rearrangement of the cellulose microfibrils, which favors the homogenization of gall tissues, at least in the beginning of the development (Magalhães et al., 2014; Suzuki et al., 2015). Homogeneous parenchyma may persist until gall maturity and senescence (Moura et al., 2008, 2009b; Isaias et al., 2011; Ferreira & Isaias, 2014; Ferreira et al., 2017a), or change during the final phases of growth and development depending on the final gall morphotype (Ferreira & Isaias, 2013; Magalhães et al., 2014; Suzuki et al., 2015).

E. Changes in the indumentum density. A dense indumentum is usually related to the increment of a boundary layer in plants, which ends up decreasing the transpiration rates and reflecting the excessive light, as well as increasing the defenses against herbivores (Schreuder et al., 2001; Carmona et al., 2011). The increment of indumentum may occur in several gall systems (Ferreira et al., 2017a), and is usually related to the protection of the gall-inducing agent against abiotic stresses and natural enemies, even though these effects must be tested (Stone & Schonrögge, 2003).

The overdifferentiation of trichomes or emergences (Fig. 2b) may be the unique process involved in the formation of *filzgalls* (Fig. 1), which are considered the simplest mite galls for

they are characterized by the formation of dense indumentum and no or few other modifications (Mani, 1964). These galls are open, and the trichomes or emergences are usually the feeding sites of eriophyids (Mani, 1964; Ferreira et al., 2017a). The cells of these emergences or hypertrophied trichomes usually have a scanty protoplasm (Mani, 1964). In *filzgalls* induced on *Miconia ibaguensis*, these cells have no other cytological features of typical nutritive cells are observed such as granulose cytoplasm and hypertrophied nucleus, but accumulate reducing sugars (Ferreira et al., 2017a).

F. Feeding and storage tissues. We are considering, in this section, storage tissues in a late sense. Therefore, we will considerate in this section three types of storage tissues in galls: common storage tissues, typical nutritive tissues, and nutritive-like tissues (*sensu* Ferreira et al. 2017a).

Important developmental alterations in histological profiles of complex galls usually involve the increment and enrichment of the gall-inducer feeding tissues (Ferreira et al., 2017a). Most complex zoocecidia presents differentiation of specialized nutritive or reserve cells (Fig. 1). Typical nutritive tissues of complex nematode, mite and insect galls have cells with dense or granulose cytoplasm, conspicuous nucleus, which store proteins, reducing sugars and/or lipids, which are sources for the inducer's nutrition (Kendall, 1930; Bird, 1961; Dropkin, 1969; Westphal et al., 1981; Ferreira et al., 2015, 2017a, 2017b; Bragança et al., 2017). These cells are in direct contact with the inducers (Fig. 2c-e, Fig. 3a), and their redifferentiation may involve the process of metaplasia (Meyer & Maresquelle, 1983; Meyer, 1987), when the cells redifferentiate into cells with higher metabolic and cytological activities, without previous dedifferentiation and proliferation. The cytoplasm granulation (Fig. 3c), observed in these nutritive cells, may be consequence of an accumulation of ribosomes and enzymes, increment of Golgi apparatus and endoplasmic reticulum, and in the number of mitochondria, as observed in other Neotropical galls (Oliveira et al., 2011; Vecchi et al., 2013; Ferreira et al., 2015). A nutritive cell may have one or several nuclei, depending on the gall-inducing or host plant species (Mani, 1964). In nematode galls, independent of the host plant or organ, the nutritive tissues may have promeristematic and totipotent cells or giant polynucleated cells (Fig. 1). Polynucleated nutritive cells are usually very hypertrophied, as those giant cells of root-knot nematode galls (Bird, 1961; Jones & Northcote, 1972; Rodiuc et al., 2014). Cyst nematode galls have syncytial nutritive tissues, formed by the induced digestion of parenchyma cell walls (Rodiuc et al., 2014). Promeristematic nutritive tissues, i.e., with totipotent cells, are observed in galls of the nematode *Ditylenchus gallaeformans* on *Miconia* spp. (Fig. 2c) (Ferreira et al., 2017a, 2017b). Distinctly from most cases, the nucleus may be absent in nutritive cells of some galls, which was reported for the lepidopteran galls on *Tibouchina pulchra* (Vecchi et al., 2013), and the cecidomyiid horn-shaped galls on *Copaifera langsdorfii* (Fabaceae) (Oliveira et al., 2011). These cells are supposedly controlled by adjacent cells, similar to phloem sieve elements controlled by companion cells.

But how have the nutritive cells been stimulated to granulate? Their conspicuous nuclei and accumulation of high-quality reserves (Fig. 3c), with few unpalatable secondary metabolites, are similarities with meristematic cells, such as vascular cambium cells (Vecchi et al., 2013), or even bud cells (Ferreira et al., 2017b). Vascular cambium cells may store starch and lipids, and respond to several plant signals to begin or to cease cell division for a while (Evert, 2006; Begum et al., 2010). Maybe the gall inducers' stimuli are responsible for the maintenance of the meristematic features of these cells, representing a convergence among galls induced by phylogenetically distant organisms. Distinct taxa of galling organisms may induce the differentiation of granulose cells, even though their galls may be histologically very distinct. Contrary to the groups cited above, the phloem-sucking insects (as Psylloidea, Coccoidea and Aphididae) induce galls with hypertrophied phloem bundles (Fig. 3b). These galls do not have typical nutritive tissues (Larew, 1981; Bronner, 1992; Ferreira et al., 2017a), reflecting the direct influence of the feeding habit on the differentiation of nutritive cells. The increment in area of phloem in phloem-sucking galls should reflect the increment of sieve elements, companion cells, and/or transfer cells, which are nutrient-rich cells, and therefore are comparable to nutritive tissues in the galls induced by other zoocecidia.

As the inner tissue compartments of galls are usually related to the nutrition of the gall-inducing agents (Bragança et al., 2017), secondary metabolites may be suppressed or at least less accumulated in such gall tissues. For instance, an impairment of accumulation of terpenes and of phenolics occurs in the nutritive tissues of the Cecidomyiidae galls on *Piper arboreum* (Bragança et al., 2017), and of Lepidopteran galls on *Marcetia taxifolia* (Ferreira & Isaias, 2013), as well as in inner storage tissues of Psylloidea galls on *Psidium* spp. (Carneiro et al., 2014a).

In general, "common storage tissues" (Fig. 1, Fig. 2c-e, Fig. 3a), with vacuolated starch-rich cells, and peripheral cytoplasm (Ferreira et al., 2017a) largely occur in galls induced by distinct taxa (Álvarez et al., 2009, 2013; Oliveira & Isaias, 2010a; Isaias et al., 2011; Carneiro & Isaias, 2014, 2015; Ferreira et al., 2017a; Richardson et al., 2017). When these storage cells are cytologically similar to the typical nutritive cells of galls, but are not directly involved in the inducer's feeding, they are called "nutritive-like tissues" (Ferreira et al., 2017a; Richardson et al., 2017). The reserves accumulated in these cells are probably used for the energetic maintenance of gall structure (Oliveira & Isaias, 2010a). Some exceptions of this general pattern may occur, for example in galls induced by *Pachypsylla* spp., a piercing-sucking Psyllidae, on *Celtis occidentalis*. This insect feeds directly on nutritive polynucleated cells developed by the dissolution of cell walls, and not in phloem cells, as usually occur in hemipteran galls (Meyer, 1987).

G. Ambrosia galls. Some Cecidomyiidae galls do not have a typical nutritive tissue (Arduin & Kraus, 2001; Dorchin et al., 2002; Rohfritsch, 2008; Sá et al., 2009), instead, their larva feed on a mycelium inoculated in plant tissues by oviposition time (Arduin & Kraus, 2001; Stone & Schonrögge, 2003; Rohfritsch, 2008; Sá et al., 2009; Chao & Liao, 2013). The nutritive resources are provided by the hyphae, which are intimately associated with the surrounding plant cells (Fig. 1), forming a nutritive 'pseudoparenchyma' (Rohfritsch, 2008). Similar to the ectomycorrhizae associated to some plant roots, the hyphae do not penetrate the cells at gall developmental site, but surround cell walls, increasing the absorption surface (Nylund & Unestam, 1982; Münzenberger et al., 2012). In ambrosia galls, pseudoparenchyma plant cells with dense cytoplasm may be observed adjacent to the inoculated mycelium (Meyer, 1987; Arduin & Kraus, 2001; Chao & Liao, 2013). Similar to the ectomycorrhizae, these enriched plant cells may provide water and nutrients to the fungi, consumed by the gall-inducing cecidomyiid. Similarities between the ectomycorrhizae and the fungi of ambrosia galls need further studies in order to elucidate the physiological and ecological functions of the fungi in the associated gall systems.

The habit of inducing ambrosia galls is considered plesiomorphic in gall-inducing Cecidomyiidae, and the derived clades of gall inducers specialize in herbivory (Roskam, 1992; Arduin & Kraus, 2001; Rohfritsch, 2008). The complexity of ambrosia galls may vary according to their associated morphospecies, and they may be histologically simple and parenchymatic, or may be complex structures consisting of a mechanical layer and outer neo-formed tissues (Chao & Liao, 2013).

H. Outer tissue compartments. In general, the galls may be divided into outer and inner tissue compartments (Bragança et al., 2017). The inner compartments of several galls comprise the typical nutritive tissues, while the outer compartment is of general occurrence, and may constitute or not a common storage tissue (Fig. 1-3).

The outer compartment usually accumulates secondary metabolites, such as phenolic derivatives (hydrolysable tannins, flavonoids, anthocyanins), alkaloids, and terpenoids (Ferreira & Isaias, 2013; Carneiro et al., 2014a; Bragança et al., 2017). These secondary metabolites are supposed to function as protective barriers against excessive sun radiation and UV light (Dias et al., 2013a, 2013b; Bragança et al., 2017), as well as against the natural enemies of the galling organism (Stone & Schonrögge, 2003; Bragança et al., 2017). The type of monoterpenes and sesquiterpenes accumulated in galls such as those induced on *Lantana camara* (Verbenaceae) determines the choice of the galling *Aceria lantanae* (Eriophyidae) for plants with red flowers (Moura et al., 2008, 2009a, 2009b). The volatile compounds are important for the recognition of host plants and for gall induction. Once in contact with host plant tissues, the differential content

of terpenes of the host plants requires an adaptive development for the inducers, which must be able to detoxify for their successful gall induction and establishment.

The cells of the gall outer compartments may have more rigid walls than those of the inner compartments due to their distinct lignin and pectin composition (Formiga et al., 2013; Oliveira et al., 2014; Carneiro et al., 2015). As the cells of the gall outer tissues are usually hypertrophied, it is also possible that the pectin composition influence water uptake to these cells. The rigidity of gall walls may also be guaranteed by cell wall lignification of sclereids redifferentiated from parenchyma cells (Álvarez et al., 2009, 2016; Carneiro et al., 2014a, 2014b, 2015; Kurzfeld-Zexer et al., 2015). The sclerenchymatic cells may also provide mechanical protection for the gall inducers against natural enemies in maturation phase (Stone & Schonrögge, 2003; Carneiro et al., 2014b).

A sclerenchyma layer with sclereids or fibers may occur between the typical nutritive tissue and the outer compartments in insect galls (Fig. 1), mainly Diptera and Hymenoptera galls (Arduin et al., 2005; Oliveira et al., 2011, 2016; Castro et al., 2012; Fleury et al., 2015; Amorim et al., 2017). The differentiation of sclerenchymatic cells may rely on the morphogenetic pattern of the host plant (Ferreira & Isaias, 2014). Mechanical layers may differentiate from the vascular cambium or pericyclic cells, similarly to the non-galled host organs (Fig. 2d) (Bedetti et al., 2013; Ferreira & Isaias, 2013). The differentiation of sclerenchyma layers surrounding the gall nutritive or nutritive-like tissues seems to be consequence of the accumulation of reactive oxygen species (ROS) due to the feeding activity of the gall inducers (Isaias et al., 2015; Oliveira et al., 2016). The ROS are captured by cinnamyl alcohols (precursors of lignins) and immobilized when deposited in cell walls (Barceló, 1997; Apel & Hirt, 2004). Outside the sclerenchymatic cells, specialized types of parenchyma, as an aerenchyma, for instance, may differentiate in gall outer compartment, and favor gas exchanges among the cells in galls deprived of stomata (Amorim et al., 2017). A collenchyma may also differentiate in gall outer layers as a conservative feature of the host organs (Amorim et al., 2017), or as a neoformed feature (Goodey, 1948).

I. Changes in dermal system. Some galls outer epidermal pavement cells may be suberized (Kraus et al., 2002) or metacutinized (Oliveira & Isaias, 2010b; Formiga et al., 2011), protecting the gall from desiccation (Isaias et al., 2013). Moreover, distinct epidermal appendices, such as trichomes, may be related to mechanical protection against natural enemies and stabilization of the microclimate inside the gall (Moura et al., 2008; Dias et al., 2013a). Stomata can also be deformed or suppressed in galls induced by nematodes, mites and insects (Goodey, 1939; Moura et al., 2008; Álvarez et al., 2009, 2016; Dias et al., 2013a; Amorim et al., 2017), which affect gas exchanges in gall outer compartments. Inner epidermal cells, limiting the chamber, in phloem-sucking insect galls may have a reduced cell surface area and thin cuticle (Dias et al., 2013a; Carneiro & Isaias, 2014), facilitating the feeding of the galling organism (Carneiro & Isaias, 2014).

As commented in other sections, metaplasia may occur during induction and development of the galls under the feeding stimuli of gall inducers. The epidermal cells of Eriophyidae and Cecidomyiidae galls may directly redifferentiate into nutritive cells, or nutritive hairs (Westphal et al., 1981; Oliveira & Isaias, 2010b), without previous dedifferentiation and proliferation of these cells (Meyer & Maresquelle, 1983).

J. Promeristems. The cell layers lining the larval chamber may keep their meristematic feature. Moreover, some galls maintain promeristems or totipotent regions capable of redifferentiating the three plant tissue systems (Ferreira et al., 2017b). The maintenance of a unique promeristematic nutritive tissue implies in the longevity of *D. gallaeformans* galls on *Miconia* spp. (Fig. 2c), once it allows the replication of several generations of nematodes in the same gall (Ferreira et al., 2017a, 2017b). Accordingly, lateral buds differentiated in rosette galls of *Pisphondylia brasiliensis* (Cecidomyiidae) on *Guapira opposita* (Nyctaginaceae) produce true chlorophyllous leaf primordia (Fleury et al., 2015). The activity of these lateral buds is distinct from that of the non-galled buds, for instead of ordinary leaves they produce modified leaves responsible for the rosette shape of the galls.

VI. Conclusions

Plant development follows the morphogenetic patterns determined in plant meristems, which however can be manipulated by galling organisms, leading to the overdifferentiation or inhibition of some plant features, as well as the differentiation of distinct cell types. Gall development obeys the inducer's feeding chemical and mechanical stimuli toward convergent anatomical traits observed in galls induced by at least 50 independently evolved animal groups. Even though galls may be considered extended phenotypes of their inducers (Carneiro et al., 2015), their host plant genome and cell machinery may be stimulated in similar ways related both to the mode of feeding and feeding sites (Fig. 1). This concept is aligned with galls as new plant organs, as stated by Shorthouse et al. (2005, p. 407): "*they* [the insect galls] *are in a sense new plant organs because it is the plant that produces the gall in response to a specific stimulus provided by the invading insect. Each species of inducer produces galls that are anatomically and physiologically different from those induced by other related species".* The combination of anatomical convergent traits in each gall system culminates in the extant gall structural diversity.

We assume galls as elegant models for developmental studies due to their constant and repetitive cycles in nature, and hope such studies may help elucidating the roles of signaling molecules in developmental processes. The studies focusing on the developmental patterns of galls induced by unrelated animal taxa on related host plant species should help elucidating the pathways on plant cell redifferentiation. We also propose that structurally simple galls, i.e., with few anatomical alterations, should be the starting model of study on gall developmental transcriptome.

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Table 1. Representative cecidozoa (gall-inducing animals) clades and general features.

Phylum	Order (n. of galling families)	Main groups	Feeding habit	Feeding tissue	Aggregation habit	Main host organs
Nematoda	Tylenchida (4)	Heteroderidea	Piercing-sucking	Giant NC	Colonial	Root
		Meloidogynidae	Piercing-sucking	Giant NC	Colonial	Root
		Anguinidae	Piercing-sucking	NT	Colonial	Leaf, Seed, Stem, Root
Arthropoda	Trombidiformes (4)	Tarsonemidae	Piercing-sucking	NT	Colonial	Leaf
		Eriophyidae	Piercing-sucking	NT	Colonial	Leaf, Bud
	Thysanoptera (2)	Phlaeothripidae	Piercing-sucking	NT	Colonial	Leaf, Bud
		Thripidae	Piercing-sucking	NT	Colonial	Leaf, Bud
	Hemiptera (11)	Psylloidea [a]	Piercing-sucking	Phloem	Solitary	Leaf
		Aphididae	Piercing-sucking	Phloem	Colonial	Leaf
		Phylloxeridae	Piercing-sucking	NT	Colonial	Leaf Root
		Adelgidae	Piercing-sucking	NT	Colonial	Bud, Leaf
		Coccoidea [a]	Piercing-sucking	Phloem or Parenchyma	Solitary	Leaf
	Coleoptera (8)	Curculionoidae [a]	Chewing	NT	Solitary	Stem, Bud, Leaf, Root
	Hymenoptera (5)	Cynipidae	Chewing	NT	Solitary / Coalescent	Leaf, Bud, Stem, Root
		Chalcidoidea [a]	Chewing	NT	Solitary / Coalescent	Leaf, Stem, Flower
		Tenthredinidae	Chewing	NT	Solitary	Leaf
	Lepidoptera (20)	Tortricoidea [a]	Chewing	NT	Solitary	Stem, Bud, Leaf
	Diptera (6)	Cecidomyiidae	Sucking disrupted-cell fluids (extracorporeal digestion)	NT	Solitary	Leaf, Bud, Stem, Flower, Fruit, Root

[a] Superfamilies with more than one important galling family. Abbreviations: NC = Nutritive cells; NT = nutritive tissue. Less representative families were suppressed. Note that there are exceptions for each case.

Sources: Mani (1964), Meyer (1987), Roskam (1992), Mound (1994), Williams (1994), Wool (2004), Raman et al. (2005), Sano & Akimoto (2011), Burckhardt & Queiroz (2012), Fernandes et al. (2012).

Table 2. Comparison among feeding habit, type of gall induction and aggregation habit.

Feeding habit	Gall induction	Aggregation habit	
		Colonial gall groups	**Solitary gall groups**
Piercing-sucking (NT)	Feeding of matrix female and offspring (by penetration in meristems, or on meristematic surfaces)	• Nematoda: Anguinidae	-
Piercing-sucking (NT)	Feeding of matrix female and offspring	• Acarina: Eriophyidae • Insecta: Hemiptera: Adelgidae	-
Piercing-sucking (NT/epidermal cells)	Oviposition inside plant tissues and/or feeding of matrix female and offspring	• Insecta: Thysanoptera	-
Piercing-sucking (phloem)	Mainly feeding of fundatrix	• Insecta: Hemiptera: Aphididae	-
Piercing-sucking (phloem)	Feeding of the nymph	-	• Insecta: Hemiptera: Psylloidea
Piercing-sucking (NT)	Feeding of fundatrix	• Insecta: Hemiptera: Phylloxeridae	-
Piercing-sucking (phloem or NT)	Feeding of the nymph	-	• Insecta: Hemiptera: Coccoidea
Chewing (NT)	Oviposition and/or feeding of the larva	-	• Insecta: Coleoptera • Insecta: Hymenoptera
Chewing (NT)	Feeding of the larva	-	• Insecta: Lepidoptera
Rasping-sucking (NT)	Feeding of the larva	-	• Insecta: Diptera: Cecidomyiidae

Sources: Felt (1936), Mani (1964), Meyer (1987), Mound (1994), Wool (2004), Raman et al. (2005), Sano & Akimoto (2011), Burckhardt & Queiroz (2012).

Table 3. Indicators of complexity in a gall.

