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INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

Nina de Castro Jorge

Galhas lenhosas em *Eremanthus erythropappus* (DC.) McLeisch: alterações anatômicas e implicações ecofisiológicas

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“Eu vou pro fronte como guerreira, nem que seja pra enfrentar o planeta inteiro. Correr a maratona, chegar primeiro, e gritar: É POR VOCÊS!”

“Deixa eu devolver o orgulho do gueto, e dar
outro sentido para a frase: **tinha que ser preto**.”

Emicida – Beira de Piscina

RESUMO

Eremanthus erythropappus (DC.) McLeisch (Asteraceae), popularmente conhecida como candeia, é uma espécie comum em áreas de transição entre Cerrado e Mata Atlântica. Na cecidologia a candeia é muito importante, sendo uma super-hospedeira de galhas com seis morfotipos associados: dois induzidos nas gemas apicais, um nas folhas e três nos caules. Dentre os morfotipos descritos, o foco desta tese são as galhas caulinares globoides induzidas por *Neolasioptera* sp. (Diptera: Cecidomyiidae). Estas galhas são rígidas, lignificadas e apesar de encontrarem-se próximas ao ápice, não comprometem as gemas apicais dos ramos hospedeiros. Durante o ciclo de vida das galhas alguns estágios de desenvolvimento são marcados, sendo eles a indução, crescimento e desenvolvimento e maturação que são caracterizados pelas etapas de manipulações dos tecidos vegetais pelo galhador. Estes estágios têm a capacidade de alterar os padrões morfogenéticos da planta hospedeira, o que promove neste sistema a marcante lignificação das galhas caulinares. Além disso, o galhador pode ainda induzir alterações bioquímicas tanto no metabolismo primário quanto no secundário, gerando compostos com potencial para intermediar as relações das galhas com outros organismos. No sistema em questão, podendo inclusive acarretar alterações no potencial alelopático da planta hospedeira. Nas galhas, as variações na dinâmica dos fitormônios orquestram o estabelecimento dos padrões teciduais peculiares que cessam com o desenvolvimento do galhador, caracterizando o estágio de senescência que se inicia com a saída do galhador. Após esta saída, as galhas em *E. erythropappus* podem ser ocupadas por formigas e fungos, o que indica que, além de conferir proteção e um microambiente favorável, as galhas podem preservar, mesmo que senescentes, um perfil químico nutricional importante que é utilizado pelas colônias de formigas e pelos fungos. As hifas dos fungos, em contato com células nutritivas remanescentes desencadeiam o estímulo para a superprodução de xilema secundário não lignificado e de um felogênio ectópico, configurando um estágio de pós-senescência. Na expectativa de gerar difusão e popularização do conhecimento científico, o estudo das galhas vazias e ocupadas por formigas serviu como tema para a produção de um livro paradidático. Este livro foi utilizado em uma escola de nível fundamental em Belo Horizonte-MG como motivador da apresentação e aplicação do método científico no ambiente escolar. O sistema foco deste trabalho se mostrou um interessante micro-labotatório de estudos do ponto de vista anatômico, ecofisiológico e educacional. As principais alterações anatômicas responsáveis pelo desenvolvimento da galha de *Neolasioptera* sp. em *E. erythropappus* ocorrem devido ao potencial de respostas do câmbio vascular e do felogênio que culminam em atributos anatômicos peculiares.

Palavras-chave: anatomia, Asteraceae, câmbio vascular, Cecidomyiidae, felogênio ectópico, desenvolvimento de galhas, divulgação científica, imunocitoquímica, galhas caulinares

ABSTRACT

Eremanthus erythropappus (DC.) McLeisch (Asteraceae), popularly known as candeia, is a common species in Minas Gerais state. In cecidology, candeia is very important, as a super host of galls with six associated morphotypes: two induced in the apical buds, one in the leaves and three in the stem. Among the morphotypes described, the focus of this thesis relies on the globoid stem galls induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae). These galls are woody, rigid, lignified and induced close to the apex, but they do not compromise the apical meristem of the host branches. During gall life cycles, some developmental stages are recognized: the induction, the growth and development and maturation stages, which are characterized by the stages of manipulation of plant tissues by the gall inducer. The gall inducer can change the morphogenetic patterns of the host plant, which promotes, in *E. erythropappus*-*Neolasioptera* sp. system, the conspicuous lignification of the stem galls. Furthermore, the gall inducer activity can promote biochemical changes in primary and secondary metabolism, generating compounds with the potential to mediate the relationships of galls with other organisms. In this system, it may even cause changes in the allelopathic potential of the host plant. In the globoid stem galls, variations in the dynamics of the phytohormones orchestrate the establishment of peculiar tissue patterns that cease with the development of the galling insect, characterizing the senescent stage, which begins with the exit of the gall inducer from the gall. After exit of the *Neolasioptera* sp., the galls on *E. erythropappus* can be occupied by ants and fungi, which indicates that, in addition to providing protection and a favorable microenvironment, the galls can preserve, even if senescent, adequate chemical and nutritional profile that are used by ant colonies and fungus. Fungal hyphae in contact with remaining nutritious cells trigger the stimulus for the overproduction of secondary non-lignified xylem cells and an ectopic phellogen, configuring a post-senescent stage. Looking forward to generating dissemination and popularization scientific knowledge, the study of ant-occupied galls served as the theme to produce a para-didactic book. This book was presented in an elementary school in Belo Horizonte city as a motivator for the presenting and applying the scientific method in the school environment. The focus of this work, proved to be an interesting micro-laboratory for studies on anatomical, ecophysiological and educational perspectives. The main anatomical changes responsible for the development of the *Neolasioptera* sp. galls on *E. erythropappus* occur due to the potential of vascular cambium and phellogen responses that culminate in the peculiar anatomical traits.

Keywords: anatomy, Asteraceae, Cecidomyiidae, ectopic phellogen, gall development, immunocytochemistry, stem galls, scientific spreading, vascular cambium

SUMÁRIO

INTRODUÇÃO GERAL

Eremanthus erythropappus (DC.) McLeisch (Asteraceae), popularmente conhecida como candeia, é uma espécie comum e em áreas de transição entre Cerrado e Mata Atlântica. São árvores de pequeno porte e folhagem densa (Mori et al. 2010) cuja madeira tem grande importância comercial, sendo utilizada na confecção de cercas por fazendeiros e para extração de óleos. O óleo de *E. eryhtropappus* é utilizado pela indústria farmacêutica e cosmética como cicatrizante e antimicrobiano (Oliveira et al. 2010). No campo da cecidologia, *E. eryhtropappus* é, também, muito importante, sendo uma super-hospedeira de galhas associada a seis morfotipos: dois induzidos nas gemas apicais, um nas folhas e três nos caules (Carneiro et al. 2009). As galhas podem ser definidas como estruturas derivadas do desenvolvimento anormal dos tecidos da planta hospedeira, que são manipulados pelos organismos galhadores (Mani 1964).

Entre os seis morfotipos descritos, as galhas globoides caulinares induzidas por *Neolasioptera* sp. (Diptera: Cecidomyiidae) (Prado e Vieira 1999), constituem o foco deste trabalho, que tem como hipótese central que as principais alterações anatômicas responsáveis pelo desenvolvimento da galha globoide caulinar de *Neolasioptera* sp. em *E. erythropappus* ocorrem devido ao potencial de respostas do câmbio vascular e do felogênio que culminam em atributos anatômicos peculiares no sistema.

As galhas caulinares globoides encontram-se próximas ao ápice dos ramos, formando intumescimentos lenhosos, rígidos e lignificados, que, contudo, não comprometem as gemas apicais dos ramos hospedeiros (Prado e Vieira 1999). A marcante lignificação das galhas caulinares é proveniente da capacidade do galhador de alterar os padrões morfogenéticos da planta hospedeira (Mani 1964, Moura et al. 2008, Oliveira et al. 2011), que é discutida no primeiro capítulo desta tese intitulado: *"Vascular traits of stem galls: cell increment vs. morphogenetic constraints in wood anatomy*". As mudanças na morfogênese de *E. erythropappus* em decorrência da indução e do estabelecimento do *Neolasioptera* sp. galhador levam a alterações nos três sistemas de tecidos, sendo que as mudanças nos tecidos condutores (Wilson 1965, Wilson e Magie 1964, Best et al. 2004) comprometem o fluxo de água nos ramos hospedeiros. De fato, a hipótese de constrição vascular postula que as alterações vasculares geram atributos funcionais que conferem prioridade ao fluxo de água para as galhas em comparação às regiões acima das galhas (Aloni et al. 1995).

Além da capacidade de alterar os padrões morfogenéticos da planta hospedeira (Mani 1964, Moura et al. 2008, Oliveira et al. 2011), o galhador pode ainda induzir alterações químicas (Raman et al. 2005) tanto no metabolismo primário quanto no secundário, incluindo variações na dinâmica dos fitormônios (Bedetti et al. 2014, Carneiro et al. 2017, Bedetti et al. 2018, Guedes et al. 2018, Kuster et al. 2020). Uma vez que o câmbio vascular é particularmente sensível ao balanço hormonal (Sirvastava 2002), a diagnose das alterações vasculares torna-se um bom modelo para avaliar a relação dos estímulos oriundos dos galhadores na atividade cambial e foi discutida no segundo capítulo desta tese, intitulado: *"The influence of CKs and IAA imbalance on water status in Eremanthus erythropappus-Neolasioptera sp. system".* Os hormônios, como citocininas e auxinas e nos tecidos galhados, desempenham um papel essencial para o crescimento e desenvolvimento das galhas (Bedetti et al. 2014), sendo o acúmulo de auxinas relacionado ao afrouxamento das paredes celulares e consequente hipertrofia celular (Best et al. 2004) e, as citocininas, por sua vez, estão envolvidas no crescimento e divisão celular, fundamentais para a hiperplasia (Best et al. 2004).

Assumimos que a presença de galhas lenhosas no caule em *E. erythropappus* provoca alterações na dinâmica estrutural e funcional do ramo galhado. Essas alterações são resultado de mudanças nas concentrações de citocininas e auxinas, provocadas pela ação do inseto galhador, que gera um efeito direto na atividade do câmbio vascular, culminando na diferenciação anormal dos tecidos vasculares da galha e nas regiões adjacentes a ela. Estas alterações promovem o desenvolvimento de um mecanismo compensatório para a manutenção do estado hídrico no sistema *E. erythropappus-Neolasioptera* sp., acarretando a priorização do fluxo de água para os caules em crescimento e desenvolvimento que hospedam galhas e para as galhas de caule.

Além da diagnose das alterações estruturais, a diagnose das mudanças químicas pode revelar metabólitos secundários neossintetizados nas galhas (Oliveira et al. 2006, Guedes et al. 2016, Jorge et al. 2018) ou mantidos em níveis semelhantes entre os órgãos galhados e não galhados (Nyman e Julkunen-Tiitto 2000, Jorge et al. 2018). A composição e aplicabilidade dos óleos essenciais de *E. erythropappus* indicam o α-bisabolol como um composto majoritário (Sousa 2003). Este sesquiterpeno é usado na medicina como agente antiinflamatório e antimicrobiano (Sousa et al. 2003) com efeitos antinociceptivos, antiinflamatórios (Sousa et al. 2008) e antiulcerogênicos (Silvério et al. 2008, 2013). Ademais, o óleo produzido pelo *E. erythropappus* tem potencial alelopático

(Pinto et al. 2019), que, contudo, mostra-se, aparentemente, ineficaz contra o ataque de herbívoros (Pires e Oliveira 2011) como o *Neolasioptera* sp.. Tendo como base estes estudos recentes sobre a composição do óleo essencial desta espécie, aqui, nos concentramos no potencial alelopático de compostos polares como um passo à frente na compreensão do efeito dos estímulos das galhas na química das plantas hospedeiras. Sendo assim, partindo da premissa de que galhas de *Neolasioptera* sp. podem potencializar a composição química de *E. erythropappus*, assumimos que o inseto galhador pode manipular o potencial alelopático da planta hospedeira e discutimos tal premissa no terceiro capítulo desta tese intitulado: *"Allelopathic potential of the extracts of non-galled stems and globoid stem galls of Eremanthus erythropappus (DC) McLeish (Asteraceae)"*. O estudo de frações químicas diversas dos extratos de galhas em comparação com aqueles dos órgãos hospedeiros podem apontar pistas sobre a mediação química ao longo do ciclo de desenvolvimento das galhas. Tal mediação pode ser tanto positiva quanto negativa e sua avaliação se dá de frente para modelos biológicos bem estabelecidos.

O ciclo de desenvolvimento das galhas é marcado por estágios que podem ser diagnosticados em macro e micro escala, sendo eles: indução, crescimento e desenvolvimento, maturação e senescência (Kraus 2009). Este último é caracterizado pela saída do inseto galhador da galha e a finalização dos estímulos estressantes nos tecidos da planta hospedeira (Rohfritsch 1992). Após a senescência, as galhas globoides de *Neolasioptera* sp. em *E. erytropappus*, servem de ninho para formigas arbóreas (Almeida et al. 2014, Santos et al. 2017, Santos et al. 2021). As galhas aqui estudadas têm função ecológica importante para a comunidade de formigas arbóreas e fungo, servindo de micro-habitat e ninho para três a onze espécies diferentes espécies de formigas, em diferentes padrões de ocupação (Almeida et al. 2014, Santos et al. 2017; Santos et al. 2021). Assumimos que características favoráveis são desencadeadas ou mantidas em galhas senescentes pela presença das formigas em seu interior, propiciando a formação de ninhos. A presença desta comunidade de formigas indica, ainda, que além de conferir proteção e um microambiente favorável, as galhas podem preservar, mesmo que senescentes, um perfil químico nutricional importante que deverá ser utilizado pelas colônias de formigas. Esta premissa é explorada no quarto capítulo desta tese, intitulado: *"The peculiar post-senescence in globoid stem galls, fungi, and arboreal ants on Eremanthus erythropappus (DC.) McLeisch (Asteraceae)"* que nos aponta que o sistema

E. erythropappus- Neolasioptera sp. serviu como modelo de estudo sobre o comportamento estrutural e funcional dos tecidos condutores em uma galha caulinar lenhosa, e sobre interferências no estágio sucessional das galhas quando ocupadas por formigas.

O capítulo final desta tese, *"The curious case of ants that live in galls: telling stories to mediate literature and science classes"*, traz o sistema tritrófico *E. erythropappus-Neolasioptera* sp.- formigas arbóreas como inspiração para a elaboração de um livro paradidático visando a divulgação científica. Os textos de divulgação podem contribuir para enriquecer o ensino de base na medida em que trazem novas questões, ampliam a visão de ciência e de mundo dos alunos e dos professores, criam recursos de ensino e localizam o conteúdo ensinado em um contexto mais abrangente (Salém e Kawamura 1996, Queiroz et al. 2012). Além disso, a habilidade de se comunicar cientificamente em suas mais diversas linguagens proporciona aos educandos o acesso a uma maior diversidade de informações, o desenvolvimento de habilidades de leitura e domínio de conceitos, além de possibilitar novas formas de argumentação e elementos de terminologia científica (Martins et al. 2001). Assumimos que o nosso texto paradidático pode contribuir para enriquecer a interação pedagógica ao apresentar interações planta-inseto que não são facilmente visíveis nos espaços formais de ensino, trazendo novos questionamentos, abrindo a visão do aluno e do professor sobre a ciência e o ambiente natural, criando recursos didáticos voltados para o contexto científico e, consequentemente, motivando os alunos.

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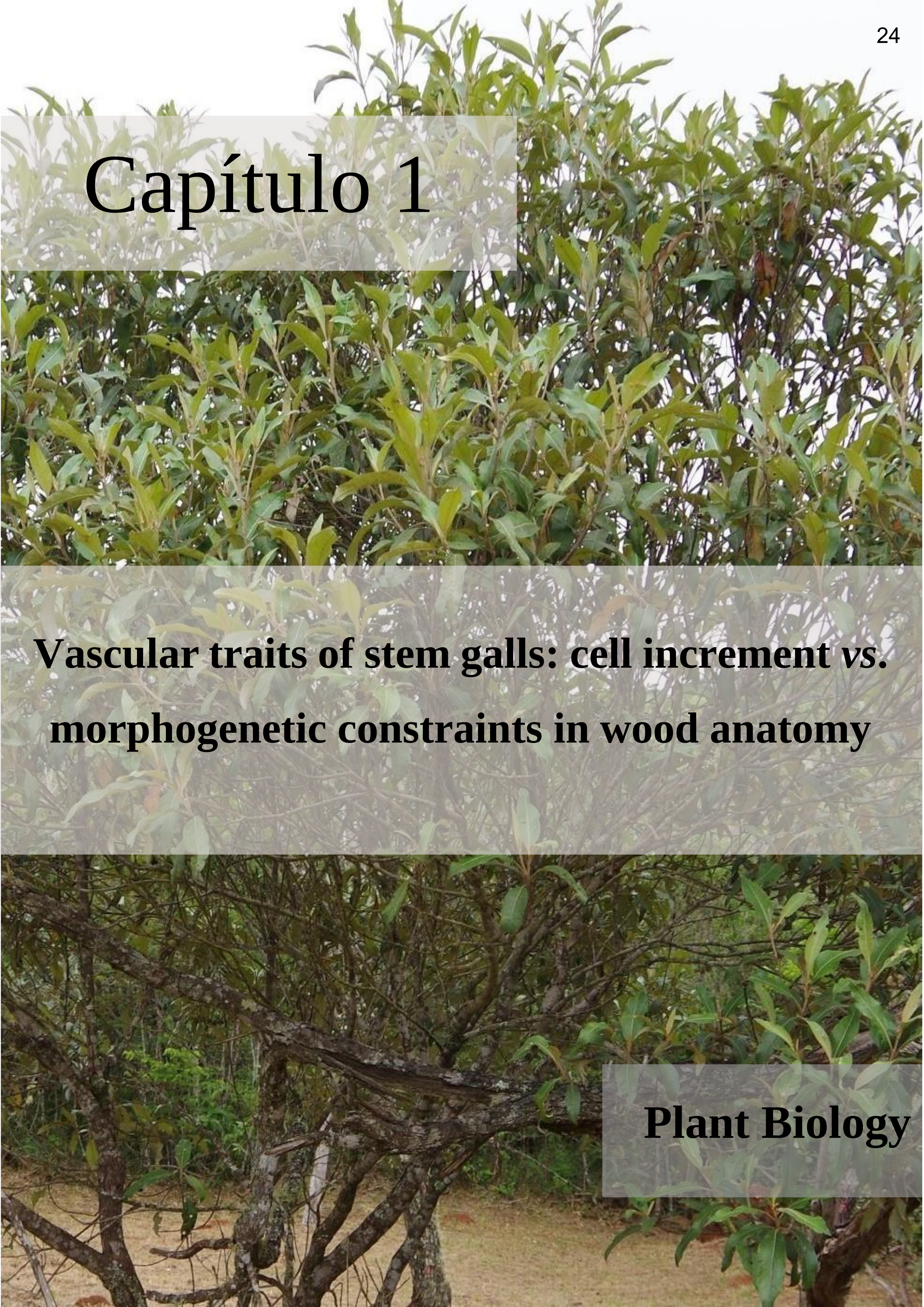

Capítulo 1

Vascular traits of stem galls: cell increment *vs.* morphogenetic constraints in wood anatomy

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RESEARCH PAPER

Vascular traits of stem galls: Cell increment *versus* morphogenetic constraints in wood anatomy

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Keywords
Auxin; Candeia; *Eremanthus erythropappus*; vessel elements; water relations.

