



New tissue organization favors water status in *Eriosoma lanigerum* galls on *Malus domestica*

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ABSTRACT

The feeding activity of *Eriosoma lanigerum* alters the stem vascular system of *Malus domestica* to such an extent that it may prioritize the water flow to its galls, as postulated by the “gall constriction” hypothesis. Accordingly, such feeding activity on host plant stems causes an asymmetrical cecidogenetic field relative to the aphid’s location along the stem axis, whose symptoms may be evaluated through the influence on the differentiation of xylem cells. From the anatomical perspective, we investigate the secondary xylem aspects of the galls on the *M. domestica* stem regions regarding the position of the aphid colonies in the radial axis. We also investigate aspects of the differentiation of xylem cells in the axial axis: below the gall, in the gall, and above the gall, to test the “gall constriction” hypothesis. In the *M. domestica* – *E. lanigerum* system, the similarity of the vessel elements in the portions above and below the gall contradicts the “gall constriction” hypothesis, however the over-differentiation of parenchyma cells and redifferentiation of abnormal vessel elements may have promoted a higher water supply to the gall than to the non-galled stem portions, functioning as a compensatory mechanism for the maintenance of the water status in gall developmental site.

Keywords: apple trees, secondary xylem, vessel elements, wooly-apple-aphid

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Introduction

The apple tree crops, *Malus domestica* (Suckow) Borkh. (Rosaceae), are susceptible to the infestation of the aphid *Eriosoma lanigerum* Hausmann, 1802 (Hemiptera: Aphididae), which results in gall induction both on roots and stems. Despite the success of the cultivation, the plants are usually infested by *E. lanigerum*, which causes the precocious decline of the crops. The development of the galls reduces the water conductivity in the affected plants, impacting the vigor of the trees. In addition, the feeding activity of the aphids in phloem cells removes carbohydrates, which can cause the *M. domestica* individual's death (Madsen & Bailey, 1958; Brown et al., 1995; Ateyyat & Al-Antary, 2009).

Gall establishment on stems may cause alterations in all plant tissue systems, which may be more conspicuous in secondary vascular tissues (Aloni et al., 1995; Best et al., 2004) due to the influence of gall induction on phellogen and vascular cambium. Vascular alterations are independent of the taxa of the organisms involved in the interaction, as they have been reported, for instance, in *Ricinus communis* L. (Euphorbiaceae) galls induced by *Agrobacterium tumefaciens* (Smith & Townsend, 1907) Conn, 1942 (Aloni et al., 1995) and in *Eremanthus erythropappus* (DC.) MacLeish (Asteraceae) galls induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) (Jorge et al., 2022). Furthermore, changes in vascular tissues have been reported in galls induced by phloem sucking-insects, such as *Pemphigus betae* Doane, 1900 (Aphididae) on *Populus angustifolia* E. James (Salicaceae) leaves (Richardson et al. 2017) and *Euphalerus ostreoides* Crawf. (Psyllidae) on *Lonchocarpus muhelbergianus* Hassl. (Fabaceae) leaflets (Oliveira et al., 2006). The alterations generated by gall induction in the plant vascular system may lead to a prioritization of water flow to the galls as postulated by the “gall constriction” hypothesis (Aloni et al., 1995). This hypothesis predicts that the stem regions below the gall have rays and vessel elements of regular size, while the gall and the region above the gall have narrower vessel elements, increased rays, and absence of fibers, which cause limited water transport to the aerial plant portions (Aloni et al., 1995; Ullrich & Aloni, 2000), resulting in a pressure increase in the gall. Such an anatomical profile in galled stems may favor the water flow to *E. lanigerum* galls, explaining the dieback of *M. domestica* branches and the decline of the crops.

Due to the asymmetrical cecidogenetic field generated by *E. lanigerum* feeding activity on *M. domestica* stems, its galls have three anatomical regions regarding the position of the aphid colonies, a proximal (PR), a median (MR), and a distal region (DR) (Fig. 1). Such regions have a decreasing degree of alterations the more distant the feeding site is. To evaluate this gradient of effects of *E. lanigerum* galls on the xylem of *M. domestica*, we investigate the aspects of the gall PR+MR, where more structural alterations are observed, and of the gall DR compared to the non-galled stems. We also

investigate isolated vessel elements in three stem portions, below the gall, the gall itself, and above the gall, to verify if the impairment to the growth of *M. domestica* trees may be a consequence of the “gall constriction” (Aloni et al., 1995). The relationship between the width and length of the vessel influences water conductivity, due to the relationship between the area and volume available for transporting water, which impacts the gall due to the action of the galling insect. Therefore, we can verify the relationship between the structural and functional impact of xylem changes in *M. domestica* caused by stimuli from *E. lanigerum*.