Complexity level	Morphogenetic steps	Examples
Simple	Parenchyma homogenization / changes in patterns of cell elongation	• Changes from dorsiventral mesophyll to homogeneous mesophyll • Changes in patterns of cell elongation
	Tissue hyperplasia	• Intense cell divisions during growth and development • Increase in number of parenchyma layers
	Overdifferentiation of trichomes or emergences	• Changes of indumentum density
	Parenchyma hypertrophy	• Parenchyma cell hypertrophy • Hypertrophy of inner secretory cells and cavities
Intermediary	Redifferentiation of a common storage tissue	• Presence of an outer parenchyma with vacuolated cells and storing starch
	Redifferentiation of a 'typical nutritive tissue'	• Cell layers surrounding gall chamber, with dense cytoplasm and feeding reserves (proteins, lipids or soluble sugars) • Giant polynucleated nutritive cells, polynucleated nurse cells, syncytial nutritive tissue (usually in nematode galls)
	Hypertrophy of vascular bundles	• Hypertrophy of phloem and xylem bundles (usually in phloem-sucking insect galls)
Complex	Redifferentiation of a 'nutritive-like tissue'	• Cell layers with dense cytoplasm, accumulation of starch and other metabolites, not directly eaten by the inducers (usually in phloem-sucking insect galls)
	Presence of mechanical layers	• Presence of sclerenchyma layers surrounding nutritive tissues • Presence of outer sclerenchyma layers
	Redifferentiation of other ground system cell types	• Differentiation of cells or tissues absent in the host organ (aerenchyma, collenchyma, hypodermis, spongy parenchyma, isolated sclereids, idioblasts, secretory tissues, etc.)
	Changes in epidermis	• Suppression of stomata differentiation / changes in stomatal pattern • Suppression of a trichome type / changes of trichome types • Stratification of epidermis • Epidermal holes/channels for re-absorption of the honeydew (some aphid galls)
	Promeristematic activity	• Axillary meristems capable of originate new leaf primordia • Nutritive cells capable of redifferentiating cells of ground, dermal and vascular systems, providing indeterminate growth

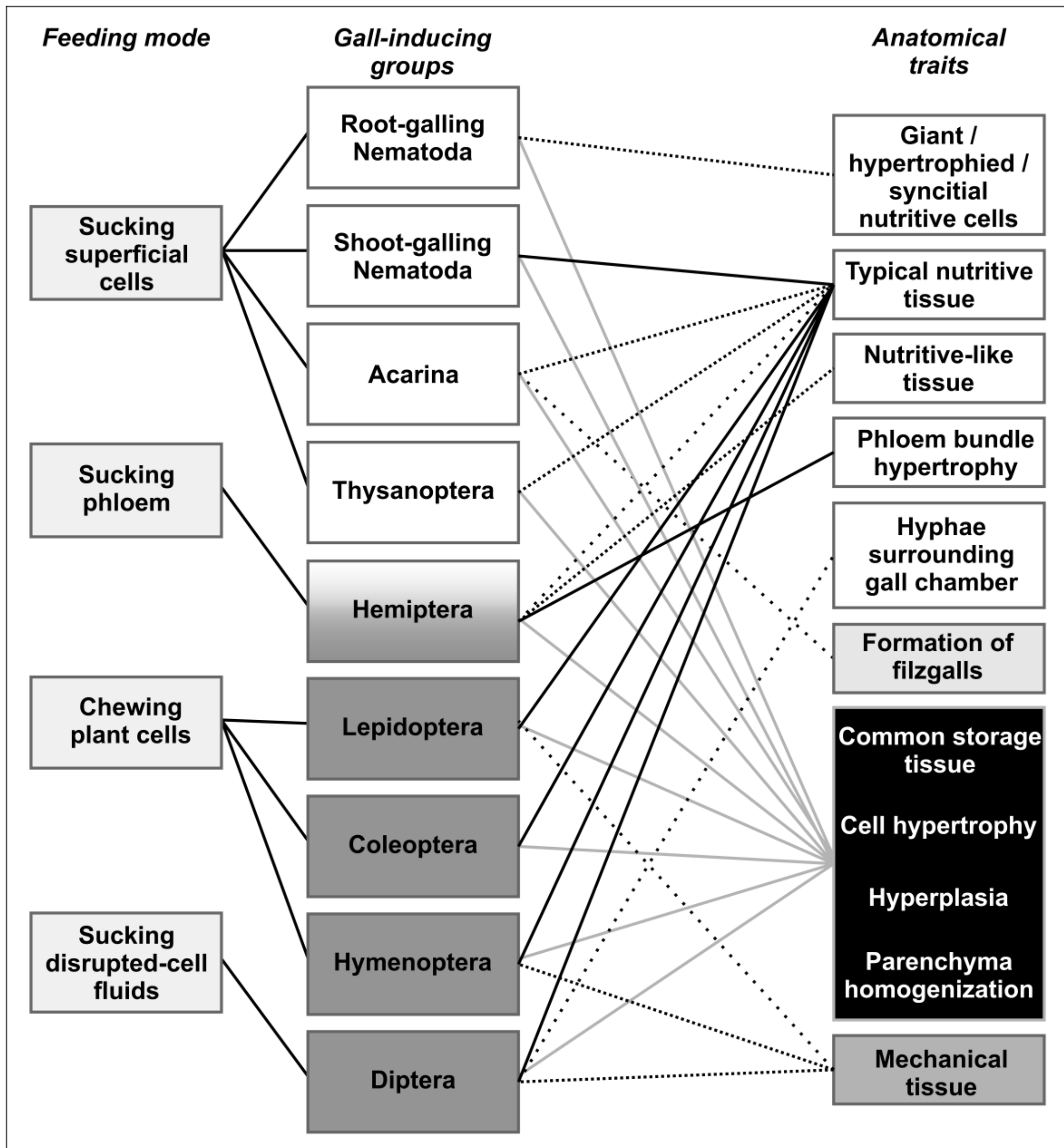

Fig. 1. Network of feeding modes, gall-inducing groups, and gall anatomical traits. In the *gall-inducing groups*, clear boxes indicate groups with colonial galls and darker boxes indicate groups with solitary galls. Uninterrupted lines indicate more frequent anatomical traits, and dashed lines indicate less frequent traits. The more spaced are the dashed lines, less frequent is the indicated feature. In *anatomical traits*, clear boxes are related to feeding tissues, the black box indicate the anatomical traits common to galls induced by all groups of animals, and the other boxes are features occurring in galls of a restricted gall-inducing taxon.

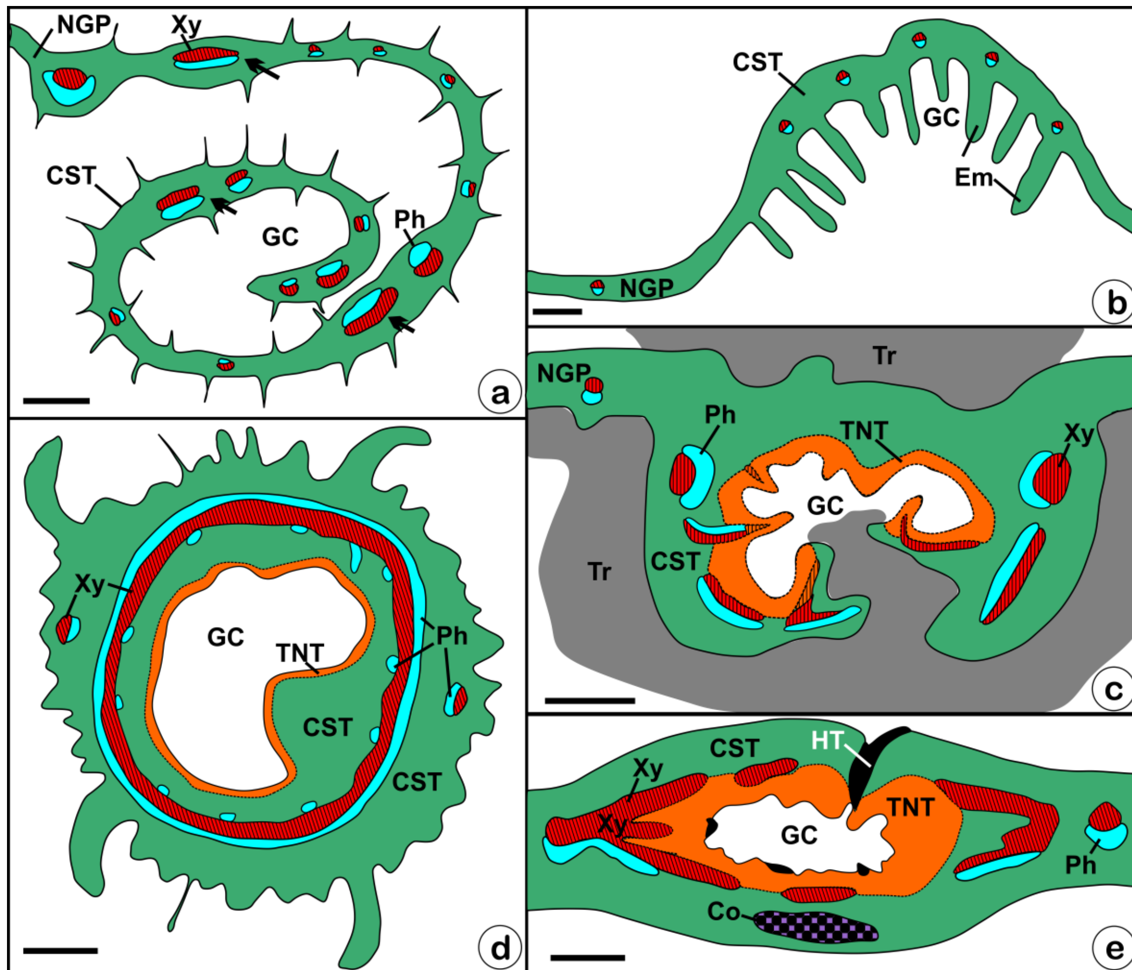

Fig. 2. Schematic drawings of cross-sectioned galls induced by insects, mites and nematodes. (a) Leaf-rolling gall induced by *Eriosoma ulmi* (Hemiptera: Aphididae) on *Ulmus minor* (Ulmaceae). Hypertrophied vascular bundles (arrows) in hypertrophic regions of this gall. (b) *Filzgall* induced by an unidentified Eriophyidae on leaves of *Miconia ibaguensis* (Melastomataceae). The overdifferentiated indumentum is formed by emergences. (c) Leaf gall induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans* (Melastomataceae). The trichomes are longer and denser when covering galls, and occur also in adaxial surface of epidermis, which does not occur in non-galled portions of leaves. (d) Fusiform stem gall induced by unidentified Lepidoptera on *Marcetia taxifolia* (Melastomataceae). (e) Lenticular leaf gall induced by *Anguina balsamophila* (Nematoda) on *Wyethia amplexicaulis* (Asteraceae). Abbreviations: CST, common storage tissue; Em: emergence; GC: gall chamber; HT: healing tissue; NGP, non-galled portion of leaf; Ph, phloem; TNT, typical nutritive tissue; Tr: trichomes; Xy, xylem. Scale bars: (a), (c), (d) 500 µm; (b) 200 µm; (e) 3mm.

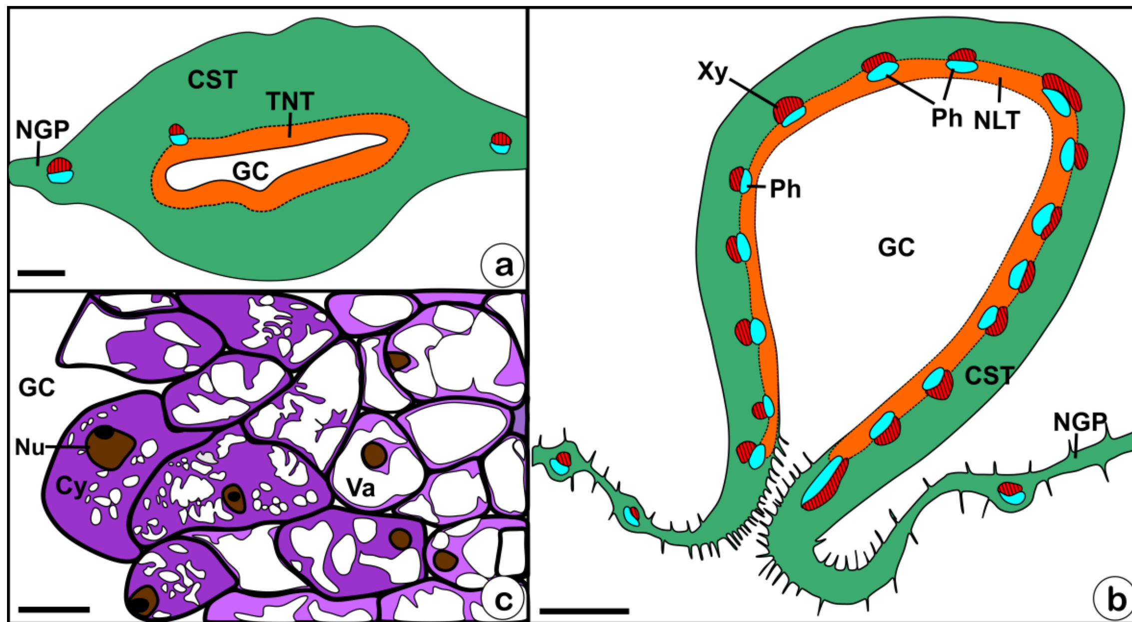

Fig. 3. Schematic drawings of galls showing histological patterns of galls and of a typical nutritive tissue. (a) Cross-sectioned lenticular gall induced by *Aceria tristriata* (Eriophyidae) on *Juglans regia* (Juglandaceae). (b) Globoid gall induced by *Tetraneura ulmi* (Aphididae) on *Ulmus minor* (Ulmaceae), longitudinal section. (c) Detail of nutritive cells of fusiform galls induced on the midribs of *Eucalyptus camaldulensis* (Myrtaceae) leaves by *Leptocybe invasa* (Hymenoptera: Eulophidae). The typical nutritive tissue shows a gradient of density of the cytoplasm (shown by darker shades of purple) towards the gall chamber; and smaller vacuoles (white) near gall chamber. Typical nutritive cells in contact with the inducer have more prominent nuclei and nucleoli. Abbreviations: CST, common storage tissue; Cy: cytoplasm; GC, gall chamber; NGP, non-galled portion of leaf; NLT, nutritive-like tissue; Nu: nucleus; Ph: phloem; TNT, typical nutritive tissue; Va: vacuole; Xy: xylem. Scale bars: (a) 200 µm; (b) 500 µm; (c) 20 µm.

Capítulo 2

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

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ARTICLE

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, whereas 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 to the types of storage tissue they have. Histological, histometric, and histochemical analyses were used to compare six gall systems with 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 into 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 a 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.

Résumé : Les Aphididae inducteurs de la galle peuvent se nourrir directement sur le phloème, alors que les Eriophyidae et les nématodes se nourrissent sur les cellules qui bordent les chambres des galles. Les auteurs assument qu'il y aura une variation dans la complexité structurale à l'intérieur des galles en fonction de chaque taxon inducteur, et que la complexité des galles pourrait être reliée aux types de tissu de stockage qu'elles possèdent. Des analyses histologiques, histométriques et histochimiques ont été utilisées afin de comparer six systèmes de galle, qui possèdent différents niveaux de complexité. Ces niveaux ne sont pas reliés au taxons, même si les galles des ériophyides sont habituellement plus simples que les galles des nématodes et des aphidiens. Les caractéristiques histologiques des galles ont permis de classer les tissus de stockage en trois types : tissus nutritifs typiques (TNT), tissus communs de stockage (TCS) et tissus ressemblant aux tissus nutritifs (TRN). Le TNT et le TRN possèdent des cellules à cytoplasme dense et à noyau important. Les cellules du TCS sont vacuolées et elles peuvent stocker l'amidon et d'autres molécules riches en énergie, comme les cellules du TRN. Contrairement au TRN ou au TCS, le TNT sert directement de source alimentaire pour les inducteurs de galle, et il est présent dans les galles induites par les nématodes et quelques galles induites par les ériophyides. Les TRN peuvent être présents dans quelques galles induites par les aphidiens, mais ils ne constituent pas le site direct d'alimentation. Les TCS sont présents chez les galles induites par les trois taxons. [Traduit par la Rédaction]

Mots-clés : galles d'aphidiens, galles d'ériophyides, galles de nématodes, tissu nutritif, cellule de stockage.

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 nonrelated 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

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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: (*i*) those induced by chewing (Lepidoptera and Hymenoptera) and scraping–sucking insects (Diptera: Cecidomyiidae); and (*ii*) those induced by sucking insects (Hemiptera) (Weidner 1961 cited in 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 (nonprominent 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. 2017). The stylets of these inducers are shorter than the stylets of the hemipterans, and they are accordingly only capable of feeding on the cells immediately adjacent to the chamber (Dropkin 1969; Westphal et al. 1981).

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 the 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 whether 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.

Materials 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 *v*/*v*) (Kraus and Arduin 1997), or with 1% safranin and 1% fast green (Johansen 1940) to distinguish cell walls with lignin or suberin, vacuoles with phenolics, and nucleus (red stained) from pectocellulosic cell walls and cytoplasm (blue stained). Some samples were embedded in glycolmethacrylate (Historesin; Leica), sectioned (5 μm), and stained with toluidine blue O (O'Brien et al. 1964) to distinguish pectocellulosic walls (purple) from lignified walls and vacuoles with phenolics (green). After staining, the slides were dehydrated and mounted in clear varnish (Paiva et al. 2006) or Entellan.

Histochemistry

Hand-made sections were treated with Lugol's reagent to detect starch (Johansen 1940), Fehling's reagent for

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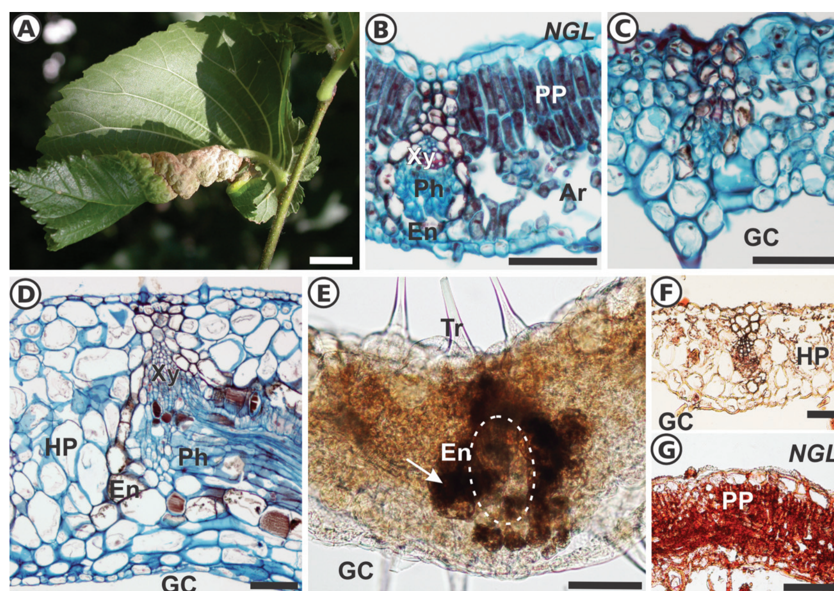
Table 1. Galls studied.

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

Fig. 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 (broken line, brown precipitates) and endodermis (arrow). (F) Low accumulation of proteins in galls. (G) Accumulation of proteins in controls. 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. Scale bars: (A) 5 mm; (B–G) 100 µm. Staining: safranin–fast green (B–D); Fehling's reagent (E); Ponceau 2R (F and G). [Colour online.]



reducing sugars (Sass 1951), Ponceau 2R for proteins (Ventrella et al. 2013), or Sudan red B for lipids (Jensen 1962).

Histometry

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 by Student *t* test using the software SigmaStat (when raw or transformed data showed a normal distribution and were homoscedastic) or a Mann–Whitney *U* 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-hoc test (normal and homoscedastic data) or Kruskal–Wallis test followed by Dunn's post-hoc test (non-normal and (or) non-homoscedastic data even after transformations). We considered the differences to be statistically significant for values of $p < 0.05$.

Results

Aphid galls

Eriosoma ulmi

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

Table 2. Histometry and histochemistry in controls and aphid galls (*Eriosoma ulmi* and *Geoica utricularia*) induced on *Ulmus minor* and *Pistacia terebinthus*.

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

Note: 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, μm²; cell layers, number. Histochemistry results: –, absence; +, ++, +++, semiquantitative levels of presence; NA, not applicable.

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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 (Figs. 1F and 1G) but no starch was detected in the gall tissues (Table 2). The storage tissues were not differentiated (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 (Figs. 2C and 2E), and is significantly hypertrophied in galls (Table 2). Both phloem bundles of the galls have secretory ducts (SD) (Figs. 2C and 2E), whereas the supernumerary bundles of the controls lack the secretory duct associated with the phloem (Fig. 2B).

Nematode galls

Ditylenchus gallaeformans on *Miconia albicans*

The globoid verrucous galls are induced on leaves (Figs. 3A and 3B), stems and inflorescences of *M. albicans* (Melastomataceae). Colonies of nematodes are surrounded by gall emergences (Figs. 3C–3E). The outer epidermis is not in contact with the colonies, and is densely covered with arachnoid trichomes (Figs. 3C–3E). 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 (Figs. 3C–3G), 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 (Figs. 3E and 3F). The cells in contact with the larvae are meristematic, and originate new emergences that grow to surround the nematode colonies (Figs. 3D and 3E). The cells of the inner parenchyma and epidermis of the nematode chamber constitute the nutritive tissue (Figs. 3F and 3G). These cells are smaller than the cells of the non-galled leaf portions (Figs. 3E and 3F; Table 4), and have a dense granulose cytoplasm, a prominent nucleus, and thin cell walls (Figs. 3F and 3G). 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* (Figs. 5A and 5B). Emergences surround the colonies of nematodes (Figs. 5B and 5C). 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 (Figs. 5B and 5C). 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 (Figs. 5C

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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			
	Eriosoma ulmi		*Geoica utricularia*		on *Miconia 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

Note: 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 (Reserves): –, absent; +, ++, +++, semiquantitative levels of presence. Cytological features: –, absent; +, present.

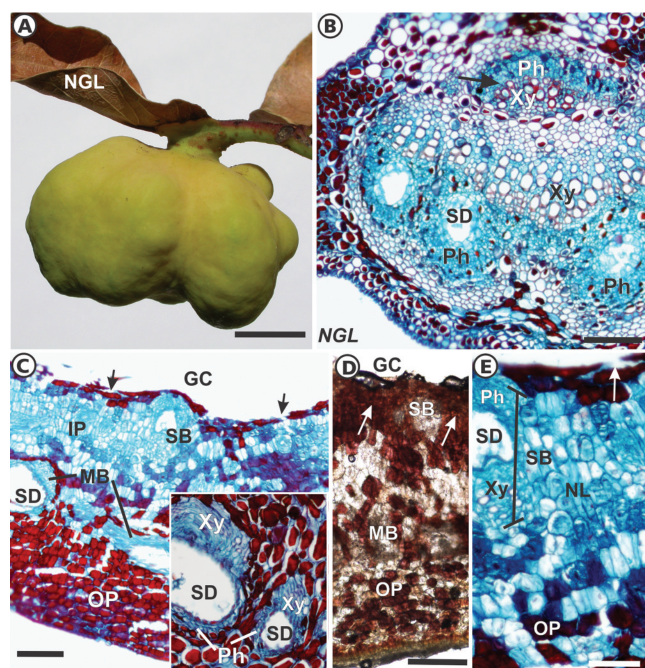

Fig. 2. Non-galled leaflet midrib and gall of *Geoica utricularia* on *Pistacia terebinthus.* (A, C, D, and E) Globoid gall associated with midrib. (B–D) Transverse sections. (B) Midrib of a non-galled leaflet (NGL), with main and supernumerary (arrow) vascular bundles. (C) Gall with main (MB) and supernumerary 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 are shown in the inset. (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). GC, gall chamber; Ph, phloem; SD, secretory duct; Xy, xylem. Scale bars: (A) 1 cm; (B and C) 100 μm; (D) 200 μm; (E) 50 μm. Staining: safranin–fast green (B, C, and E); Fehling's reagent (D). [Colour online.]

and 5D). 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 (Figs. 5A and 5B). 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 (Figs. 6A and 6B), with a dense indumentum on the abaxial surface (Figs. 6C and 6D), where the mites live and feed. Sometimes a dense indumentum may also develop on the adaxial surface. The galls have homogeneous parenchyma (Figs. 6D and 6E), with no alterations in the number of cell layers or in the thickness of the mesophyll compared with 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 (Figs. 6E–6G). The emergences are considerably larger and denser than in the controls (Figs. 6D and 6E) (Table 5). The mites feed on vacuolated cells of the epidermis located among and on the bases of the emergences (Figs. 6E and 6F; 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).

