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Original Article

New functional tissues induced by a galling lepidopteran on stems of *Marcetia taxifolia* (Melastomataceae): An analysis based on cell wall composition and dynamics

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ABSTRACT

The plant cell walls are dynamic structures, whose pectin composition varies according to the developmental and functional stages and in response to external factors. Gall inducers change the functional profiles of their host organs, and herein, we explore how stem cell walls respond to gall development, particularly in the nutritive tissues consumed by the lepidopteran larva. The pectin composition of the non-galled stems of *Marcetia taxifolia* (Melastomataceae) in primary and secondary growth and of Lepidoptera stem galls in distinct stages of development were studied by immunocytochemistry. During the development of the stems of *M. taxifolia*, the changes in protein and pectin composition suggest that young stems are growing and elongating with the help of extensins, arabinogalactan-proteins (AGPs), and homogalacturonans (HGs). In the galls, the predominance of galactans indicates changes in the pectin composition, allowing cell wall stiffness in hyperplasic tissues. The chewing stimuli of the Lepidoptera induce the synthesis of galactans, arabinans, and HGs in nutritive cells, allowing the cell wall flexibility during the hyperplasic process and the porosity necessary for the translocation of water and nutrients toward the gall chamber.

Keywords: Cell walls, Insect galls, Lepidoptera, *Marcetia taxifolia*, Nutritive tissue, Stem anatomy.

Introduction

The cell wall is an important compartment in plant cells, whose variable composition determines tissue functions (Lionetti *et al.*, 2012; Cosgrove, 2016; Boanares *et al.*, 2018). Pectins, as key cell wall components, mediate interactions with external factors (Agoda-Tandjawa *et al.*, 2016; Wu *et al.*, 2018; Liu *et al.*, 2021). Pectins are complex and diverse structural carbohydrates that contain galacturonic acid (Caffall & Mohnen, 2009), and are essential for cell wall properties such as flexibility, elasticity, stiffness, porosity, and hydration (Knox *et al.*, 1990; Cosgrove, 2016). The most abundant pectins in the plant cell walls are the homogalacturonans (HGs), which are synthesized in a methyl-esterified form (Knox *et al.*, 1990). However, during tissue development, these HGs may be de-methyl-esterified by pectin methyl-esterases (PMEs) (Lionetti *et al.*, 2012), exposing negative charges that bind with calcium ions.

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The binding of Ca^{2+} with de-methyl-esterified HGs forms pectic gels that rigidify and reinforce the cell walls (Knox *et al.*, 1990; Sabba & Lulai, 2005). This reinforcement is important to control the calcium input and the cell maturity (Mastroberti & Mariath, 2008; Douchiche *et al.*, 2010). The HGs linked to the calcium ions are essential in the middle lamellae, where they promote cell adhesion (Knox *et al.*, 1990; Knox, 1992; Albersheim *et al.*, 2010). During fruit softening, for example, the pectate lyases and endopolygalacturonases cleave mainly the low methyl-esterified HGs, promoting cell wall loosening (Micheli, 2001; Marín-Rodriguez *et al.*, 2002).

Plant cell walls may also be rich in rhamnogalacturonans I (RG-I) and II (RG-II), which are groups of ramified and heterogeneous pectin polymers (Knox *et al.*, 1990; Albersheim *et al.*, 2010). The RG-I are branched polymers of rhamnose and galacturonic acid (Moore *et al.*, 2008; Albersheim *et al.*, 2010). The lateral chains of the RG-I may be replaced by arabinans and galactans (Albersheim *et al.*, 2010). The arabinans confer reinforcement with flexibility and are essential to maintaining the hydration of the cell walls during the ordinary development of some tissues and under stress (Moore *et al.*, 2008; Lahaye *et al.*, 2020). The galactans seem to be involved in cell elongation (McCartney *et al.*, 2003) and the extensibility of cell walls (Jones *et al.*, 1997). When combined, arabinans, galactans, and HGs may also contribute to an increase in cell wall porosity (Zykwinska *et al.*, 2005).

Other important compounds in the cell walls are glycoproteins, such as the arabinogalactan proteins (AGPs) and the extensins. The AGPs operate as cell signals during cell differentiation, embryogenesis, and tissue development, and are directly involved in cell divisions (Showalter, 1993; Seifert & Roberts, 2007; Albersheim *et al.*, 2010). The extensins are glycoproteins that act on the strengthening of the cell walls during the development (Sabba & Lulai, 2005), as well as in the defense against pathogens (Showalter, 1993; Gao & Showalter, 1999; Leroux *et al.*, 2011). These glycoproteins seem to increase the cross-linking between pectins, which may contribute to the cell wall's defense against infections by limiting the mobility of pathogens, depending on specific peroxidase activity (Showalter, 1993; Castilleux *et al.*, 2020).

The cell fate determination in response to external factors involves changes in the dynamics of cell wall composition (Cosgrove, 2016; Wu *et al.*, 2018; Liu *et al.*, 2021), alongside other factors such as cytoplasmic alterations. In this context, the interactions between insects and plants have been proven to cause cell redifferentiation (*sensu* Lev-Yadun, 2003) and, consequently, the redirection of cell structural and functional features. These new features imply the establishment of cytological and histochemical peculiarities, as observed in several insect galls (Oliveira & Isaias, 2010; Oliveira *et al.*, 2010; Ferreira & Isaias, 2013; 2014; Vecchi *et al.*, 2013; Ferreira *et al.*, 2015; 2019; 2022; Rezende *et al.*, 2019; Nobrega *et al.*, 2021). These changes depend on the alterations in cell wall composition (Formiga *et al.*, 2013; Oliveira *et al.*, 2014; Carneiro *et al.*, 2014; Teixeira *et al.*, 2018; Martini *et al.*, 2019; Bragança *et al.*, 2020; Ferreira *et al.*, 2020; Silva *et al.*, 2021; Nogueira *et al.*, 2024; Santos *et al.*, 2024), which set the neoformed tissues of insect galls into elegant models to elucidate the involvement of the cell walls in developmental processes.

The changes in cell walls in galls depend not only on plant cell potentials to respond to external stress factors but also on the species-specific stimuli of the gall inducers (Formiga *et al.*, 2013; Oliveira *et al.*, 2014; Ferreira *et al.*, 2020). For instance, in *Bacharis reticularia* DC., a super host of gall inducers, the extensins were detected only in the pocket gall morphotype, which has major structural alterations compared to the host organ (Formiga *et al.*, 2013). The role of the extensins was related to the wall strengthening in the new design assumed by the gall (Formiga *et al.*, 2015). Accordingly, changes in pith cell elongation, as occurs in the development of nutritive cells in stem lepidopteran galls (Ferreira & Isaias, 2013; Santos *et al.*, 2024), should demand shifts in the set of pectins in cell walls.