Materials and methods

Sampling

Our model of study, *M. domestica* cv. ‘Eva’, was developed by the Instituto Agronômico do Paraná (IAPAR) for cultivation in regions with mild winter, such as in Minas Gerais state, Brazil (Oliveira et al., 2011; 2014). Samples of non-galled stems and stem galls were collected from four-year-old individuals (n = 5) of *M. domestica* cv. Eva grafted on ‘M9’ rootstock in a commercial orchard in the municipality of Ervália, Minas Gerais State, Brazil (20°52’02” S, 42°38’41” W), and were fixed in FAA (formalin, acetic acid, 50 % ethanol, 1:1:18) (Johansen, 1940).

Dissociation of vessel elements

Fragments (0.25 cm²) of the non-galled stems and of the stem portions below and above the galls, and of the galls in PR+MR and DR (n = 5 per sample, a total of 25 samples) were submitted to cell dissociation. The samples were washed three times in tap water and immersed in 50 % sodium hypochlorite, which was changed several times for approximately three days until the samples were soft to dissociate. The samples were then washed in tap water, stained in 0.5 % safranin for 24 h, submitted to cell manual dissociation, and washed in tap water. The slides (n = 3 per sample) were mounted with Kaiser’s jelly glycerin (Kraus & Arduin, 1997). These slides were used for measuring the length and width of vessel elements (n = 10 vessel elements per slide, a total of 300 measurements) using the AxioVision 7.4 software (Carl Zeiss® Microscopy GmbH, Jena, Germany).

The number of vessel elements in an image area of 8 mm² was counted in transverse sections of the non-galled stems and of the stem galls (n = 5 individuals, 10 images per individual). The density of vessel elements per xylem area was obtained by dividing the average number of vessel elements per the image of the xylem area (8 mm²) and adjusting to vessels per mm². The anatomical, cytometric, and histometric analyses were performed on images obtained with a Leica® ICC50HP digital camera coupled to a Leica® DM500 light microscope.