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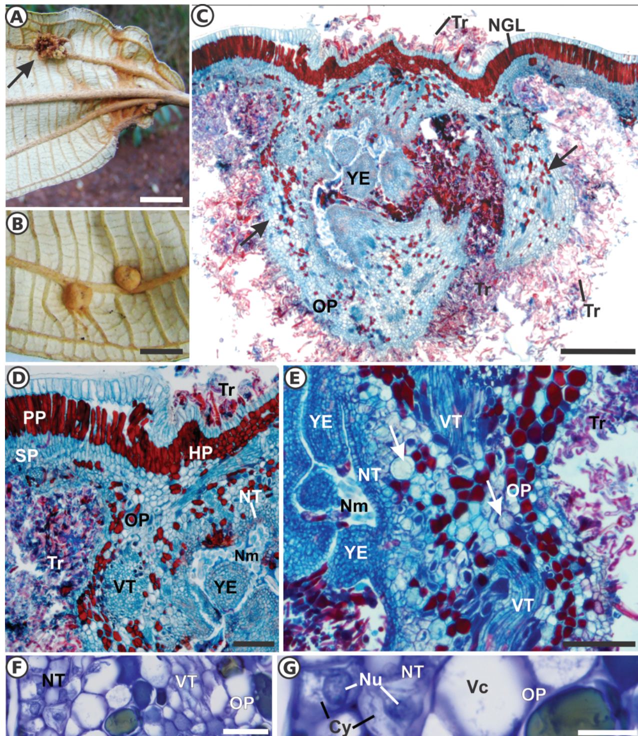
Fig. 3. Galls of *Ditylenchus gallaeformans* on *Miconia albicans.* (A and B) Globoid galls induced on abaxial surface of *M. albicans.* (A) Older verrucous gall (arrow). (B) Younger gall. (C) Gall with overlapped curved emergences (arrow). (D) Transition zone between non-galled leaf with palisade and 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 and G) Differences among cells of nutritive tissue, with denser cytoplasm and prominent nucleus, and outer parenchyma, with larger and vacuolated cells. 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. Scale 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 O (F and G). [Colour online.]



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Aceria tristriata

The galls are small and lenticular (Figs. 7A and 7B). 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 (Figs. 7D–7F), and accumulate a considerable amount of proteins (Fig. 7E) and some oil drops (Table 3).

Table 4. Histometry and histochemistry in controls and nematode galls (*Ditylenchus gallaeformans*) induced on *Miconia albicans* and *M. ibaguensis*.

	D. gallaeformans – *M. albicans*		*D. gallaeformans* – *M. ibaguensis*	
	Control	Gall	Control	Gall
Wall thickness	143.25b	302.12a	174.09b	409.65a
Adaxial/outer epidermis				
Cell area	604.65a	148.54b	237.82a	168.11b
Trichomes	–	+++	–	+++
Parenchyma				
Cell layers	5.67b	15.39a	8.20b	20.20a
Cell area				
Adaxial	701.50a	528.67a	960.71a	883.92a
Abaxial	139.61b	84.78b	312.81b	128.13c
Starch	++	+	++	+
Reducing sugars	+	+++	+	+++
Proteins	+	+	+	+
Oil	+	–	+	–
Abaxial/nutritive epidermis				
Cell area	92.37a	63.92b	151.77a	113.83b
Trichomes	++	–	++	–

Note: 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, μm²; cell layers, number. Histochemistry results (starch, reducing sugars, proteins, oil): –, absence; +, ++, +++, semiquantitative levels of presence.

The non-nutritive parenchyma cells are not in contact with the mites, and have large vacuoles and a peripheral cytoplasm (Figs. 7D and 7G). Starch and reducing sugars accumulate in the outer parenchyma cells (Tables 3 and 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 with nem-

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Fig. 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. 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. Scale bars: (A) 200 μm; (B) 50 μm; (C) 500 μm. Staining: safranin–astra blue (A); Lugol (B); Fehling's reagent (C). [Colour online.]

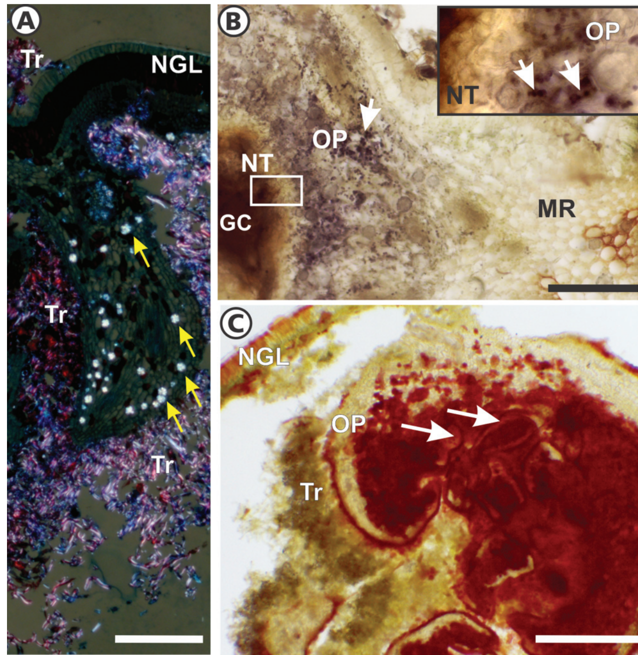
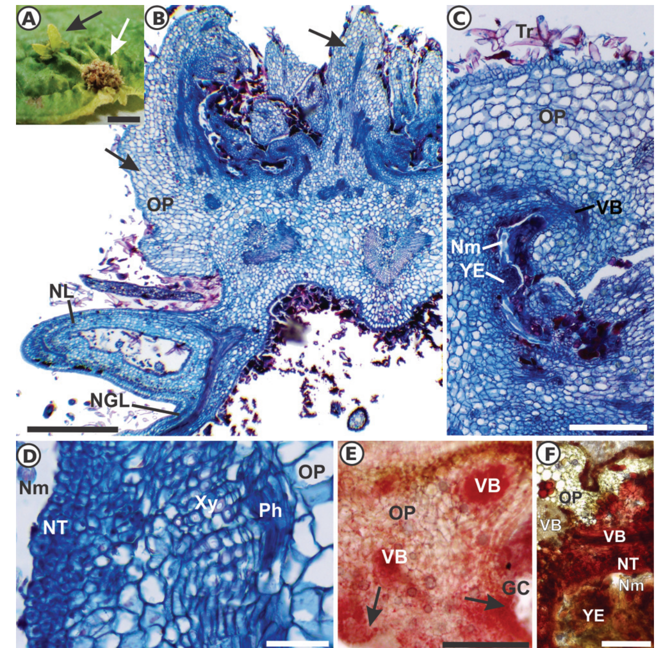


Fig. 5. Galls of *Ditylenchus gallaeformans* on *Miconia ibaguensis*. (A) Globoid verrucous galls (white arrow) induced on adaxial surface, with leafy neoformations (black arrow). (B–F) Transverse sections. (B) Gall with curved vascularized emergences (arrows) and leafy neoformation. (C) Details of mature 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. 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. Scale bars: (A) 5 mm; (B) 500 μm; (C, E, and F) 200 μm; (D) 50 μm. Staining: safranin–astra blue (B–D); Ponceau 2R (E), and Fehling's reagent (F). [Colour online.]



atode 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; Kutsukake et al. 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, which is a novelty by comparison with the other gall systems (Ferreira et al. 2017). 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 developmen-

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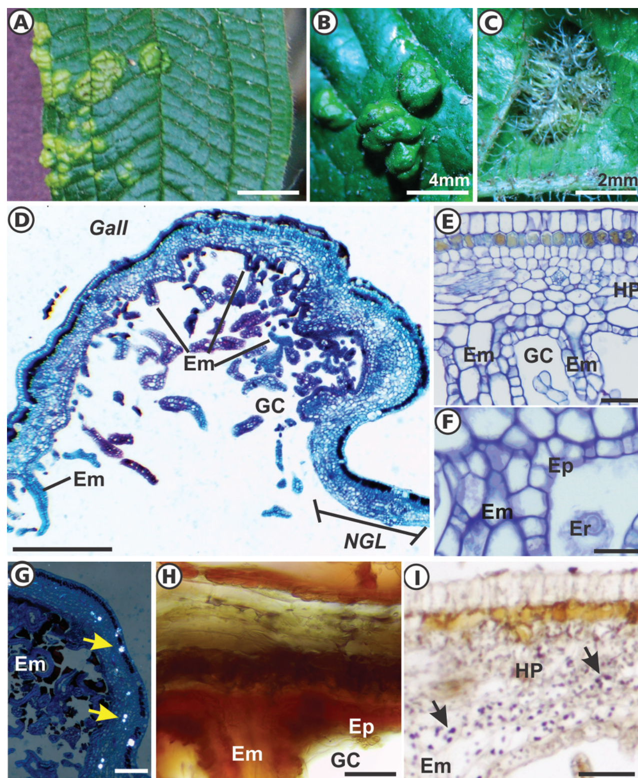
Fig. 6. Gall of Eriophyidae on *Miconia ibaguensis*. (A–C) Blister-shaped galls, with overdifferentiated indumentum in abaxial region (C). (D–I) Transverse sections of galls. (D) Blister gall in continuity with non-galled leaf region (NGL). Several neoformed emergences are visible in abaxial region. (E and 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. Em, emergences; Er, eriophyid; Ep, epidermis; GC, gall chamber; HP, homogeneous parenchyma; NGL, leaf non-galled region; NT, nutritive tissue; OP, outer parenchyma. Scale bars: (A) 1 cm; (B) 4 mm; (C) 2 mm; (D) 500 μm; (E, H, and I) 50 μm; (F) 25 μm; (G) 200 μm. Staining: astra blue–safranin (D and G); toluidine blue O (E and F); Fehling's reaction (H); Lugol (I). [Colour online.]



tal 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 accordance 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

Table 5. Histometry and histochemistry in controls and eriophyid galls induced on *Miconia ibaguensis* and *Juglans regia*.

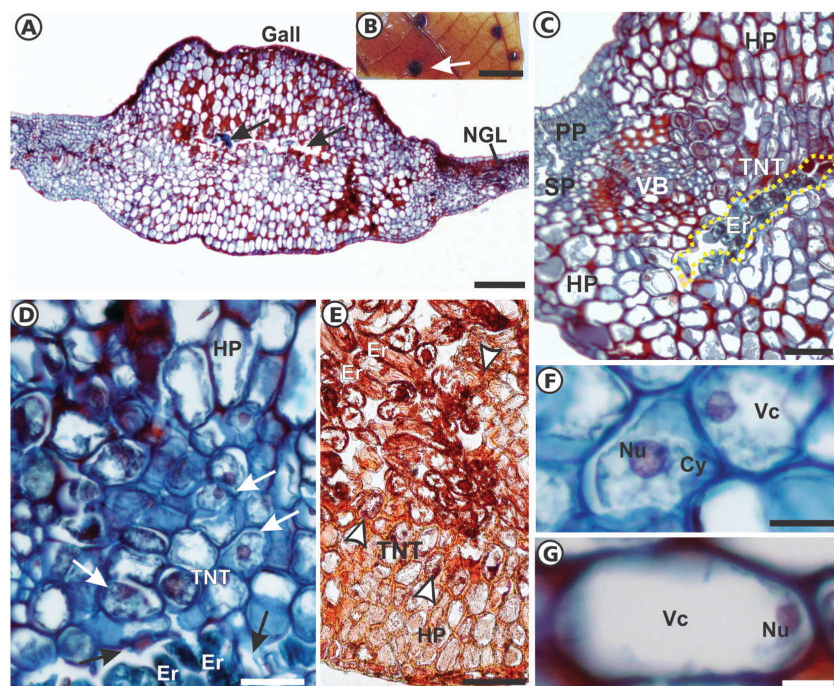
	Eriophyidae – *M. ibaguensis*		*Aceria tristriata* – *J. regia*	
	Control	Gall	Control	Gall
Wall thickness	174.09a	154.63a	127.42b	782.06a
Adaxial epidermis				
Cell area	237.82a	328.17a	241.07b	393.90a
Parenchyma				
Cell layers	8.20a	7.60a	5.80b	18.70a
Cell area				
Adaxial	960.71a	319.02b	197.75b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

Note: 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 (starch, reducing sugars, proteins, oil): –, absence; +, ++, +++, semiquantitative levels of presence.

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. 2017; 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 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 neoforma-

Fig. 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. 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. Scale bars: (A) 200 μm; (B) 5 mm; (C and E) 100 μm; (D) 50 μm; (F and G) 10 μm. Staining: safranin–fast green (A, C, D, F, and G); Ponceau 2R (E). [Colour online.]



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tion 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*, which is 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). Our proposal for standardized terminology 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. 2017), 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, which is 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).

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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, 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 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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Capítulo 3

Totipotent nutritive cells and indeterminate growth in galls of *Ditylenchus gallaeformans* (Nematoda) on reproductive apices of *Miconia*

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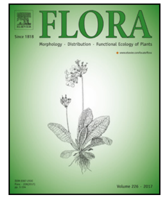

Original Research

Totipotent nutritive cells and indeterminate growth in galls of *Ditylenchus gallaeformans* (Nematoda) on reproductive apices of *Miconia*

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ABSTRACT

The majority of gall-inducing nematodes lives in the soil, and induces galls on roots. Nevertheless, some nematodes are able to climb up the stems and induce galls on aerial plant organs. This is the case of *Ditylenchus gallaeformans* (Oliveira et al., 2013), which induces galls on the inflorescences of *Miconia albicans* (Sw.) Steud. (1841) and *Miconia ibaguensis* (Bonpl.) Triana (1871) (Melastomataceae). Under the influence of *D. gallaeformans*, the flowers of *M. albicans* and *M. ibaguensis* do not differentiate *in situ*. Instead of flowers, the axes of the galled inflorescences are surrounded by emergences with nutritive tissues lining the larval chambers. The nutritive tissues are meristem-like, but distinct from other galls, they have a promeristematic capability. In other words, the nutritive tissues of these galls have totipotent cells, originating new covering emergences with dermal, ground and vascular tissues. Therefore, these galls have an indeterminate growth, which is a novelty for galls in general. Additionally, *D. gallaeformans* induces a long-distance impact on fruits, which have increased number of carpels. Such long-distance effects indicate that *D. gallaeformans* is a peculiar colonial parasite, which may compensate the damages of gall inducing mechanisms by favoring, at least partially, its host plant fitness. The number of carpels is a conservative character in most plant species, and the promeristems are maintained only in apical and lateral buds of normal plants. However, *D. gallaeformans* is capable of manipulating these conservative features in its host plants, and the signaling factors involved in these interactions deserves special attention.

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1. Introduction

Plant galls are abnormal neoformed structures induced by parasitic organisms on their host plants (Krusberg, 1963; Mani, 1964; Raman, 2011). The gall inducer – host plant relationship is usually species-specific, because each galling species induces galls in one (or few) host species (Mani, 1964; Meyer, 1987; Raman, 2011). Many groups of living organisms, such as bacteria, virus, fungi, plants, insects, mites, and nematodes can induce galls (Mani, 1964). The galls may be induced by the feeding activity of the parasites or, in the case of some insects, by oviposition (Meyer, 1987; Oliveira et al., 2016). Gall formation involves distinct developmental steps, including hyperplasia, cell hypertrophy, tissue redifferentiation, and/or changes in accumulation of primary and secondary metabolites (Goodey, 1948; Mani, 1964; Meyer, 1987; Bronner, 1992; Rohfritsch, 1992; Moura et al., 2008; Oliveira et al., 2010; Ferreira and Isaias, 2014, 2013; Carneiro and Isaias, 2015). Distinct types of abnormal growths may be produced with a specific balance of the developmental steps: leaf-rolling, leaf-folding, pouch (invagination of leaf lamina), organ intumescence, and covering galls (with neoformation of emergences, which cover the inducers) (Mani, 1964; Meyer, 1987; Rohfritsch, 1992). The emergences are plant organ appendages, "which consist of epidermal as well as subepidermal tissues", and may be vascularized or not (Fahn, 1990).

Nutritive tissues where inducers directly feed are common in galls, except in those induced by phloem-sucking insects (Hemiptera). Nutritive cells store proteins, lipids or reducing sugars, depending on the gall-inducer's *taxon* (Kostoff and Kendall, 1929; Bronner, 1992; Ferreira and Isaias, 2014; Oliveira et al., 2016). Nutritive tissues usually have cells with thin walls, prominent nucleus, and active metabolism, and are constantly replaced during the inducer's feeding (Kostoff and Kendall, 1929; Goodey,

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1948; Larew, 1981; Ferreira et al., 2015). Therefore, they may be considered meristem-like tissues, which originate exclusively new nutritive cells (Ferreira and Isaias, 2013). Gall growth and development occur before the complete tissue differentiation, mainly in cell outer layers (Rohfritsch, 1992). Accordingly, galls are considered structures with determinate growth, because they have a limited growth phase (Krusberg, 1963; Meyer, 1987; Raman, 2011). As they need energy to their own growth, maintenance, and to the feeding of the gall inducers, galls are strong sinks of photoassimilates (Jankiewicz et al., 1969; Larson and Whitham, 1991; Castro et al., 2012). For instance, if part of the photoassimilates flow to the galls rather than to the growing flowers or fruits, they may be harmful for the host plant reproduction.

Phytoparasitic nematodes usually live in the soils, and feed by piercing and sucking the plant cells (Weischer and Wyss, 1976; Wyss, 1997, 2002). The most advanced herbivore nematodes are endoparasites, which migrate across plant tissues, and belong to the order Tylenchida (Wyss and Grundler, 1992). When capable of inducing histological alterations, they are considered galling nematodes, which usually attack a broader range of host plant species than insects and mites (Mani, 1964). Moreover, distinct histological organization is expected in each host species (Goodey, 1948; Mani, 1964). As they are soil inhabitants, most of galling nematodes parasite roots (Mani, 1964; Meyer, 1987), where they may induce very specific and distinct cytological changes. For instance, galls induced by root-knot nematodes (*Meloidogyne* spp.) have giant, polynucleated, protein-rich nutritive cells redifferentiated from the vascular parenchyma (Bird, 1961; Yousif, 1979; Finley, 1981; Di Vito et al., 2004); cyst nematodes (*Heterodera* spp.) induce the formation of a syncytial nutritive tissue by digestion of cell walls (Dropkin, 1969; Jones and Northcote, 1972); and *Xiphinema* spp. (Dorylaimida) are ectoparasites and induce galls with several hypertrophied nurse cells (=nutritive cells) in root cortical parenchyma (Weischer and Wyss, 1976; Wyss, 2002). In spite of these peculiarities, nematode root galls do not grow after the differentiation of the nutritive cells, and so are considered to have determinate growth.

Nematodes do not exclusively parasite roots; some nematodes can climb stems, and induce galls on aerial shoots (Mani, 1964; Skinner et al., 1980). Few galling nematodes of aerial parts are known and studied when compared with root galling nematodes (Meyer, 1987). After actively ascending the stems, the nematodes usually penetrate the young meristematic aerial organs and induce the formation of crypts (=chambers) lined with nutritive cells, where the colonies feed (Goodey, 1939, 1948; Mani, 1964; Watson and Shorthouse, 1979; Skinner et al., 1980; Wyss, 2002). These nematodes live into the galls until the leaves enter senescence and die, and then they return to the soils to find another host plant. Sometimes, they may live in the dry plant tissues or soils for several months or years, since most of them are resistant to desiccation (Mani, 1964). Each galling nematode species have their own adaptations, but they usually have both parthenogenetic and sexual reproduction, and freely live in the soil for variable periods (Mani, 1964; Meyer, 1987). Moreover, galling nematodes occasionally cause alterations at a distance (tele-effects), such as the development of abnormal inflorescences away from the sites of infection (Mani, 1964). However, such long-distance effects have never been thoroughly studied.