Distinct of leaves, the stems elongate predominantly in an anticlinal direction, which could involve the highly methyl-esterified homogalacturonans (HGs) and galactans (Hwang & Kokini, 1991; McCartney *et al.*, 2003; Xu *et al.*, 2011). Consequently, stem galls are good models to test the role of pectins in cell elongation and the fate of the cell wall composition because the functional alterations occur mainly in a group of cells. In this context, the nutritive cells derived from pith cells in the Lepidoptera-induced stem galls on *Marcetia taxifolia* (A.St.-Hil.) DC. (Melastomataceae) (Fig. 1A) (Ferreira & Isaias, 2013; Ferreira *et al.*, 2015) are interesting models for examining how pectin dynamics contribute to the cellular adaptations that support gall development. *Marcetia taxifolia* is a plant adapted to the vegetation of Campos Rupestres (rocky fields) in Minas Gerais, Brazil. It is suited to extreme sunlight exposure and drought conditions during the day, cold nights, and foggy mornings (Alves *et al.*, 2014; Oliveira *et al.*, 2016). Based on studies on the same host plant-galling insect system, it is possible to establish parallels among cytological (Ferreira *et al.*, 2015), histochemical peculiarities (Ferreira & Isaias, 2013), and cell wall functional composition of non-galled stems and lepidopteran galls. We expect that the cytological features of cell walls of gall nutritive tissues, with multivesicular bodies and thin and polylamellated cell walls (Ferreira & Isaias, 2013; Ferreira *et al.*, 2015) are associated with a pectin composition that allows cell replication and nutrient availability to the gall inducer. We anticipate that the stress induced by the gall-inducing Lepidoptera alters the cell wall composition and dynamics of non-galled organs. Additionally, we expect the gall nutritive tissue to show high levels of HGs, galactans, and arabinans due to its hyperplasic characteristics.

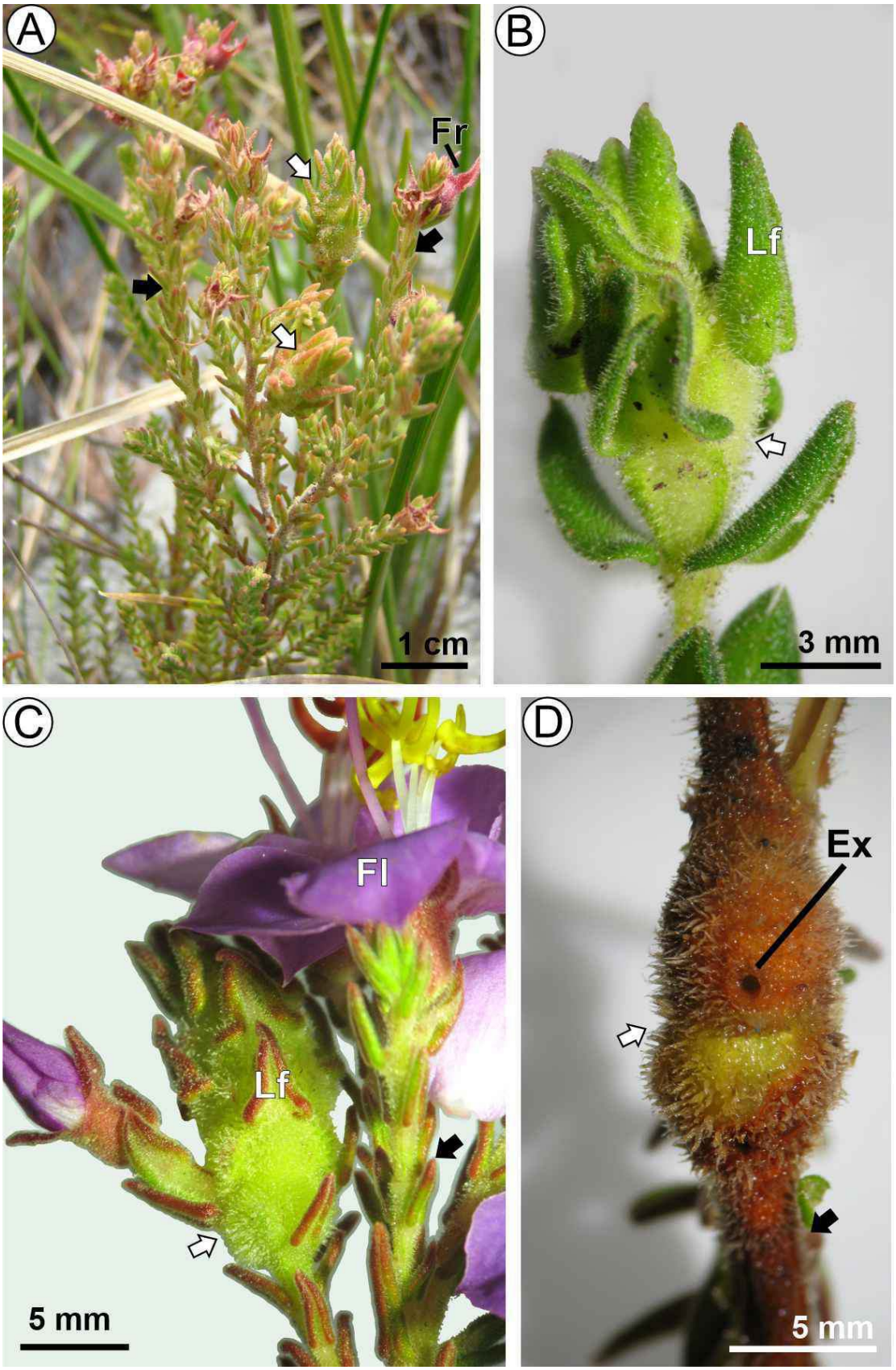


Figure 1. Stem galls on *Marcetia taxifolia* (Melastomataceae). **A)** Plant habit and stem galls. **B)** Gall in the growth and development phase (GGD). **C)** Gall in the maturation phase (GM). **D)** Gall in the senescence phase (GS). Black arrows: non-galled stems; White arrows = galls. Abbreviations: Ex = exit hole; Fl = flower; Fr = fruit. Lf = leaf.

Material and Methods

Sampling. Stems of *Marcetia taxifolia* (A.St.-Hil.) DC. (Melastomataceae) in primary growth (SP), secondary growth (SS), and fusiform stem galls induced by a microlepidopteran in the growth and development phase (GGD) (Fig. 1B), maturation (GM) (Fig. 1C), and senescence (GS) (Fig. 1D) were collected (n = 4 per category) from distinct individuals of the "crystal-pink" population (*sensu* Gardoni *et al.*, 2007) (19º17'52,5" S 043º35'27,8" W; 1321 m) at Serra do Cipó, Minas Gerais state, Brazil. The developmental phases were defined according to Ferreira & Isaias (2013), as follows: the GGD had 2–4 mm thickness, the GM was larger (4–6 mm), and the GS had the presence of an exit hole. A voucher of the fertile material (specimen with flowers) and with the Lepidoptera stem galls (with the gall inducer) was deposited in the BHCB herbarium under the number 161778.