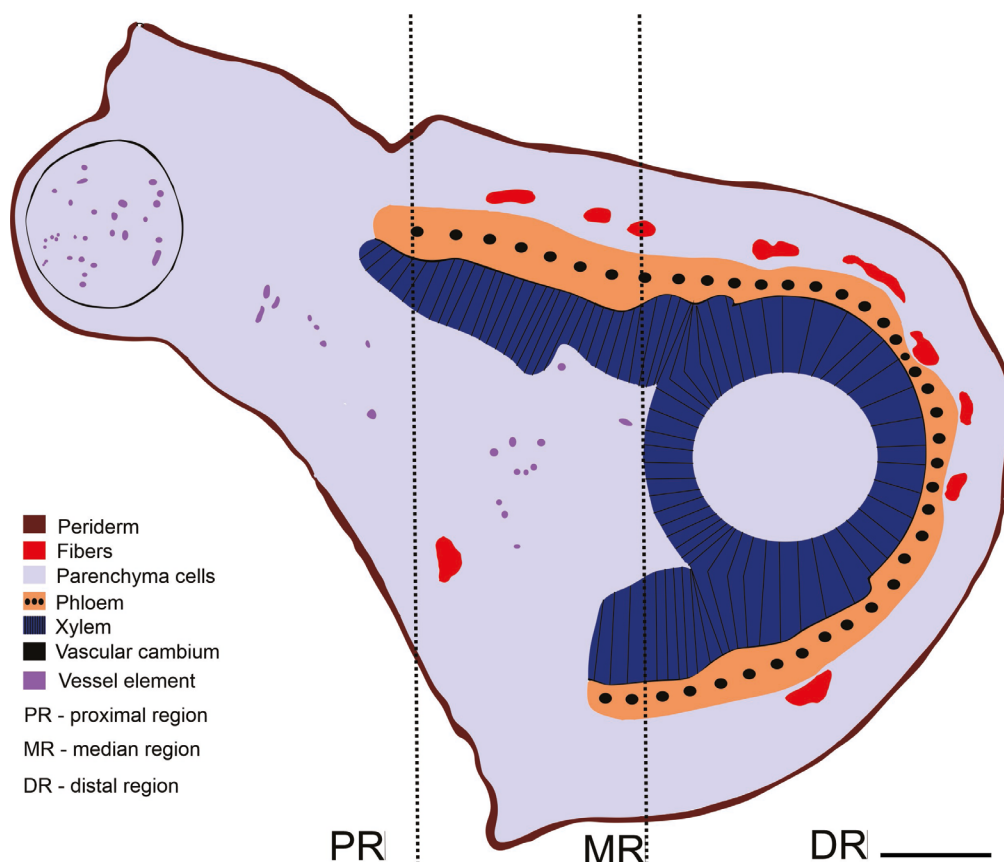


Figure 1. Anatomy scheme of stem galls in *Malus domestica* induced by *Eriosoma lanigerum*.

Statistical analyses

Parametric data were compared using the Student's T-test (for two categories) or one-way ANOVA (for three or more categories), followed by Tukey's test. Non-parametric data were compared with the Mann-Whitney test (for two categories) and Kruskal-Wallis' test (for three or more categories), followed by Dunn's test. All statistical analyses are conducted using SigmaStat® (Systat Software, Inc., Chicago, Illinois), while graphs are generated in GraphPad Prism 8.0®. A significance level of $p = 0.05$ is applied to all tests.

Results

Analyses of vessel elements

The analysis of the galled stem portions: above the gall, gall, and below the gall, evidenced similarities and differences regarding the vessel element (VE) dimensions. The VE differ in length ($p < 0.001$) and width ($p = 0.009$) between the stem portion below the gall and in the gall,

and they also differ in length ($p < 0.001$) and width ($p = 0.005$) between the gall and the stem portions above the gall (Fig. 2A-B).

The average density of the VE in the non-galled stems (3.5 mm^{-2}) is different from that of the galled stems (4.9 mm^{-2}) ($p = 0.008$) (Fig. 3A). The VE in the non-galled stems are significantly longer ($p \leq 0.001$) and narrower ($p = 0.008$) than the VE in the gall proximal + median regions, but they are similar to those of the gall distal region in length ($p = 0.171$) and width ($p > 0.05$). The VE in the gall distal region are narrower than those of the gall proximal + median regions in length ($p < 0.001$) and width ($p < 0.05$) (Fig. 3B). The average dimensions of the VE in the non-galled stems are $142.9 \pm 13.7 \mu\text{m} \times 11.03 \pm 0.65 \mu\text{m}$, while the average dimensions of the VE in the gall distal region are $126.3 \pm 11.2 \mu\text{m} \times 10.8 \pm 12 \mu\text{m}$ (Fig. 3C).

The VE of the non-galled stems and of the gall distal region have various-sized appendices in one or both extremities, the perforation plates are simple and the pits are opposite (Fig. 3C). The VE observed in the gall proximal + median regions have displaced perforation plates, the majority without appendices (Fig. 3C); their average dimensions are $52.5 \pm 3.2 \mu\text{m} \times 16.12 \pm 3.2 \mu\text{m}$ (Fig. 3C).