Ditylenchus gallaeformans (Oliveira et al., 2013) (Tylenchida: Anguinidae) induces galls on leaves, stems, and inflorescences of several Melastomataceae species, mainly in the genus *Miconia* (Oliveira et al., 2013). Distinct from the other galling nematodes, *D. gallaeformans* is a migratory ectoparasite, which does not penetrate plant tissues. It is sexually dimorphic and both males and females may be found into the galls, where mating occurs (Santin, 2008; Oliveira et al., 2013). The eggs are laid into gall chamber, where up to 11,000 individuals may co-exist (Santin, 2008). The life cycle

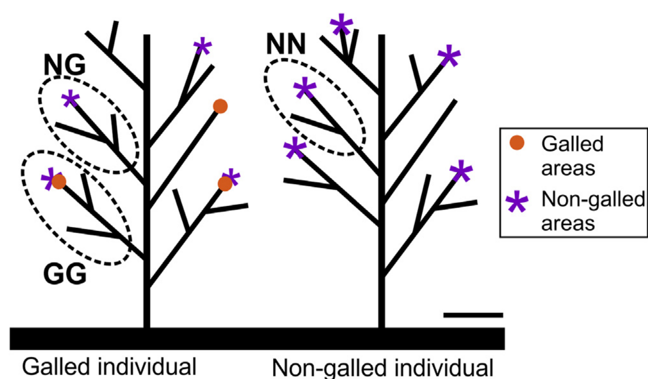

Fig. 1. Schematic drawing of *Miconia* spp. individuals with and without galls occurring in the same population, and showing the relative distance of the non-galled and galled inflorescences. This drawing was based on a population of *M. albicans* occurring on Parque Municipal das Mangabeiras, Belo Horizonte city, Brazil, where plants are ca. 2.50 m height. Galled plants have shoots with non-galled inflorescences (NG) and shoots with galled inflorescences (GG). Non-galled plants have only non-galled shoots (NN). Bar: 50 cm.

of *D. gallaeformans* lasts 19 days, and up to 14 generations may be produced in one year. The juveniles have four stages, but at the second stage they are already able to migrate and induce new galls. From the fourth stage, juveniles may survive in anhydrobiosis for several months, and can be dispersed by the wind. They migrate to other plants and induce new galls in the wet season (Santin, 2008). Even though the host plants of *D. gallaeformans* are native in South America, they are invasive species in Hawaii and other pacific islands (Oliveira et al., 2013). As they can be cultivated in laboratory, such nematodes were suggested as biological controls (Oliveira et al., 2013), but further effects in the fitness of host plants need to be evaluated.

Currently, we studied the developmental and histochemical changes induced by *D. gallaeformans* in reproductive structures of *Miconia albicans* (Sw.) Steud. (1841) and *M. ibaguensis* (Bonpl.) Triana (1871). Such plants are native shrubs of Brazilian savannah, with perennial individuals, widespread in Central and South America (Viana et al., 2013). Both species are apomictic, with bisexual flowers, and their fruits may be dispersed by birds, rodents, and ants (Santos et al., 2012; Silveira et al., 2013). This work shows novel pathways of plant manipulation induced by a gall-inducer, never reported for any other studied gall. The following questions are assessed: (1) how do *D. gallaeformans* galls develop, and which are the histological processes defining gall growth? And (2) are there long-distance effects caused by *D. gallaeformans* in reproductive shoots of *M. albicans* and *M. ibaguensis*?

2. Material and methods

Non-galled and galled reproductive apices (Fig. 1) ($n \geq 5$ for each category) were collected from three populations of *Miconia albicans* and *M. ibaguensis* (Melastomataceae). Collection sites are located at Parque das Mangabeiras (Belo Horizonte, MG, Brazil, 19°56′41.7″S 43°53′52.9″W), Parque Estadual Serra Verde (Belo Horizonte, MG, Brazil, 19°47′31.6″S 43°57′29.8″W) (Portugal-Santana and Isaias, 2014), and Parque Nacional da Serra do Cipó (Santana do Riacho, MG, Brazil, 19°14′17.5″S 43°31′03.6″W). Voucher specimens of the galled material were deposited at the BHCB Herbarium (Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil) under the numbers 169,416 and 169,417.

Fresh samples were transported to the Laboratory of Plant Anatomy (Dept. of Botany, UFMG), fragmented, and fixed in 2.5% glutaraldehyde and 4.5% formaldehyde in 0.1 M phosphate buffered saline, pH 7.2 (Karnovsky, 1965). Fixed fragments of inflorescences,

flower buds, and fruits were dehydrated in *n*-butyl series, and embedded in Paraplast® (Johansen, 1940; Kraus and Arduin, 1997). The blocks were sectioned in a rotary microtome (Leica Jung Biocut) (14 μm); the sections were deparaffinized (Kraus and Arduin, 1997), stained with 0.45% astra blue and 0.05% safranin (Bukatsch, 1972; Kraus and Arduin, 1997), and mounted with coverslips using colorless varnish (Paiva et al., 2006). In order to detect primary metabolites in gall tissues, fresh galled inflorescences were hand-sectioned using razor blades. These hand-made sections were immediately submitted to Fehling's reagent for the detection of reducing sugars (Sass, 1951), Ponceau 2R for the detection of proteins (Ventrella et al., 2013), Lugol's reagent for the detection of starch (Johansen, 1940), and Sudan red B for the detection of lipids (Jensen, 1962). All tests were analyzed under light microscopy, and blank-sections were used for comparison.

In order to verify the existence of long-distance effects of the galling stimuli, we compared fruits developed in galled and non-galled individuals. Fresh immature fruits obtained from: individuals without galls (NN, fruits of non-galled individuals); inflorescence shoots without galls in galled individuals (NG, fruits of non-galled shoots of galled individuals); and inflorescence shoots with galls (GG, fruits of galled inflorescences) (Fig. 1) of *M. albicans* and *M. ibaguensis* were compared ($n \geq 6$ individuals; at least 5 fruits per individual). The samples were transversely hand-sectioned with razor blades, mounted on slides and analyzed under light microscope. The carpel number in fruits of NN, NG and GG was counted, and compared by Kruskal-Wallis analysis of variance, followed by Dunn's test in SigmaStat®, considering $\alpha = 0.05$.

3. Results

3.1. Morphology of inflorescences and galls

The reproductive apices of *M. albicans* (Fig. 2A) and *M. ibaguensis* develop into long, terminal scorpioid panicles, with indeterminate growth. The reproductive axes elongate during the differentiation of the floral meristems into flower buds. The galled inflorescences (Fig. 2B–D) have shortened axes, and do not commonly develop flowers or fruits, except for few non-affected areas (Fig. 2B–C), where fruits similar to those of the non-galled inflorescences differentiate. The galled inflorescences form large groups of coalescent protuberances, which are brown in *M. albicans* (Fig. 2B), and light green in *M. ibaguensis* (Fig. 2C–D). During senescence, the galls turn gray (*M. albicans*) or black (*M. ibaguensis*).

3.2. Anatomy of the non-galled inflorescences

The axes of the non-galled inflorescences are covered either by a dense indumentum of arachnoid trichomes in *M. albicans*, or stellate trichomes and emergences in *M. ibaguensis* (Fig. 3A). The

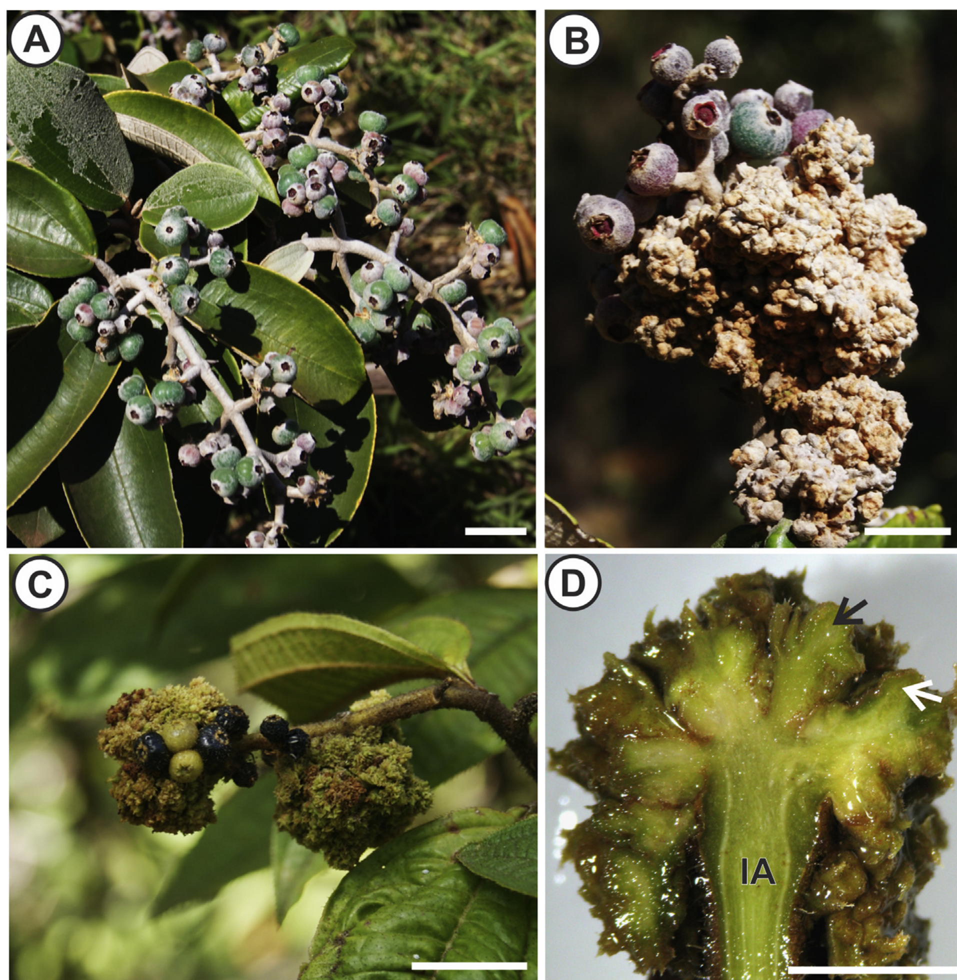

Fig. 2. Inflorescences on *Miconia albicans* and *M. ibaguensis* (Melastomataceae). (A–B) *M. albicans.* (A) Non-galled axes with developing immature fruits (pink) and mature fruits (jade-green). (B) Galled axis with some immature (pink) and mature (green) fruits. (C–D) *M. ibaguensis.* (C) Galled axis with some immature (green) and mature (black) fruits. (D) Longitudinal section of a galled inflorescence under stereomicroscope, showing the neoformed regions (arrows) that shelter nematodes. Bars: A–C = 2 cm; D = 0.5 cm. Abbreviations: IA = inflorescence axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

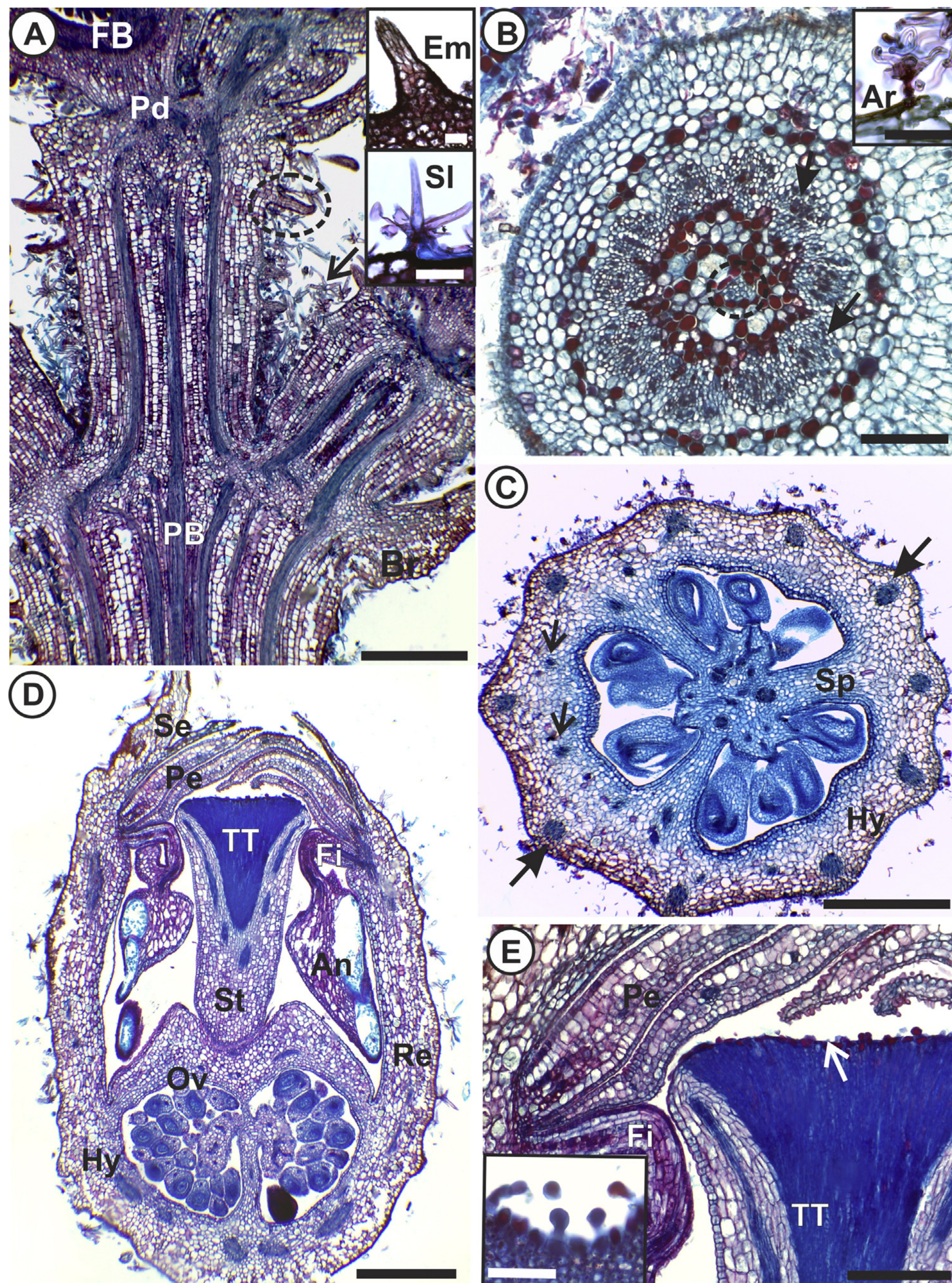

Fig. 3. Anatomy of non-galled inflorescences of *Miconia albicans* and *M. ibaguensis* (Melastomataceae). (A, D, E) *M. ibaguensis*. (B, C) *M. albicans*. (A) Longitudinal Section (LS) of the inflorescence axis, showing the pith bundles and the vascularization of the floral buds through the pedicel. The axes and hypanthia are covered by emergences (dotted circle; Em) and several stellate trichomes (arrow; Sl). (B) Transverse Section (TS) of the receptacle, with a vascular cylinder composed of 10 vascular bundles (arrows) and a discrete pith bundle (dotted circle), and detail of an arachnoid trichome (Ar). (C) TS of the median region of the hypanthium, showing the 10 receptacular vascular bundles (closed arrows). Open arrows point the vascular bundles of the carpels. (D) LS of a floral bud, showing the hypanthium formed by the fusion of the ovary and expanded receptacle. The expanded receptacle bears the petals, sepals and filaments with the bases located at the upper floral bud. (E) LS of the floral bud, showing the upper region of the style, transmitting tissue, stigma (arrow) and simultaneous divergence of the petals and filaments. Detail: papilose cells of the stigma, in a transverse section. Abbreviations: An = anther; Br = floral bracts; Fi = filament; Hy = hypanthium; Ov = ovary; PB = pith bundles; Pd = pedicel; Pe = petal; Re = receptacle; Se = sepal; Sg = stigma; Sp = septum; St = style; TT = transmitting tissue. Staining: safranin and astra blue. Bars: A, C, D = 500 μm, B, E = 200 μm, Details = 50 μm.

epidermis is uniseriate in both species, and covers a 10–12-layered cortical parenchyma, with crystalliferous idioblasts containing druses. The vascular cylinder is continuous, amphiphloic, and delimits a parenchymatic pith, with interspersed bicollateral bundles.

The flowers of *M. albicans* and *M. ibaguensis* have pedicels, receptacle (Fig. 3B), and sepals densely covered by trichomes, but the petals, stamens and carpels are glabrous. The pedicels have a unique vascular cylinder, which diverges into 10 vascular bundles in the receptacles (Fig. 3B). The flower has a receptacular inferior ovary, i.e., the receptacle is urn-shaped and fused with the lower and median regions of the ovary wall, forming the hypanthium (Fig. 3C–D). The hypanthium develops up to the level of the stigma, and the sepals, petals, and stamens are inserted at the upper limit of the urn-shaped receptacle (Fig. 3D). Each filament folds over itself,

Table 1
Number of carpels in fruits occurring upon NN (non-galled individuals), NG (non-galled inflorescences of galled individuals) and GG (galled inflorescences) of *Miconia albicans* and *M. ibaguensis* (mean ± standard deviation).

	Number of carpels			Statistics	
	NN (control)	NG	GG	H	p-value
M. ibaguensis	3.00 ± 0.00	3.13 ± 0.19	3.21 ± 0.18*	6.776	0.034
M. albicans	3.00 ± 0.00	3.20 ± 0.15*	3.13 ± 0.15	7.069	0.029

* Significant difference ($p < 0.05$) when compared with the control. Kruskal-Wallis test (H), followed by Dunn's test.

and hides the anther between the upper ovary and the inner side of the receptacle (Fig. 3D).

The ovary is 3-locular, with axillary placentation (Fig. 3C), and the carpels are multi-ovulated. The flower has a single style, with a solid transmitting tissue at the center (Fig. 3D–E). The upper portion of the style is hollow, and the transmitting tissue is in contact with the stigma, which is composed by papilose secretory cells (Fig. 3E).

The sepal, petal and stamen traces diverge from the receptacle at the level of the stigma (Fig. 3D). Each of the 10 stamens are vascularized by a single vascular bundle, i.e., by the vascular traces diverged from the 10 receptacular bundles. The anthers are bithecate and tetrasporangiate. The receptacular bundles alternate, and vascularize the petals and sepals. The 5 petals are free and the 5 sepals are almost totally fused, with free apices.

3.3. Fruits and flowers on non-galled and galled individuals

The fruits of the non-galled individuals (NN), of the non-galled inflorescences on galled individuals (NG), and of the galled inflorescences (GG) of *M. albicans* and *M. ibaguensis* are structurally similar. They are complex fruits, composed by tissues originated from the ovary (the true fruit), and tissues originated from the receptacle (the false fruit). The mesocarp is formed by hypertrophied parenchyma cells interspersed by some brachysclereids, with the 10 vascular bundles of the hypanthium, surrounded by 4–10 layers of pericyclic fibers.

Changes on the number of carpels (from three to four), of stamens (from 10 to 12), and of the receptacular bundles (from 10 to 12) occur on some flowers and fruits of the GG and of the NG of *M. albicans* and *M. ibaguensis* (Fig. 4). In *M. ibaguensis*, the increment in number of carpels per fruit is significant in GG when compared with the controls (NN). In *M. albicans*, the fruits of NG have a significant increment in the number of carpels when compared with NN (Table 1).

3.4. Anatomy of the galled inflorescences of Miconia albicans and M. ibaguensis

The colonies of *Ditylenchus gallaeformans* infest the meristematic reproductive apices of *M. albicans* (Fig. 5A) and *M. ibaguensis*, i.e., inflorescences with elongating axes and flower meristems undergoing differentiation. Vascularized emergences, originated either from the cortical ground meristem of the undifferentiated axes or from the domes of the differentiating floral meristems, surround the colonies of the Nematoda (Figs. 5–7). The galled inflorescences may bear galls, viable flower buds, and mature flowers and fruits.

The emergences in the galls of *D. gallaeformans* are formed either by cell proliferation in the protoderm and cortical ground meristem of the inflorescence axes, or by the redifferentiation of the floral meristems (Fig. 5A–C). These emergences are curved, and cover the larval chambers (Figs.5A–C; 6 ; 7A–C). The outer epidermis of the emergences is not in contact with the nematodes and is densely covered by arachnoid trichomes in *M. albicans* (Figs.5B; 6), and

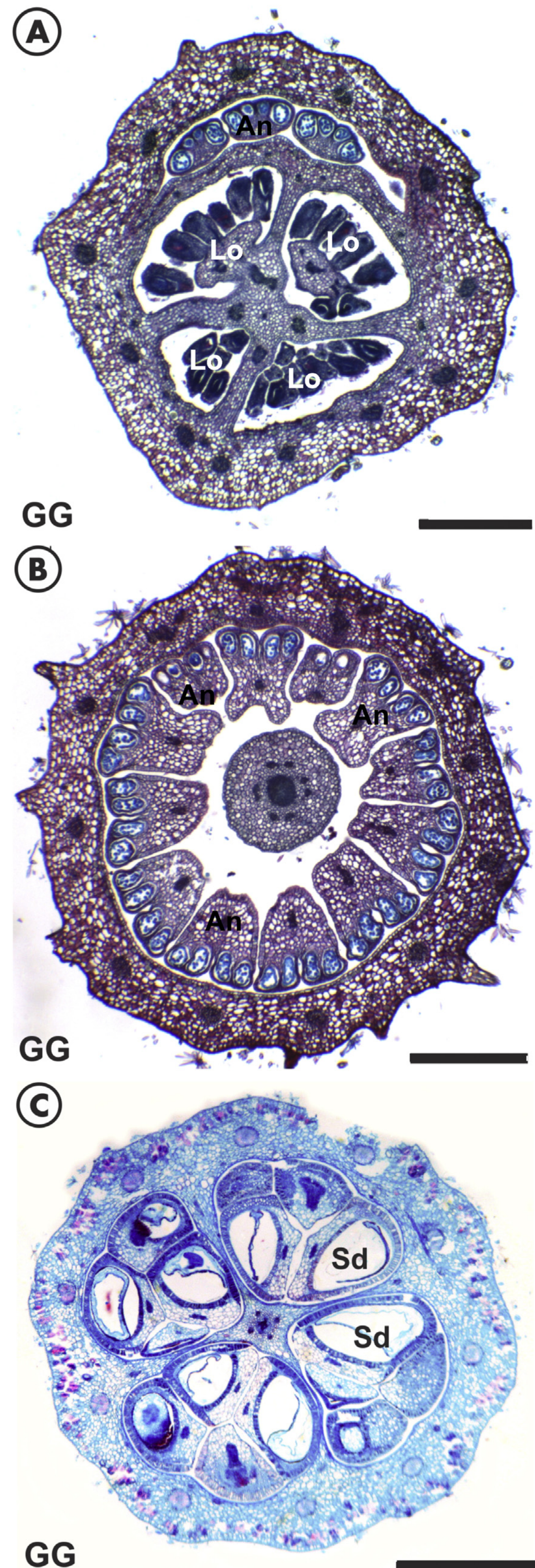

Fig. 4. Altered floral buds and fruit developed on a galled inflorescence (GG). (A) Transverse Section (TS) of the basal region of a floral bud of *Miconia ibaguensis*, with 4 carpels, 4 locules and 12 receptacular bundles. (B) TS of the median region of floral bud of *M. albicans*, with 12 anthers and 12 receptacular bundles. (C) TS of the median region of an immature fruit of *M. albicans*, with 4 carpels and 10 receptacular bundles. Abbreviations: An = anther; Lo = locule; Sd = seed. Staining: safranin and astra blue. Bars: A–B = 500 μm; C = 1 mm.