Fixation and microtomy. Hemi-sectioned fragments of each sample were fixed on 2.5% glutaraldehyde and 4.5% formaldehyde in 0.1M, pH 7.2 PBS (phosphate buffered saline) (Karnovsky, 1965), dehydrated in *n*-butyl-series, and embedded in Paraplast® (Kraus & Arduin, 1997). The blocks were sectioned in a rotary microtome (12 µm) and fixed on the slides with Haupt adhesive (Haupt, 1930; Kraus & Arduin, 1997). The slides were dried on a 42º C hotplate, deparaffinized with butyl-acetate, and hydrated in ethyl-series (Kraus & Arduin, 1997). For anatomical analyses, the hydrated slides were stained with a 9:1 mixture of 0.5% astra blue and 0.5% safranin (Bukatsch, 1972), dehydrated in ethyl series followed by butyl acetate (Kraus & Arduin, 1997), and mounted in Acrilex® colorless varnish (Paiva *et al.*, 2006). Another set of fixed plant fragments was dehydrated in ethyl series and embedded in glycol methacrylate Leica Historesin®. The historesin blocks were sectioned in rotary microtome (7 µm), and the slides were stained with toluidine O blue (O'Brien *et al.*, 1964) and mounted in Entellan®.

Immunocytochemical analyses. Sections of Paraplast blocks were affixed in slides, deparaffinized, and hydrated. The hydrated slides were incubated for 30 min in 3% powder milk Molico® diluted in 0.1M PBS, pH 7.1, followed by incubation for two hours in monoclonal antibodies (MAbs) (Table 1) diluted in 3% powder milk/PBS (1:10). The MAbs were obtained from the Centre for Plant Sciences, University of Leeds, Leeds, UK. As a control, the slides were incubated in 3% powder milk in PBS without the MAbs. The slides were washed 5x in PBS, and incubated in a secondary antibody conjugated to FITC (fluorescein isothiocyanate) diluted in 3% powder milk/PBS (1:100) and kept in the dark for two hours. The slides were washed 5x in PBS, mounted with coverslips on 50% glycerin, and sealed with nail polish. The slides were analyzed under the Confocal Microscope Zeiss 510 META, Carl Zeiss, Germany, with 488 nm excitation of an argon laser and 505–530 nm emission filter.

Measurements of fluorescence and statistical analyses. The fluorescence peaks in the cell walls of distinct plant tissues were measured with the software MacBiophotonics ImageJ (Chomicki *et al.*, 2014). The fluorescence intensities were calculated by the grayscale methodology (Gy = Gray value) along cell walls (from 5 cells each section; n = 3 samples) given by the software (Chomicki *et al.*, 2014; Nogueira *et al.*, 2024). The values obtained were compared by ANOVA in the SigmaStat® software, considering $p \leq 0.05$ (Figs. S1-S3). Based on statistical analyses, the intensity of labeling was considered negative (-), weak (+), moderate (++), and intense (+++).

Table 1. Primary monoclonal antibodies (MAbs) used in the labeling of cell wall epitopes.

MAbs	Epitopes	References
LM1	Extensins	Sabba & Lulai (2005), Leroux *et al.* (2011)
LM2	Arabinogalactan-proteins (AGPs)	Smallwood *et al.* (1996)
LM5	(1→ 4) β-D-galactan	Jones *et al.* (1997)
LM6	(1 → 5) α-L-arabinan	Willats *et al.* (1998)
JIM5	Methyl-esterified (up to 50%) homogalacturonans (HGs)	Knox *et al.* (1990), Willats *et al.* (2000)
JIM7	Methyl-esterified (35-90%) homogalacturonans (HGs)	Knox *et al.* (1990), Willats *et al.* (2000)

Results

The stems of *M. taxifolia* on primary growth (SP) have an unstratified epidermis and 3-4 cell layers of homogeneous parenchyma on the cortex (Fig. 2A). The cortical layers have four wings containing chlorophyllian spongy parenchyma and small vascular traces (Fig. 2A). A continuous vascular cylinder, undivided in bundles, with phloem, xylem, and intraxylary phloem, surrounds a parenchymatic pith (Fig. 2A). Epitopes of extensins, recognized by LM1, were detected in all tissues of the SP (Table 2). These epitopes were intensely detected mainly in cell membranes of the cortical parenchyma and cell walls of the xylem, and moderately in the phloem and pith cell walls (Fig. 2B; Table 2). Epitopes of AGPs, recognized by LM2, were intensely detected in the cell walls and membranes of the xylem cells, moderately detected in the cortex, and weakly in the phloem and pith (Fig. 2C; Table 2). The epitopes of galactans, recognized by

LM5, were weakly detected in the cell walls of all tissues except the phloem, where they were moderately labeled (Table 2). The epitopes of arabinans were weakly labeled by LM6 in all tissues except the xylem, where they were intensely labeled, mainly in inner cell wall layers of fibers and parenchymatic rays (Fig. 2D; Table 2). High methyl-esterified HGs, recognized by JIM7, were moderately detected in the cortex, phloem, xylem, and pith, while the low methyl-esterified HGs were moderately labeled by JIM5 only in the epidermis (Table 2).

The secondary growth in the stems (SS) is established by a typical activity of the vascular cambium, generating secondary xylem with lignified parenchyma rays, and gelatinous fibers (Fig. 3A). The LM1 does not label the extensins in SS, while the LM2 intensely labeled the epitopes of AGPs in the xylem (in the middle lamellae), moderately in the cortex, and weakly in phloem and pith (Table 2). The epitopes of galactans, recognized by LM5, were moderately detected in the phloem, and weakly detected in the cortex (Table 2). The epitopes of arabinans recognized by LM6 were intensely detected in the xylem, mainly in gelatinous fibers, and weakly in the epidermis, cortex, phloem, and pith, especially in the inner layers of cell walls (Fig. 3B; Table 2). The epitopes of the high methyl-esterified HGs were intensely detected in the epidermis, cortex, and phloem, and moderately detected in the pith (Fig. 3C; Table 2), while the low methyl-esterified HGs were intensely detected in the phloem (Fig. 3D), moderately detected in the pith, and weakly detected in the epidermis, cortex (Fig. 3E), and xylem (Table 2). In the pith cells, the HGs were marked mainly in the inner cell wall layers (Fig. 3C).