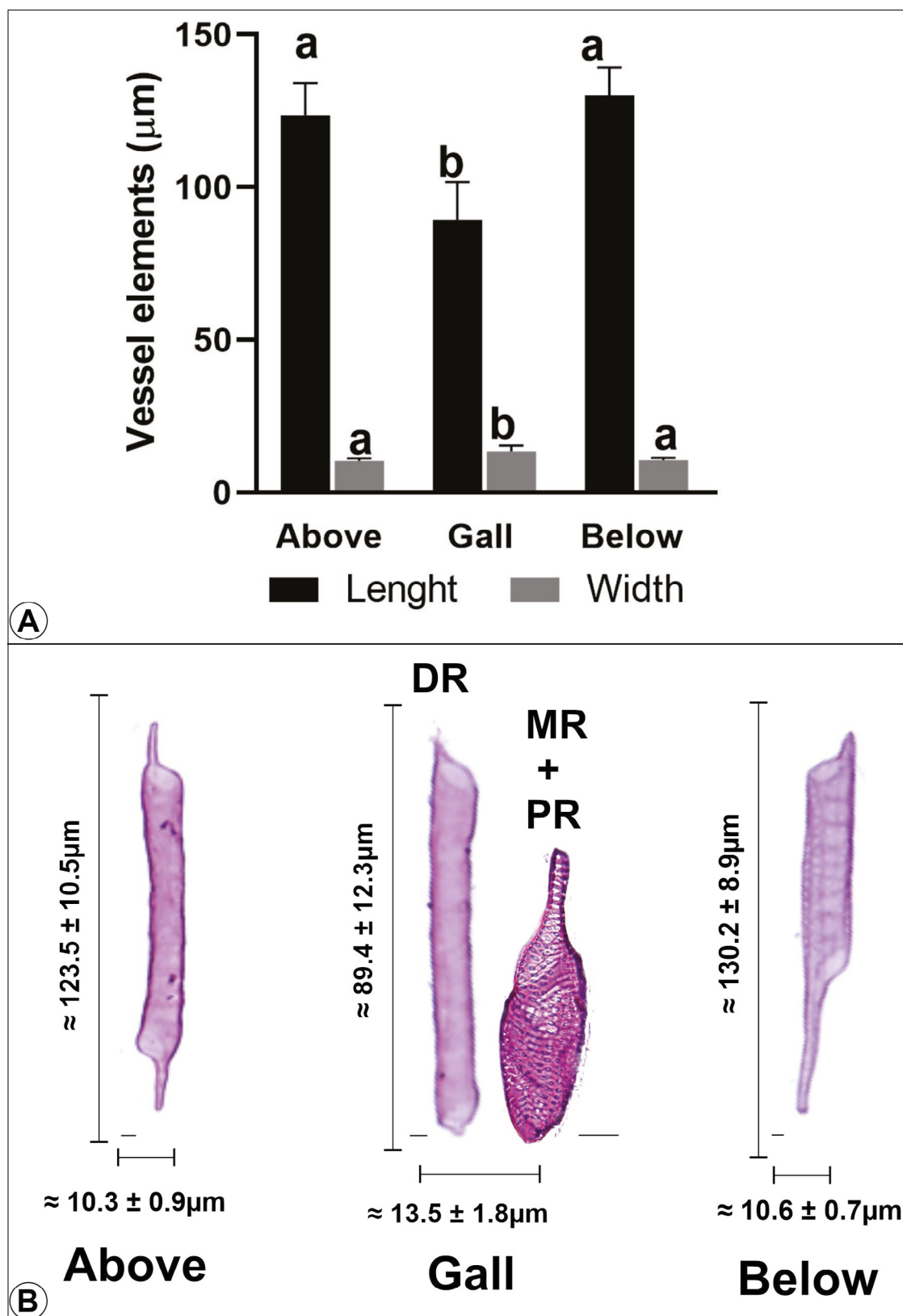


Figure 2. Vessel element features of *Malus domestica*-*E. lanigerum* system. A: Mean length and width of VE in the three regions of galled stems (one-way ANOVA and Tukey's test). B: Vessel element (VE) dimensions in the three stem portions. The gall VE may have simple perforation plates in DR and abnormal VE with displaced perforation plates and appendices in the PR+MR. Different letters indicate statistically distinct means, while identical letters indicate statistically similar means. PR = proximal region. MR = median region. DR = distal region. Bars: B above and below - 200μm, gall DR - 100μm, gall MR+PR - 50μm.