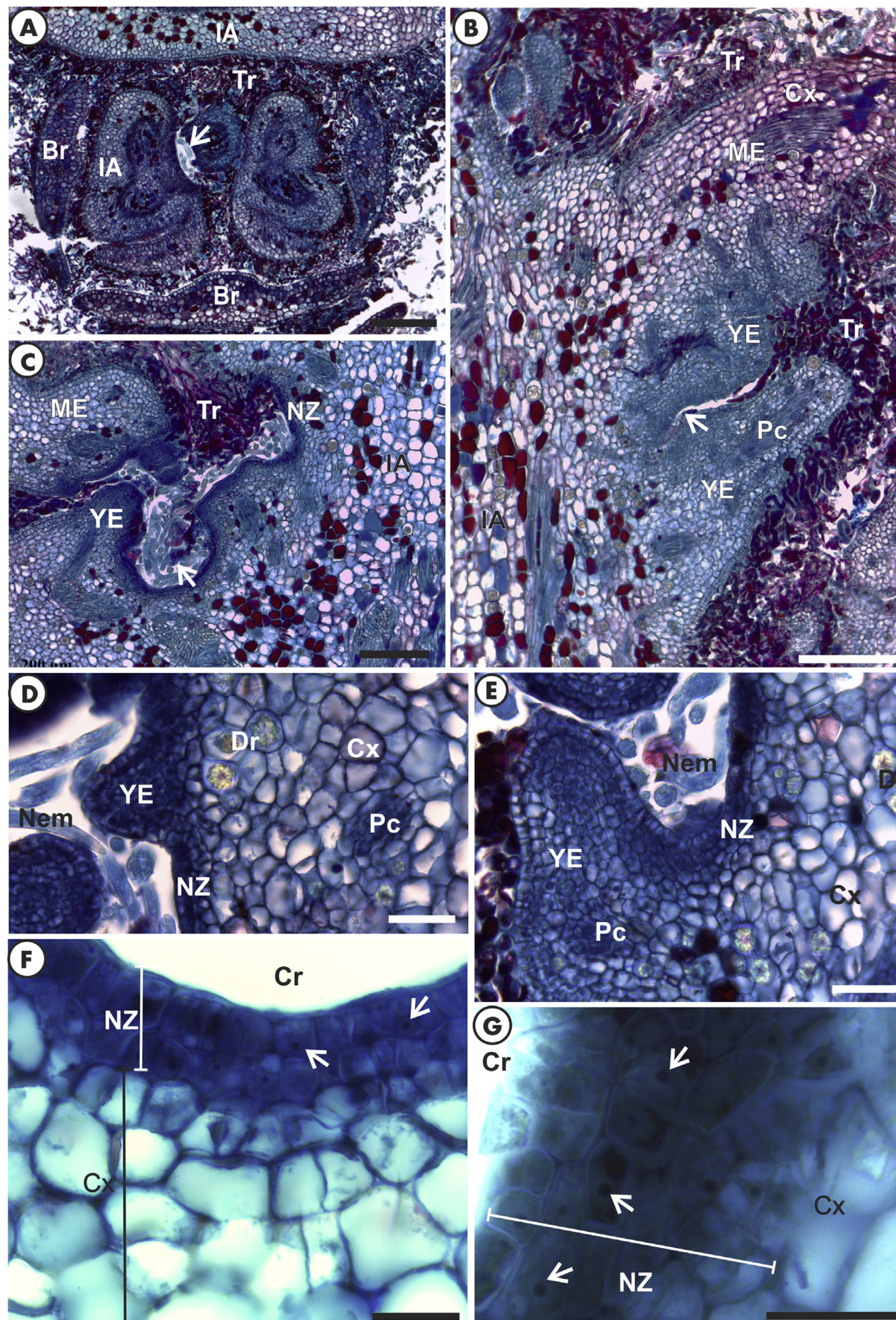

Fig. 5. Anatomy of the inflorescences of *Miconia albicans* (Melastomataceae) galled by *Ditylenchus gallaeformans* (Nematoda). (A) Transverse Section (TS) of meristematic inflorescence axis infected by nematodes (arrow). (B–C) Longitudinal Section (LS) of a galled inflorescence. Mature Emergences (ME) have hypertrophied parenchyma in the cortex and nutritive cells surrounding the nematode chambers (arrow). Nutritive Zone (NZ) may differentiate new vascularized Young Emergences (YE). (D–E) NZ around nematode chambers, originating some meristematic young emergences. (F–G) Details of NZ around nematode chambers. The nutritive cells have evident nuclei and nucleoli (arrows) and dense cytoplasm. The cortex cells are vacuolated and hypertrophied. Abbreviations: Br = floral bracts; Cr = chambers; Cx = cortex; Dr = druses; IA = inflorescence axis; Nem = nematodes; Pc = procambium; Tr = arachnoid trichomes. Staining: safranin and astra blue. Bars: A– C = 200 μm; D–E = 50 μm; F–G = 20 μm.

stellate trichomes in *M. ibaguensis* (Fig. 7A). Under the outer epidermis, there is a 10–15-layered cortical parenchyma that accumulates starch (Table 2), and has interspersed phenolic and crystalliferous idioblasts (Figs.5D–E; 7D). Mature emergences are vascularized by collateral bundles. The inner core of the curved emergences, close to the nematodes, is formed by a 3–5 layered nutritive tissue, whose small cells have evident nuclei and nucleoli, and dense cytoplasm (Figs.5F–G; 7D). The uniseriate epidermis covering the larval chamber is also part of the nutritive zone, and has cells with dense cytoplasm, fragmented vacuoles and evident nuclei. There is no histochemically detectable accumulation of lipids or starch in the nutritive zone, but reducing sugars (Fig. 7E) and proteins (Fig. 7F) were detected surrounding the chambers (Table 2).

Some cells of the nutritive zone divide anticlinally and periclinally, originating new emergences with protodermal, procambial, and ground meristem cells (Figs.5C–E; 7B–E). These neoformed

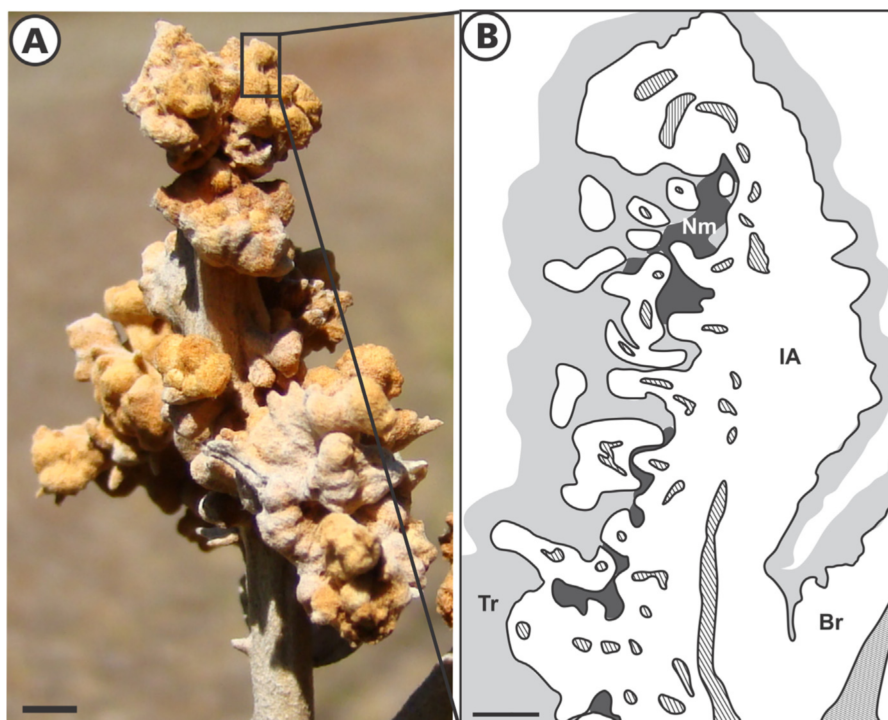

Fig. 6. Schematic drawing of a portion (shown in A) of a galled inflorescence of *Miconia albicans* induced by *Ditylenchus gallaeformans* (Nematoda) on longitudinal section. The dark-shaded regions in (B) show the occurrence of the colonies of nematodes (Nm) surrounded by meristematic nutritive tissues. The light-shaded regions in (B) show the arachnoid-trichomes covering (Tr) sealing gall apertures. The galled inflorescence axes (IA) may have inflorescence bracts (Br). Striped regions correspond to vascular tissues, showing that the mature emergences surrounding the colonies of nematodes are vascularized. Bars: A = 0.3 cm; B = 500 μm.

Table 2
Primary metabolites detected in inflorescence galls induced by *Ditylenchus gallaeformans* on *Miconia* spp.

Metabolites	Tests	Gall regions
Lipids	Sudan red B	–
Starch	Lugol	+ Outer parenchyma
Reducing sugars	Fehling	+ Nutritive tissue + Vascular bundles
Proteins	Ponceau 2R	+ Nutritive tissue + Vascular bundles

(+) positive reaction. (−) negative reaction in all tissues.

emergences mature, providing new nutritive surfaces for the feeding of *D. gallaeformans* larvae, and covering the colonies (Fig. 6). Independently of their sizes, all observed galls have neoformed nutritive emergences, which allow the growth and survival of the Nematoda colonies. Mature emergences eventually undergo senescence, when the nutritive cells accumulate polyphenols, differentiate phellogen and suber, thus characterizing a healing process.

4. Discussion

Ditylenchus gallaeformans causes direct and long-distance effects on the reproductive organs of *Miconia* spp. Changes in the number of carpels in fruits of galled individuals were observed, both in galled and non-galled shoots, indicating effects of *D. gallaeformans* at a distance. The property of inducing the redifferentiation of totipotent cells in nutritive tissues, leading to the formation of new covering emergences, culminates in the indeterminate growth of *D. gallaeformans* galls, which is a novel report on the development of galls.

4.1. Development of non-galled reproductive structures on Miconia *spp.*

Inferior ovaries fused with the urn-shaped hypanthium, and the development of complex fruits, i.e., fruits developed from ovary fused with other structures (*cf.* Cortez and Carmello-Guerreiro, 2008) are common features of the Melastomataceae. The receptacular inferior ovaries, such as those of *M. albicans* and *M. ibaguensis*, have also been observed in *Marcetia taxifolia* (Melastomataceae) (Ferreira and Isaias, 2014). Therefore, either receptacular ovaries or appendicular ovaries may occur in the Melastomataceae. For instance, appendicular ovaries were reported to occur in *Memecylon edule*, *Me. guinensis*, *Me. capitellatum*, *Rhexia marina* and *Tibouchina semidecandra* (Satyavathi et al., 1991), which differ from receptacular ovaries by the vascularization. While the receptacular hypanthium is only vascularized by the receptacular bundles, the appendicular hypanthium has the traces of sepals, petals and stamens arranged concentrically in the hypanthium wall (Mauseth, 1988; Costello and Motley, 2004).

4.2. Long-distance effects of Ditylenchus gallaeformans

Long-distance effects related to galling stimuli are rarely reported, but some groups of gall-inducers, including nematodes, fungi and insects, are known to promote long-distance effects (Mani, 1964; Meyer and Maresquelle, 1983; Sopow et al., 2003). The long-distance effects induced by *D. gallaeformans* were detected both in *M. albicans* and *M. ibaguensis*, with significant alterations in the number of carpels in galled plants. Further physiological studies should detect the primary causes of long-distance effects in

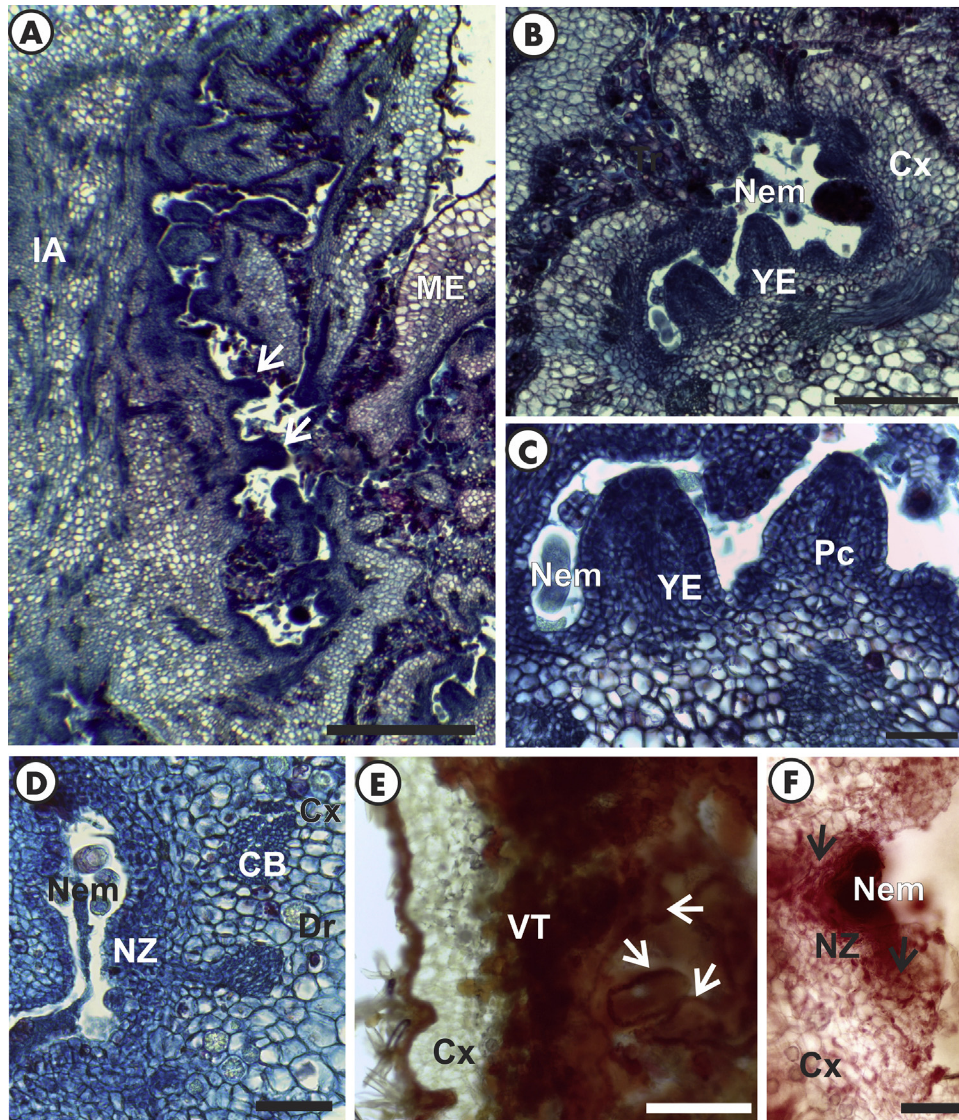

Fig. 7. Anatomy of the inflorescences of *Miconia ibaguensis* (Melastomataceae) galled by *Ditylenchus gallaeformans* (Nematoda). (A) Longitudinal section of a galled inflorescence. Mature Emergences (ME) are vascularized and have hypertrophied parenchyma in the cortex and nutritive cells around the nematode chambers. Young Emergences (YE) (arrows) emerge from cell divisions in nutritive zone. (B–D) Details of Nutritive Zone (NZ) around nematode chambers originating YE through cell divisions. YE are meristematic, and the procambium may be observed in some of them. The nutritive cells have dense cytoplasm, while the cortical cells are vacuolized and hypertrophied. (E) Reducing sugars concentrated in NZ (arrows) and vascular tissues. (F) Proteins concentrated in NZ (arrows). Abbreviations: CB = collateral bundles; Cx = cortex; Dr = druses; IA = inflorescence axis; Pc = procambium; Nem = nematodes; Tr = stellate trichomes; VB = vascular tissue. Staining: A–D: safranin and astra blue. E: Fehling's reagent. F: Ponceau 2R. Bars: A = 500 μm; B, E = 200 μm; C–D, F = 50 μm.

response to *D. gallaeformans* galling habit. Stressing factors caused by gall development may involve oxidative stress, source-sink relationships, and also changes in hormonal rates or in gene expression (Oliveira et al., 2016). Our results indicate that these responses may vary according the host plant species. Why shoots of *M. albicans* more distant from the site of gall induction have more affected fruits, while in *M. ibaguensis* the closest the shoots are, the higher are the alterations in fruits, remains to be investigated. Alterations in the development of flowers and fruits were associated with the imbalance of growth regulators (Bilderback, 1972; Raghavan, 2012; Williams, 1994). Hormonal changes were reported in several gall systems, including those induced by nematodes (Krusberg, 1963; Bird and Koltai, 2000; Favery et al., 2016) and insects (Byers et al., 1976; Elzen, 1983; Mapes and Davies, 2001a, 2001b; Leite et al., 2007; Bedetti et al., 2014), and therefore the changes in growth regulators may be involved in the long-distance effects of *D. gallaeformans*.

4.3. *Direct impacts of* D. gallaeformans

The main impact of *D. gallaeformans* occurs on reproductive meristematic apices, where the potential reactiveness of the cells is higher, which is an essential factor for the differentiation of galls (Weis et al., 1988). *D. gallaeformans* has a peculiar habit, since it induces galls on plant surfaces, instead of penetrating plant tissues (Goodey, 1948; Watson and Shorthouse, 1979; Meyer, 1987; Wyss, 2002; Di Vito et al., 2004; Perry and Moens, 2011). The common pattern of differentiation of the inner protoderm is inhibited, and the maintenance of its meristematic potential is revealed by the development of new emergences. The meristematic nature of the cells is crucial for the indeterminate growth of *D. gallaeformans* galls on *Miconia* spp.

The indeterminate growth of the galls on *M. albicans* and *M. ibaguensis* contradicts the common sense that Nematoda and Insecta galls have determinate growth (Krusberg, 1963; Mani,

1964; Raman, 2011). The adaptive value of the indeterminate growth consists in providing protection and nutrition (Price et al., 1987; Stone and Schonrogge, 2003) to the colonies of *D. gallaeformans* for longer periods. The meristematic cells in *D. gallaeformans* galls are typical nutritive cells (*sensu* Kostoff and Kendall, 1929; Larew, 1981), with dense cytoplasm, and storage of proteins and reducing sugars. These nutritive cells differ from the cells of nematode root galls, which are polynucleated and very large (giant cells) (Bird, 1961; Yousif, 1979; Weischer and Wyss, 1976; Finley, 1981; Di Vito et al., 2004). The nutritive cells of Nematoda galls induced in aerial plant parts may be as small as the meristematic cells reported herein (Goodey, 1939, 1948; Mani, 1964; Watson and Shorthouse, 1979; Skinner et al., 1980; Larew, 1981), but the ability to redifferentiate other tissues, and new structures, such as the covering emergences, has not been previously reported.

Current data on nematode galls point out their structural variability, as well as the widespread occurrence of nutritive tissues in animal-induced galls (insects, mites and nematodes) (cf. Kostoff and Kendall, 1929; Kendall, 1930; Bronner, 1992; Moura et al., 2008; Oliveira et al., 2010; Ferreira et al., 2015, 2017). However, non-nutritive galls have been reported for phloem-sucking insects (namely, Hemiptera: Aphididae and Psylloidea), as a consequence of their special feeding sites, the phloem cells (Larew, 1981; Bronner, 1992; Álvarez et al., 2009, 2013; Isaias et al., 2011), even though other storage tissues not related with feeding are common (Carneiro and Isaias, 2015; Ferreira et al., 2017).

The maintenance of the meristematic nature of the nutritive tissue is common in galls. However, the nutritive tissues in galls are usually programmed to originate new nutritive cells, and theoretically their meristematic capability is limited (see Kostoff and Kendall, 1929; Goodey, 1948; Larew, 1981; Ferreira and Isaias, 2014, 2013; Oliveira et al., 2016). *D. gallaeformans*, on the other hand, is capable of inducing nutritive cells to behave as promeristematic cells, with unlimited totipotency. The signaling factors secreted by the nematodes, or other stressing factors, must be investigated in order to elucidate how they maintain totipotent meristematic sites in mature organs. In the usual developmental patterns of plants, totipotency is restricted to promeristems, capable of originating ground meristem, procambium, and protoderm, each one responsible for originating a restrict range of tissues (Lev-Yadun, 2003). Promeristems are present only in plant apical and lateral buds, and the maintenance of promeristematic cells in galls of *D. gallaeformans* must involve specific signaling patterns, distinct from the other studied galls induced by nematodes, insects, or mites.

5. Conclusions

The development of colonial galls induced by *D. gallaeformans* on *M. albicans* and *M. ibaguensis* reveals important novelties concerning the developmental patterns of galls. Firstly, the nematodes inhibit the development of flowers *in situ*, and induce the overdifferentiation of meristematic nutritive emergences, which enable the indeterminate growth to the galls. The indeterminate growth in galls was herein reported for the first time, and it enables the survivorship of several generations within the galls, increasing the fitness of *D. gallaeformans*. Distinct from other gall inducers, *D. gallaeformans* induces the redifferentiation of promeristematic nutritive cells, with the capability of differentiating a large range of plant cell lineages. Secondly, these galling nematodes induce direct (*in situ*) and long-distance effects on reproductive apices. Even though galled inflorescences decrease the ability of host plants to produce seeds, the supranumeric carpels in fruits of galled plants should potentially increase seed production as a mechanism of compensation. The long-distance effects of *D. gallaeformans*, and the maintenance of promeristematic cells in mature galls are good study models, for specific biochemical signaling must be involved in these physiological effects. The number of carpels is a conservative character in most plant species, and the promeristems are maintained only in apical and lateral buds of normal plants. Therefore, the influx of hormones and the stressing factors that may cause these alterations should be thoroughly studied to evaluate how the nematodes can manipulate conservative features in plants. In addition, the reproductive potential of infected plants *versus* non-infected plants should be compared (e.g., number of fruits produced by individual, mean fruit size, mean number of seeds per fruit, efficiency of dispersion by distinct dispersers, and seed germination rates) in order to attest the real value of *D. gallaeformans* as a biological control tool for invasive Melastomataceae.