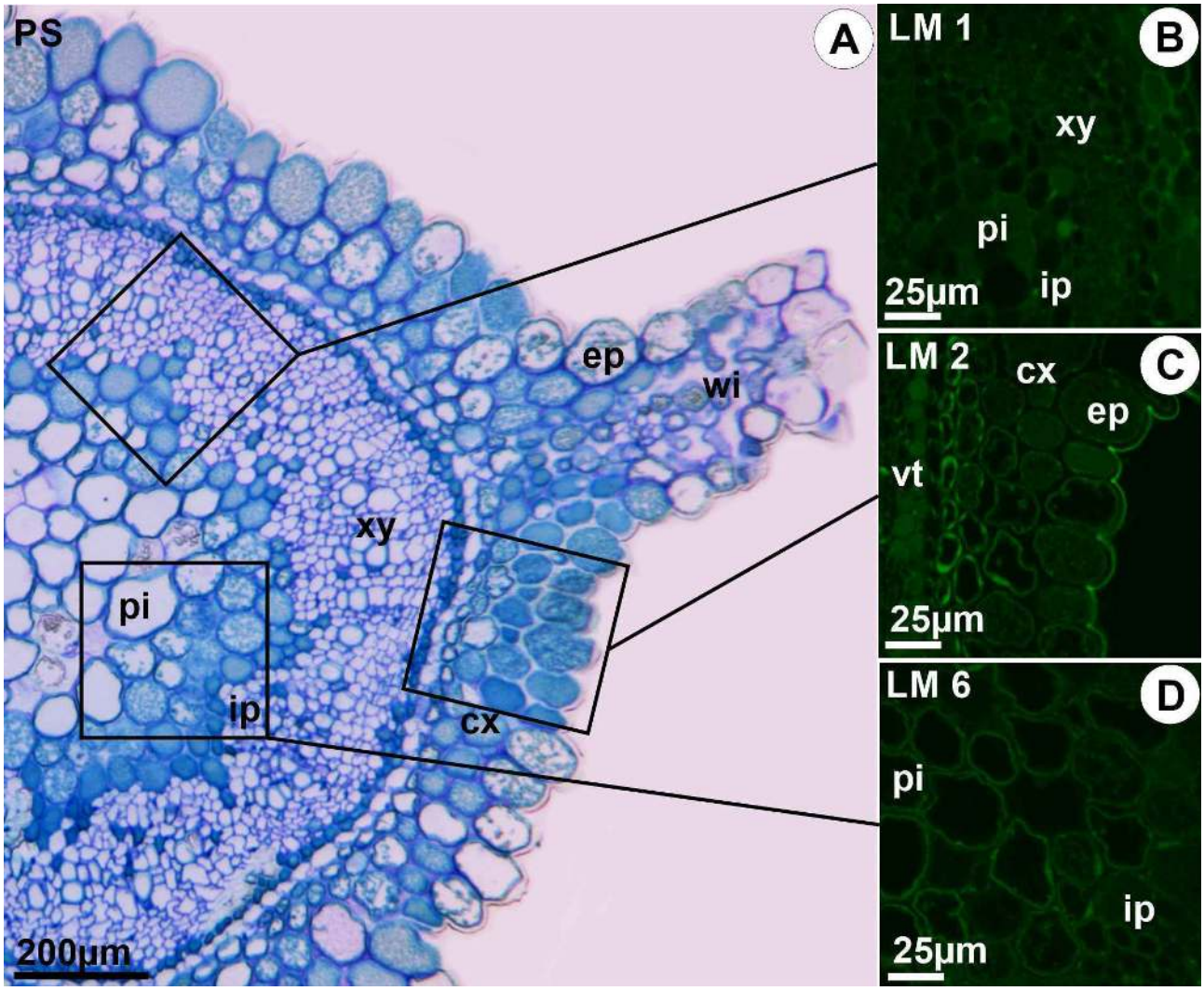


Figure 2. Distribution of extensins and some pectins in stems in primary growth (SP) in *Marcetia taxifolia* (Melastomataceae). **A)** Light micrograph with SP stained with toluidine O blue. **B)** Extensins marked by LM1 in intraxylary phloem (ip), pith (pi) and xylem (xy). **C)** Arabinogalactan-proteins (AGPs) marked by LM2 are shown in cell walls and membranes of the epidermis (ep) and cortical parenchyma (cx) of SP. **D)** Arabinans marked by LM6 are shown in the pith (pi) and intraxylary phloem (ip) of SP. Abbreviation: wi = one of the four wings that occurs in the stems of *M. taxifolia*.

Table 2. Intensity of labelling in distinct tissues based on grayscale values.

Organs and tissues	Extensins (LM1)	AGPs (LM2)	Galactans (LM5)	Arabinans (LM6)	High ME HGs (JIM7)	Low ME HGs (JIM5)
Stems in primary growth (SP)						
Epidermis	++	-	+	+	-	+
Cortex	+++	++	+	+	+	-
Phloem	+	+	++	+	+	-
Xylem	+++	+++	+	+++	+	-
Pith	+	++	+	+	+	-
Stems in secondary growth (SS)						
Epidermis	-	-	-	+	+++	+
Cortex	-	++	+	+	+++	+
Phloem	-	+	++	+	+++	+++
Xylem	-	+++	-	+++	-	+
Pith	-	+	-	+	++	++
Gall in growth and development (GGD)						
Epidermis	-	-	++	+	-	+
Cortex	-	-	++	+	-	+
Phloem	+	-	+++	+	-	-
Xylem	++	-	++	+	++	-
Nutritive tissue	+	+	++	+	-	-
Gall in maturation (GM)						
Epidermis	-	-	+	+	-	-
Cortex	-	-	+	-	-	+
Phloem	-	-	+	+	-	+
Xylem	+	-	+	+	-	+
Nutritive tissue	-	-	++	+	++	+++
Gall in senescence (GS)						
Epidermis	-	-	+	+	+	-
Cortex	-	-	+	+	++	-
Phloem	-	-	++	+	+	-
Xylem	-	-	+	+	-	+
Nutritive tissue	-	-	++	+	++	-

Labelling: (+) = weak; (++) = moderate; (+++) = intense; (-) = negative.

Abbreviations: AGPs = arabinogalactan-proteins; HGs = homogalacturonans; ME = methyl-esterified.

The stem galls are induced by a larva of an unidentified species of Lepidoptera in the pith of the stem in primary growth. The gall develops from cell hypertrophy and hyperplasia of the epidermis, cortical, vascular, and pith parenchyma (Fig. 4A). The larva lodges into a larval chamber within the pith cells, which are distinct from those of the stem, for they are hyperplasic in several layers, and redifferentiate into nutritive cells. The galls in growth and development phase (GGD) have an unstratified epidermis with dense and hypertrophied trichomes, a cortex with parenchymatic hypertrophic cells, a continuous vascular cylinder with phloem, xylem, and intraxylary xylem, and a hyperplasic nutritive tissue (Fig. 4A). The epitopes of extensins were moderately detected in the xylem of GGD, and weakly detected in the phloem and nutritive tissue (Table 2). The epitopes of AGPs were weakly detected only in the nutritive tissue (Table 2). The epitopes of galactans were intensely detected in phloem and moderately detected in the epidermis, cortex, xylem, and nutritive tissues (Table 2; Fig. 4B).