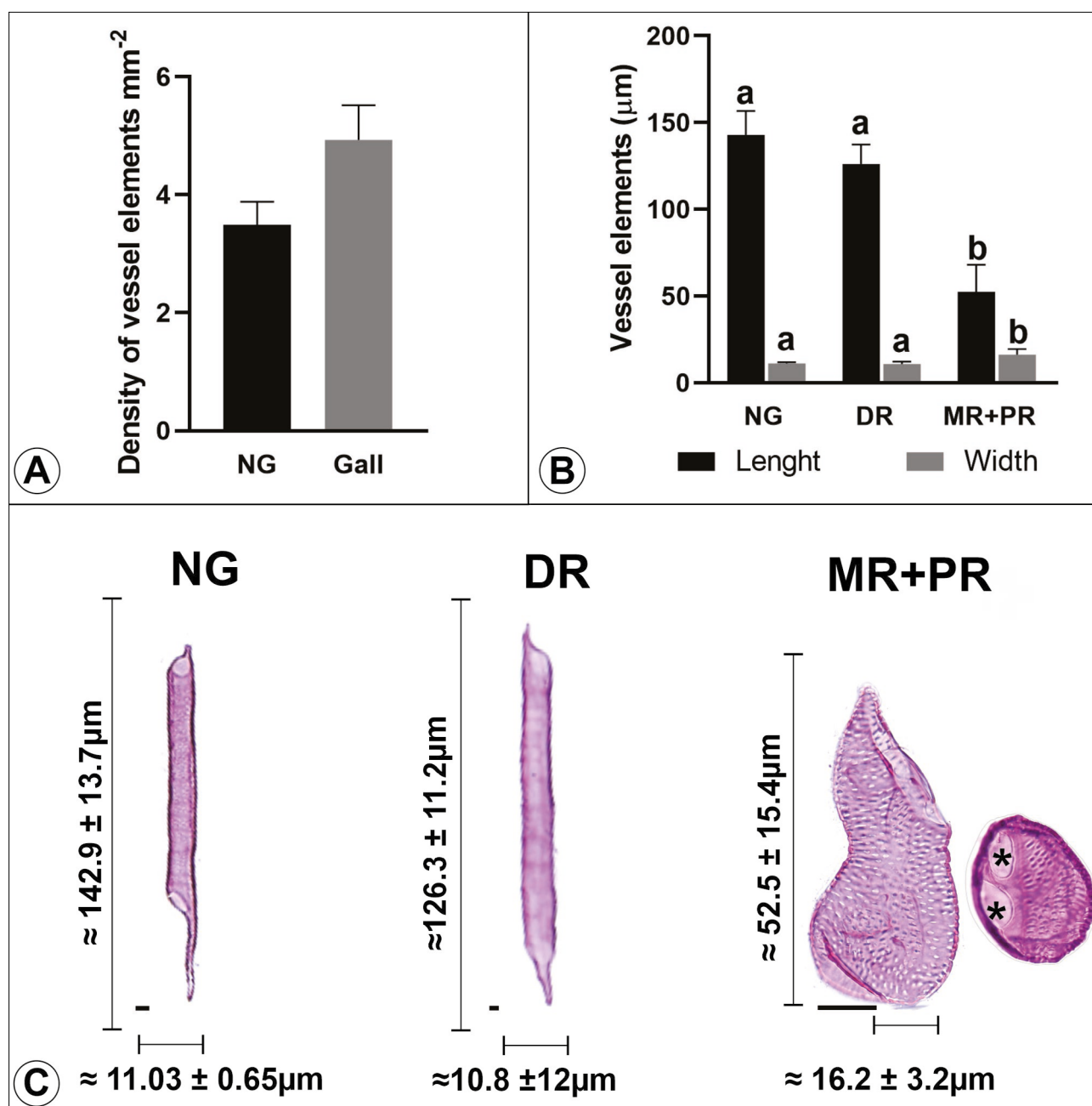


Figure 3. Xylem features of *Malus domestica*-*E. lanigerum* system. A: Density of VE per area (mm^{-2}) in non-galled stems and stem galls. B: Mean length and width of VE in non-galled stems, and in gall DR, and PR+MR (one-way ANOVA and Tukey's test; Kruskal-Wallis and Dunn's test). C: Mean length and width of VE in non-galled stems, and in gall DR, and PR+MR. VE in NGS and gall DR have with simple perforation plate, while the VE in gall PR+MR have a large and displaced perforation plate (asterisk). Different letters indicate statistically distinct means, while identical letters indicate statistically similar means. PR = proximal region. MR = median region. DR = distal region. Bars: B - $50\mu\text{m}$.



Discussion

The galls induced on *M. domestica* by the colonies of *E. lanigerum* result from abnormal divisions of the vascular cambium with pronounced effects observed in secondary xylem organization, which are the focus of current investigation. The main diagnostic features observed in the secondary xylem are an increment in the differentiation of abnormal vessel elements, the reorientation of the cell axis, and the over-differentiation of parenchyma cells (Freitas, 2022; Nogueira et al., 2024).