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ABSTRACT

- *Eremanthus erythropappus* hosts globoid stem galls induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) close to the stem apex, which do not compromise the shoot apical meristem (SAM). We hypothesize that maintenance of the SAM, as well as the increasing number of leaves per branch and of galled stem lengths and diameters, are a consequence of alterations in vascular cells and, consequently, in the priority for water flow from non-galled stems to the stem galls. Our study focuses on the globoid stem galls to evaluate if gall induction and development promote changes in structure and function of secondary xylem cells.
- Anatomical, cytological, histometric and physiological methods were used to analyse non-galled stem branches (NGS), mature globoid stem galls and stem portions below and above the galls.
- These analyses revealed that vessel elements are larger in stem galls and in stem portions above the galls. Under *Neolasioptera* sp. induction activity, the vascular cambium of *E. erythropappus* produces less numerous but larger vessel elements and overproduces parenchyma cells.
- Contrary to the vascular constriction hypothesis proposed for bacterial galls, the vascular traits of the *Neolasioptera* sp. stem galls on *E. erythropappus* result in priority for water flow to galls and the non-galled portions above the galls, allowing the maintenance of galled stem growth and development.

INTRODUCTION

Eremanthus erythropappus (Asteraceae), a super host of galling herbivores, is associated with six gall morphotypes: two induced in apical buds, one in leaves and three in stems (Carneiro *et al*. 2009). One of these gall morphotypes is induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) 1999, close to the stem apex, but does not compromise the shoot apical meristem (SAM) (Prado & Vieira 1999). These galls are woody, rigid and apparently lignified, and the host stem increases in length, diameter and in number of leaves per branch (Prado & Vieira 1999).

Because of gall induction, alterations to vascular tissues may occur (Wilson & Magie 1964; Wilson 1965), which have been demonstrated, for instance, in bacteria-induced galls in *Vaccinium macrocarpon* (cranberry) (Best *et al*. 2004). In this plant–bacteria system, cells external to the vascular cambium hypertrophy and, together with tissue hyperplasia, result in a swelling in the stem with an increment of periderm (Best *et al*. 2004). The xylem vessels, close to the gall developmental site and differentiated in the year of gall induction, are narrow and dense compared to those of non-galled stems of the same age. The sieve tube elements apparently differ between the gall and the non-galled portion of the stem of *V. macrocarpon* (Best *et al*. 2004). A similar result was observed in *Agrobacterium tumefaciens-induced* galls in stems of *Ricinus communis* L., where the vascular tissue is altered in galls in relation to the non-galled stems. Features, such as narrow xylem vessels above the gall development site, increased rays and absence of fibres, in this case support the hypothesis of vascular constriction and explain the mechanism that gives priority to the flow of water between growing and developing galls over that of the host shoot organs located above the gall (Aloni *et al*. 1995; Aloni & Ullrich 2007).

We consider that in host plant-galling insect systems, water flux is prioritized for the growing and developing stems hosting galls and to the stem galls, which favours gall development. To test this hypothesis, we analysed the globoid stem galls induced by *Neolasioptera* sp. on *E. erythropappus* and examined: (i) if gall induction promotes the development of additional neoformed vascular tissues in response to the increasing needs of the growing gall; and (ii) the functional consequences of the gall-inducing activity over the development of secondary plant tissues. We also evaluated the functional traits of the stem galls that contribute to water priority flow to stems with galls during growth and development and mature stages compared to non-galled stems of a similar age.

MATERIAL AND METHODS

Samples of non-galled stem branches and globoid stem galls of *E. erythropappus* during growth and development and mature stages at 15–18 mm (n ≥ 15), in the vegetative stage, were collected from five individuals previously and randomly marked in the field. The samples comprise parallel (galled and non-galled) sub-terminal stem branches, two to three internodes below the apex, collected between 2017 and 2019 from a population of *E. erythropappus* in Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais, Brazil (20°26′04.3″ S, 43°30′37.4″ W). Gall morphotype characterization follows Isaias *et al*. (2013).

ANATOMICAL ANALYSIS

Gall development and wood anatomy

For gall developmental analysis, samples of non-galled stem branches (NGS), globoid stem galls during growth and development (soft texture) and mature (woody aspect) stages, and stem portions below and above the galls (5 cm from the gall) (n ≥ 5 per sample) were collected (Fig. 1). For secondary growth analysis, non-galled stem branches in the vegetative stage and mature globoid stem galls (n ≥ 5) were collected and sectioned in three anatomical planes (transverse, radial longitudinal and tangential longitudinal). These samples were fixed in FAA (formalin, acetic acid, 50% ethanol, 1:1:18) (Johansen 1940), dehydrated in an ethanol series (70%, 96% and absolute), followed by immersion in isoamyl acetate, and embedded in Paraplast® (Missouri, USA) (Álvarez *et al*. 2009). The samples were sectioned (18 μm) in a rotatory microtome (Leica 2035 BIOCUT®; Wetzlar, Germany), deparaffinized and stained in aqueous Astra blue-safranin 9:1 (v/v) (Bukatsch 1972). The slides were mounted with varnish Acrilex® (São Paulo, Brazil) (Paiva *et al*. 2006) and images were obtained under a photomicroscope (Leica DM 500®; Wetzlar, Germany) with a coupled digital camera (Leica ICC50 HP®; Wetzlar, Germany).

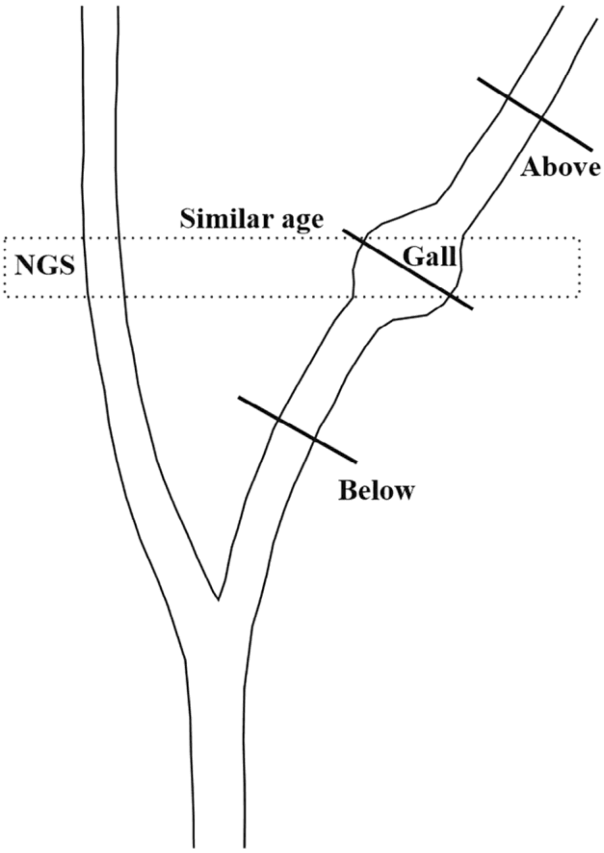

Fig. 1. Diagram of the regions analysed in galled stems and non-galled stems on *Eremanthus erythropappus*. NGS, non-galled stem; Below, regions below the gall development site; Above, regions above the gall development site. Dashed line, gall and region in non-galled stem of similar age.

Ultrastructural analysis

Non-galled stem branches and globoid stem galls during growth and development and mature stages (n = 5) were fixed in 4% Karnovsky reagent in 0.1 M phosphate buffer (pH 7.2) for 24 h (Karnovsky 1965), post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2), dehydrated in an ethanol series (Johansen 1940) and embedded in Araldite® (Luft 1961). The material was cross-sectioned in a Ultramicrotome Leica UC6, contrasted with uranyl acetate and lead citrate (Reynolds 1963), and examined in a G2-20-SuperTwin FEI 200 kV transmission electron microscope at the Center of Microscopy at the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil (http://www.microscopia.ufmg.br).

Analyses of vessel elements

In each of the five trees examined, fragments of non-galled and galled stem branches were collected. These fragments (1 cm^3, comprising regions with phloem and xylem) were divided in four samples: (i) non-galled stem branches, (ii) stem mature galls and galled stem portions (iii) below and (iv) above the galls (n = 5 per sample). These fragment samples were fixed in Karnovsky reagent, washed three times and immersed in Jeffrey mixture (10% chromic acid: 10% nitric acid, 1:1 v/v), changed every time complete oxidation was observed (approximately 3 days), washed in tap water (three times), stained in safranin 0.5% for 24 h, submitted to manual dissociation, washed and mounted with Kaiser's jelly glycerine (Jeffrey 1919). The lengths and widths of vessel elements in each of the four samples were measured (n = 10 per tree, a total of 50 different vessel elements per sample) using the AxioVision 7.4 software (Carl Zeiss Microscopy, Jena, Germany).

The average number of vessel elements per area of vascular tissue was calculated from photographs of transverse sections of the xylem area (8 mm^2) (n = 10 per sample) in each individual and with five repetitions.

Water potential measurements

The water potential of non-galled and galled stem branches of nine individuals of similar age was determined using a pressure chamber (Model 600; PMS, USA) (Scholander *et al*. 1965). The water potential of the individuals was measured at predawn and midday.

Statistical analyses

Parametric data were compared using Student's *t*-test (two categories: non-galled stems and galls). Non-parametric data were tested with the Kruskal-Wallis test (three categories: galls and stem regions below and above the galls) followed by Tukey's test. The tests were performed with R software (R Core Team 2015) considering $P \leq 0.05$.

RESULTS

Anatomical alterations due to gall development and wood anatomy

The induction of the stem galls in *E. erythropappus* is concomitant with the initiation of secondary growth. The redirection of the ordinary vascular cambium activity culminates with an over-differentiation of parenchyma cells instead of vessel elements and fibres (Fig. 2A and B). However, despite the increased and altered vascular cambium activity, other regions with secondary xylem and phloem differentiate (Fig. 2B). During the maturation stage, there is an increment in the secondary xylem but with areas of non-lignified parenchyma (Fig. 2B). Around the larval chamber, three to five layers of nutritive cells differentiate (Fig. 2B), the gall inducer lives inside the larval chamber, feeding on the nutritive tissue until development is com- plete. After which the insect leaves the gall through an exit channel. The parenchyma cells during gall development are larger (Fig. 2C) than those of non-galled stem branches (Fig. 2D) and have thin and sinuous walls, as well as large vacuoles (Fig. 2C). The nucleus occupies a parietal position, and it is possible to observe mitochondria and chloroplasts (Fig. 2E), phenolic compounds (Fig. 2F) and vesicular bodies (Fig. 2G). In longitudinal sections, it is also possible to observe areas of secondary xylem parenchyma among the vessel elements due to the redirection of normal development of the vascular cambium. Most of the cells around the larval chamber are parenchymatic and only a few vascular elements are observed (Fig. 2H and I).

Non-galled stem branches contain isolated vessels aligned in a radial pattern (Fig. 3A) distinct from the stem galls, whose vessels may be aligned in a radial pattern or form clusters of four or more vessels (Fig. 3B). Discrete phloem elements and primary phloem fibre strands are observed in non-galled and galled stems; however, secondary phloem fibre strands differentiate from the vascular cambium only in galled stems (Fig. 3B). Rays with mixed procumbent and square cells occur in non-galled stem branches and stem galls (Fig. 3C and D); there is a distinct increment in ray width due to an increase in the number of cell layers. The rays in non-galled stem branches are uniseriate (Fig. 3E), and in stem galls they are multiseriate with three to four cell layers (Fig. 3F).

Gall induction did not affect stem axial growth (Fig. 4A), but subtle anatomical changes above (Fig. 4B), in the galls (Fig. 4C) and below (Fig. 4D) the galls were observed,

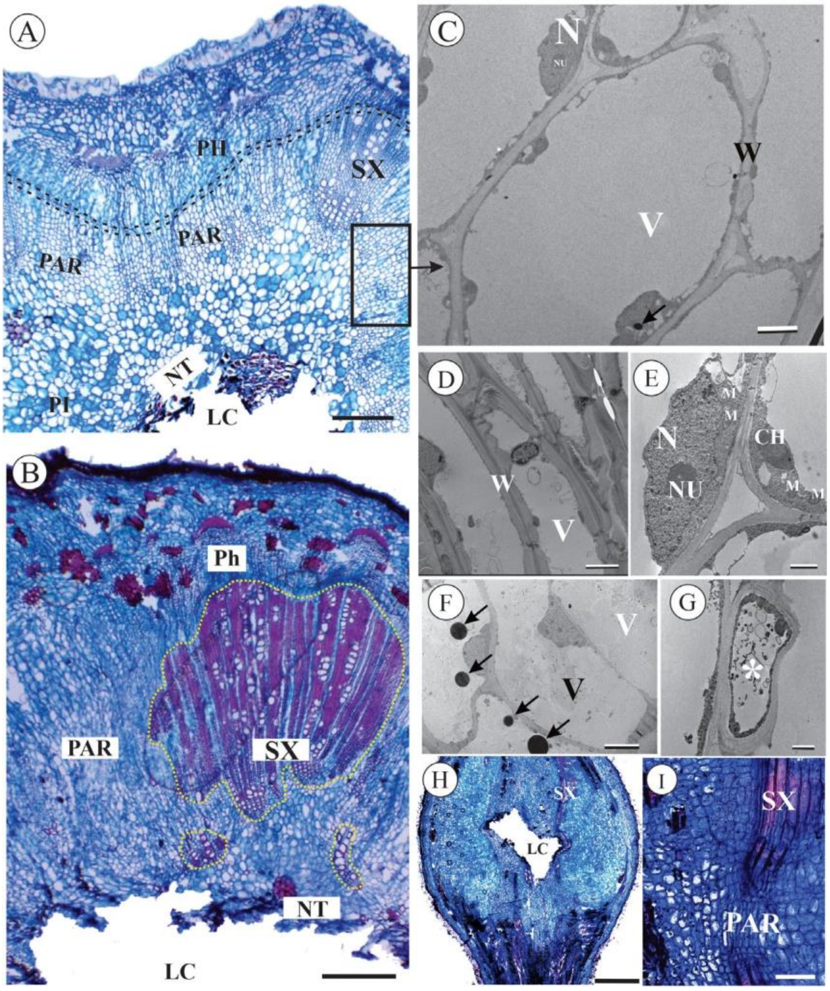

Fig. 2. Developmental anatomy of globoid stem galls in *Eremanthus erythropappus* and cytology of non-galled stem and gall. A–G: Transverse sections. A and B: Anatomy in light microscopy. C–G: Cytology in transmission electron microscopy. H and I: Longitudinal sections. A: Young gall; detail of altered cambium differentiation resulting in overproduction of parenchyma cells instead of fibres and vessel elements. B: Mature gall evidencing lignified cells alternating with parenchyma cells in secondary xylem. C: Parenchyma cells of a young gall with large vacuole, thin and sinuous cell walls, and nucleus in parietal position. D: Cells of non-galled stems evidencing the vacuoles and straight cell walls. E–G: Young gall. E: Detail of a cell, showing nucleus and nucleolus, mitochondria and chloroplasts. F: Parenchyma cell storing phenolic compounds inside the vacuoles. G: Vesicular bodies. H, I: Mature galls. H: General aspect in longitudinal section. I: Detail of secondary xylem interrupted by parenchyma cells. *Vesicular bodies; Arrow, phenolic compounds; CH, chloroplasts; Dashed black line, cambial zone; Dashed yellow line, regions with secondary xylem differentiation with lignified cells; LC, larval chamber; M, mitochondrion; N, nucleus; NT, nutritive tissue; NU, nucleolus; PAR, non-lignified parenchyma cells of secondary xylem; PH, phloem; PI, pith; SX, secondary xylem; V, vacuole; W, cell wall. Scale bars: (A, H): 200 µm; (B, I): 50 µm; (C, D, F): 5 µm; (E, G): 2 µm.