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Capítulo 4

Beyond the defensive role, phenolics influence both oxidative stress and photosynthetic metabolism in nematode and eriophyid leaf galls

Beyond the defensive role, phenolics influence both oxidative stress and photosynthetic metabolism in nematode and eriophyid leaf galls

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Abstract

The concentration of phenolic compounds usually increases in insect galls, where they are related to the defense against natural enemies. However, the strict anti-herbivore role of these compounds has been questioned. Phenolics may also mediate acid-induced cell wall growth and avoid the increment of reactive oxygen species accumulation in gall tissues. Current hypothesis is that the increased levels of phenolics are involved in the oxidative homeostasis, but also in photosynthesis maintenance in galls, which is herein tested in the galls induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans* and *M. ibaguensis* (Melastomataceae), and by an unidentified Eriophyidae (Acarina) on *M. ibaguensis*. Vacuolar phenolics increased in the three gall systems, but the investment in apoplastic phenolics and carotenoids differ. Galls induced by *D. gallaeformans* on *M. albicans* and by the Eriophyidae on *M. ibaguensis* have increased accumulation of apoplastic phenolics, which is related to the control of phospholipid peroxidation. The galls induced by *D. gallaeformans* on *M. ibaguensis* have higher carotenoid contents, which is related to the maintenance of non-photochemical quenching. The investment in carotenoids and/or in polyphenols in nematode and eriophyid galls represents mechanisms to maintain oxidative homeostasis, avoiding premature cell death and allowing basal photosynthetic activity. Analyzing galls induced by the same Nematoda on the two species of *Miconia*, and galls induced by two distinct

inducers on *M. ibaguensis*, the role of both gall-inducing stimuli and host plant physiological peculiarities in gall metabolism is attested.

Keywords: apoplast, carotenoids, chlorophyll fluorescence, polyphenols, reactive oxygen species

Introduction

Galls are neoformed structures induced by specific parasites on their host plants (Mani 1964). As they are specific, they are considered extended phenotypes of galling organisms, which control gall morphogenesis and metabolism (Dawkins 1982; Carneiro et al. 2015). Host plant growth and metabolism is altered under influence of the associated parasites (Isaias et al. 2015; Oliveira et al. 2016), and an increase of phenolic compounds contents is observed in several insect galls (Abrahamson et al. 1991; Hartley 1998, 1999; Nyman & Julkunen-Tiitto 2000; Motta et al. 2005; Formiga et al. 2009; Guedes et al. 2016). The increment of polyphenol contents has been traditionally related to the defense against natural enemies of the galling organisms (Hartley 1998; Nyman & Julkunen-Tiitto 2000; Motta et al. 2005). However, phenolics in insect galls have been related to an increase of IAA (indol-3-acetic acid), influencing processes of cell hypertrophy and hyperplasia (Abrahamson et al. 1991; Hori 1992; Hartley 1999; Bedetti et al. 2014, 2017; Tooker & Helms 2014; Suzuki et al. 2015; Carneiro et al. 2017). They are also involved in the antioxidant system by preventing the premature senescence of gall tissues (Gopinathan & Suresh 1985; Isaias et al. 2015; Oliveira et al. 2016, 2017; Kot et al. 2017).

Despite the high oxidative stress in their tissues, occasioned by greater growth and respiration rates, some green galls can maintain photosynthetic metabolism (Bogatto et al. 1996; Fay et al. 1993; Oliveira et al. 2011, 2017; Castro et al. 2012; Carneiro et al. 2014a, 2017), which may have an important role for the maintenance of gall tissue homeostasis (Isaias et al. 2015), and avoidance of hypercarbia and hypoxia (Pincebourde and Casas 2016; Oliveira et al. 2017). Oxidative stress in plants usually causes a reduction in chlorophyll fluorescence rates (Krause & Weis 1984; Demmig-Adams & Adams 1992; Maxwell & Johnson 2000). Plant organs under increased oxidative stress have decreased photochemical efficiency, due to the decreased capability of preventing the damages caused by the saturating light (Maxwell & Jonhson 2000). However, the conversion of sunlight into chemical potential by photosystem II (photochemical reactions), the chlorophyll fluorescence, and the energy dissipation as heat, promoted by carotenoids,

are important mechanisms of light dissipation in plants (Demmig-Adams & Adams 1992), avoiding oxidative stress.

Oxidative stress is also related to the peroxidation of membrane phospholipids and oxidation of proteins (DeLong et al. 2002; Pinto et al. 2007), which may affect cell physiology and culminate in programmed cell death (PCD) (Knight et al. 2001). Accordingly, the primary roles of polyphenols may involve dissipation of oxidative stress induced both by abiotic and biotic factors (Close & McArthur 2002), while the anti-herbivore role could be effective just for the attack of generalists (Carmona et al. 2011).

Current work investigates three leaf gall systems: two of them are induced by *Ditylenchus gallaeformans* Oliveira et al. 2013 (Nematoda: Anguinidae) on *Miconia albicans* (Sw.) DC. and *M. ibaguensis* (Bonpl.) Triana, and the third one is induced by an unidentified Eriophyidae (Acarina) on *M. ibaguensis*. The galls induced by *D. gallaeformans* on *M. albicans* and *M. ibaguensis* are formed by parenchymatic vascularized emergences covering chambers, sheltering nematode colonies. Each emergence has inner nutritive tissues with promeristematic activity, i.e., capable of producing new emergences, providing indeterminate growth to these galls (Ferreira et al. 2017a, 2017b). The galls induced by the eriophyid on *M. ibaguensis* are simple galls with dense indumentum and determinate growth, usually facing the abaxial surface. The dense indumentum is formed by non-vascularized emergences, whose epidermal cells are the sites of the mites feeding (Ferreira et al. 2017a). Currently, we compare gall metabolism under the influence of distinct host plants and distinct gall-inducing species. We expect that the increase of polyphenol contents should be related to the control of oxidative stress and to the maintenance of a basal photosynthetic metabolism in tissues of leaf galls induced by nematodes and mites. As we compare the responses of distinct host plants, *M. albicans* and *M. ibaguensis*, to the same parasites, *D. gallaeformans*, and to the similar host plants, *M. ibaguensis*, to distinct parasites, *D. gallaeformans* and the Eriophyidae, we also discuss if gall metabolism is an extended phenotype of their inducers or if plant machinery is determinant for gall features.

Material and methods

Collections. Samples of *M. albicans* and *M. ibaguensis* (Melastomataceae) were collected from populations located at the Ecological Station (EEco) of the Universidade Federal de Minas Gerais (UFMG), in Belo Horizonte, Brazil (19°52’29” S; 43°58’21” W; 886 m).

Leaves galled by *D. gallaeformans* (Nematoda: Anguinidae) on both *Miconia* spp., and the control-leaves, i.e., the correspondent opposite non-galled leaves of the same pair of leaves, were collected. The control-leaves and leaves galled by an unidentified Eriophyidae (Acarina) on *M. ibaguensis* (Melastomataceae) were collected in a population located near the Center of Didactic Activities 1 (CAD-1) of UFMG (19°51'56" S; 43°57'57" W; 854 m). As explained below, in some analyses, the galled leaves were divided into non-galled portion of galled leaves (NGP), corresponding to the non-affected portions of the galled leaves; non-galled portion close to gall edge (NGCG), corresponding to the non-galled portion of galled leaves in contact with the galls (0.5 cm around the galls); and galls (the infested portions).

Impacts on leaf area. Control (mature) leaves and galled leaves (with mature galls) (10 per sample) were collected (n = 10 individuals), scanned with a scale bar, and their areas measured with the AxioVision® software. The affected area (sum of the areas with galls) of the galled leaves was measured, and the mean percentage of affected leaf area was calculated as the affected leaf area/total leaf area.

Relative water content. Discs (26.75 mm^2 each) of control-leaves and galls (10 per sample) were cut (n = 10 individuals), and immediately weighted for the determination of fresh weight (FW). The samples were immersed in distilled water for 24h at 4 ºC, dried in with a towel paper, and weighted one more time for the determination of turgid weight (TW). Then, the samples were dried in a 60 ºC stove after 24h and 48h, and weighted for the determination of dry weight (DW). Relative water content (RWC) was obtained following the relations (FW - DW)/(TW - DW) (França et al. 2012).

Chlorophyll and carotenoid contents. Chloroplast pigments were extracted using dimethyl sulfoxide (DMSO) (Barnes et al. 1992). Leaf discs (3 per sample) (26.75 mm^2 each) of control-leaves and galls were collected (n = 10 individuals), weighted and immersed in 5 mL of DMSO in amber bottles until complete extraction (about 24h). DMSO was added to the solution, reaching 10 mL of volume. The solution was analyzed in the spectrophotometer using triplicates, under the following wavelengths: 480 nm, 649 nm and 665 nm. Chlorophyll *a* and *b* and carotenoid contents were calculated according to Wellburn (1994) and were expressed in $\mu g\ mg^{-1}$ of fresh mass.

Chlorophyll fluorescence. Control and galled leaves (3 per sample) were collected (n = 5 individuals), maintained in the dark for 1h, and immediately taken to the Laboratory of Plant Anatomy for analyses in the Handy FluorCam FC 1000-H / Photon Systems Instruments®. Different photosynthetic parameters were measured for comparisons among controls and galled leaves: minimum fluorescence in dark-adapted state (F_0), maximum fluorescence in dark-adapted state (F_m), maximum PSII quantum yield in the dark-adapted state (F_v/F_m; where $F_v = F_m – F_0$,), PSII operating efficiency [$(F'_m–F')/F'_m$]; where F'_m is the fluorescence signal when all PSII centers are closed in the light-adapted state and F' is the measurement of the light-adapted fluorescence signal, instantaneous fluorescence decline ratio in light (R_{fd}) and steady-state non-photochemical quenching (NPQ) [$(F_m–F'_m)/F'_m$] (Genty et al. 1989; Oxborough 2004). The parameters were measured on the control leaves, non-galled portions of galled leaves (NGP), non-galled portions up to 0.5 cm of the gall edge (NGCG), and on galls induced by nematodes and eriophyids.

Histochemical analyses. To detect phenolic accumulation, fragments of control-leaves and of the galls were fixed in 2.5% glutaraldehyde and 4.5% formaldehyde in phosphate buffer (0.1 M; pH 7.2) (Karnovsky 1965), dehydrated in butanol series, embedded in Paraplast, and sectioned in rotary microtome (12 µm) (Kraus & Arduin 1997). The slides were deparaffinized with butyl acetate, hydrated in an ethanol series, stained with 3% Iron(III) chloride (Kraus & Arduin 1997), and mounted with colorless varnish (Paiva et al. 2006).

For detection of main ROS (reactive oxygen species) accumulation sites, hand-made transverse sections of fresh galls and control-leaves were submitted to 0.5% DAB (3,3-diaminobenzidine) for 15 and 30 min in the dark (Rosseti & Bonatti 2001), and analyzed under a light microscope Leica ICC50 HP (Leica, Wetzlar, Germany). The images were compared in order to detect the main sites of peroxidase activity.

Levels of lipid peroxidation. Control-leaves, NGP and galls (3 per sample) were collected (n = 5 individuals), in the end of the rainy season (March-April), and immediately immersed in liquid nitrogen (N_2). The material was taken to laboratory, and 0.2 g of each sample was macerated in liquid N_2 using 20 mg of PVPP (polyvinylpolypyrrolidone). An aliquot (1 mL) of 0.01% butylated hydroxytoluene (BHT) (w/v) in 80% ethanol was added to each sample. The homogenate was centrifuged at 3,000 g for 10 min, and the

supernatant was collected and maintained at 4 ºC. The supernatant (25 µl) was added to 25 µL of methanol, and other 25 µL was separately added to 25 µL of 10 mM TFF (triphenylphosphine) in methanol, in order to eliminate the background from other substances that could overestimate the results. The mixture was homogenized and stored at room temperature for 30 min. An aliquot of FOX reagent (1 mL) (90% methanol, 110 mM $HClO_4$, 4 mM BHT, 2 mM ammonium iron(II) sulfate, and 150 µM xylenol disodium salt orange) was added to each sample, the mixtures were incubated for 10 min, and therefore they were read in quadruplicates in microplate reader at 560 nm (DeLong et al. 2002; Pinto et al. 2007, modified by Faria 2010). The phospholipid hydroperoxides were quantified (nmol g^{-1} of dry mass), according equivalents of hydrogen peroxide (H_2O_2) (Merck) in the concentrations of 0 to 320 µm.

Quantification of phenolics. Control-leaves, galls, and NGP were collected (5 per sample from n = 5 individuals), in the end of rainy season, and immediately immersed in liquid N_2. The material was taken to the Laboratory of Plant Anatomy, and 0.1 g of each sample was macerated in liquid N_2. An aliquot of 500 µL of methanol at 4º C was added to each sample, the samples were vortexed, and centrifuged at 12,000 g for 5 min. The supernatant was collected and placed in clean tubes. The whole process was repeated two times with 250 µL of absolute methanol each. The extract was used to quantify the soluble phenols. An aliquote (250 µL) of 2M NaOH was added to the remaining pellet of the methanolic extraction, and maintained at 70 ºC for 16 h. Then, 250 µL of 2M HCl was added to each tube, which were centrifuged at 12,000 g for 5 min. The supernatants were used to quantify the phenolics associated to cell walls. The extracts of soluble and cell wall-associated phenolics were separately diluted in water (20 µL of solution + 980 µL of distilled water, 1:50, of *M. ibaguensis* samples; and 5 µL of solution + 995 µL of distilled water, 1:200, of *M. albicans* samples), and 100 µL of Folin-Ciocalteau reagent was added to each sample. After 5 min, 600 µL of a saturated solution of Na_2CO_3 in 1M NaOH solution was added. The samples were incubated for 1-24 h, and therefore they were read in quadruplicates in a microplate reader at 725 nm, using a chlorogenic acid standard-curve with concentrations from 0 to 250 µM. After calculation of $\varepsilon = A/C$, where A was the mean absorbance of chlorogenic acid curve, and C was the mean concentration of chlorogenic acid used in the calculation of the curve, the polyphenol concentrations of each sample (c) was calculated in mg g^{-1} of fresh mass, by the equation $c = [(A/\varepsilon)$ (DF/fresh mass)$]$, where DF is the dilution factor, DF = 50 for *M. ibaguensis* samples and

DF = 200 for *M. albicans*. The concentrations in mg g^{-1} of dry mass were given after simultaneous calculation of correspondent dry mass for each category. The extractions and calculations were performed according to Gurr et al. (1992).

Statistical analyses. The means were compared using Student's t test, or Analyses of Variance (ANOVA) followed by Tukey's post-test in the software SigmaStat®. The data in percentage were transformed by logit linear transformation (Warton & Hui, 2011) before statistical comparisons. For normality and homoscedastic conditions, some data were transformed using log10. When data did not satisfy these presupposes, they were analyzed by Kruskall-Wallis test followed by Dunn's post-test, considering $P < 0.05$. Mean and standard deviations of (gall value - control value)/(control value) obtained from each individual were used to calculate the proportional changes of the measured parameters from the control-leaves to the galls.

Results

Leaf area impacted by galls. The total leaf areas of *M. albicans* and *M. ibaguensis* were not significantly altered by *D. gallaeformans* galling activity (Fig. 1). However, an evident reduction of approximately 15% of area was observed in leaves galled by the Eriophyidae on *M. ibaguensis* (Fig. 1). *D. gallaeformans* infested an average of 1.5% (± 1.31) and 2.25% (± 2.89) of leaf areas on *M. ibaguensis* and *M. albicans*, respectively. The Eriophyidae infested an average of 22.86% (± 25.58) in *M. ibaguensis* leaf area.

Chloroplast pigments and relative water contents. The chlorophyll *a* and *b* contents decreased significantly in leaves galled by both animal species (Fig. 2A-B), but the carotenoid contents did not differ in *D. gallaeformans*-induced galls on *M. albicans*, when compared to the controls. Carotenoid contents were similar between the controls and the Eriophyidae-induced galls, however, it increased approximately 65% in *D. gallaeformans*-induced galls on *M. ibaguensis* (Fig. 2C). The relative water content (RWC) was similar in the controls and in the *D. gallaeformans* galls on *M. albicans*. In *M. ibaguensis,* the RWC was higher in the controls than in the galled leaves induced by *D. gallaeformans* and the Eriophyidae (Fig. 2D).

Chlorophyll fluorescence and non-photochemical quenching. The chlorophyll fluorescence rates (F_0 and F_m) decreased in *D. gallaeformans* galls, however, in the Eriophyidae galls, these rates were similar to the control leaves (Fig. 3A-B). Also, no differences were found in NGP and NGCG in the observed parameters (Fig. 3A-B).

The maximum PSII quantum yield (F_v/F_m), and the PSII operating efficiency $(F'_m–F')/F'_m$ of *D. gallaeformans* and the Eriophyidae galls, and respective NGP and NGCG were similar in *M. ibaguensis*. In *M. albicans,* F_v/F_m and $(F'_m–F')/F'_m$ decreased in galls, but they did not differ in NGP and NGCG (Fig. 3C-D).

The fluorescence decline ratio (R_{fd}) did not change in *M. albicans* galls, but it decreased in *D. gallaeformans* and Eriophyidae galls on *M. ibaguensis* in comparison to the controls (Fig. 3E). Even though R_{fd} was smaller in *M. ibaguensis* galls, these ratios were not altered in NGP and NGCG (Fig. 3E). The non-photochemical quenching (NPQ) was smaller in galls induced by *D. gallaeformans* on *M. albicans*, and in the galls induced by the Eriophyidae on *M. ibaguensis.* No changes were observed in NPQ in *D. gallaeformans*-induced galls on *M. ibaguensis* (Fig. 3F). The NPQ of NGP and NGCG was similar to the controls in all cases (Fig. 3F).

Polyphenol and ROS accumulation sites. The reactive oxygen species (ROS) were intensely detected in the spongy and palisade parenchymas, and in the phloem of *M. albicans* control leaves (Fig. 4A). In galls induced by *D. gallaeformans* on *M. albicans,* the ROS was intensely detected in the vascular bundles, and in the cell walls of common storage tissue, typical nutritive tissues, and the neoformed emergences (Fig. 4B). The ROS color reaction was more intense in palisade and spongy parenchymas of non-galled leaves of *M. ibaguensis*, but moderate in midrib cortex and phloem (Fig. 4C). In galls induced by *D. gallaeformans,* the reaction was intense in in the vascular bundles and trichomes. In the common storage tissue, typical nutritive tissue, and neoformed emergences, a strong color reaction was detected in cell walls (Fig. 4D). In galls induced by the Eriophyidae on *M. ibaguensis,* the ROS were intensely detected in common storage tissue, and in the emergences (Fig. 4E).

Polyphenols were intensely detected in both epidermal surfaces and in the mesophyll of control leaves of *M. albicans* (Fig. 5A). Polyphenols also accumulated in several layers of common storage tissue and in the trichomes of *D. gallaeformans* galls (Fig. 5B). A moderate staining was also observed in nutritive tissues of these galls (Fig. 5C). In *M. ibaguensis*, phenolics were intensely detected in the parenchymas and

epidermal cells of the control leaves (Fig. 5D). They were also detected in the common storage tissue and epidermal cells of Eriophyidae galls (Fig. 5E), and in the stellate trichomes, common storage tissue, and nutritive tissue of *D. gallaeformans* galls on *M. ibaguensis* (Fig. 5F).

Phospholipid peroxidation. The concentrations of peroxided phospholipids were higher in all studied galls when compared to controls (Fig. 6A). No differences between the control plants and the NGP were observed (Fig. 6A). An increase of approximately 7% of peroxided phospholipids was observed in galls induced by *D. gallaeformans* on *M. albicans*. Peroxided phospholipids increased approximately 42% in *D. gallaeformans* galls on *M. ibaguensis* galls, and 15% in the Eriophyidae galls on *M. ibaguensis*.

Polyphenol contents in galls. There was an increase in soluble phenolics in all galls when compared to the control leaves (Fig. 6B). The soluble phenol contents increased approximately 30% in *D. gallaeformans* galls on *M. albicans* and *M. ibaguensis*, and 37% in the Eriophyidae galls. There were no differences between the NGP and the control leaves. The contents of cell wall-associated phenolics increased in *D. gallaeformans* galls on *M. albicans* (60%) and in the Eriophyidae galls on *M. ibaguensis* (30%), but no increase was detected in *D. gallaeformans* galls on *M. ibaguensis* galls (Fig. 6C).

Metabolic alterations. Galls induced by *D. gallaeformans* on *M. albicans* leaves had the smallest alteration of phospholipid peroxidation (+7%). The maintenance of the R_{fd} occurred only in *D. gallaeformans* galls on *M. albicans* (Fig. 7A). In these galls, there was an increase of soluble polyphenols and polyphenols associated to the walls (Fig. 7A). In galls induced by *D. gallaeformans* on *M. ibaguensis*, which had the greatest alteration of phospholipid peroxidation (+38%), the content of polyphenols associated to the walls was maintained, but the content of soluble polyphenols increased (Fig. 7B). In those galls induced by the Eriophyidae on *M. ibaguensis,* an increase of 15% of phospholipid peroxidation was followed by an increase of soluble polyphenols and polyphenols associated to the walls (Fig. 7C).