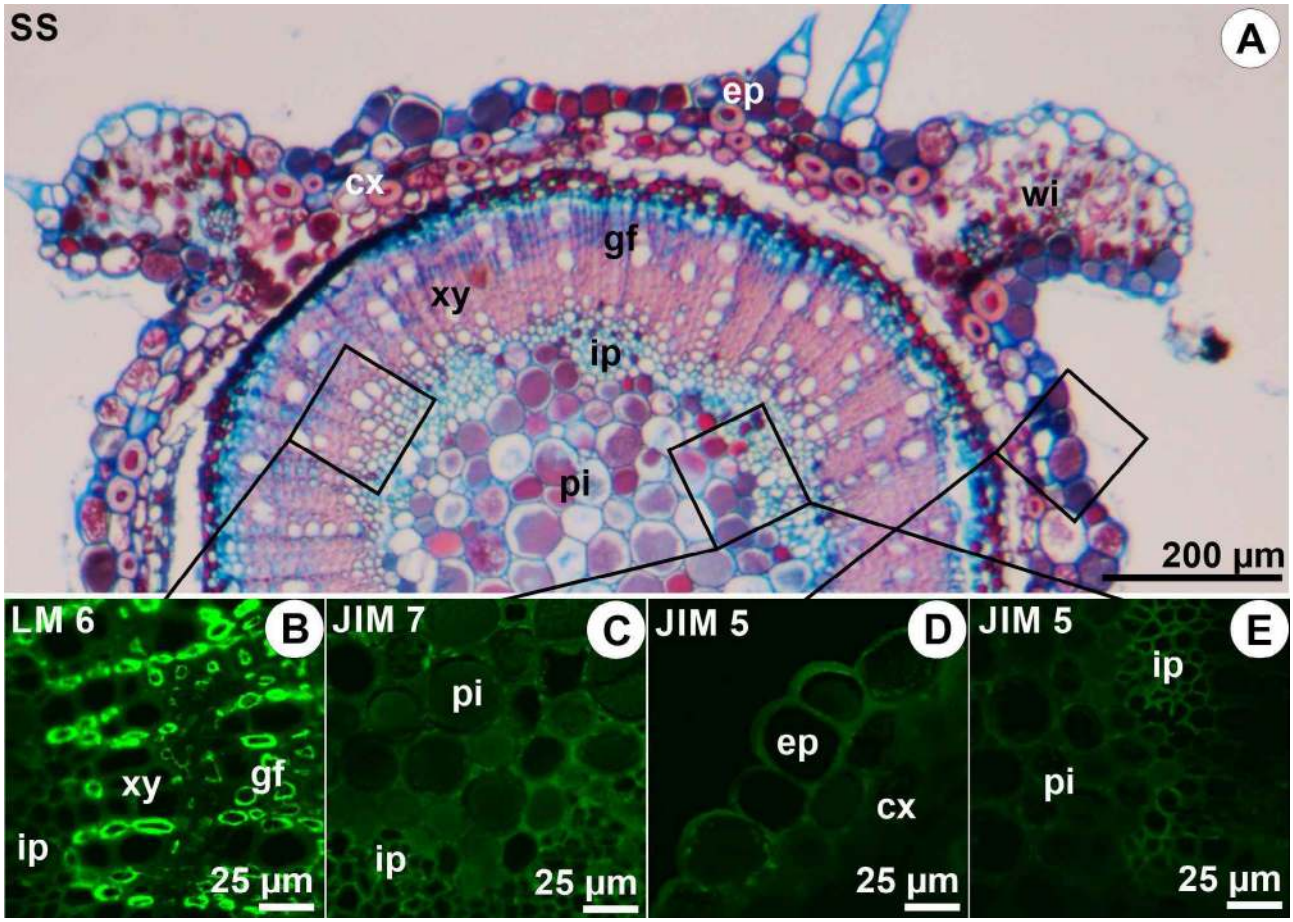


Figure 3. Distribution of some pectins in stems in secondary growh (SS) of *Marcetia taxifolia* (Melastomataceae). **A)** Light micrograph with SS stained with safranin and astra blue. **B)** Arabinans marked by LM6 in secondary xylem (xy), gelatinous fibers (gf), and ray parenchyma of SS. **C)** High methyl-esterified HGs (homogalacturonans) labeled by JIM7 in pith (pi) and intraxylary phloem (ip) of SS. **D-E)** Low methyl-esterified HGs (homogalacturonans) labeled by JIM5 in the epidermis (ep), intraxylary phloem (ip), and pith (pi) of SS. Abbreviation: wi = one of the four wings that occurs in the stems of *M. taxifolia*.

The epitopes of arabinans were weakly detected by LM6 in all tissues (Fig. 4C-D; Table 2). The epitopes of the high methyl-esterified HGs were moderately detected in the xylem, while those of the low methyl-esterified HGs were weakly detected in the epidermis and cortex (Table 2).

The galls in the maturation phase (GM) have vascular cambium activity, and lignification of some cortical cells, which are hypertrophied (Fig. 4E). The nutritive tissue is multilayered, with dividing cells into the outermost layers and vacuolated cells in the innermost layers in contact with the larva (Fig. 4E). On this phase, the epitopes of extensins were weakly detected only in the xylem, while the epitopes of AGPs were not detected in any cell type (Table 2). The epitopes of galactans were moderately detected in the nutritive tissue, and weakly detected in the epidermis, cortex, phloem, and xylem (Table 2). The epitopes of arabinans were weakly detected in the epidermis, phloem, xylem, and nutritive tissue (Fig. 4F; Table 2). The high methyl-esterified HG epitopes were moderately detected only in nutritive tissue, while those of the low methyl-esterified HGs were intensely detected in the nutritive tissue, and weakly detected in the cortex, phloem, and xylem (Table 2).

The galls reach senescence (GS) at the beginning of the lepidopteran pupal development, and the cells of the nutritive tissue enter autolysis (Fig. 5A). During this phase, the epitopes of extensins and AGPs were not detected. The epitopes of galactans were moderately detected in phloem and nutritive tissue, and weakly detected in the cortex and xylem (Table 2). The epitopes of arabinans were weakly detected in all tissues (Table 2). The high methyl-esterified HG epitopes were moderately detected in the cortex and nutritive tissue (Fig. 5B) and weakly detected in the epidermis and phloem (Fig. 5C; Table 2), while the epitopes of the low methyl-esterified HGs were detected only in the xylem (Table 2).