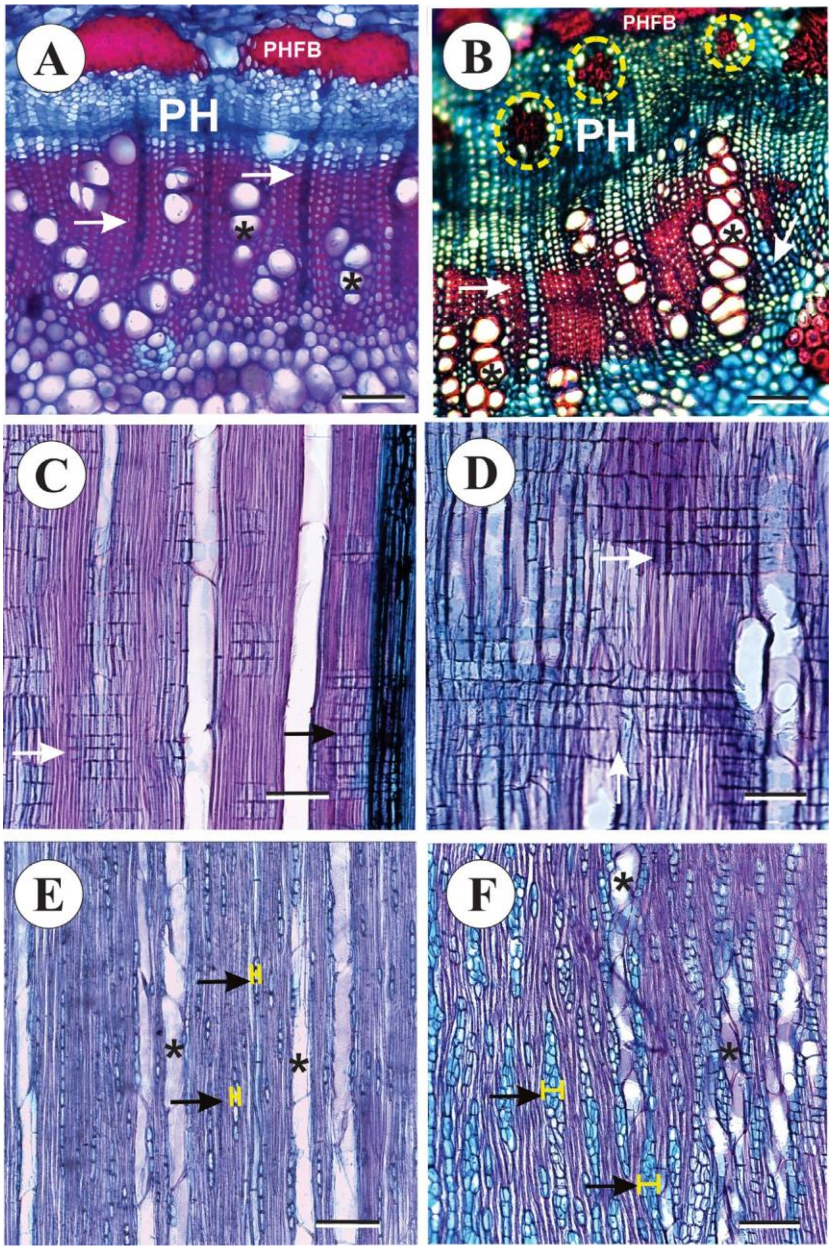

Fig. 3. Secondary growth in stems and stem galls on *Eremanthus erythropappus*. A, C, E: Non-galled stems. B, D, F: Stem galls. A, B: Transverse sections. C, D: Radial longitudinal sections. E, F: Tangential longitudinal sections. A: Vessel elements isolated and in radial pattern, the ray width is one or two cells. B: Vessel elements arranged in radial pattern and in clusters of four or more, the ray width has multiple cells; overproduction of phloem cells occurs. C, D: Rays with mixed procumbent and square cells in non-galled stem and galled tissues. E: Uniseriate rays and vessel elements (yellow line). F: Multiseriate rays and vessel elements revealing subtle increment in number of ray cells (yellow line), and arrangement of vessel elements. *Vessel elements; Arrow, rays; Dashed circle, phloem secondary fibres; PH, phloem; PHFB, phloem primary fibre; Scale bar: 50 μm.

especially in vessel and ray arrangement. The vessels are arranged in a dendritic pattern and grouped in multiples of four or more vessel elements in the regions above the galls (Fig. 4B and B^1) and in the gall tissues (Fig. 4C and C^1). However, in regions below gall development sites (Fig. 4D and D^2), the vessel arrangement is radial and similar to that of non-galled stem branches. In gall development sites, an increase in divisions of the vascular cambium initials favours the formation of non-lignified parenchyma cells over fibres and vessel elements (Fig. 4C), and a subtle increment in the number of ray cells (Fig. 4C^2).

Vessel element analyses

The vessel elements in non-galled stem branches and galls of similar age maintain similar lengths ($P = 0.7$) and widths ($P = 0.6$) (Fig. 5A). The vessel elements of non-galled stem branches have a length and width (207.9 9 39.9 μm) similar, on average, to the stem galls (200.0 9 41.2 μm).

The vessel element lengths above (233.8 μm) and below (190.2 μm) the galls are statistically different from each other ($P = 0.0003$). In addition, vessel element widths in stem portions above (42.2 μm) and below (34.1 μm) the galls are also statistically different from each other ($P = 0.0030$) (Fig. 5A and B). The vessel element lengths above the galled portions and in the galls (218.4 μm) are similar ($P = 0.3417$) (Fig. 5A–C) but differ between stem galls and stem portions below the galls ($P = 0.0301$) (Fig. 5A and B). The vessel element widths in portions below (34.1 μm) and in galls (41.2 μm) are also different ($P = 0.00008$) (Fig. 5A–C).

The average number of vessel elements in non-galled stem branches (3.2 mm^{-2}) is higher than in the stem galls (2.1 mm^{-2}) ($P = 0.0001$) (Fig. 5D). The average number of vessel elements per area in portions above (2.3 mm^{-2}) and in

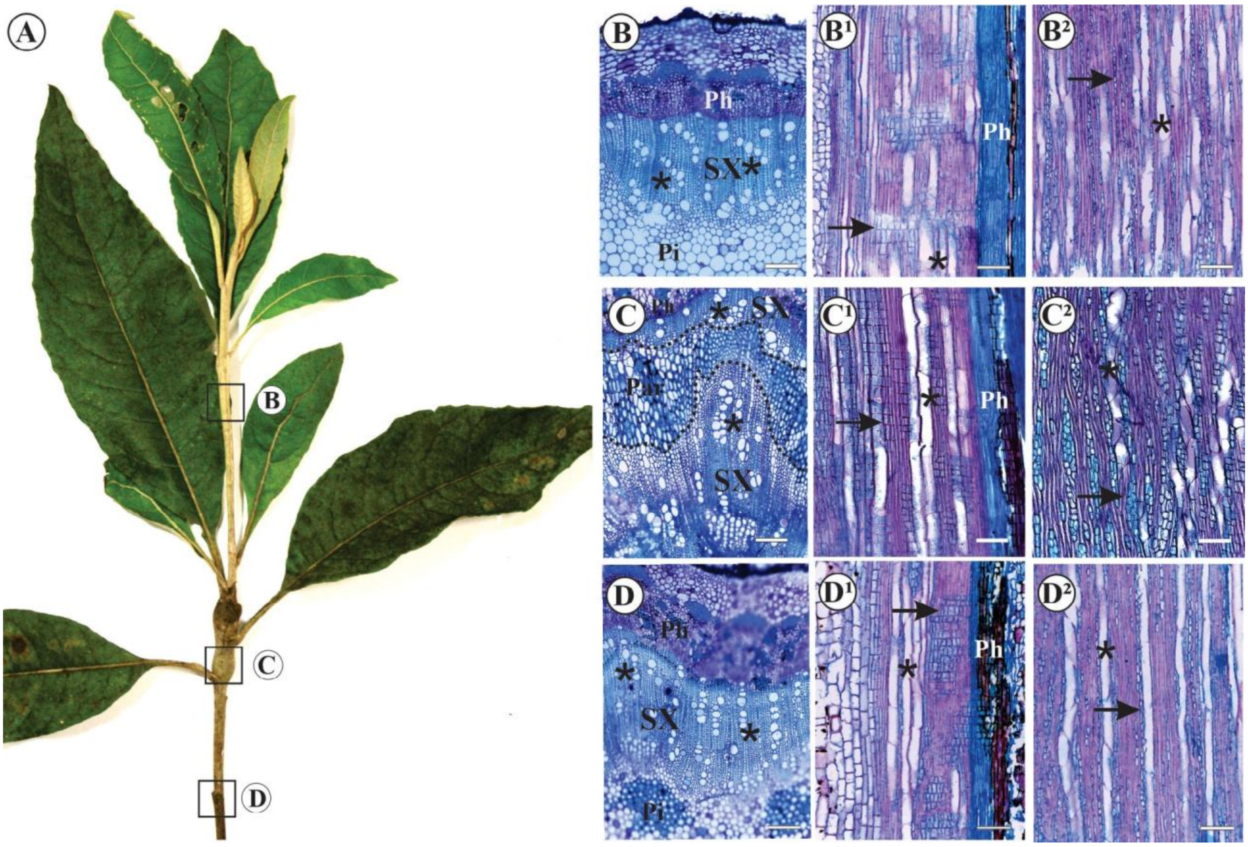

Fig. 4. *Eremanthus erythropappus* galled stem (A) with galled region above the gall (B), stem gall (C) and galled region below the gall (D). B–D^2: Anatomy of galled stem regions. B–B^2: Galled stems above the gall. B: Transverse section showing parenchymatic pith, vessels in radial and diagonal patterns, secondary phloem and fibres, parenchymatic cortex and periderm. B^1: Radial longitudinal section evidencing rays with procumbent and square cells, secondary xylem with vessel elements and secondary phloem. B^2: Tangential longitudinal section with uniseriate rays and vessel elements. C–C^2: Stem galls. C: Transverse section with clusters of four or more vessels and increment in non-lignified parenchyma cells. C^1: Radial longitudinal section showing rays with procumbent and square cells, vessel elements and secondary phloem. C^2: Tangential longitudinal section evidencing multiseriate rays with 2–3 cells and vessel elements. D–D^2: Galled stems below the gall. D: Transverse section evidencing parenchymatic pith, vessels in radial pattern, secondary phloem and fibres, and parenchymatic cortex. D^1: Radial longitudinal section evidencing rays with procumbent and square cells, vessel elements and secondary phloem. D^2: Tangential longitudinal section showing uniseriate rays and vessel elements. *Vessel elements; arrow, rays; Dashed line, region with changes in cambium differentiation; Par, parenchyma cells; Ph, phloem; Pi, pith; SX, secondary xylem. Scale bars: (A) 5 cm; (B–D^2) 200 μm.

the galls (2.0 mm^{-2}) is similar ($P = 0.0683$), but the density of vessel elements is higher in portions below the galls (2.8 mm^{-2}) ($P = 0.0001$) (Fig. 5E).

Water potential

The predawn water potential is −0.71 MPa in non-galled stem branches and −0.58 MPa in galled stems (Fig. 6). The midday water potential is −1.26 MPa in non-galled stem branches and −0.95 MPa in galled stems (Fig. 6). Both predawn and midday water potentials are statistically similar in non-galled and galled stems at predawn ($P = 0.1272$) and midday ($P = 0.1438$).

DISCUSSION

Plants perform different structural and physiological adjustments, such as changes in their tissue systems, hydraulic architecture and/or in transpiration regulation, to cope with environmental stresses (Sperry *et al*. 2002; Martínez-Vilalta *et al*. 2014). The new physiological status of a gall developmental site demands alterations in the cytological profile of the gall tissues, which has been documented in several host plant–galling herbivore systems (Bayer 1992; Schörongge *et al*. 2000; Oliveira *et al*. 2006). The development of *Neolasioptera* sp. galls on *E. erythropappus* results from changes in divisions of the vascular cambium initial cells, with an increase in the differentiation of xylem parenchyma cells. Similar to other host plant–gall inducer systems (Aloni *et al*. 1995; Arduin & Kraus 2001; Guedes *et al*. 2018), secondary xylem experiences dramatic changes in fibre differentiation, while the differentiation of vessel elements is inhibited at the site of gall development. The main alteration in *E. erythropappus* cytological profile concerns parenchyma cells, which are larger and more numerous in gall development sites than in the non-galled stems. The over-differentiation of parenchyma cells with sinuous and thin cell walls indicates their hyperplasic and hypertrophic potential (Oliveira & Isaias 2010) and implies increased water storage.

Like a compensation mechanism, the diameter of vessel elements is enhanced, whereas the differentiation of

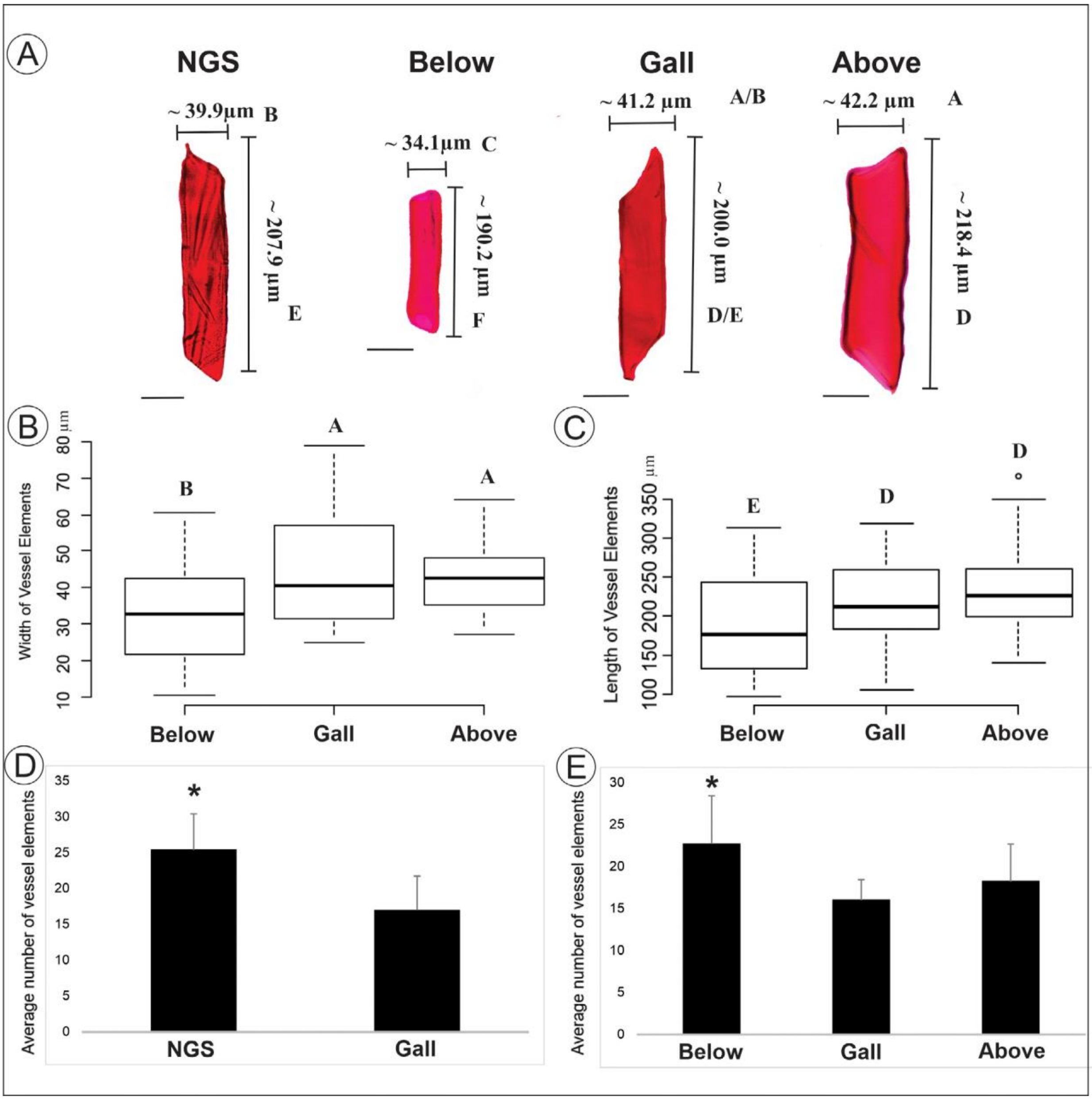

Fig. 5. Vessel element analyses in non-galled stems, galled stems and galls of *Eremanthus erythropappus*. A: Dissociated vessel elements of non-galled stems and of stem regions below and above the galls, and of the galls. Numbers refer to average width and length of the vessel elements. B, C: Vessel element measurements. Boxplot: bisecting lines: median width or length of vessel elements; whiskers: SD; dot in (C): outlier. B: Average width of vessel elements. C: Average length of vessel elements. D, E: Average number of vessel elements in non-galled stems and in stem regions below and above the galls, and in stem galls. Graph bar: whiskers: 6¼SD; *: significant statistic differences. D: Average number of vessel elements between non-galled stems and stem galls. E: Average number of vessel elements among non-galled regions, galled stems and stem galls. D: Student’s *t*-test (for two categories: non-galled stems and galls); B, C, E: Kruskal-Wallis test (for three categories: galls and stem regions below and above the galls) followed by Tukey’s test; results followed by different letters differ statistically at 0.5%. Scale bars: 50 µm. Above, stem region above the gall; Below, stem regions below the gall; NGS, non-galled stem.

parenchymatic rays and vessel elements and their arrangement and dimensions may undergo inconspicuous changes (Tyree & Zimmermann 2002). These alterations occur to retain the equilibrium in total water supply to the plant body (Tyree & Zimmermann 2002). In the galls on *E. erythropappus*, the similarity in vessel element dimensions between non-galled stem branches and stem galls, and the difference in dimensions between different regions of galled stems, indicate that gall effects are also basipetally oriented. The orientation of the effects of gall development, compared to standard patterns in non-galled stem branches, follows the polar stimulating streams of high auxin and ethylene levels synthesized in gall tissues (Aloni *et al*. 1989, 1995; Aloni 2013). Auxins also control the characteristics of xylem cells, *e.g*. length, diameter and cell lumen dimensions (Uggla *et al*. 1998; Arend *et al*. 2002). The effects of auxin concentration in *Neolasioptera* sp. galls on *E. eryhtropappus* result in larger vessel elements arranged in clusters. Similar results were found in *Populus* sp. under the effect

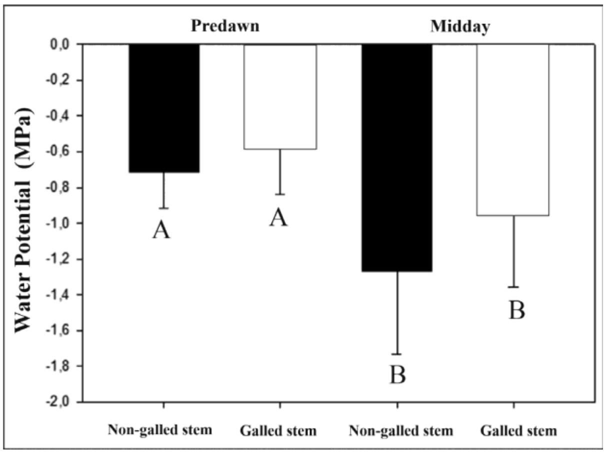

Fig. 6. Water potential of non-galled stems and galled stems of *Eremanthus erythropappus* of similar age at two different times of day: *predawn* and *midday*. Bars: water potential in non-galled stems or in galled stems. Whiskers: SD. Student's *t*-test (for two categories: non-galled stem and galled stems) applied, results followed by similar letters are statistically similar at 0.5%. Both predawn and midday water potentials are statistically similar between non-galled and galled stems at predawn and midday.

of phytotropin and morphactin auxin transport inhibitors (Junghans *et al*. 2004). The histolocalization of phenolic compounds in parenchyma cells with sinuous and thin walls has been related to the regulation of cell expansion and division through an imbalance of phenolic compounds and auxins (Hori 1992; Bedetti *et al*. 2014). These features are observed in various hemipteran galls (Hori 1992) and in Cecidomyiidae-induced galls on *Piptadenia gonoacantha* (Bedetti *et al*. 2014).