The increase of carotenoid contents occurred only in galls induced by *D. gallaeformans* on *M. ibaguensis*, and in these galls, the NPQ was maintained (Fig. 7B). In the other studied galls, the maintenance of the carotenoid contents was followed by the significant reduction of NPQ (Fig. 7A, C). The maintenance of F_m occurred only in the

Eriophyidae galls on *M. ibaguensis* (Fig. 7C). The chlorophyll contents decreased significantly in all studied galls (Fig. 7A-C).

Discussion

The increase in phenolic contents may be related to the enhancement in plant tissue antioxidant defenses (Close & McArthur 2002; Isaias et al. 2015; Oliveira et al. 2016, 2017), as observed in the three gall systems on *Miconia* spp. We propose that cell wall associated phenolics are involved in counterbalancing the increased oxidative stress due to the galling activity and gall growth. Apoplastic peroxidases catalyze the reaction between hydrogen peroxide and polyphenols, by depositing phenolic polymers in the cell walls, and protecting cellular membranes and important molecules against peroxidation and oxidation by reactive oxygen species (ROS) (Apel & Hirt 2004). The lignin biosynthesis, additionally, is another metabolic process which consumes ROS and phenolic substrates (Apel & Hirt 2004). Phenolics have been proposed to avoid the irreversible oxidative damages in cellular machinery of insect galls (Isaias et al. 2015; Oliveira et al. 2016), which may lead to cell death (Møller et al. 2007). An increased activity of polyphenol-oxidases was related to the impairment of ROS and a major accumulation of polyphenols in insect gall tissues (Gopinathan & Suresh 1985; Kot et al. 2017). Herein, the reaction with DAB revealed major accumulation of ROS in cell walls, which lead us to infer the relation of polyphenols and ROS accumulation in nematode and eriophyid galls. This is also especially important in galls induced by *D. gallaeformans*, because they have indeterminate growth and a long life cycle (Ferreira et al. 2017a, 2017b). Even though *D. gallaeformans* galls on *M. albicans* and *M. ibaguensis* are histologically similar (Ferreira et al. 2017a), current results demonstrate that the host plant physiological responses are distinct not only according to galling herbivore species, but also to the host plant species. Similar responses in galls induced by the same galling herbivore were expected, since it is common to consider galls as extended phenotypes of their inducers (Dawkins 1982; Carneiro et al. 2015). Nevertheless, the developmental and physiological responses in galls are dependent of plant machinery, and therefore the galls may also be considered extended phenotypes of the plants (Ferreira et al. 2018).

The ROS are supposed to be important signals in gall induction, generated by the injuries caused by the gall-inducing organisms, and by the increased respiration rates required for cell growth and proliferation. The increment of ROS signalizes

developmental responses and other metabolic alterations, such as the observed alterations in phenolics biosynthesis (Isaias et al. 2015). If the ROS bursts reach irreversible levels, plant cells may enter in PCD, interrupting gall establishment. Thus, the investment in antioxidant strategies would allow the maintenance of living and functional cells and of the life cycle of the galling organisms (Oliveira et al. 2016). The galls of *D. gallaeformans* on *M. albicans* have the lowest increase of oxidative stress among the three gall systems studied, which is demonstrated by the minor increase in phospholipid peroxidation (+7%), and by the maintenance of fluorescence decline ratio (R_{fd}). This relative stability is followed by a major production of soluble (vacuolar) polyphenols (+32%), and those associated to the cell walls (apoplastic) (+60%), corroborating the initial hypothesis about the antioxidant role of phenolics. The decreasing chlorophyll *a* and *b* contents are followed by a decrease in maximum (F_v/F_m) and operating PSII quantum yield [$(F'_m-F')/F'_m$] in *D. gallaeformans-M. albicans* system. These photosynthetic parameters are linked to a reduction in chlorophyll contents, which has been associated to a dilution of pigments by cell hypertrophy in insect galls (Oliveira et al. 2011, 2017; Carneiro et al. 2014a, 2014b; Huang et al. 2014a; Malenovský et al. 2015). However, the similarity of the RWC between the non-galled leaves and the galls of *D. gallaeformans* on *M. albicans* indicates that the reduction of chlorophyll contents is not simply a case of pigment dilution. In these galls, the reduction in chlorophyll fluorescence (F_0 and F_m) and in NPQ should be associated with mechanisms of photochemical dissipation and increment of apoplastic and vacuolar phenolics, which may maintain the oxidative homeostasis (Maxwell & Johnson 2000; Close & McArthur 2002). Vacuolar polyphenols are important in antioxidant dissipation responses in plants exposed to excessive sunlight (Di Ferdinando et al. 2014). The antioxidant and photoprotective apparatus provided by the vacuolar and apoplastic polyphenols in *D. gallaeformans-M. albicans* galls supports a basal photosynthetic activity, which additionally has been proposed to avoid hypoxia and hypercarbia in insect gall tissues (Pincebourde & Casas 2016; Oliveira et al. 2017).

The physiological alterations of the galls induced by *D. gallaeformans* on *M. ibaguensis* are distinct from those of the galls on *M. albicans*. In *D. gallaeformans-M. ibaguensis* system, the increase in carotenoid contents (+70%) is followed by the maintenance of NPQ, corroborating the role of carotenoids in the prevention of photodamage. Carotenoids are important in light dissipation by transference or by light conversion in heat in xanthophyll cycle (Demmig-Adams & Adams 1992; Demmig-Addams et al. 1996). The increasing carotenoid contents and similar NPQ seem to be

especially important to the maintenance of the maximum quantum yield (F_v/F_m) and the operating efficiency of PSII [$(F'_m–F')/F'_m$]. Therefore, the decline in chlorophyll contents is compensated by the increase in carotenoid and soluble polyphenols (+24%), which maintains the efficiency of the PSII. The increment of carotenoid/chlorophyll ratios prevents the inactivation of the PSII (photoinhibition) caused by the oxidative stress in some insect galls (Huang et al. 2014b, 2015). The maintenance of a basal photosynthetic metabolism in *D. gallaeformans-M. ibaguensis* galls is linked to an increase in carotenoid and soluble polyphenols, which dissipate excessive light and allow the control of O_2 and CO_2 concentrations in gall tissues. These galls had no significant increase in apoplastic phenolics, which explains the highest oxidative stress in their tissues (+38% in phospholipid peroxidation and -24% in R_{fd}), when compared to other gall systems studied herein. Therefore, the apoplastic polyphenols are important to the maintenance of the oxidative homeostasis in galls, where the inducer's feeding activity and constant cell growth and replication generate more ROS (Isaias et al. 2015, Oliveira et al. 2016).

The phospholipid peroxidation (+15%) and R_{fd} (-40%) in Eriophyidae galls on *M. ibaguensis* indicate an intermediate level when compared to the galls of *D. gallaeformans*, which is also true for the accumulation of apoplastic (+39%) and soluble phenolics (+32%). As expected for the anatomically simplest of the three galls (Ferreira et al. 2017a), the physiological impacts of the eriophyid cause a minor reduction in chlorophyll contents. The oxidative impacts are significant, but do not affect chlorophyll fluorescence (F_0 and F_m) and PSII yield, which may be associated to the photoprotective effects of increased soluble phenolic contents (Close & McArthur 2002; Di Ferdinando et al. 2014). In fact, the accumulation of vacuolar phenolics demonstrated by the histochemical reaction to Iron(III) chloride occurs mainly in the upper cell layers of the control leaves and galls. The high infestation of the Eriophyidae on *M. ibaguensis* causes a significant reduction in leaf area, as observed in other gall systems (Constantino et al. 2009; Nasareen & Ramani 2014), but does not affect the fluorescence rates and the PSII yield as *D. gallaeformans* does.

Distinct from Eriophyidae-*M. ibaguensis* and *D. gallaeformans-Miconia* spp., the effects of other eriophyids and insects on non-galled portions of infested leaves and galls affect the fluorescence and PSII yield, which is directly related to infestation levels (Samsone et al. 2012). Therefore, *M. albicans* and *M. ibaguensis* are able to constrain the effects of the galling colonies to gall developmental sites, preventing additional oxidative damages in photosynthetic machinery. The non-alteration of phospholipid peroxidation

levels and phenolic contents in non-galled portions of galled leaves may be related to antioxidant and photoprotective strategies of the host plants. The restricted impacts of the galls on *M. albicans* and *M. ibaguensis* demonstrate the capability of these plants to control the increment of ROS in adjacent cells, due to their antioxidant systems, including the high production of polyphenols. Accordingly, plants capable of increasing polyphenol and carotenoid production during the galling herbivore attack and gall development should be less affected by irreversible oxidative stress and premature cell death.

The host plant metabolic specificities determine distinct responses in the physiological impacts of the gall inducers. The responses observed in galls induced by the same parasite, *D. gallaeformans*, on the two host plant species, *M. albicans* and *M. ibaguensis*, reveal two strategies: first, an increase of apoplastic polyphenols as a regulator of oxidative homeostasis, and second, an increase of carotenoid contents, which leads to an increase in excessive energy dissipation. Both strategies are important to maintain a basal photosynthetic metabolism and to avoid hypercarbia and hypoxia in gall tissues. The antioxidant role of apoplastic phenolics avoids the triggering of oxidative burst in the apoplast, which may consequently prevent programmed cell death and premature gall senescence. Distinct from the common sense about the galling parasite control over its host plants, the distinct physiological responses induced by *D. gallaeformans* on *M. albicans* and *M. ibaguensis* demonstrate the crucial role of the host plant physiological machinery in the determination of gall phenotypes. Therefore, galls are not strictly the extended phenotypes of their inducers, and their features reflect peculiar physiological responses of their host plants.

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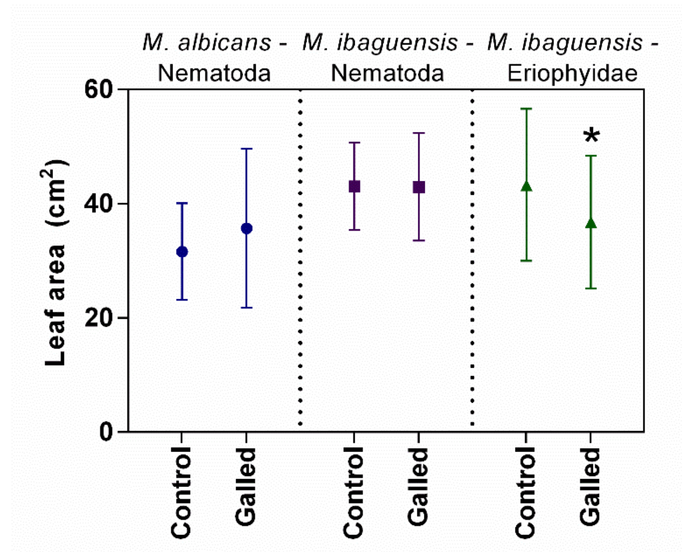

Figure 1. Leaf area (cm^2) of controls and leaves galled by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans*; by *D. gallaeformans* on *M. ibaguensis*; and by an unidentified Eriophyidae on *M. ibaguensis.* The bars indicate the means and standard deviations. The asterisk indicates significant difference ($P < 0.05$).

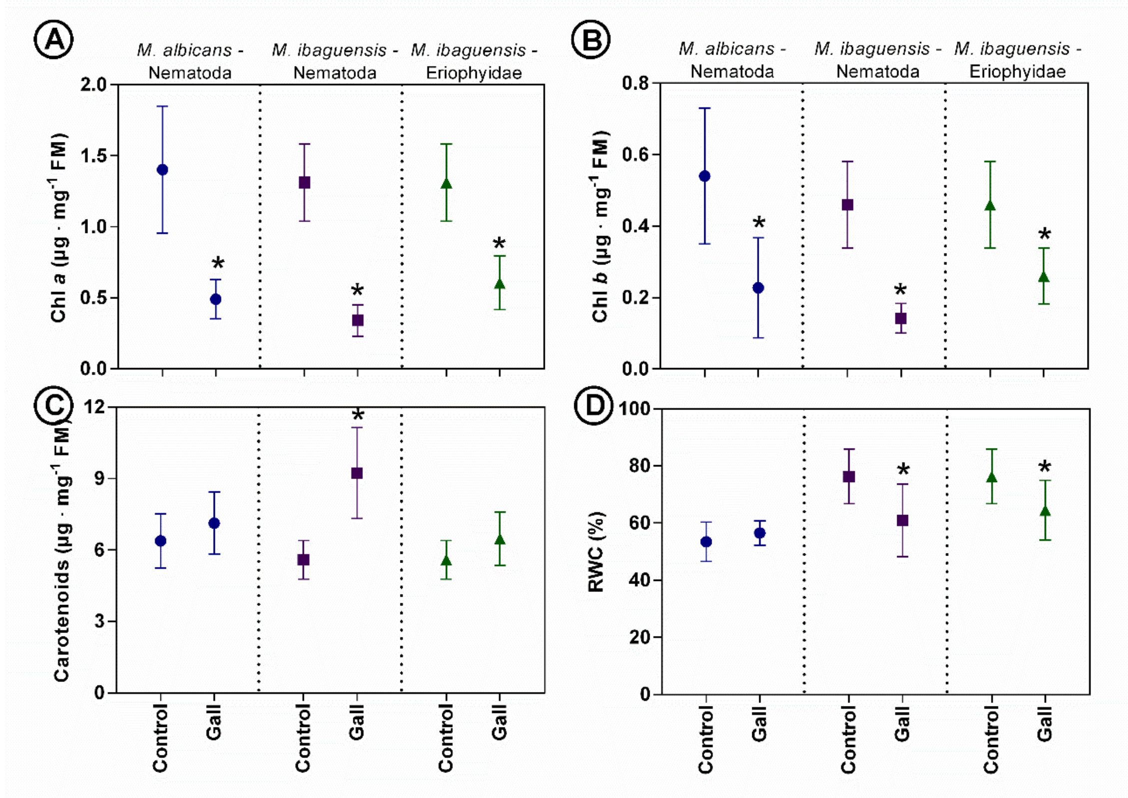

Figure 2. Chloroplast pigments (µg g^{-1}) and relative water content (%) in leaves and galls induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans*; by *D. gallaeformans* on *M. ibaguensis*; and by an unidentified Eriophyidae on *M. ibaguensis*. *A*- chlorophyll *a*. ***B*-** chlorophyll *b*. ***C*-** carotenoids. ***D*-** relative water content (RWC). **Bars** indicate the means and standard deviations. **Asterisks** indicate significant differences ($P < 0.05$).

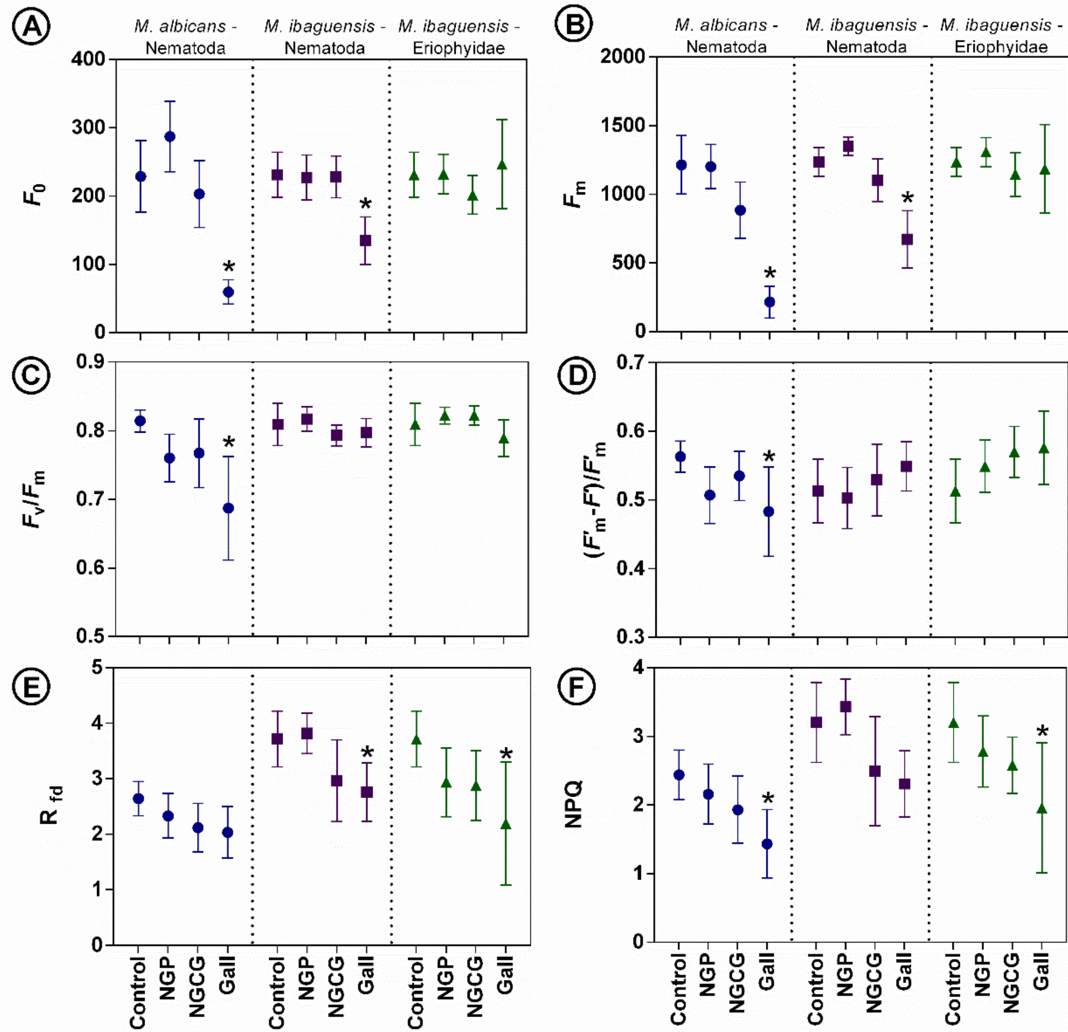

Figure 3. Photosynthetic parameters in leaves, non-galled portions of galled leaves (NGP), non-galled portions close to gall edge (NGCG), and galls induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans*; by *D. gallaeformans* on *M. ibaguensis*; and by an unidentified Eriophyidae on *M. ibaguensis*. *A-* F_0 (minimum fluorescence in dark-adapted state). ***B-*** F_m (maximum fluorescence in dark-adapted state). ***C-*** F_v/F_m (maximum quantum yield of photosystem II). ***D-*** $(F'_m–F')/F'_m$ (PSII operating efficiency). ***E-*** R_{fd} (instantaneous fluorescence decline ratio in light). ***F-*** NPQ (instantaneous non-photochemical quenching during light adaptation). **Bars** indicate the means and standard deviations. **Asterisks** indicate significant differences ($P < 0.05$).

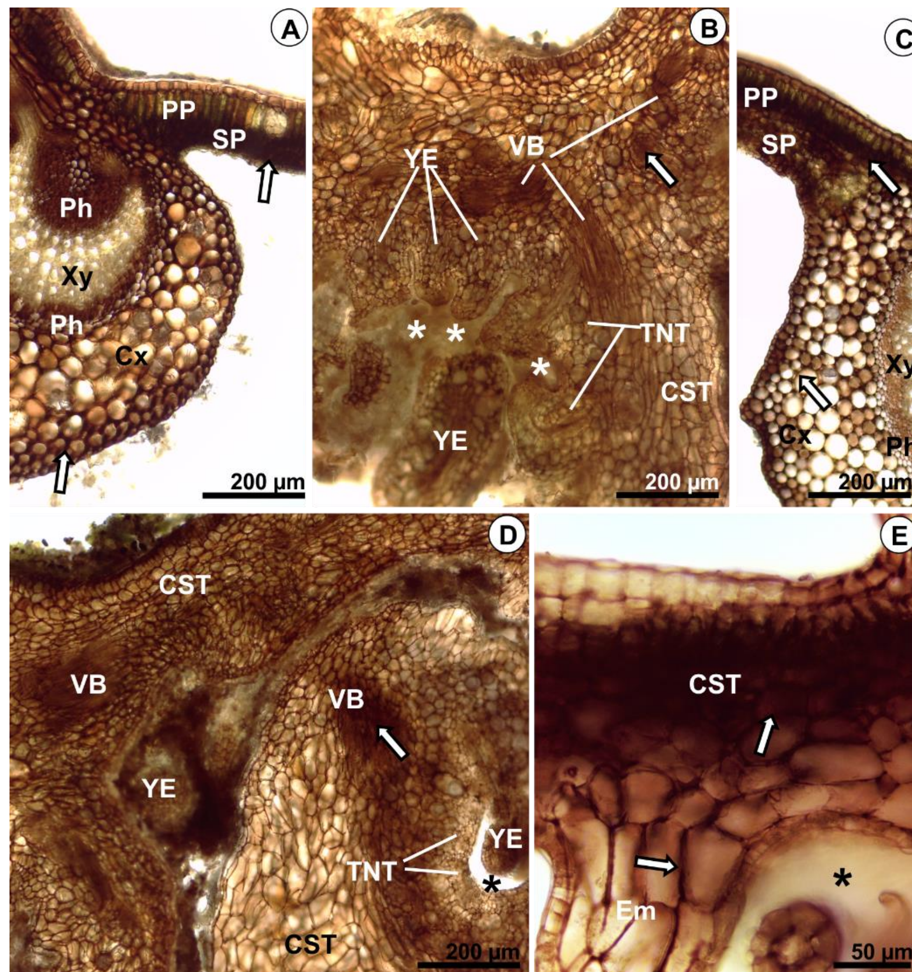

Figure 4. Histochemistry of reactive oxygen species (ROS) in leaves and galls of *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans* and *M. ibaguensis*, and Eriophyidae galls on *M. ibaguensis*. Transverse sections. The asterisks indicate the gall chambers; the arrows indicate accumulation of ROS. ***A-B-*** *M. albicans.* ***A-*** Midrib and mesophyll of control leaves. ***B-*** Nematode gall. ***C-F-*** *M. ibaguensis.* ***C-*** Midrib and mesophyll of control leaves. ***D-*** Nematode gall. ***E-F***- Eriophyid gall. **Abbreviations:** ***CST,*** common storage tissue; ***Cx,*** cortex; ***Em,*** emergence; ***Ph,*** phloem; ***PP,*** palisade parenchyma; ***SP,*** spongy parenchyma; ***TNT,*** typical nutritive tissue; ***VB,*** vascular bundle; ***Xy,*** xylem; ***YE,*** young emergences. **Staining:** 0.5% 3,3′-diaminobenzidine (DAB).