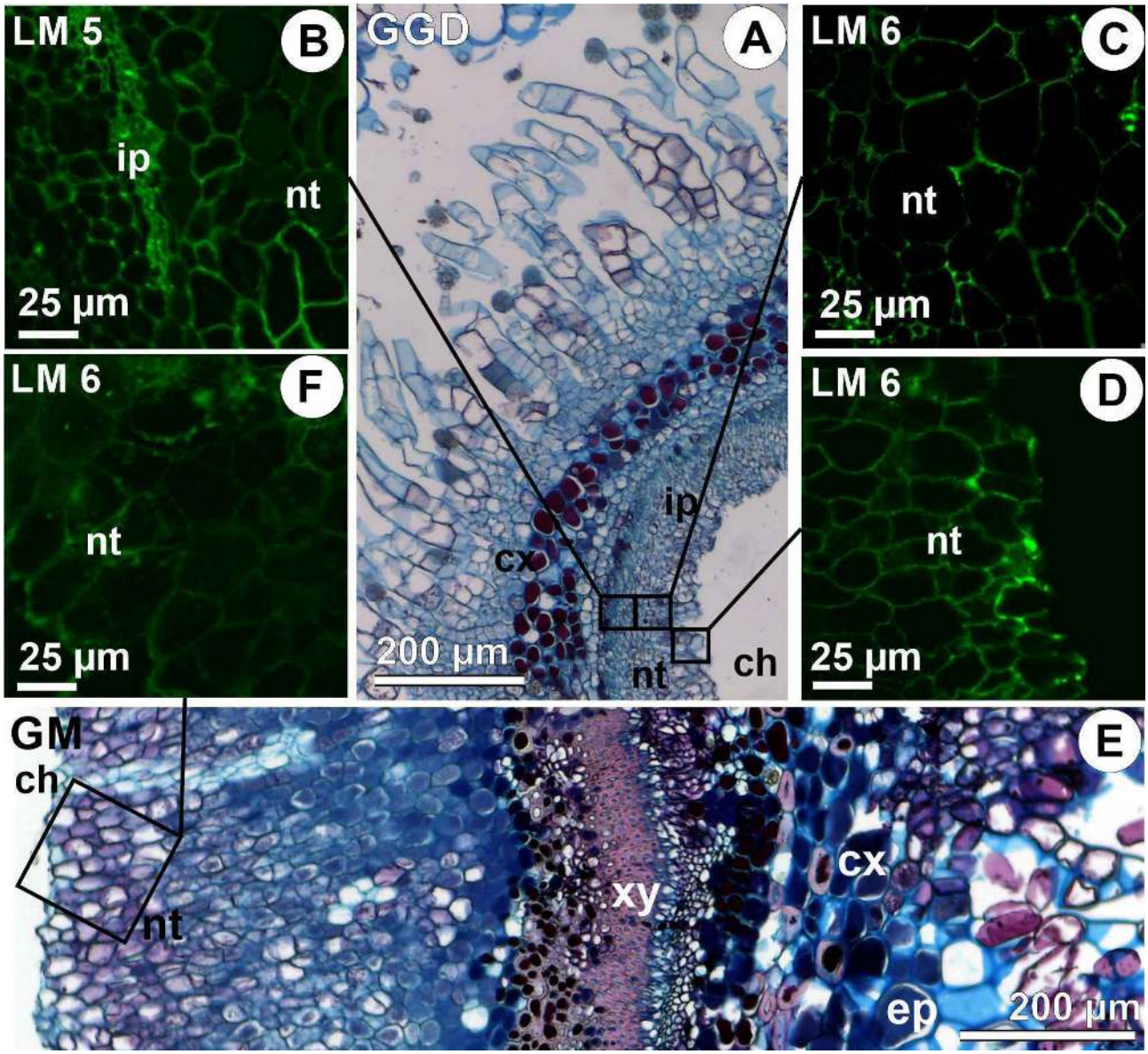


Figure 4. Distribution of some pectins in galls in the growth and development phase (GGD) and maturation (GM) induced by Lepidoptera on *Marcetia taxifolia* (Melastomataceae). **A)** Light micrograph with transversal section of GGD. **B)** Galactans marked by LM5 in intraxylary phloem (ip) and nutritive tissue. **C-D)** Arabinans marked by LM6 are shown in the nutritive tissue (nt) of GGD. **E)** Transversal section of GM stained with safranin and astra blue. **F)** Arabinans in the nutritive tissue (nt) of GM. Abbreviations: ch = larval chamber; cx = cortex; ep = epidermis; xy = xylem.

Discussion

Microlepidopteran galls on *M. taxifolia* induce hypertrophy in the epidermis and cortex, along with hyperplasia and redifferentiation of pith cells into nutritive tissues. These alterations lead to the stem swelling without drastic changes in tissue arrangements, in accordance with the results found by Ferreira & Isaias (2013). The cell walls of the nutritive tissues, derived from pith parenchyma in non-galled stems, show distinct composition, confirming previous descriptions of cell redifferentiation in *M. taxifolia* galls (Ferreira & Isaias, 2013). The walls of gall nutritive cells demonstrate a richer chemical profile than the other tissues, with a combination of arabinans, galactans, and low and high methyl-esterified HGs, similar to the secondary phloem in SS. The clear distinction in the pectin composition of the galls denotes the development of a new functional design, as previously proposed on the morphological basis for other insect galls (Shorthouse *et al.*, 2005; Oliveira & Isaias, 2010; Oliveira *et al.*, 2011; Ferreira & Isaias, 2014). The epitopes of arabinans were intensely detected in the xylem of non-galled stems, especially on gelatinous fibers, but they were