The over-production of axial parenchyma cells, larger and multiseriate rays, and the increased diameter of the stem can be related to ethylene effects, as described in *Populus* sp. (Junghans *et al*. 2004). Ethylene, a gaseous hormone produced in stressful situations (Yang & Hoffman 1984; Bleecker 1999), such as gall development, may naturally regulate auxin fluxes (Suttle 1988; Junghans *et al*. 2004).

The structural and hormonal adjustments in stem galls and their host shoots can result in a new pattern of hydraulic conductivity (Aloni *et al*. 1995; Aloni 2013). According to the gall constriction hypothesis, the differentiation of narrow vessels in host stems above the gall development sites results in priority of water supply to the gall over host stem portions located above the galls. Consequently, a growing gall should retard the development of its host shoot (Aloni *et al*. 1995; Aloni 2013). However, the growth of the stem portions above the stem galls on *E. erythropappus* is not compromised, and leaves, flowers and fruits develop in galled stems, which can also host successive galls (Prado & Vieira 1999).

For *E. erythropappus*, a low density of vessel elements in the galls is related to maximum efficiency in water transport, because even at low density, the vessels are wider both in galls and in the stem portions above the galls. Such compensation caused an increase in length and width in the stem branches and in the number of leaves per branch, as suggested by Prado & Vieira (1999). The combination of a low density of wide vessels is contrary to the high density of narrow vessels observed in *Agrobacterium* galls (Aloni *et al*. 1989, 1995; Aloni 2013), with different effects on water transport.

In *A. tumefaciens,* the vascular constriction causes priority to water flow to the growing and developing galls over that of the host shoot located above the gall. However, in *E. erythropappus* the water conductance flows primarily to the galled stems. Hydraulic conductivity directly depends on the diameter of the vessel elements (Tyree & Zimmermann 2002); hence, the differentiation of wide vessels in the stem portions above and in the galls is more efficient in long-distance water transport than the narrow vessels, as suggested by Tyree *et al*. (1994) and Tyree & Zimmermann (2002). Such wide vessels guarantee long term and normal development of the stems, despite hosting the *Neolasioptera* sp. galls. Theoretically, a small number of wide conduits are more efficient than many narrow conduits (McCulloh *et al*. 2010) and may also be related to maintenance of the shoot meristem activity above the galls.

The cellular and tissue dimensions, together with the vitality of the host stem branches, indicate an adequate water flow. Consequently, the cytological profile of the *E. erythropappus*–*Neolasioptera* sp. system results in higher water status, and consequent increased hydration in galled than in non-galled stems (Chaumont & Tyerman 2014).

Gall induction promotes the development of additional neoformed vascular tissues in response to the increasing needs of the growing gall; however, the neodifferentiated cells follow a different pattern: there is an increase in parenchyma cells in detriment to vessel elements and lignified cells. The alterations in secondary xylem pattern in the *E. erythropappus*–*Neolasioptera* sp. stem galls do not support the constriction hypothesis proposed by Aloni *et al*. (1995). The overproduction of parenchyma with large vacuoles capable of storing water and the changes in vessel element dimensions promote higher priority to water supply to galls and the non-galled stem portions above the galls, allowing maintenance of growth and development in the galled stem.

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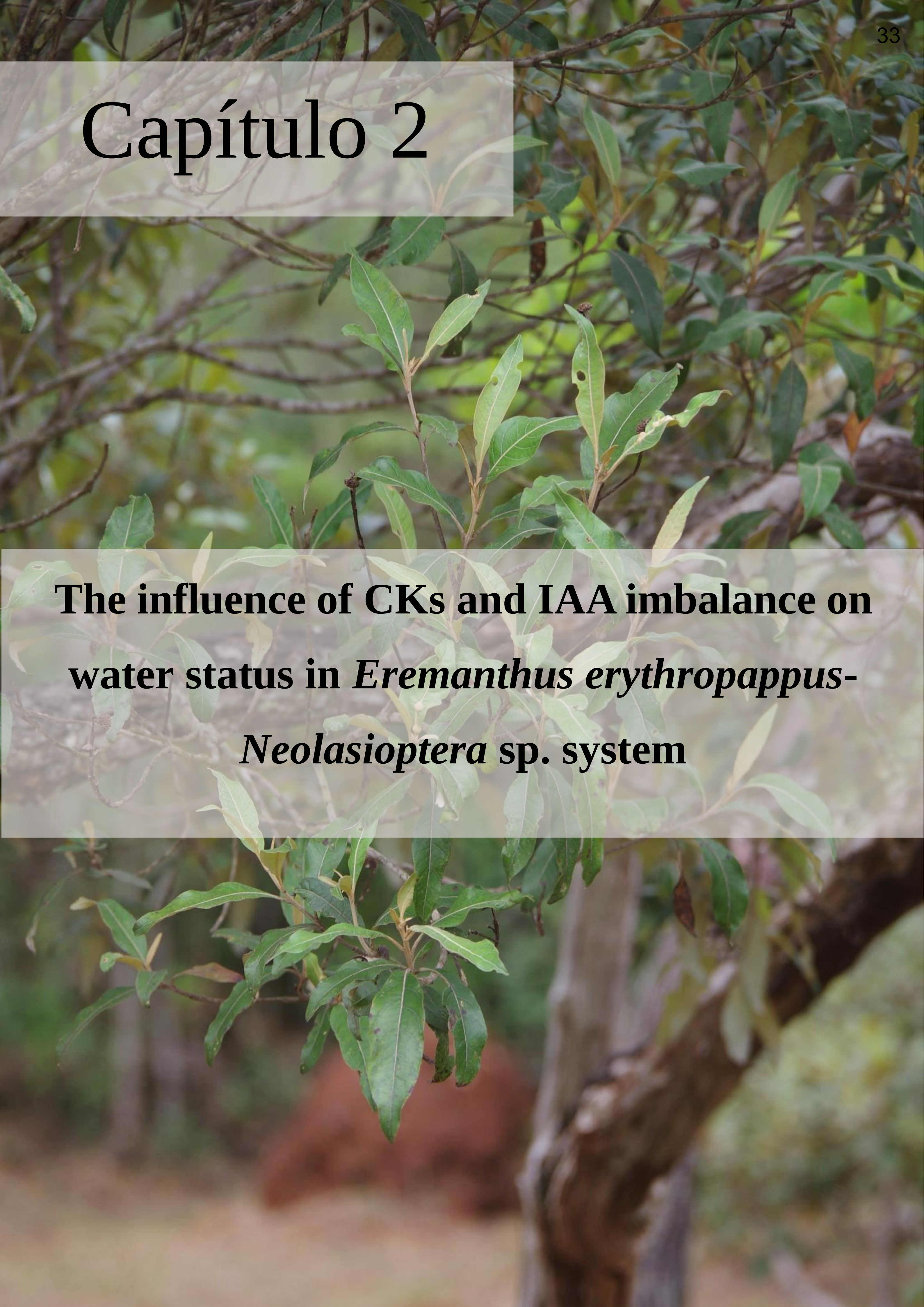

Capítulo 2

The influence of CKs and IAA imbalance on water status in *Eremanthus erythropappus*-*Neolasioptera* sp. system

The influence of CKs and IAA imbalance on water status in *Eremanthus erythropappus-Neolasioptera* sp. system

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Abstract

Stem galls structural traits may be caused by deviations in ordinary cambial activity, which may result in alterations in the organization of parenchyma, fibers, and vessel elements. Accordingly, the stem galls induced by *Neolasioptera* sp. on *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae) have non-differentiation of fibers, abnormal vessel elements, and overproduction of parenchyma cells. Such alterations may be related to the dynamics of accumulation of phytohormones, such as auxins and cytokinins, in gall tissues, as these phytohormones regulate the common processes of cell hypertrophy and hyperplasia. We mapped the sites of accumulation of CKs and IAA in of non-galled stem branches in vegetative stage and of globoid stem galls in mature stage using polyclonal antibodies. The samples were divided in four groups: (1) non-galled stem branches, (2) globoid stem galls in mature stage, and stem portions (3) below and (4) above the galls. The epitopes of CKs were labeled in xylem and phloem cell walls of non-galled stems, and of the stem portions above the gall. These epitopes were also labeled in cell walls of the nutritive tissue and xylem of stem galls, and in the cell walls of xylem in portions below the stem gall. The epitopes of IAA were immunolocalized neither in non-galled stems nor in mature galls, but they were labeled in the content of xylem cells of the galls and of stem portions below the galls, and in cell walls of phloem, xylem, and fibers of stem galls. Current results relate the differentiation of larger vessel elements and the overproduction of parenchyma cells to the imbalance of CKs and IAA in the stem portions below and above the galls and in the stem galls with direct effects on vascular cambium activity.

Keywords: abnormal cambium activity, stem galls, vessel elements

Introduction

Stem galls have peculiar structural traits because of alterations in cambial activity (Sirvastava 2002), and as a demand for functional adjustments on the stem tissue systems. As a peculiar vascularization trait, the stem galls induced by *Neolasioptera* sp. on *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae) is constituted by less numerous but larger vessel elements in comparison to the non-galled stems. Accordingly, the overproduction of parenchyma cells relates to the higher water potential in the stem galls than in the non-galled stems (Jorge et al. 2022). Both the larger vessel elements and the overproduction of parenchyma cells result from alterations in the activity of the vascular cambium, which is particularly sensitive to auxins (Sirvastava 2002, Jorge et al. 2022). The accumulation of auxins in gall tissues may cause the loosening of cell walls and consequent cell hypertrophy (Best et al. 2004). In addition, another phytohormones, the cytokinins, are involved in cell growth and division, fundamental for hyperplasia (Best et al. 2004). The accumulation of auxins (IAA) and cytokinins (CKs) in gall tissues is essential for the growth and development of galls, as they regulate the common processes of cell hypertrophy and hyperplasia (Bedetti 2014). We hypothesize that the cytometric changes on the vessel elements in the globoid galls on *E. erythroappus*, described by Jorge et al. (2022), involve alterations in the dynamics of the two phytohormones, CK and IAA, which regulate the abnormal vascular cambium activity in gall developmental site.

Commonly, the influence of the galling insects on host plant tissues refers to alterations in the dermal and ground tissue systems as responsible for the new morphogenetic patterns (Mani 1964, Rohfritsch 1992, Moura et al. 2008, Oliveira et al. 2011) and for the new biochemical profiles (Raman et al. 2005). Even though it is not much explored, the vascular system is sensitive to the galling insects, which is herein explored in the stem galls on *Eremanthus erythropappus,* popularly known as “Candeia”, a common species in Minas Gerais, Brazil (Mori et. al. 2010). Close to the apex of the stem branches, woody, rigid, and lignified galls are formed, which do not compromise the apical buds of the host branches (Prado and Vieira 1999, Jorge et al. 2022). These galls are common and induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) (Prado and Vieira 1999).

Our study focuses on the immunolabeling of CKs and IAA in non-galled stems, stem galls, and in the stem portions bellow and above the galls. The discussion is guided by the relationship among the histolocalization of the growth regulators, the anatomical traits, and the hydric status in the distinct stem portions and in the stem galls.

Material and methods

Host plant species and sampling

Samples of non-galled stem branches in vegetative stage and of globoid stem galls in mature stage, with 15-18 mm of width (n ≥ 5), were collected from individuals of *E. erythropappus* sequentially numbered along the Chapel’s trail (*“Trilha da Capela”*) in Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais state, Brazil (20º26’04.3”S 43º30’37.4”W). The samples comprise parallel sub-terminal stem branches (galled and non-galled), with similar ages, 2-3 internodes below the apex. The samples were divided in four groups: (1) non-galled stem branches (NGS), (2) globoid stem galls in mature stages (woody aspect), and stem portions (3) below and (4) above the galls (5cm away from the galls) ($n \geq 5$ per sample) (Fig. 1). The characterization of the gall morphotype followed Isaias et al. (2013).

Cytokinin and auxin immunolocalization

The immunolocalization of cytokinins (transzeatine riboside, CKs) and auxins (Indole-3-acetic acid, IAA) were performed as described by Zhang et al. (2014) and Bedetti et al. (2018). For the detection of CKs, samples of the four groups ($n = 3$) were washed in 10mM PBS, and fixed in a solution containing 91% 10mM PBS, 4% paraformaldehyde, and 5% glutaraldehyde, at 4°C for 15h. For the detection of IAA, samples of the four groups ($n = 3$) were pre-fixed in EDAC/100 mM phosphate-buffered saline (PBS) (pH 7.0), at 4°C, for 1h, and transferred to the fixative (91% 10mM PBS, 4% paraformaldehyde, and 5% glutaraldehyde) at 4°C for 15h. For the detection of CKs, the pre-fixation was suppressed because paraformaldehyde should link to CKs bases (*N*6-isopentenyladenine (ip), DHZ, and zeatin) in plant tissues (Zhang et al. 2014, Dewitte et al. 1999). After fixation, all samples were dehydrated in an ethanol series (70%, 96%, absolute), followed by three immersions in isoamile acetate for 15, 30 and 30 min, and embedding in Paraplast® (Alvarez et al. 2009). Transverse sections (18 µm) were obtained in a rotatory microtome (Leica 2035 BIOCUT®), deparaffinized with butyl acetate, hydrated in an ethanol series, and washed in 10 mM PBS. The sections were incubated in blocking solution constituted of 93.4% 10 mM PBS, 0.1% Tween-20, 1.5% glycine, and 5% bovine serum albumin (BSA) for 45 min. Afterwards, the sections were washed in regular-salt rinse solution (RSR) containing 99% 10 mM PBS, 0.1% Tween-20, 0.9% BSA for 5 min. Then, the sections were treated with 99.1% 10 mM PBS, 0.8% Triton X-100, and 0.1% BSA. Subsequently, the tissue sections were incubated separately in the primary rabbit anti-trans-zeatin riboside (ZR) antibody (AS09 414) (Agrisera®, Vännäs, Sweden), for cytokinin detection, or in the primary rabbit anti-IAA polyclonal antibody (AS09 445) (Agrisera®, Vännäs, Sweden), diluted 1:40 in PBS, for auxin detection (Livanos et al. 2016) at room temperature for 2 h, in the dark. For control, the primary antibodies were suppressed. Then, the sections were washed in RSR and 10 mM PBS and incubated in the secondary antibody anti-rabbit IgG (whole molecule) – FITC antibody produced in goat (F9887) (Sigma Aldrich®, St. Louis, Missouri), diluted 1:40 in PBS, at room temperature for 2 h. After washing three times in RSR and PBS, the slides were mounted in 50% glycerin, analyzed, and photographed under a fluorescence microscope (Leica® DM 2500 LED), with blue excitation light (450-490 nm) and green emission light (515 nm), coupled to a digital camera (Leica® DFC 7000T).

Results

Cytokinin immunolocalization

In the NGS tissues, the epitopes of CK-trans-zeatin riboside were moderately labeled by AS09 414 in the cell walls of xylem and in the cell content and cell walls of the phloem (Fig. 2A-B). In galled stems, the CK-trans-zeatin riboside were labeled in the stem regions above and below the galls, and in gall tissues (Fig. 2C-H). The epitopes of Ck-trans-zeatin riboside were moderately labeled by AS09 414 in cell content of xylem and phloem in the stem portions above the gall (Fig. 3C-D). In gall tissues, the AS09 414 strongly labeled the epitopes of Ck-trans-zeatin riboside in cell walls of nutritive tissue (Fig. 3E) and in cell walls and cell content of xylem (Fig. 3F). The epitopes of CKs were weakly labeled by AS AS09 41 in cell content of xylem in the stem regions below the gall (Fig. 3G-H).