The similarity of the dimensions of the vessel elements in the portions below and above the stem galls indicates a restricted amplitude for the cecidogenetic field, leading the xylem cells to increase the water supply to the galls on *M. domestica*. Distinctly, variations in vascular tissues in other host plant-gall inducer systems, such as *Ricinus communis*-*Agrobacterium tumefaciens*, have been observed in the stem portions above and below the gall, which supported the gall constriction hypothesis (Aloni et al., 1995; Ullrich & Aloni, 2000). In *M. domestica*-*E. lanigerum* system, the neo-formed vessel elements in the gall PR+MR are different from those observed above and below the gall. Such neo-formed cells, potentially along with xylem parenchymatic cells, increase the storage of water in the gall developmental site, since new vascular tissues perform an increase in water transport, thus providing the energy needs of the galling insect (Bragança et al., 2025). The over-differentiation of xylem parenchyma cells is also accompanied by a higher average number of vessel elements in the stem galls than in the non-galled stems on *M. domestica*, similar to the observations on the *Agrobacterium*-induced galls (Aloni et al., 1995; Aloni, 2013). The higher number of both parenchyma cells and vessel elements is restricted to the gall PR+MR, and confers not only water storage but also an increment in the potential translocation of water inside the gall. This water increment generates a favorable environment for the development of colony of gall inducers, which are in fact, exophytophagous and actively feed on xylem cell content (Sandanayaka & Hale, 2003; Sandanayaka et al., 2003; Hao et al., 2020; Zhou et al., 2021). In the light of the microenvironment hypothesis (Price et al., 1987; Stone & Schönrogge, 2003), the gall cannot protect the galling insect from most of the outer abiotic factors, except for the water stress (Cull & Van Emden, 1977; Spiller et al., 1990; Stone & Schönrogge, 2003). The new tissue organization guarantees increasing water accumulation, which buffers the gall inducers not only for hydric stress (Oliveira et al., 2017; Martini et al., 2020) but also for excess internal heating (Martinez, 2009; Miller III et al., 2009).

The impairment in vigor of the trees attacked by *E. lanigerum* sometimes results in plant death (Brown et al., 1995; Ateyyat & Al-Antary, 2009). As the *E. lanigerum* gall induction and development on *M. domestica* affects the differentiation of xylem cells, it can explain the reduction in

water conduction in affected plants reported in the literature (Ateyyat & Al-Antary, 2009). The over-differentiation of parenchyma cells in insect galls has been associated with processes of hyperplasia and hypertrophy (Oliveira & Isaías, 2010), which can be influenced by ethylene action over vascular cambium cells (Junghans et al., 2004). Ethylene is a phytohormone produced under stressful situations (Yang & Hoffman, 1984), such as gall induction (Aloni et al., 1998). The more parenchyma cells differentiate in stem galls, the higher the water storage in the galled stems is, as observed in *E. erythropappus* stem galls (Jorge et al., 2022), corroborating the harmful effect of the galls on the development of *M. domestica* plants herein diagnosed.

The abnormal vessel elements in *E. lanigerum* galls, whose size and shape are distinct from those of the vessel elements of the non-galled stem portions and exhibit 'wound' like characteristics, such as asymmetry and shorter length (Bartlem et al., 2014), can originate from the redifferentiation of parenchyma cells (Bartlem et al., 2014; Anand & Ramani, 2021; Beltrame et al., 2022; Bragança et al., 2025). The abnormal vessel elements alter the fluid dynamics, favoring the water supply to the galls. This redifferentiation relates to hormone transport and concentration, mainly to auxins (Aloni et al., 1995; Best et al., 2004; Aloni, 2013; Dolzblasz et al., 2018; Bragança et al., 2021), which leads us to infer that the feeding activity of *E. lanigerum* is modulating the auxin transport in the gall developmental site. The auxin transport, the activity of receptors, and their influx in xylem parenchyma cells seem to orchestrate the differentiation of vessel elements and parenchyma cells in the gall PR+MR. Further, the reorientation of vessel elements observed in the gall PR+MR may imply a high-water supply to the galled stem portions.

In the *M. domestica* – *E. lanigerum* system, the main alteration in the secondary xylem is the over-differentiation of parenchyma cells, which also contributes to the redifferentiation of vessel elements. The similarities in the dimensions of the vessel elements in the axial axis, i.e., in the portions above and below the galled stems, do not support the "gall constriction" hypothesis proposed by Aloni et al. (1995). The conservative features of the vascular cylinder in galled stems contrast to the changes in the radial axis in the proximal-median regions of the galls. Such changes indicate the maintenance of water status in gall tissues and favor the aphid's water balance, even though it does not corroborate the constriction hypothesis.

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Authors' Contributions

All authors contributed to the study conception and design. Mariana Freitas, Gener Penso, Edgard Picoli, and Rosy Isaías did the field sampling. Mariana Freitas, Ravena Nogueira, Ana Maria Santos, Edgard Picoli, and Rosy Isaías analyzed the structure. All authors analyzed the data, wrote the manuscript, read, and approved the final manuscript

Conflict of Interest

The authors have no conflict of interest.

Data availability:

The data of this study are available from the first author, MSCF, upon reasonable request.