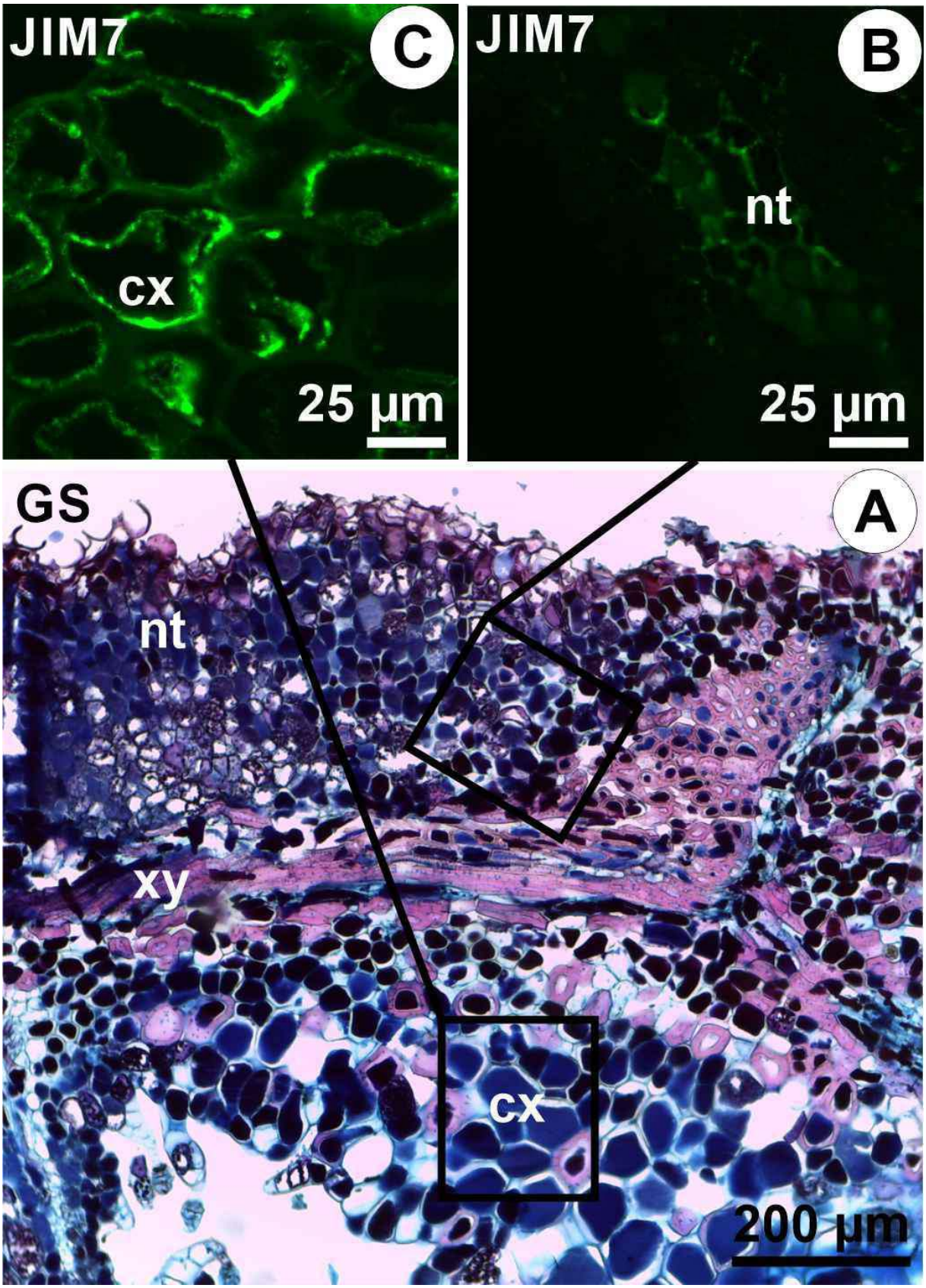


Figure 5. Distribution of some pectins in senescent galls (GS) of *Marcetia taxifolia* (Melastomataceae). **A)** Light micrograph with transversal section of GS stained with safranin and astra blue. **B-C)** High methyl-esterified HGs (homogalacturonans) labeled by JIM7 in the cytoplasm of cortical cells and cell walls of nutritive tissue in GS. Abbreviations: cx = cortex; nt = nutritive tissue; xy = xylem.

weakly detected in galls. The immunocytochemical profiles of *M. taxifolia* stem in primary and secondary growth are markedly distinct from those of lepidopteran galls in growth and development, maturation, and senescent phases, demonstrating changes in functional profiles in galled condition. While the epitopes of arabinogalactan proteins (AGPs), extensins, and arabinans were more intensely detected in the non-galled stems, the galactans were more intensely detected in gall tissues, revealing changes toward cell wall reinforcement in hyperplasic conditions. The epitopes of HGs were widely labeled in the non-galled tissues, especially in the pith, but additional pectins were detected in the nutritive tissue, which enhances porosity and reinforces the cell walls, facilitating the flow of water and macromolecules toward the gall inducer (Silva *et al.*, 2021), while also providing strength to the cell walls in response to the chewing habits of the lepidopteran larva. This reinforcement is crucial as it helps maintain the structural integrity of the tissue, allowing it to withstand the mechanical stress caused by feeding, which in turn supports the gall's overall health and functionality.

As expected, the nutritive cells of the Lepidoptera galls on *M. taxifolia* demonstrate a great variety of intensely detected pectins, as also observed in galls of *Palaeomystella oligophaga* Becker & Adamski (Lepidoptera) on *Macairea radula* (Bonpl.) DC. (Melastomataceae) (Santos *et al.*, 2024). The meristematic nature of the storage nutritive tissue (Ferreira *et al.*, 2015), which replaces the cells of the typical nutritive tissue, is associated with the galactan composition of the cell walls and with the presence of the multivesicular bodies associated with cell membranes (Ferreira *et al.*, 2015). Distinct from other insect galls, the lepidopteran galls have an intense hyperplasia of nutritive cells also in the maturation phase, due to the voracious chewing habit of the larva (Ferreira *et al.*, 2015; 2022). Therefore, the combination of galactans, arabinans, and high methyl-esterified HGs confer flexibility and stability to the hyperplasic cells in different gall systems (Carneiro *et al.*, 2014; Teixeira *et al.*, 2018). Moreover, this composition confers porosity to the cell walls (Zykwinska *et al.*, 2005), allowing the free flow of macromolecules and water between the storage and the typical nutritive tissues of the lepidopteran galls (Santos *et al.*, 2024). Such patterns were also observed in the nutritive tissues of mite and nematode galls (Ferreira *et al.*, 2020), nutritive-like parenchyma of aphid galls (Nogueira *et al.*, 2024), and senescent eriococcid galls (Silva *et al.*, 2021), demonstrating the importance of this combination of pectins for the water and metabolite translocation in galls. In the case of *Espinosa nothofagi* (Pteromalidae, Hymenoptera) gall tissues, although no pectins or hemicelluloses were detected in the nutritive tissues by immunocytochemistry, the presence of galactans, arabinans, and HGs in the outer storage tissues was linked to facilitating nutrient flow to the nutritive cells (Guedes *et al.*, 2025). Such combination can also explain the opposite gradients of carbohydrates and lipids in *M. taxifolia* galls, accumulated differentially in the two zones of the nutritive tissue (Ferreira & Isaias, 2013), suggesting a conversion of starch into reducing sugars, followed by their conversion into lipids, which are together considered the main food source of energy to the galling lepidopterans (Guedes *et al.*, 2023; Martins *et al.*, 2023).

The developmental changes in the cell walls, accessed by immunocytochemical analysis, and detected in the galls, may indicate new functional aspects not revealed by the histological analyses. The detection of galactans was more intense in the GGD than in the non-galled stems, which relates to cell wall stiffening during elongation and expansion (Hwang & Kokini, 1991; McCartney *et al.*, 2003; Xu *et al.*, 2011). The hyperplasic tissues in galls have galactan-rich cell walls (Carneiro *et al.*, 2014; Teixeira *et al.*, 2018), as herein observed in the GGD, for the epidermis, cortex, and nutritive tissues of *M. taxifolia* galls (Ferreira & Isaias, 2013). The maintenance of galactans in the nutritive tissues in the GM corroborates their importance in hyperplasic tissues, since nutritive cells still divide during gall maturation, but have shifted elongation patterns in comparison with the pith cells (Ferreira & Isaias, 2013). On the other hand, the high detection of the epitopes of galactans in the walls of phloem cells is enough to confer the stiffening and porosity of these walls, as proposed by Zykwinska *et al.* (2005), which have been related to the detection of galactans and arabinans in other galls.