Auxin immunolocalization

The epitopes of IAA were immunolocalized neither in non-galled stems (NGS) nor in mature galls, but they were weakly labeled by AS09 445 in xylem cell content in the stem portions above the galls (Fig. 3A-B) Also, the epitopes of IAA were strongly labeled by the AS09 445 in the cell content and cell walls of xylem, and were moderately labeled in cell walls of phloem, and fibers (Fig. 3C-D) in the stem portions below the galls.

Discussion

As a result of gall induction, alterations in the division of cambium initials may occur (Wilson 1965, Wilson and Magie 1964), as observed due to the development of *Neolasioptera* sp. galls on *Eremanthus erythropappus*. In this host plant-galling herbivore system, the changes in vascular cambium activity result in an increase in the differentiation of xylem parenchyma cells (Jorge et al. 2022).

The CKs, as positive regulators of cell divisions in the vascular cambium (Rahimi et al. 2020), are responsible for the maintenance of physiological juvenility in plant organs (Davies and Gan 2012, Thomas 2013). Accordingly, the immunolabeling of CKs by AS09 41 in xylem cells in galls and stem portions above and below the galls indicates that the xylem cells may transport CKs in galled stems, as reported for other plant organs (Kubo et al. 2010, Lacombe and Achard 2016, Ferreira et al. 2019). The transport of Cks was not interrupted by the action of the galling herbivore, and the flow of cytokinins from adjacent stem portions can maintain the physiological juvenility of the gall, increase the sensitivity of cambium cells to auxins, and induce the proliferation of parenchyma cells originated from cambium activity. Such physiological traits do not impair the development of *E. erythropappus* stems in response to gall induction and establishment (Prado and Vieira 1999, Jorge et al. 2022).

The imbalance of the high concentration of CKs and low concentration of IAA estimated by the intense immunohistolocalization of CKs in *E. erythropappus* galls is remarkable. Such high detection relates to intense hyperplasia, to cell cycle progression (Joonghyuk et al. 2021), and to the increased circumference of the galled organ, as observed in fusiform galls on *I. ingoides* (Bragança et al. 2021). Even though the CKs can regulate the transport of IAA in the vascular cambium (Aloni 2001), the epitopes of IAA were not detected by the AS09 445 in *E. erythropappus* gall tissues. Such non-detection of the epitopes of IAA is associated to an increase in hyperplasic sites (*cf.* Jorge et al. 2022) in the gall developmental sites. Nevertheless, the strong labeling of IAA by the AS09 445 in the walls of parenchyma cells and fibers and in the content of xylem cells in stem portions below the galls relates to the changes in the dimensions of the vessel elements reported by Jorge et al. (2022). In *E. erythropappus-Neolasioptera* sp. system, the vessel elements are smaller and narrower in the stem portions bellow the galls than in the stem galls and in the stem portions above the galls (Jorge et al. 2022), a difference which, however, does not support the constriction hypothesis proposed by Aloni (1995).

The dynamics of CKs and IAA in the galls on *E. erythropappus* reinforces the role of these hormones as activators of compensatory mechanisms in vascular tissues toward supplying the water demand of the galls. It is worthwhile to highlight that the differences in the dimension of the vessel elements occur only in the stem portions below the galls (Jorge et al. 2022). Such differences indicate the control of the two phytohormones on the patterns of differentiation of vascular cells, as observed in leaf galls on *I. ingoides* (Bragança et al. 2021). Herein, we reinforce the basipetal effects of gall development over the ordinary developmental patterns of the non-galled stem branches, which follow the polar stimulating streams of high auxin levels synthesized in gall tissues (Aloni et al. 1995, 1989 and 2013, Jorge et al. 2022). The cytological traits developed under the abnormal cambial activity promote a higher priority of water supply to the galled stems than to the non-galled stems (Jorge et al. 2022).

Conclusion

The dynamics of IAA and CKs labeled by the polyclonal antibodies in the galls induced by *Neolasioptera* sp. and in the adjacent stem tissues of *E. erythropappus* indicates the role of hormonal influx in the differentiation of gall vascular tissues. Different from what is observed in several galls, in *E. erythropappus*, the auxin flow seems to be interrupted in gall developmental site, with direct effects on vascular cambium activity. The cytometric changes on the vessel elements and predominance of parenchyma cells in the globoid stem galls on *E. erythroappus* result from the imbalance of CKs and IAA, which generate a compensatory mechanism toward maintaining the water status in *Eremanthus erythropappus*-*Neolasioptera* sp. system.

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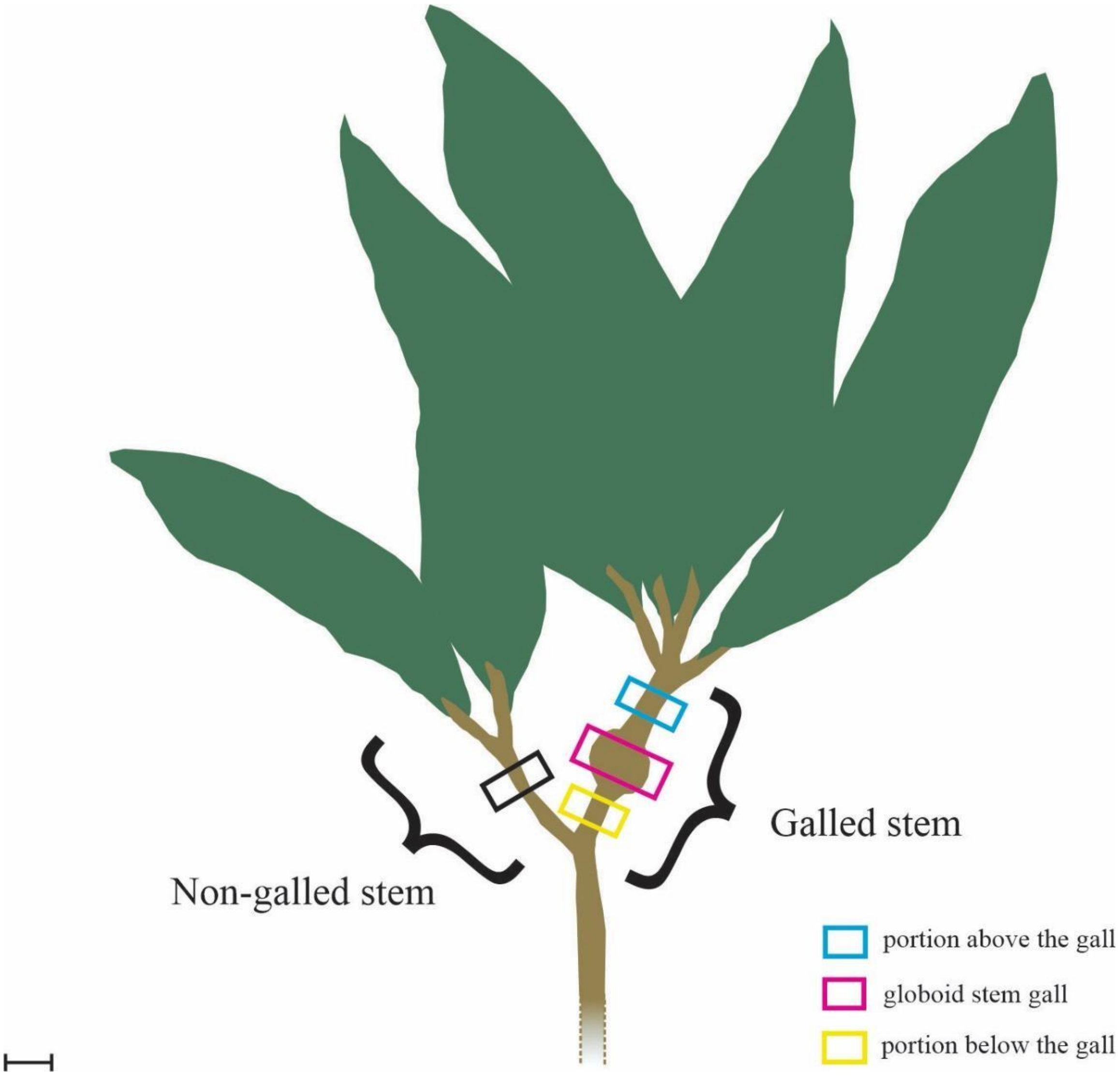


Figure 1. Diagram of *Eremanthus erythropappus* stem branches indicating the regions analyzed in non-galled stems, galled stems (black box), in the three stem portions: above the galls (blue box), galls (pink box) and bellow the galls (yellow box). **Scale bar:** 1cm

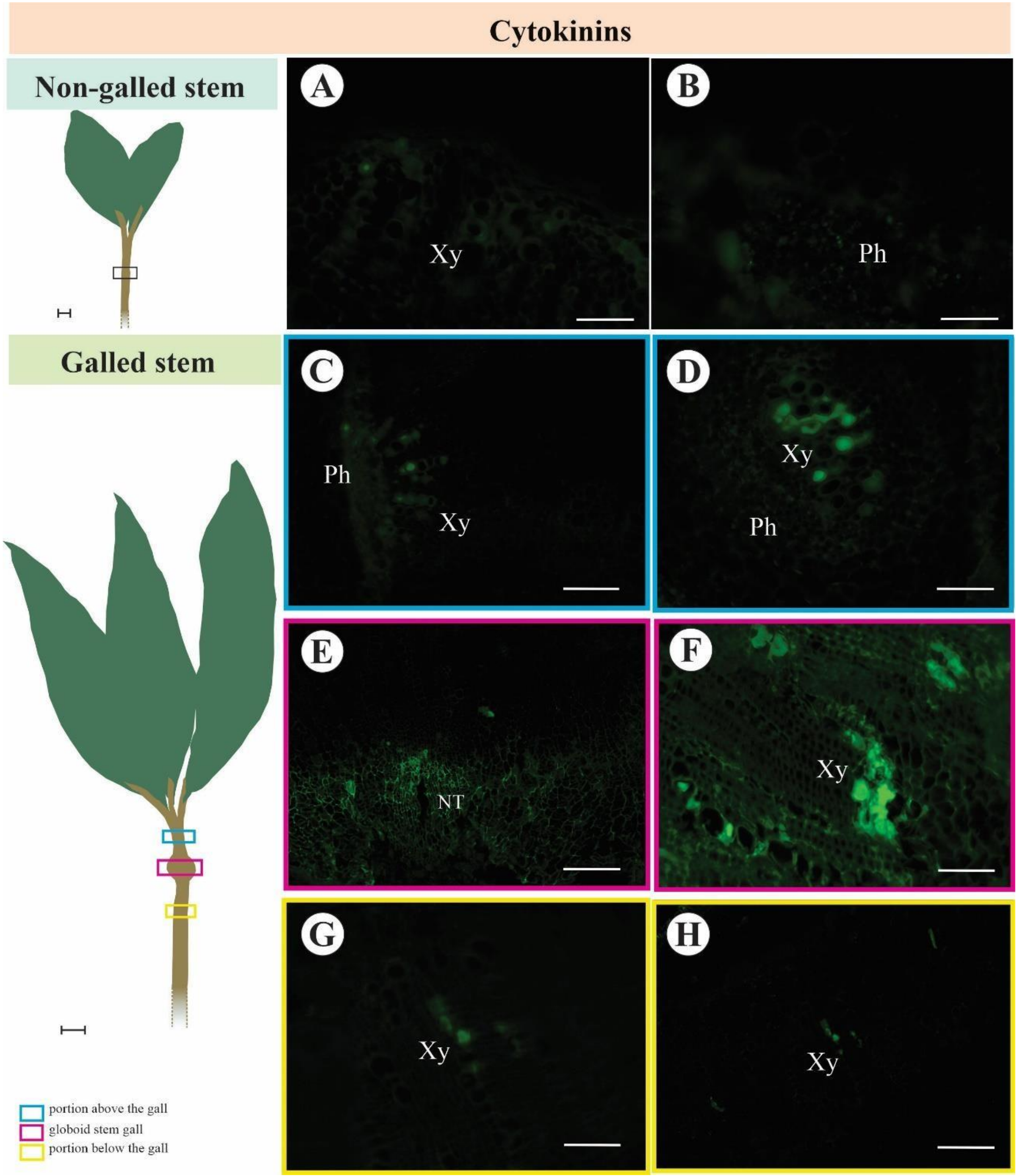


Figure 2. Ck-trans-zeatin riboside immunolocalization by AS09 414 on *Eremanthus erythropappus* non-galled stems and in galled stems portions. **(A-B)** Non-galled stem. **(C-H)** Galled stems. The epitopes of CKs were moderately labeled by AS09 414 in the cell walls of **(A)** xylem **(Xy)** and in the cell content of **(B)** phloem **(Ph)**. **(C-D)** Stem portions above the gall. The epitopes of CKs were moderately labeled in xylem **(Xy)** and phloem **(Ph)** cell content. **(E-F)** Stem galls. The epitopes of CKs were strongly labeled in the cell walls of nutritive tissue **(NT)** in **(E)** and in cell walls and cell content of xylem **(Xy)** in (F). **(G-H)** Stem portions below the gall. The epitopes of CKs were weakly labeled in cell content of xylem **(Xy)**. **Scale bar:** 1cm, A-H: 50 µm. Black box: non-galled stem portion.

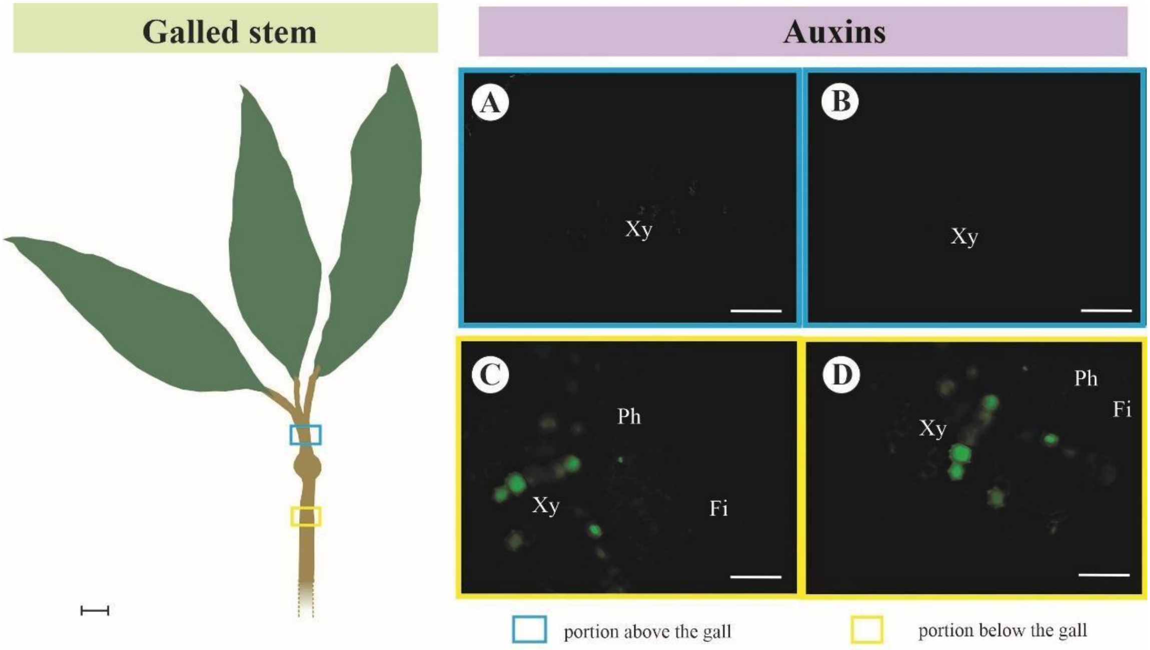
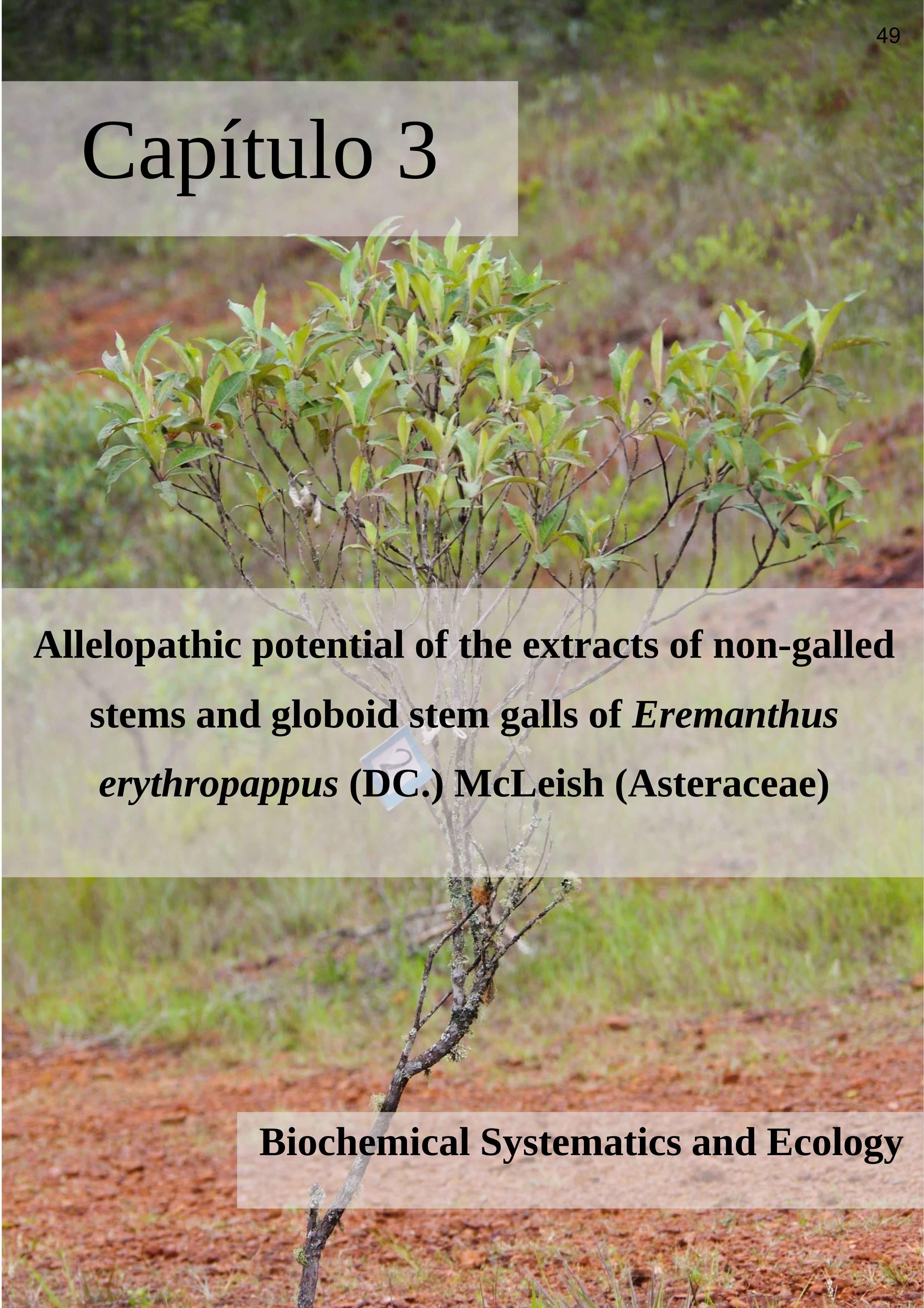


Figure 3. Indole-3-acetic acid immunolocalization by AS09 445 on Eremanthus erythropappus galled stem portions. (A-B) Stem portions above the galls. The epitopes of IAA were weakly labeled in xylem **(Xy)** cell content. (C-D) Stem portions below the galls. The epitopes of IAA were strongly labeled in cell walls and cell content of xylem **(Xy)** and they were moderate labeled in cell content and cell walls of phloem **(Ph)**, and fibers **(Fi)**. Scale bar: 1cm, A-D: 50 µm.