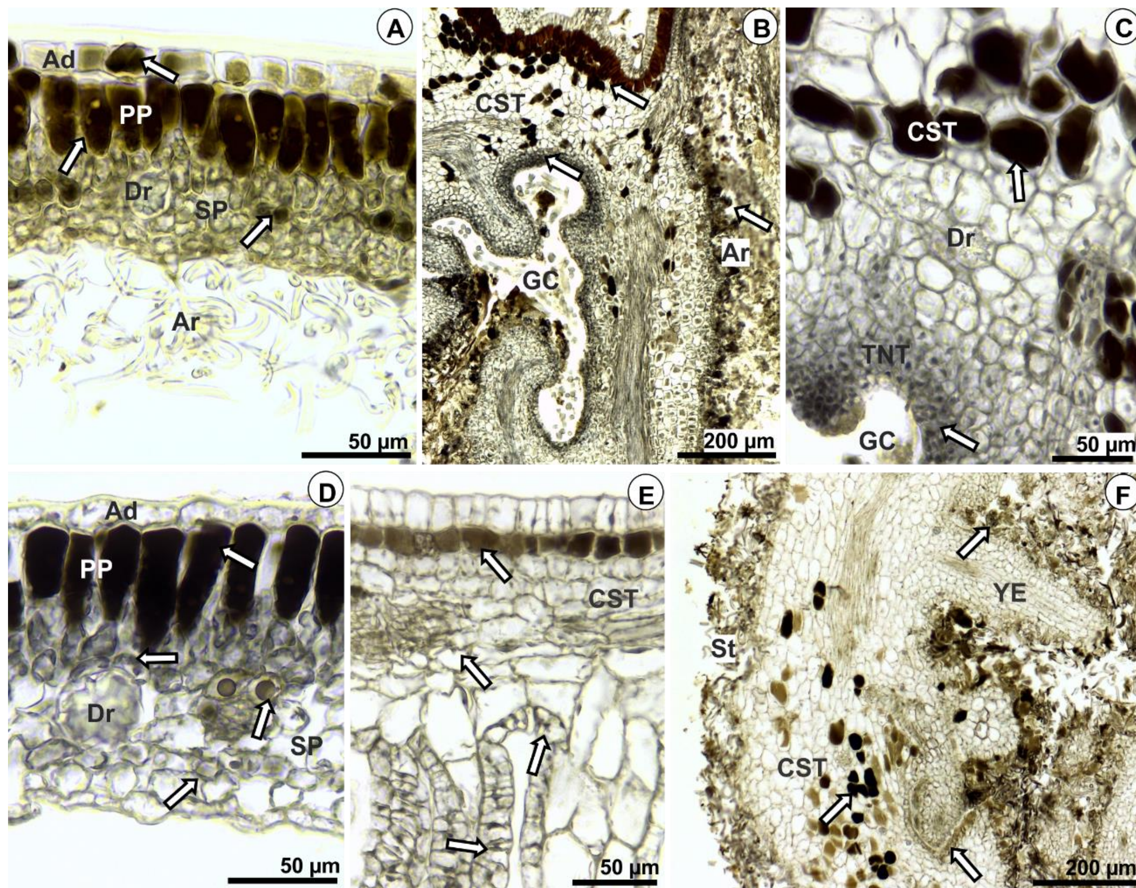

Figure 5. Histochemistry of polyphenols in leaves and galls induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans*; by *D. gallaeformans* on *M. ibaguensis*; and by an unidentified Eriophyidae on *M. ibaguensis.* Transverse sections. The arrows indicate positive reaction. ***A-C-*** *M. albicans.* ***A-*** Mesophyll of control leaves. ***B-C-*** Nematode gall. ***D-F-*** *M. ibaguensis.* ***D-*** Mesophyll of control leaves. ***E-*** Eriophyid gall. ***F***- Nematode gall. **Abbreviations:** ***Ad,*** adaxial epidermis; ***Ar,*** arachnoid trichomes; ***CST,*** common storage tissue; ***Dr*****,** druses; ***Em,*** emergence; ***GC,*** gall chamber; ***PP,*** palisade parenchyma; ***SP,*** spongy parenchyma; ***St,*** stellate trichomes; ***TNT*****,** typical nutritive tissue; ***YE,*** young emergences. **Staining:** 3% Iron(III) Chloride.

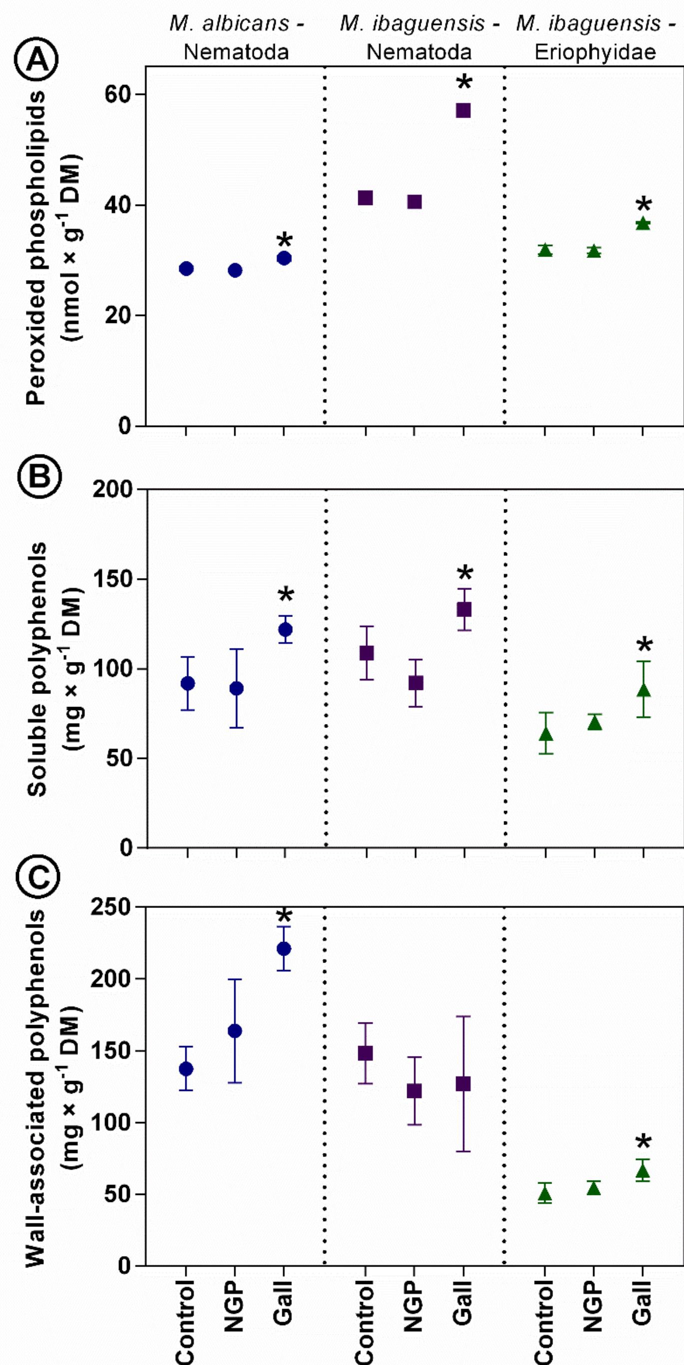

Figure 6. Polyphenol (mg g^{-1}) and peroxided phospholipid (nmol g^{-1}) contents in leaves, non-galled portions of galled leaves (NGP), and galls induced by *Ditylenchus gallaeformans* (Nematoda) on *Miconia albicans*; by *D. gallaeformans* on *M. ibaguensis*; and by an unidentified Eriophyidae on *M. ibaguensis*. *A-* Peroxided phospholipids. ***B-*** Soluble phenolics. ***C-*** Cell wall associated phenolics. **Bars** indicate the means and standard deviations. **Asterisks** indicates significant differences ($P < 0.05$).

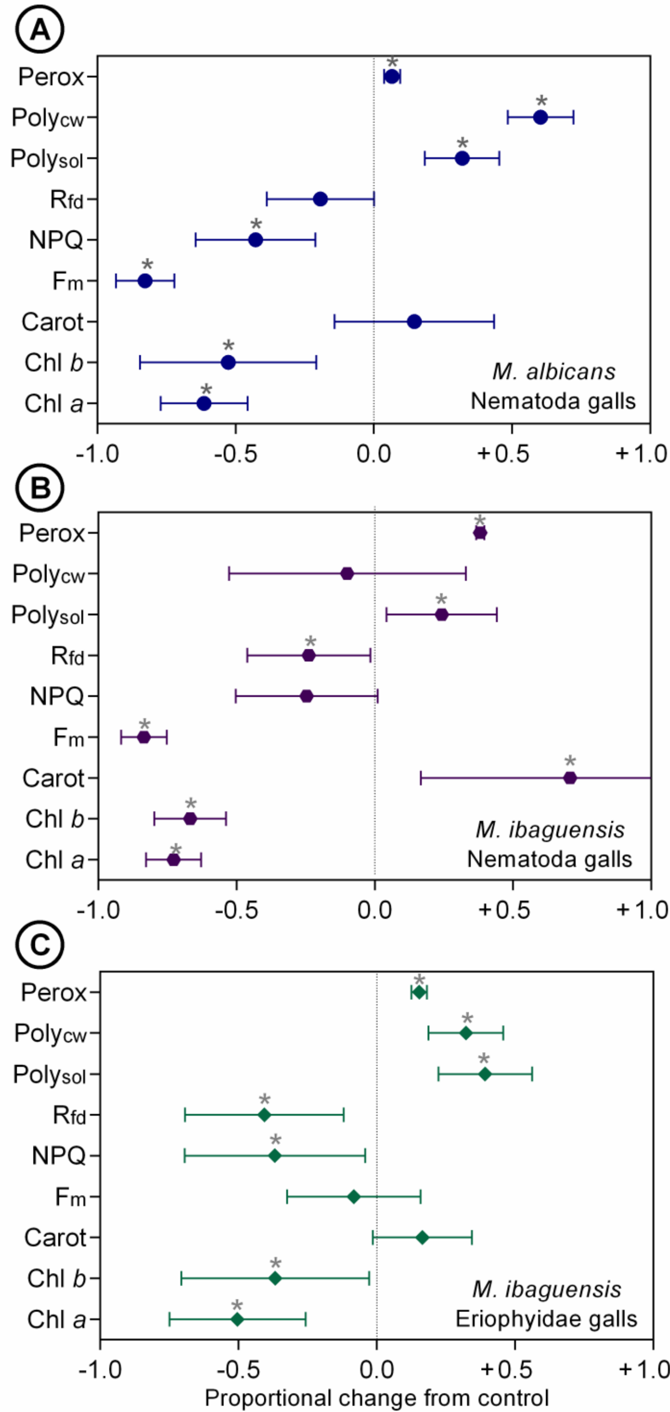

Figure 7. Metabolic alterations (mean and standard deviation) in galls when compared to non-galled leaves. ***A-*** *Miconia albicans-Ditylenchus gallaeformans* (Nematoda) galls; ***B-*** *Miconia ibaguensis-D. gallaeformans* galls; ***C-*** *M. ibaguensis*-Eriophyidae (Acarina) galls. **Parameters:** Perox: peroxided phospholipids; $Poly_{CW}$: polyphenols associated to the cell walls; $Poly_{sol}$: soluble polyphenols; NPQ: non-photochemical quenching during light adaptation; F_m: maximum fluorescence; Carot: carotenoid content; Chl *b*: chlorophyll *b* content; Chl *a*: chlorophyll *a* content. **Asterisks** indicate statistical differences ($P < 0.05$).

CONSIDERAÇÕES FINAIS

Galhas induzidas por animais, de modo geral, apresentam muitas peculiaridades anatômicas determinadas pelo modo de alimentação do galhador, os locais sobre os quais estes se alimentam, bem como seu táxon. Galhas induzidas por nematódeos, por sua vez, apresentam tecidos nutritivos bastante especializados, que variam de acordo com a espécie indutora. Aquelas induzidas por *Ditylenchus gallaeformans* apresentam tecidos nutritivos promeristemáticos, característica aqui reportada pela primeira vez para zoocecídias. Essa característica, tipicamente relacionada às células da planta hospedeira, é fundamental para a manutenção da vida útil mais longa destas galhas, e para o valor adaptativo destas para as colônias de nematódeos indutores.

Muito embora as especificidades das galhas sejam comumente atribuídas às espécies de galhadores, galhas induzidas por nematódeos, ácaros e insetos podem apresentar características convergentes, tais como hipertrofia celular, aumento da densidade do indumento, hiperplasia, homogeneização do parênquima, a diferenciação de um tecido comum de reserva (TCR) e de tecidos nutritivos típicos (TNT). Tais similaridades indicam produtos da manipulação do potencial ontogenético das plantas hospedeiras. Contudo, há limites impostos a esta manipulação, ligados à diferenciação celular e tecidual de cada espécie vegetal. Como exemplos destes limites, podemos citar os diferentes tipos de tricomas tectores observados em galhas induzidas por um mesmo galhador, *Ditylenchus gallaeformans*, em *Miconia albicans* e *M. ibaguensis*, e a manutenção de estruturas secretoras imersas no floema de *Pistacia terebinthus* em galhas induzidas por *Geoica utricularia.*

De forma inesperada, a ação de *D. gallaeformans* sobre duas espécies vegetais hospedeiras, *Miconia albicans* e *M. ibaguensis*, resulta no aumento no número médio de carpelos nas plantas hospedeiras, quando comparadas às plantas não infestadas. Contudo, em *M. albicans*, o aumento no número médio de lóculos por fruto é observado em ramos não galhados de indivíduos galhados. Em *M. ibaguensis*, o aumento no número médio de lóculos por fruto é significativo em frutos presentes nos ramos galhados. Ademais, as respostas diferenciais à ação dos indutores nas espécies cogenéricas aqui estudadas se estendem às respostas metabólicas, nas quais os sistemas antioxidantes também variam de acordo com a planta hospedeira, sendo distintas em *M. albicans* e *M. ibaguensis*

atacadas por *D. gallaeformans.* Enquanto nas galhas em *M. albicans,* o investimento na produção de polifenóis no apoplasto previne a peroxidação dos fosfolipídios de membranas, nas galhas induzidas em *M. ibaguensis,* a prevenção à peroxidação se dá pelo investimento na produção de carotenoides, o que permite a maior dissipação de energia excessiva. De qualquer maneira, ainda que o teor de clorofilas nas galhas de *D. gallaeformans* seja reduzido, ambos os mecanismos de dissipação de energia permitem que o fotossistema II permaneça ativo, favorecendo também a manutenção de uma taxa basal de fotossíntese.

Adicionalmente, a análise de galhas de indutores diferentes na mesma planta hospedeira, como é o caso das galhas de *D. gallaeformans* e Eriophyidae em *M. ibaguensis* permitiram registrar tanto divergências quanto convergências anatômicas, histoquímicas e fisiológicas. Deste modo, a contribuição deste trabalho ao estado da arte das interações animal-planta que culminam no desenvolvimento de galhas reside no fato de que galhas são fenótipos anatômicos e fisiológicos determinados tanto pela espécie de galhador, quanto pela espécie de planta hospedeira, sendo, portanto, ambos os organismos associados agentes protagonistas na determinação fenotípica da galha.

Do ponto de vista metabólico, avançamos pelo entendimento de que galhadores como os nematódeos, capazes de induzir galhas em espectros mais amplos de plantas hospedeiras, possuem maior capacidade de se adaptar a hospedeiros com potencialidades anatômicas e metabólicas distintas. Embora tratando-se de espécies distintas, as *Miconia* spp. adaptadas a condições de estresse hídrico e luminoso, possuem mecanismos distintos de dissipação de radicais livres, os quais são potencializados nas interações com Nematoda e Acarina galhadores, garantindo seu estabelecimento e a sobrevivência prolongada das células das galhas.

Tendo em vista o conhecimento gerado por esta tese em relação aos sistemas de *Miconia* com ácaros e nematódeos galhadores, o próximo desafio é compreender como plantas hospedeiras filogeneticamente próximas respondem à influência dos animais na expressão de caracteres como a composição da parede celular (pectinas e hemiceluloses), características citológicas dos tecidos de reserva, acúmulo de auxinas e citocininas, e atividade de enzimas ligadas ao metabolismo dos carboidratos. Estes estudos permitirão testar a hipótese de que as potencialidades das plantas hospedeiras são determinantes na estrutura e fisiologia das galhas, mediando o foco do protagonismo entre indutores e plantas hospedeiras.

CONSIDERACIONES FINALES

Las agallas inducidas por animales, de manera general, presentan muchas peculiaridades anatómicas determinadas por el modo de alimentación del individuo gallícola, los sitios sobre los cuales estos se alimentan, y el taxón del inductor. Las agallas inducidas por nemátodos presentan tejidos nutritivos muy especializados, que varían de acuerdo con la especie inductora. Específicamente, las agallas inducidas por *Ditylenchus gallaeformans* poseen tejidos nutritivos promeristemáticos, característica reportada aquí por primera vez para zoocecidias. Esta característica, típicamente relacionada con las células de la planta hospedera, es fundamental para el mantenimiento de la vida útil más extensa de estas agallas, y por el valor adaptativo que le confiere para las colonias de nemátodos inductores.

Aunque las especificidades de las agallas son comúnmente atribuidas a las especies gallícolas, las agallas inducidas por nemátodos, ácaros e insectos pueden presentar características convergentes, tales como hipertrofia celular, aumento de la densidad del indumento, hiperplasia, homogenización del parénquima, diferenciación de tejido común de reserva (TCR) y de tejidos nutritivos típicos (TNT). Dichas similitudes indican la manipulación del potencial ontogenético de las plantas hospederas. Sin embargo, hay límites impuestos a esta manipulación, ligados a la diferenciación celular y tisular de cada especie vegetal. Como ejemplos de estos límites, podemos citar los diferentes tipos de tricomas tectores observados en agallas inducidas por una misma especie gallícola, *Ditylenchus gallaeformans*, sobre *Miconia albicans* y *M. ibaguensis*, y la manutención de estructuras secretoras inmersas en el floema de *Pistacia terebinthus* en agallas inducidas por *Geoica utricularia*.

De manera inesperada, la acción de *D. gallaeformans* sobre las dos especies de plantas hospederas, *Miconia albicans* y *M. ibaguensis*, resulta en un aumento del número medio de carpelos en las plantas hospederas, con respecto a las plantas no infestadas. Sin embargo, en *M. albicans* se observó el aumento del número de lóculos por fruto en ramas sin agallas de individuos infestados por agallas. En *M. ibaguensis*, el aumento del número medio de lóculos en frutos sobre ramas con agallas fue significativo. Además, las respuestas diferenciales a la acción de los inductores en las especies congéneres aquí estudiadas, se extienden a respuestas metabólicas, en las cuales los sistemas antioxidantes

también varían de acuerdo con la planta hospedera, siendo distintas en *M. albicans* y *M. ibaguensis* atacadas por *D. gallaeformans*. En las agallas de *M. albicans* la inversión en la producción de polifenoles en el apoplasto previne la peroxidación de fosfolípidos de membranas, mientras que en las agallas inducidas en *M. ibaguensis* la prevención de la peroxidación ocurre por la inversión en la producción de carotenoides, lo que permite una mayor disipación de energía excesiva. De igual forma, aunque el contenido de clorofilas en las agallas de *D. gallaeformans* fue reducido, ambos mecanismos de disipación de energía permiten que el fotosistema II permanezca activo, lo que favorece además la manutención de una tasa basal de fotosíntesis.

Adicionalmente, el análisis de las agallas inducidas por distintas especies en un mismo hospedero, como en el caso de *D. gallaeformans* y Eriophyidae sobre *M. ibaguensis*, ha permitido registrar tanto divergencias como convergencias anatómicas, histoquímicas y fisiológicas. De este modo, la contribución de este trabajo para el estado del arte de la interacción animal-planta, especialmente la formación de agallas, reside en el hecho de que las agallas son fenotipos anatómicos e fisiológicos determinados tanto por la especie de animal gallícola, como por la especie de hospedero, siendo, por lo tanto, ambos organismos protagonistas del fenotipo de las agallas.

Desde el punto de vista metabólico, hemos avanzado en el entendimiento de que los inductores, como nematodos capaces de inducir agallas en un espectro más amplio de plantas hospederas, poseen mayor capacidad de adaptarse a hospederos con diferentes potencialidades anatómicas y metabólicas. Aunque, son especies distintas, las *Miconia* spp. están adaptadas a condiciones de estrés hídrico y luminoso, por lo que poseen mecanismos distintos de disipación de radicales libres, los cuales son potencializados durante las interacciones con gallícolas como Nematoda y Acarina, lo que garantiza el establecimiento y supervivencia prolongada de las células de las agallas.

Teniendo en cuenta el conocimiento generado por esta tesis, en los sistemas de *Miconia* con ácaros y nemátodos gallícolas, el próximo reto está en comprender como los hospederos filogenéticamente relacionados responden a la influencia de los animales en la expresión de caracteres como la composición de la pared celular (pectinas y hemicelulosas), características citológicas de los tejidos de reserva, acumulación de auxinas y citocininas, y la actividad de enzimas ligadas al metabolismo de los carbohidratos. Estos estudios permitirán testar la hipótesis de que las potencialidades de

las plantas hospederas son determinantes en la estructura y fisiología de las agallas, mediando el protagonismo entre inductores y plantas hospederas.