Several studies have suggested that the low methyl-esterified HGs occur mainly in mature organs because the de-methyl-esterification releases the HG molecules sites that may bind to the calcium ions and confer rigidity to the plant cell walls (Knox *et al.*, 1990; Sabba & Lulai, 2005; Mastroberti & Mariath, 2008; Formiga *et al.*, 2013; Carneiro *et al.*, 2014; Oliveira *et al.*, 2014). The epitopes of HGs, in general, were slightly detected in the SP and the GGD, with a predominance of high methyl-esterified form. The loss of methyl-esterified groups in mature organs has been associated with the reinforcement of the cell walls in plant organs (Mastroberti & Mariath, 2008), including galls in the maturation phase (Formiga *et al.*, 2013; Carneiro *et al.*, 2014; Oliveira *et al.*, 2014). This imbalance of HG forms may be associated with the defense against mechanical damage. However, in *M. taxifolia* stem galls, the epitopes of the HGs occur in weak intensity when compared to the non-galled stems, except for the nutritive cells. This peculiar chemical alteration of the lepidopteran stem galls on *M. taxifolia* in relation to the non-galled stems can be compensated by the presence of galactans, giving a distinct functional profile to the stem galls.

An increase of the high methyl-esterified HGs, both from SP to SS, and from GGD to GM, was also observed in the host leaves and associated galls on *Baccharis dracunculifolia* DC. (Asteraceae) (Oliveira *et al.*, 2014) and *Psidium myrtoides* O.Berg (Myrtaceae) (Carneiro *et al.*, 2014). The recycling of low methyl-esterified HGs and the production of new

high methyl-esterified HGs in *M. taxifolia* likely maintain protection and rigidity in mature cell walls. Also, the detection of high methyl-esterified HGs in galls induced by Lepidoptera on *M. taxifolia* in higher intensity in senescent phase (GS) than in the GM and GGD may be indicative that there is a decrease in the activity of the PMEs due to the galling stress. The increase in the levels of high methyl-esterified HGs in senescent galls was also observed in other insect galls (Oliveira *et al.*, 2014; Carneiro *et al.*, 2014) and does not seem to be a consequence of the insect mode of feeding. The decline of the PMEs activity has been attributed to an increase of ethylene in ripening fruits (Awad & Young, 1980; Kanellis *et al.*, 1989; Wegrzyn & MacRae, 1992; Pelloux *et al.*, 2007), which is also related to ROS signaling in several plant processes (D'Haeze *et al.*, 2003; Desikan *et al.*, 2005). ROS and ethylene are important to induce the first steps of gall development and the maintenance of its structure (Isaias *et al.*, 2015). The signaling of ROS followed by ethylene production can explain the detection of high methyl-esterified HGs in the senescent lepidopteran galls on *M. taxifolia*.

The cell walls of the primary growth stem (SP) of *M. taxifolia* have more intense detection of the epitopes of extensins and arabinogalactan-proteins (AGPs) than the SS and galls. The extensins may reinforce and confer elasticity to the cell walls during the growth and development phase, as well as control programmed cell death (PCD), and pathogens infection, as previously proposed (Showalter, 1993; Gao & Showalter, 1999; Chaves *et al.*, 2002; Seifert & Roberts, 2007; Albersheim *et al.*, 2010; Leroux *et al.*, 2011). Primary stems have flexible and strong cell walls, for they are structures in the elongation process, which sustains new shoots and leaves. Thus, cell flexibility is important for the host organ elongation, without the collapse of the tissues. After the initiation of the secondary growth in the stems (SS), there is a weak expression of extensins, but maintenance of the AGPs in the secondary xylem. This chemical profile should be a consequence of the end of the cell elongation in the SS, where the xylem fibers and parenchyma cells seem to be alive. The AGPs, which were highly detected in these cells of the SS, seem to play a defensive role against PCD. Additionally, the arabinans with intense peaks in the cell walls of xylem cells in PS and SS, when linked to the RG-II, are responsible for the defense against drought stress (Moore *et al.*, 2008). The presence of arabinans in the inner layers of gelatinous fibers of *M. taxifolia* supports the proposal of Evert (2006), for the G-layers of such type of cells can absorb a great quantity of water. The absence of gelatinous fibers in galls, along with the weaker detection of arabinans in xylem cells, may indicate alterations in this functional aspect within the galled portions. Additionally, the presence of arabinans in nutritive and storage tissue cells is crucial for maintaining water in gall cells (see Guedes *et al.*, 2025), which is necessary for the continuity of cell divisions and growth. Further investigation is needed into potential compensatory mechanisms for the hydration of gall tissues, including possible changes in vascular cell histometry and density both inside and adjacent to the galls (Nobrega *et al.*, 2023).

In summary, the galling activity of lepidopteran larvae on *M. taxifolia* stems induces significant alterations in cell structure and biochemical composition, particularly in the pectin profiles of stem tissues. The hypertrophy and hyperplasia of nutritive cells lead to increased accumulation of galactans, enhancing flexibility and structural reinforcement of the galled tissues. The accumulation of HGs, galactans, and arabinans in storage and nutritive cells facilitates water and nutrient flow, supporting gall development. However, the reduced detection of arabinans in galled xylem suggests a potential impairment of the host plant's water storage capabilities, which may negatively impact its resilience to environmental stressors. Further investigations are necessary to explore compensatory mechanisms in the hydration of gall tissues and their ecological implications.

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Authors' Contribution

BGF: conceptualization, formal analysis, methodology, visualization, writing – original draft, writing – review and editing; DCO: conceptualization, formal analysis, writing – original draft, writing – review and editing; RMSI: conceptualization, supervision, funding acquisition, project administration, resources, writing – original draft, writing – review and editing.

Conflict of interest

The authors declare that there are no conflicts of interest.

Supplementary Materials

The following online material is available for this article:

Figure S1. Mean distribution of low methyl-esterified homogalacturonans (HGs) (JIM5) and high methyl-esterified HGs (JIM7) per tissue and per organ in stems in primary growth (SP), secondary growth (SS), galls in

growth and development phase (GGD), galls in maturation phase (GM) and galls in senescence phase (GS) in *Marcetia taxifolia* (Melastomataceae).

Figure S2. Mean distribution of extensins (LM1) and arabinogalactan-proteins (LM2) per tissue and per organ in stems in primary growth (SP), secondary growth (SS), galls in growth and development phase (GGD), galls in maturation phase (GM) and galls in senescence phase (GS) in *Marcetia taxifolia* (Melastomataceae).

Figure S3. Mean distribution of galactans (LM5) and arabinans (LM6) per tissue and per organ in stems in primary growth (SP), secondary growth (SS), galls in growth and development phase (GGD), galls in maturation phase (GM) and galls in senescence phase (GS) in *Marcetia taxifolia* (Melastomataceae).

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