Capítulo 3

Allelopathic potential of the extracts of non-galled stems and globoid stem galls of *Eremanthus erythropappus* (DC.) McLeish (Asteraceae)

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Allelopathic potential of the extracts of non-galled stems and globoid stem galls of *Eremanthus erythropappus* (DC) McLeish (Asteraceae)

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ABSTRACT

Eremanthus erythropappus (DC) McLeisch (Asteraceae), commonly known as "candeia", is a native species of Minas Gerais state, Brazil, frequently used in medicine as an anti-inflammatory and antimicrobial agent. *Eremanthus erythropappus* hosts six different gall morphotypes, whose associated inducers manipulate plant cells and tissues and stimulate the neo-synthesis, maintenance, or inhibition of both primary and secondary metabolites on their host plant organs. Based on the premise that galls can potentialize the chemical composition of their associated host plants, our study investigated the allelopathic potential of methanolic extracts of *E. erythropappus* non-galled stems and globoid stem galls on *Lactuca sativa* seeds. We also studied the anatomical alterations induced on lettuce plantlets submitted to the methanolic extracts to describe diagnostic features related to the allelopathic effect. Five pentacyclic triterpenes constituted the major class of the identified compounds, followed by volatile oils, alkane hydrocarbons, alkanes, and ketones. The germination rates were statistically different between the treatments, and among the treatments, and the controls (water and methanol). The pentacyclic triterpenes of the extracts of the non-galled stems and the stem galls have a powerful allelopathic effect on the germination of *L. sativa*; a higher effect than that of the sesquiterpenes of the essential oils previously described for the leaf chemical compounds. The galling *Neolasioptera* sp. activity on *E. erythropappus* stems elicited the synthesis and accumulation of new chemical compounds, two triterpenes (ϒ-taraxasterol, and β-amyrone) and one alkane (tricosane), and inhibited the synthesis of compounds previously related to extracts of *E. erythropappus* stems, i.e., two triterpenes (A-neooleana-3(5),12-diene, and lupenone) and one ketone (2(1H) naphthalenone). These extracts altered the differentiation of root parenchyma cells of *L. sativa* germinated plantlets, but our premise was not corroborated as the galling activity did not potentialize the allelopathic effects.

1. Introduction

Eremanthus erythropappus (DC) McLeisch (Asteraceae) leaves produce an essential oil with potential allelopathic effects (Pinto et al., 2019), but ineffective against herbivore attacks as its vegetative organs are frequently attacked by galling insects. Among galling insects associated to *E. erythropappus*, undescribed species fo *Neolasioptera* induces globoid galls on stem branches (Prado and Vieira 1999). Galling insects can manipulate plant cells and tissues by the interaction with the host plant secondary metabolism and phytohormones (Bedetti et al. 2014, 2017; Carneiro et al., 2017; Kuster et al., 2020). Accordingly, the galling *Neolasioptera* sp. may manipulate the allelopathic potential of *E. erythropappus* host stems. Such manipulation may result in the neo-synthesis of secondary metabolites (Oliveira et al., 2006; Guedes et al., 2016; Jorge et al., 2018) or in the maintenance of the synthesis of primary and secondary metabolites in similar levels (Nyman and Julkunen-Tiitto 2000; Jorge et al., 2018).

Eremanthus erythropappus essential oils are mainly composed of α-bisabolol, a sesquiterpene used in medicine as an anti-inflammatory and antimicrobial agent (Sousa et al., 2003) and revealed anti-nociceptive, anti-inflammatory (Sousa et al., 2008), and antiulcerogenic effects (Silvério et al., 2008, 2013). Currently, we deviate from the essential oils and focus on the potential allelopathy of polar compounds of non-galled stems and stem galls, as a step forward in understanding

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the effect of the galling stimuli on the chemical of host plants.

The allelochemical compounds can affect crucial physiological processes and induce anatomical changes in the target plantlets (Inderjit and Duke 2003; Isik et al., 2016). Based on the premise that *Neolasioptera* sp. galls can potentialize the chemical composition of their associated host *E. erythropappus* plants, we tested the allelopathic potential of methanolic extracts of the non-galled stems and the globoid stem galls on the seeds of *Lactuca sativa*, an indicative model species. We also described diagnostic anatomical features related to the allelopathic effect of the methanolic extracts on *L. sativa* plantlets. Three focuses guided our discussion: (1) the chemical characterization of the methanolic extracts of non-galled stems and globoid stem galls toward identifying potential allelopathic compounds, (2) the effect of the allelochemicals of the methanolic extracts on *L. sativa* seed germination, and (3) the anatomical alterations in plantlets submitted to the methanolic extracts.

2. Material and methods

Non-galled stems and mature globoid stem galls were collected in September 2018 from 20 different individuals of *E. erythropappus* (Fig. 1A and B), sequentially numbered along the chapel trail (= trilha da capela) a mountain field where "candeia" trees are abundant and dominant, in Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais, Brazil (20°26′04.3″ S 43°30′37.4″ W).

2.1. Extract preparation

A set of mature globoid stem galls were dissected in laboratory conditions to remove the galling insects. Samples of non-galled stems and globoid stem galls (10 g) were macerated with 250 mL of 100% methanol for seven days, at room temperature. The underivatized methanol extracts of the two samples were filtered through Whatman® No 1 filter paper and dried under reduced pressure in a rotary evaporator (Heidolph Liaborota 4000) coupled to an empty pump (v-700-Buchi) at 40 °C, obtaining 8.916 g of stem gall dry extract and 9.712 g of non-galled stem dry extract.

2.2. Bioassay

For the bioassay, 8 g of stem gall and non-galled stem dry extracts were redissolved in 80 mL of 100% methanol. Then, 3 mL of each methanolic extracts were added to Petri dishes ($n = 4$ per extracts and controls) covered with Whatman® No 1 filter paper disc. The Petri dishes were placed in a fume hood, and after methanol evaporation, 3 mL of distilled water were added (Aguilera et al., 2015a). Two controls were used, one with methanol and one with distilled water. For the first, 3 mL of methanol was added to the filter paper disks in the Petri dishes, which were placed in a fume hood until total solvent evaporation, then 3 mL of distilled water were added. For the latter, 3 mL of distilled water were added to the filter papers in the Petri dishes. On each Petri dish, *L. sativa* seeds were homogeneously distributed ($n = 30$), for each treatment and controls. The *L. sativa* seeds were purchased at the local Agroflora market in the city of Concepción, Chile. The Petri dishes were sealed with Parafilm® to prevent evaporation and were incubated for seven days in a growth chamber at 20 °C, relative humidity of 70–75% with a light/dark cycle of 12/12 h. After seven days, the germination percentage in each treatment and controls were evaluated (Aguilera et al., 2015a).

2.3. Extraction and identification of chemical compounds

The dry extracts of the stem galls and of the non-galled stems were fractionated in a chromatographic column packed with an ion-exchange resin (Sephadex® LH-20). Each dry extract (50 mg) was resuspended in 250 mL of deionized water and the components of each extract were separated according to their polarity. Fractionation was done by passing the samples through a column with three solvents in decreasing polarity: water, hexane, and ethyl acetate. The hexane and ethyl acetate fractions were dried under reduced pressure in a rotary evaporator coupled to an empty pump at 40 °C.

The identification of the compounds of hexane and ethyl acetate fractions of the extracts of the non-galled stems and of the stem galls was performed by gas chromatography coupled to mass spectrophotometry (GC-MS). For this, 10 mg of hexane and ethyl acetate dry extracts were diluted in 300 μL of ethyl acetate. The Agilent® 7890A gas chromatograph is equipped with a splitless injector (250 °C) and an Agilent® 5975C mass detector, a capillary column of fused silica type HP5-MS (30 m × 0.25 mm internal diameter, x 0.25 mm thick) and helium gas (constant flow of 1 ml min-1). The separation was carried out under the following characteristics: Temperature: 250 °C; Detector (mass): 280 °C; Oven: initial 100 °C for 5 min, increasing to 8 °C/min up to 250 °C, and maintained for 15 min. The adjustment of the detector as a scanner varied from 50 to 500 amu, and the flow of carrier gas (electronic grade helium) was 1 mL/min. The samples were analyzed in triplicate. The tentative identification of the compounds was done by matching the mass spectra with the records in NIST 05 (NIST/EPA/NIH MASS 2005 Spectral Library). Compound identification was tentatively assigned when the overlap with the database was ≥90% match. The percentage of compounds in the extracts were calculated based on the total area of the GC-MS peaks.

2.4. Statistical analysis

The germination data were compared using Chi-square test. The tests were performed with R software considering $p \leq 0.5$ (RStudio Team, 2021).

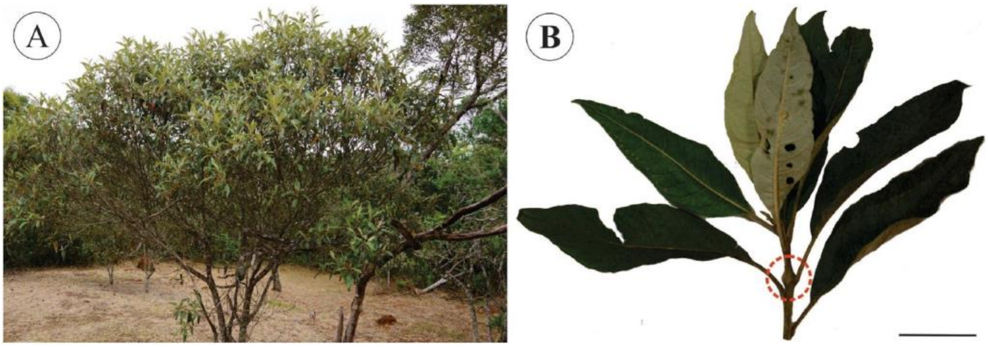

Fig. 1. *Eremanthus erythropappus* tree and a vegetative branch with subapical globoid stem gall. **(A)** Habitus of *E. erythropappus* tree on chapel trail (= trilha da capela) in Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais, Brazil. **(B)** A globoid stem gall (dashed circle). **Scale bar:** 5 cm.

2.5. Anatomical analysis

Root apices of germinated plantlets (n≥5) were fixed in Karnovsky's solution (1965), dehydrated in an *n*-butyl series, and embedded in Paraplast® (Kraus and Arduin 1997). The material was sectioned (18 μm) in a rotatory microtome (Leica 2035 BIOCUT®), deparaffinized, and stained in Astra blue-safranin 9:1 (v/v) (Bukatsch 1972, modified to 0.5%). The slides were mounted with varnish Acrilex® (Paiva et al., 2006), and the images were obtained with a photomicroscope (Leica DM 500®) with a coupled digital camera (Leica ICC50 HP®).

3. Results

3.1. Chemical compounds

Pentacyclic triterpenes constituted the major class of the identified compounds of the extracts of non-galled stems and stem galls, followed by three alkanes, an alkene, and a ketone (Table 1). In a similar pattern, friedelan-3-one, hentricontane, 1,19-eicosadiene, and nonscosane were detected in non-galled stems and stem galls (Table 1 and Figure Supplementary 1). The two triterpenes (A-neooleana-3(5),12-diene and lupenone) and one ketone (2(1H) naphthalenone) were detected in the non-galled stem samples (Table 1). Two different triterpenes (ϒ-taraxasterol and β-amyrone) and one alkane (tricosane) were exclusively detected in the extracts of stem galls (Table 1 and Figure Supplementary 2). Other minor compounds were detected (Table Supplementary 1 and 2), occupying less than 1.5% of the chromatogram peak area.

3.2. Bioassay

Both the extracts of non-galled stems and stem galls significantly inhibited the germination of *L. sativa* seeds. The extract of non-galled stems conferred the highest percentage of inhibition (9.16%), which differed significantly from the extract of stem galls (24.16%) ($p \leq 0.0001$) (Fig. 2).

3.3. Anatomical analysis

The apex of germinated plantlets submitted to extracts of the non-galled stems and stem galls had necrotic regions (Fig. 3A). This necrosis occurred in all the primary root cells but did not affect the epidermis (Fig. 3B and C). The two extracts – of non-galled stems and stem galls – caused different alterations in root cells regarding cell expansion, which differs among the cortical parenchyma cell layers (Fig. 3B and C). In germinated plantlets submitted to the extracts of non-galled stems (Fig. 3B), the 4–6 layers of parenchymatic cells are homogeneous and periclinally elongated close to the procambium (Fig. 3B). In germinated plantlets submitted to the extract of stem galls, the cortical parenchyma cells are organized in two layers. The outermost layer has hypertrophied anticlinally elongated cells, while the innermost 4–5 layers are homogeneous (Fig. 3C).

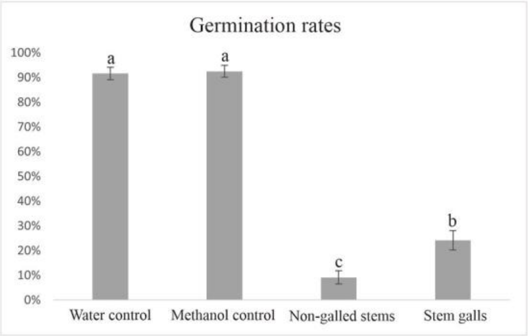

Fig. 2. Germination rate of *Lactuca sativa* seeds under the methanolic extracts of non-galled stems and stem galls. Different letters mean statistical differences for $p \leq 0.05$.

4. Discussion

Gall inducers can stimulate or inhibit the synthesis of secondary metabolites (Nyman and Julkunen-Tiitto 2000; Oliveira et al., 2008) in their host plant organs, as is valid for the *Neolasioptera* sp. associated with *E. erythropappus*. The galling *Neolasioptera* sp. elicited the synthesis and accumulation of new chemical compounds, such as the pentacyclic triterpenes ϒ-taraxasterol and β-amyrone, and the alkane tricosane on *E. erythropappus* stem galls. Accordingly, the *Neolasioptera* sp. could also had the ability to inhibit the synthesis of compounds of ordinary occurrence in *E. erythropappus* stems, such as the triterpenes A-neooleana-3 (5), 12-diene and lupenone, and the ketone 2(1H)naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl).

Both the methanolic extracts of non-galled stems and stem galls of *E. erythropappus* had inhibitory effects on *L. sativa* germination. The differences on the chemical composition and concentration of allelochemicals between the extracts of non-galled stems and stem galls probably determined the higher inhibition of germination of the seeds

Table 1

Major chemical compounds of the methanolic extracts of non-galled stems and stem galls on *Eremanthus erythropapus*.

Compounds	Molecular formula	MW (g/mol)	RT (min)	RA (%)		Nature of compounds
				Non-galled stem	Stem galls	
Friedelan-3-one	$C_{30}H_{50}O$	426.00	34.362/ 34.350	16.12	3.52	Pentacyclic triterpenes
Hentriacontane	$C_{31}H_{64}$	436.80	24.998/ 24.986	14.32	3.65	Alkane
A-Neooleana-3(5),12-diene	$C_{30}H_{48}$	408.70	33.468	11.62	–	Triterpene
1,19-Eicosadiene	$C_{20}H_{38}$	278.50	22.102	7.02	5.86	Alkene (diene)
Nonacosane	$C_{29}H_{60}$	408.80	22.646	5.82	–	Alkane
lupenone (Lup-20(29)-en-3 one)	$C_{30}H_{48}O$	424.70	30.165	4.66	–	Pentacyclic triterpenes
2(1H)naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	218.33	32.104	3.04	–	Ketone
ϒ-Taraxasterol(Urs-20-en-3-ol,(3.β.,18.α.,9.α.)-)	$C_{30}H_{50}O$	426.70	33.581	–	12.34	Pentacyclic triterpenes
β-Amyrone	$C_{30}H_{48}O$	424.00	32.173	–	2.75	Pentacyclic triterpenes
Tricosane	$C_{23}H_{48}$	324.60	20.976	–	2.02	Alkane

Notes: MW, molecular weight from GC–MS data; RT, Retention times; RA, Relative peak area (peak area relative to total peak area per fraction), (—) absent.

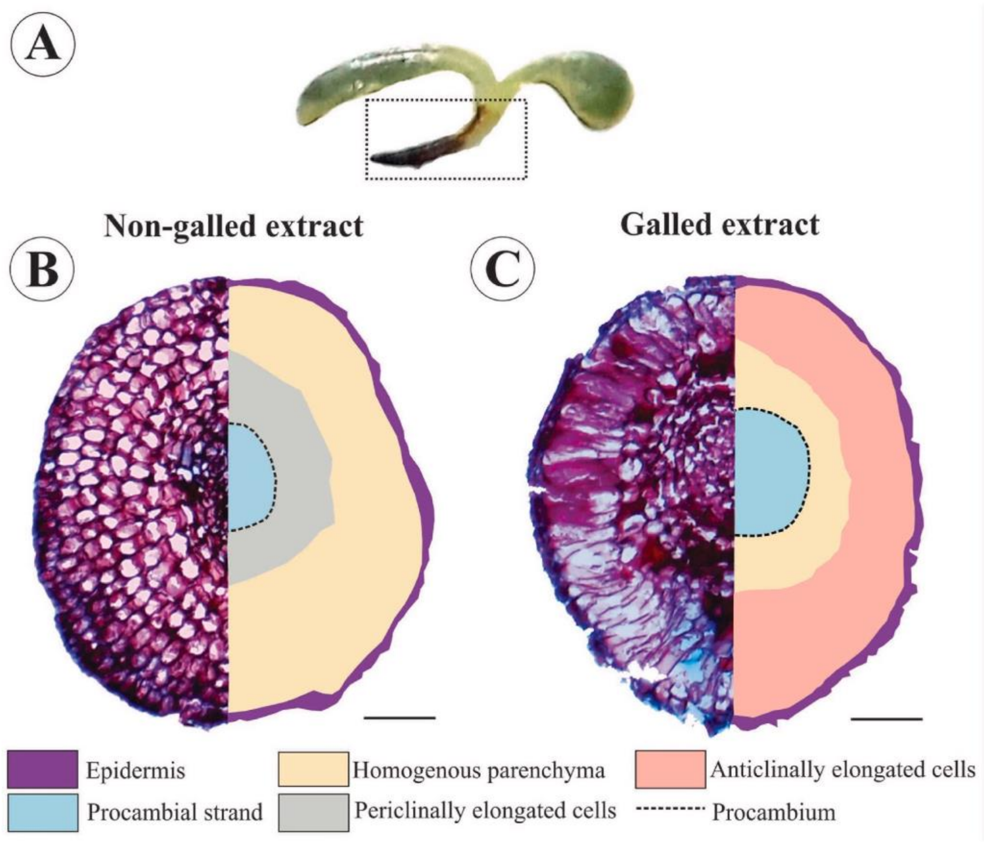

Fig. 3. Effects of extracts of non-galled stems and stem galls of *Eremanthus erythropappus* on *Lactuca sativa* plantlets. **(A)** Plantlet submitted to the extract of non-galled stems evidencing the necrotic apex. **(B–C)** Anatomy of the primary root apex of *L. sativa* in transverse section. **(B)** Unaffected epidermis, 4–5 layers of homogeneous parenchyma cells, 2–3 layers of parenchyma with periclinally elongated cells next to the procambial strand in a germinated plantlet submitted to the extract of non-galled stems. **(C)** Unaffected epidermis and outer most parenchyma cell layer with hypertrophied and anticlinally elongated cells. The inner most layers next to the procambial strand in a germinated plantlet submitted to extract of galled stem extract are homogeneous. **Scale bar:** 200 μm.

submitted to the non-galled stem extract. This inhibition may be attributed to volatile sesquiterpenes of the essential oil of *E. erythropappus* leaves (Pinto et al., 2019) but may also be influenced by the constitutive pentacyclic triterpenes detected herein. In fact, the allelopathic effect of the pentacyclic triterpenes on the germination of *L. sativa* was higher than the effect of the sesquiterpenes of the essential oil of the leaves. Such assumption was based on the minimal effect of *E. erythropappus* essential oil on lettuce germination (Pinto et al., 2019).

The pentacyclic triterpenes are C30 compounds with demonstrated allelopathic activity (Macías et al., 2019), as the friedelan-3-one (Santos et al., 2008; Ghosh et al., 2010; Freitas et al., 2015), the ϒ-taraxasterol (Watanabe et al., 2014; da Silva et al., 2019), and the lupenone (Macías-Rubalcava et al., 2007) detected in *E. erythropappus*. The concomitant presence of the friedelan-3-one and friedelin, both in the extracts of non-galled stems and stem galls, can be responsible for the allelopathic effect of the methanolic extracts. The pentacyclic triterpene friedelin detected in *E. erythropappus* is a component of the bark tissues of many plant families (Han et al., 2019). This triterpene reduced the germination of *Oriza sativa*, *Triticum aestivum*, and *Pisum sativum* (Ghosh et al., 2010), *Amaranthus leucocarpus, Echinochloa crusgalli* (Castañeda et al., 1992), and *L. sativa*, as well as the growth of *Allium cepa* (Freitas et al., 2015).

Although lupane triterpenes are known as growth promoters of *L. sativa* (Macías et al., 1994; 1997), such group of triterpenes, specially lupenone, has already been reported as inducers of allelopathic stress in seedlings of lettuce (Aguilera et al., 2015b, Macías et al., 2020), *Mimosa pudica*, and *Senna obtusifolia* (Luz et al., 2010). Considering the high percentage of inhibition of germination of the non-galled stem extract, where lupenone was detected, it is probable that this triterpene contributes to the allelopathic effect of the non-galled stem extract of *E. erythropappus*.

The ϒ-taraxasterol, the major constituent of the methanolic extract of stem galls, has been isolated from some weeds and its allelopathic potential was reported (Cerdeira et al., 2013; da Silva et al., 2019). The three pentacyclic triterpenes, lupenone, taraxerol, and friedelin, isolated from the leaves of *Sebastiania adenophora* inhibited the root growth of *Lycopersicon esculentum* (23%–49%) and *E. crusgalli* (28%–78%) (Macías-Rubalcava et al., 2007).

As previously discussed, the allelopathic effect of the major compounds of the extracts of non-galled stems and stem galls has been verified. However, a possible synergistic effect with minority compounds cannot be discarded (Table S 1 and 2), as has been considered for herbicide tests (Macías et al., 2019). Synergistic chemical interactions in plant extracts can induce greater phytotoxicity than isolated pure compounds (Zhang et al., 2012; Chotsaeng et al., 2017 and Jiang et al., 2020). The interaction of gall-inducing herbivores with their host plant metabolism results in alterations on plant tissues (Oliveira et al., 2008). The anatomical alterations induced by the phytotoxic substances in *E. erythropappus* extracts in the radicle of *L. sativa* may indicate a symptomatologic pattern. Although some lettuce seeds germinated, all root apices were necrotic, which compromised the survival of the plantlets. Root necrosis is a common effect in plantlets subjected to allelochemical stress (Aguilera et al., 2017; Carvalho et al., 2019) as described for lettuce plantlets (Aguilera et al., 2015a) and for Chilean native plantlets (Aguilera et al., 2015b) subjected to allelochemicals from *Acacia dealbata*.

The morphological damage induced by the methanolic extracts on *L. sativa* radicles was accompanied by changes at the tissue and cellular levels. The shape and structure of the *L. sativa* root cells were affected by the allelochemicals released from the extracts of non-galled stems and stem galls of *E. erythropappus*. The irregularly shaped cortical cells, and the collapsed cells are diagnostic of the allelochemical effects reported for radicle anatomy (Oliveira et al., 2008, Aguilera et al., 2015a, b, c). The mechanism of the allelopathic activity of the triterpenes is unknown (Macías et al. 2007), but the disruption of the cell membranes demonstrated for the friedelan (González-Coloma et al., 2011), a component of the extracts of non-galled stems and stem galls on *E. erythropappus*. may indicate a detergent-like effect, producing alterations at the membrane level (Duke and Oliva 2004).

As gall inducers usually manipulate the chemical composition of their host organs for their nutrition and protection (Price et al., 1998), the *de novo* synthesis of taraxasterol, an antifeedant (Yang and Lin 2017) and molting inhibitor (Jordon-Thaden and Louda 2003), in the stem galls of *E. erythropappus*, can be related to a defensive strategy of the *Neolasioptera* sp..

5. Conclusion

The activity of *Neolasioptera* sp. on *E. erythropappus* stems elicited the synthesis and accumulation of new chemical compounds and inhibited the synthesis of compounds previously related to the *E. erythropappus* stem. The original phytotoxic properties of the host stems and the neo-synthesis of compounds due to gall stimuli may be redirected toward the galling insect defensive benefit. Our premise that the galling stimuli would potentiate the phytotoxicity of the host stems was not supported because the allelopathic potential of the host plant is reduced due to the activity of the galling *Neolasioptera* sp..

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Author statement

Nina Jorge and Rosy Isaias did the field sampling. Nina Jorge, Lubia Guedes, Narciso Aguilera and José Becerra did the extraction and identification of chemical compounds Nina Jorge, Lubia Guedes, Narciso Aguilera did the bioassay. Nina Jorge, Lubia Guedes and Rosy Isaias analyzed the structure. Nina Jorge, Lubia Guedes, Narciso Aguilera, José Becerra and Rosy Isaias analyzed the data, wrote, revised and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2021.104379.

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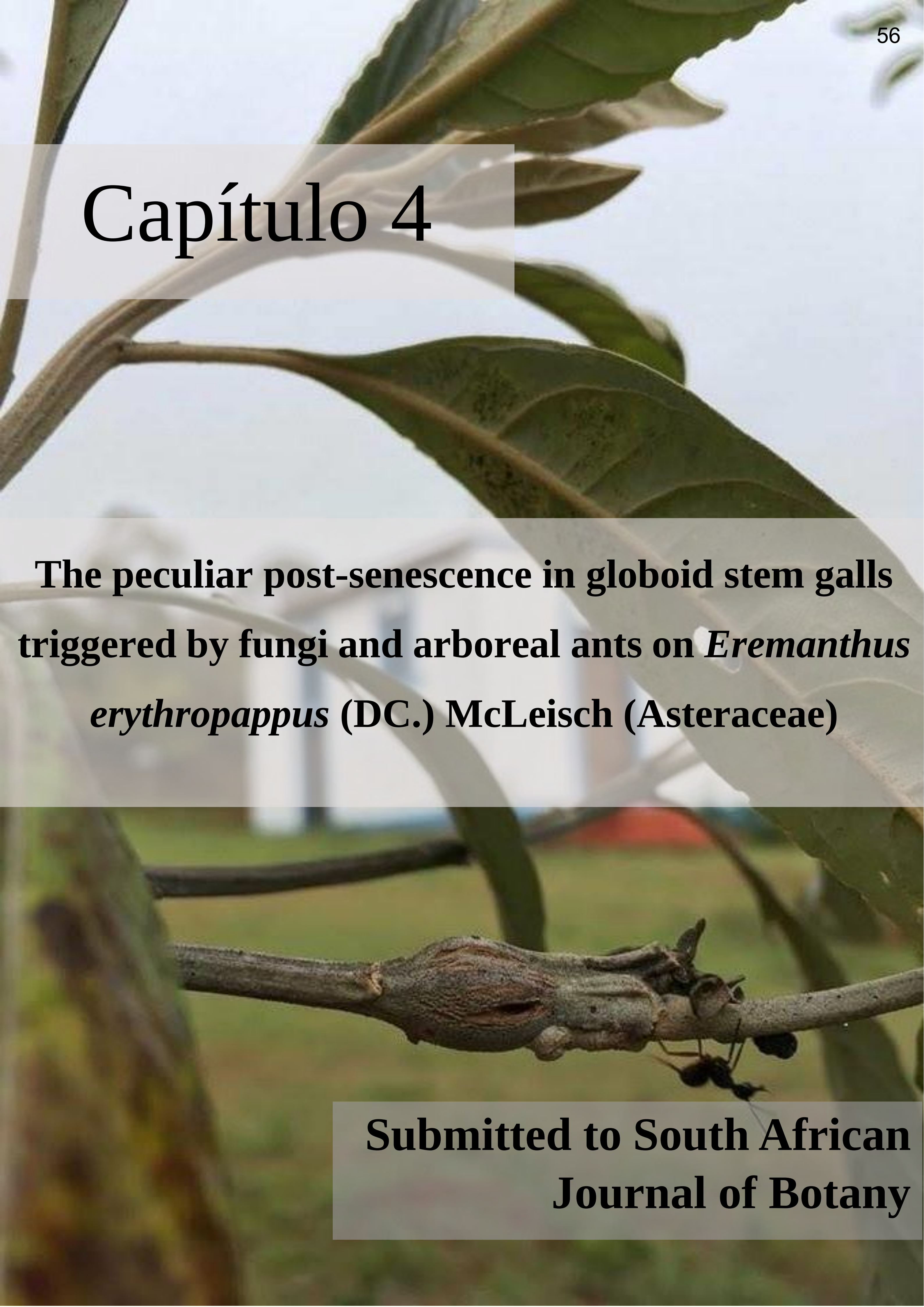

Capítulo 4

The peculiar post-senescence in globoid stem galls triggered by fungi and arboreal ants on *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae)

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The peculiar post-senescence in globoid stem galls triggered by fungi and arboreal ants on *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae)

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Contribution of authors

All authors contributed to the study conception and design. Nina Jorge, Mariana Freitas, Fernando Vale and Rosy Isaias did the field sampling. Heraldo Vasconcelos did the identification of the ants. Nina Jorge, Mariana Freitas, Fernando Vale and Rosy Isaias analyzed the structure. All authros analyzed the data, wrote the manuscript, read and approved the final manuscript.

Abstract

Eremanthus erythropappus (DC.) McLeisch (Asteraceae), popularly known as “candeia”, is a common species in Minas Gerais state – Brazil and hosts six gall morphotypes. Our focus relies on the diagnosis of how one of these morphotypes, the globoid stem galls induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) under senescence, can still react to the biotic stimuli of ants and fungi. The senescent globoid galls can be found in two conditions: (1) empty-galls characterized by the exit channel scar closed by plant tissue development, and (2) ant-occupied galls characterized by open exit channel, and the gall chamber occupied by ants and fungi. We hypothesize that favorable anatomical traits triggered in senescent galls on *E. erythropappus* may favor the formation of peculiar ant-nest galls. The anatomical analyses reveal that phellogen activity along the exit channel completely closes the empty-galls after the escape of the galling *Neolasioptera* sp.. The ant-occupied galls host six different genera of ants, whose movement in and out of the gall prevent the closing of the exit channel and allow the invasion of fungal hyphae. The gall-ant-fungi interaction relies on the ants mechanically maintaining the opening of the exit channel previously dug by the galling *Neolasioptera*, while the fungi stimulate the metabolism of the cells in the gall developmental site. Such interaction of the three taxa is responsible for the tissue responses and the maintenance of the peculiar ant-nest galls.

Keywords: Gall-ant-fungi interaction, ectopic phellogen, *Neolasioptera* sp., senescent gall

1. Introduction

Gall tissues are sinks of plant metabolites, which may favor fungal proliferation after the escape of the gall inducer (Bissett and Borkentl, 1988; Wilson, 1995; Muñoz-Adalia et al., 2019). The fungal invasion may create microhabitats for opportunistic inquilines, such as predators, parasitoids, and secondary inhabitants (Mani, 1964), which find high humidity and limited air circulation inside the gall (Stone and Schonrögge, 2003). Abandoned senescent galls may shelter secondary inhabitants, mainly arthropods (Mani, 1964), which can use the galls either as refugees to avoid the attack of natural enemies, to escape from unfavorable environmental conditions, or as nest sites (Sanver and Hawkins, 2000; Yamazaki and Sugiura, 2008; Sugiura and Yamazaki, 2009). In *Eremanthus erythropappus*, senescent globoid stem galls can be found in two conditions: (1) empty-galls characterized by the scar of the exit channel closed by plant tissue development, and (2) ant-occupied galls characterized by the open exit channel and the chamber occupied by ants and fungi. *E. erythropappus* (DC.) McLeisch (Asteraceae), popularly known as “candeia”, is a common tree species in southeastern Brazil (Mori et al., 2010), where it hosts at least six gall morphotypes (Carneiro et al., 2009). One of these galls is induced close to the apex of the stem branches by an undescribed species of *Neolasioptera* (Diptera: Cecidomyiidae) (Prado and Vieira, 1999). These galls are woody and tough (Prado and Vieira, 1999) due to the process of lignification during maturation (Jorge et al., 2022), and do not compromise the development of the shoot apical meristem (Prado and Vieira, 1999; Jorge et al., 2022).

Surveys in several areas of Minas Gerais state in Brazil revealed that 5.5-14% of the *E. erythropappus* galls are occupied by ants belonging to 11 different species (Almeida et al., 2014; Santos et al., 2017; Santos et al., 2021). The selection of a gall by arboreal nesting-ants depends on the size of the cavity entrance (Novais et al., 2017), but the morphological adaptability of the internal environment of these nest-galls has not been evaluated. Accordingly, we hypothesize that the cells and tissues of the senescent galls on *E. erythropappus* may respond to the activity of the arboreal ants as a post-gall reaction toward a favorable microenvironment for ant colonies. To test this hypothesis, we focus on the structural comparison between empty-galls and ant-occupied galls to evaluate the occurrence of tissue dynamics after the escape of adult *Neolasioptera* and under the ant-fungi impact.

2. Material and methods

Senescent globoid stem galls (n = 227) were collected in May-August 2018 in 20 individuals of *E. erythropappus*, sequentially numbered along the chapel trail (= trilha da capela) in Itacolomi State Park (Parque Estadual do Itacolomi), Ouro Preto, Minas Gerais, Brazil (20º26'04.3" S 43º30'37.4" W). First, the maximum width and length of each gall were measured using a digital caliper and used to calculate the volume of each gall by the formula = $4.\pi.r^3/3$. Then, the galls were dissected in the laboratory and separated into two groups: empty-galls and ant-occupied galls (Figure 1), whose ant inhabitants were collected, fixed in 70% ethanol, and identified at the Universidade Federal de Uberlândia (UFU), where the vouchers were deposited. After the measurements and classification, the volume of the galls was compared using a paired *t*-test in R software.

Empty-galls (n = 10) and ant-occupied galls (n = 10) were fixed in FAA (formalin, acetic acid, 50% ethanol, 1:1:18) (Johansen, 1940), dehydrated in an *n*-ethyl series (70%, 96% and absolute), followed by incubation in isoamyl acetate (Álvarez, 2009), and embedded in Paraplast® (Kraus and Arduin, 1997). The samples were sectioned (18 µm) in a rotatory microtome (Leica 2035 BIOCUT®), deparaffinized, and stained in Astra blue-safranin 9:1 (v/v) (Bukatsch, 1972, modified to 0.5%). The slides were mounted with varnish Acrilex™ (Paiva et al., 2006). For the staining of fungi, the empty-galls ($n \geq 10$) and the ant-occupied galls ($n \geq 10$) were embedded in Leica® historesin, sectioned (20 µm) in a rotatory microtome (Leica 2035 BIOCUT®), stained with 5% cotton blue in lactophenol for 20 min, and counter-stained in 1% safranin for 10s. In such a test, the fungi hyphae were stained in blue (Marques et al., 2013) and the plant cells were stained in red. The slides were observed in a photomicroscope (Leica DM 500®) with a coupled digital camera (Leica ICC50 HP®).

3. Results

Ants inhabited forty-two of the 227 collected galls (i.e., 21.15%). Seven different genera of ants were recorded, of which the most common were *Myrmelachista*, *Camponotus*, and *Nesomyrmex* (Table 1). In the galls from three *E. erythropappus* individuals, the inhabitants consisted of entire ant colonies (a founding queen plus a few workers, larvae, and pupae); in six galls, the only inhabitant was a queen, whereas, in 10 galls, the inhabitants were only workers or workers and brood (larvae and pupae). The mean volume of the ant-occupied galls ($\bar{x}$= 287.56) did not differ from that of the empty-galls ($\bar{x}$= 354.23) (t-test, p = 0.97).

Gall senescence in *E. erythropappus* begins by pupation and culminates with the escape of the adult galling *Neolasioptera* through an exit channel (Fig. 1a), which can be closed by plant tissue development, configuring the group of empty-galls (Fig1b). The ant-occupied galls have the gall chamber inhabited by ants and fungi hyphae (Fig. 1c). The empty-galls are closed after the escape of the galling insect (Fig. 2a), and a scar identifies the closure zone. Internally, cells from phellogen origin fill the exit channel (Fig 2b). The closure zone has a suberized dermal system with 3-4 layers of phelloderm, necrotic parenchyma, and remnant portions of the nutritive tissue (Fig. 2b-c).

In the ant-occupied galls, phellogen activity in the exit channel is maintained (Fig. 3a), and 8-10 layers of phelloderm are produced. The movement of the ants in and out of the galls prevents the closing of the exit channel, allowing the invasion of the fungal hyphae (Fig. 3b). Inside the gall chamber, the fungal hyphae intersperse to the remnant portions of the nutritive tissue (3c-d), and the gall parenchyma is necrotic. The primary xylem is maintained, and an abnormal ectopic phellogen activity differentiates phelloderm layers surrounding the larval chamber. In addition, it is possible to observe the overproduction of non-lignified secondary xylem in a radial pattern (Fig. 3c). The ant-occupied galls are open and histologically active.

4. Discussion

The *E. erytropappus*-Cecidomyiidae gall-fungi-ant system herein reported is unique as it does not actively involve the galling *Neolasioptera*, which has reached the adult phase and has left the gall. So, instead, the ants and the fungi opportunistically take advantage of the gall induced and developed under the stimuli of the *Neolasioptera* but have peculiar mechanical and metabolic strategies to keep the ant nest-galls active.

Ant-occupied galls on *E. erythropappus* represent 14% of the galls found in *Itacolomi* State Park, MG-Brazil (Almeida et al., 2014), 5.5% of the galls found in seven regions of the state of Minas Gerais (Santos et al., 2017), and 9.5% of the galls found in *Serra do Cipó* State Park, MG-Brazil (Santos et al., 2021). Herein, we reported 21.15% of the galls occupied by ants in *Itacolomi* State Park, MG-Brazil. The ant-occupied galls have been reported as nests for eight (Almeida et al., 2014), eleven (Santos et al., 2017), three (Santos et al., 2021), or five, here reported, different genera of arboreal ants. The most common genera found in *E. erythropappus* galls was *Myrmelachista* (Santos et al., 2017; Santos et al., 2021), followed by *Nesomyrmex* and *Camponotus.* For the first time, *Camponotous* was described occupying galls on *E. erythropappus* with queen, workers, and brood. The anatomical traits of the empty-galls on *E. erythroppapus* relate to the typical pattern of senescent galls (Ferreira and Isaias, 2013; Formiga et al., 2014), where tissue development is interrupted due to the lack of stimuli of the galling *Neolasioptera*. The closure of the exit channel due to phellogen activity and the production of suber and phelloderm cells is crucial for avoiding pathogen invasion (Biggs, 1992), and for the continuity of the non-galled stem portions, above the gall (Carneiro et al., 2009; Jorge et al., 2022). The phellogen activity in the globoid stem galls results in suberized cell layers lining the exit channel, a process that may be influenced by external conditions (humidity, light, temperature, excess of oxygen) or by traumatic stimuli (Biggs, 1992).

In the ant-occupied galls, the mechanical impact of ants going in and out is crucial for triggering the main difference between the empty-galls and the ant-occupied galls, i.e., the maintenance of the nest-entrance channel open. The passage of the ants mechanically triggers the activity of the phellogen (Bloch, 1941), which is an ordinary trait of the dermal system of *E. erythroppapus* stems. Phellogen activity is stimulated toward cell divisions and growth, culminating in tissue regeneration along the herein termed functional ant-channel. As a result of the mechanical stimuli, the original phellogen can be replaced by a traumatic phellogen due to a process of meristematic activation within the exposed non-conducting phloem (Fortes et al., 2004), from which new tissue layers differentiate (Natividade, 1950; Inácio et al., 2018; Lopes et al., 2020). From the anatomical point of view, the induction of a traumatic phellogen along the escape channel is a particular case of development post-gall senescence in *E. erythropappus*-*Neolasioptera* sp. system. In such a system, the stimulus of the external stress triggers the reassumption of plant cell cycles.

The presence of fungi hyphae in the gall chamber was not related to any specific ant species inhabiting the nest-gall, which suggests that the maintenance of the gall opening after senescence favors fungi invasion. Fungi invasion may have occurred by hyphae development from the bark toward the gall chamber, and the contact of the fungi with the cells lining the chamber stimulates plant cell responses. The stable microhabitat, with good humidity, limited air circulation, and nutritional metabolites, inside the galls (Bissett and Borkent, 1988; Wilson, 1995, Muñoz-Adalia et al., 2019) is explored by the ants, probably, with the intermediation of fungi infestation.

The senescence process in plant tissues relates to natural and subtle structural alterations (Fink, 1999); however, the fungi and the ant occupation changes the senescent dynamics in the *Neolasioptera* galls on the stems of *E. erythropappus*. The traumatic phellogen activity is stimulated and prevents the complete closure of the gall, which enters into a post-gall senescence stage. The temporal relationship necessary for the interaction among gall tissues, ants, and fungi seems precise, as after the closure of the exit channel by phellogen activity, the ant entrance, and fungi invasion do not occur.

In such a perspective, the establishment of the ant nest-galls requires a neo-stimulation from the ants or the fungi, as the *Neolasioptera* is not involved in the metabolic stimuli in ant-occupied galls. The ants were found exclusively in abandoned galls; then, they were considered secondary inhabitants (Santos et al., 2021), which opportunistically occupy the galls and obtain shelter, as is the case of the ants, and food, as is the case of the fungi.

The primary anatomical response in this peculiar plant-ant-fungi interaction in the globoid stem galls on *E. erythropappus* is the over-activation of a traumatic phellogen after their typical pattern of closing the escape channel. This opportunistic use of galls by secondary inhabitants are common in insect galls (Fernandes et al., 1988; Araújo et al., 1995; Crawford et al., 2007); however, records of ant nests with queens and larvae in galls are rare (Wheeler and Longino, 1988; Craig et al., 1991; Araújo et al., 1995). Even considering its low frequency (21.15%), the occupation of the galls by different ant colony members may indicate exploration of the niche and the increasing of the species richness and abundance of local communities (Cornelissen et al., 2016). Furthermore, gall occupation by founding queens or just workers and immatures indicates the potential formation of new nests or the expansion of colonies by polydomy (Debout et al., 2007). Accordingly, the presence of the fungi turns the globoid galls on *E. erythropappus* into actual nests, since in addition to an opportunistic shelter, it provides food for the ants (Klimes et al., 2012; Almeida et al., 2014).

Eremanthus erythropappus was previously described as a “non-myrmecophyte” species (Oliveira and Brandão, 1991; Mackay and Whalen, 1998; Oliveira and Pie, 1998), once no ant nests had been previously found in it, and the most common ant species inhabiting the plant have also been found in the soil (Rosumek, 2008). The rarity of ant nests in *E. erythropappus* trees suggests that the exudates of the extrafloral nectaries or some other type of food resource would attract the ants to the plant species. Peculiarly, the fungi inside the chamber in *E. erythropappus* ant-occupied galls digest nutritive and parenchyma cells. The product of this digestion may be consumed by the ants and may be the alternative food resource that potentially attracts ants, as reported by Rosumek (2008).

The comparison of the empty-galls and the ant-occupied galls revealed that the hyphae of the fungi in contact with remnant nutritive cells trigger the stimuli for the overproduction of non-lignified secondary xylem (Jorge et al., 2022). Also, the activation of the ectopic phellogen results in 4-5 layers of phelloderm cell toward the larval chamber and preserves the microhabitat condition for the ant-colonies. Accordingly, our hypothesis is corroborated as the *E. erythropappus* cells and tissues respond to the mechanical activity of the ants by stimulating the differentiation of an ectopic phellogen. Also, the fungi trigger the gall senescent cells to remain metabolic active.

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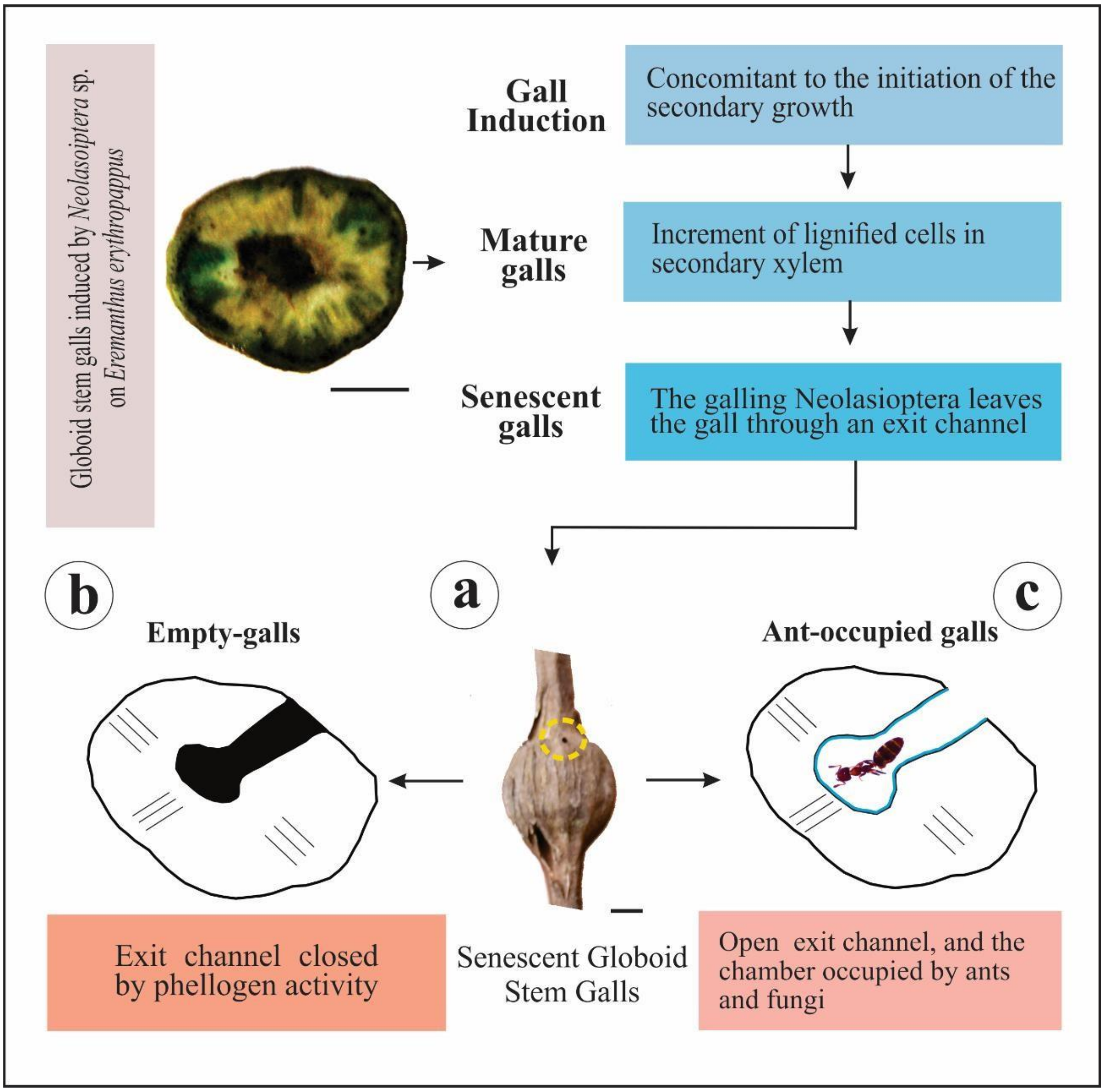


Fig. 1 Senescent galls on *Eremanthus erythropappus*. **(a)** Stem with a senescent gall. **(b-c)** Schematic representations **(b)** of an empty-gall closed by plant tissue development, and **(c)** of an ant-occupied gall. **Dashed line:** exit channel; **Blue line:** fungi. Scale bar: 3cm

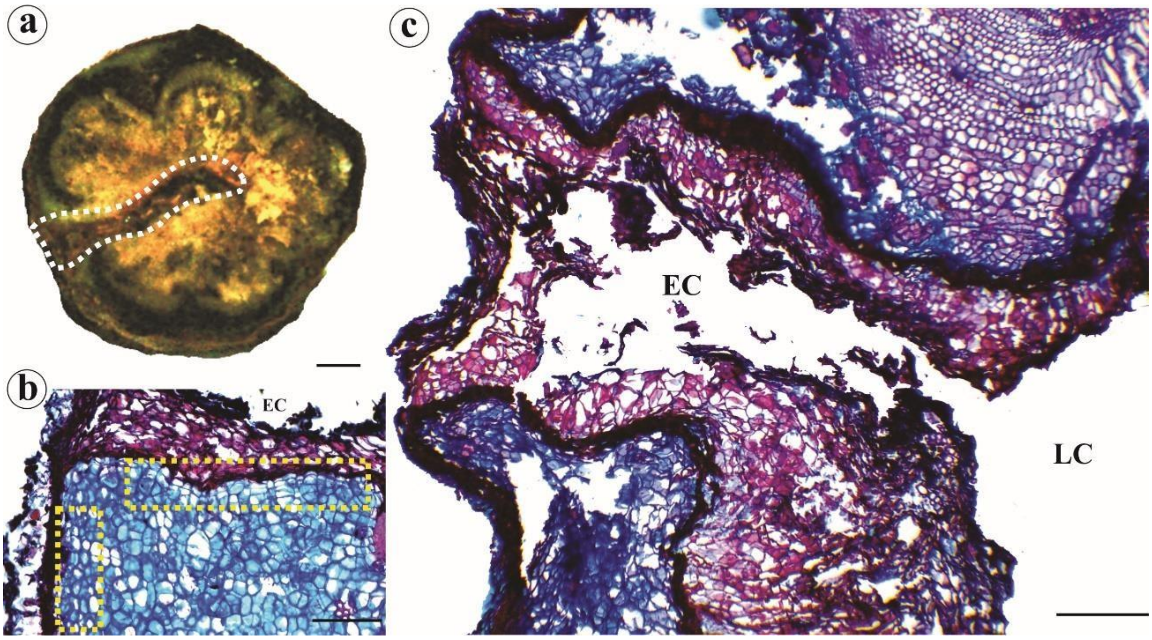


Fig. 2 *Eremanthus erythropappus* empty-galls. **(a)** Hemi-section of a gall evidencing the escape channel (dashed line). **(b-c)** Gall anatomy. **(b)** Phellogen activity (dashed box) producing phelloderm and suber. **(c)** Gall with non-lignified xylem cells, necrotic parenchyma, remnant portions of the nutritive tissue, and suber and necrotic cells lining the exit channel. **EC:** exit channel; **LC:** larval chamber. **Scale bar:** **(a)** 0,5 cm **(b)** 200 µm **(c)** 500µm.

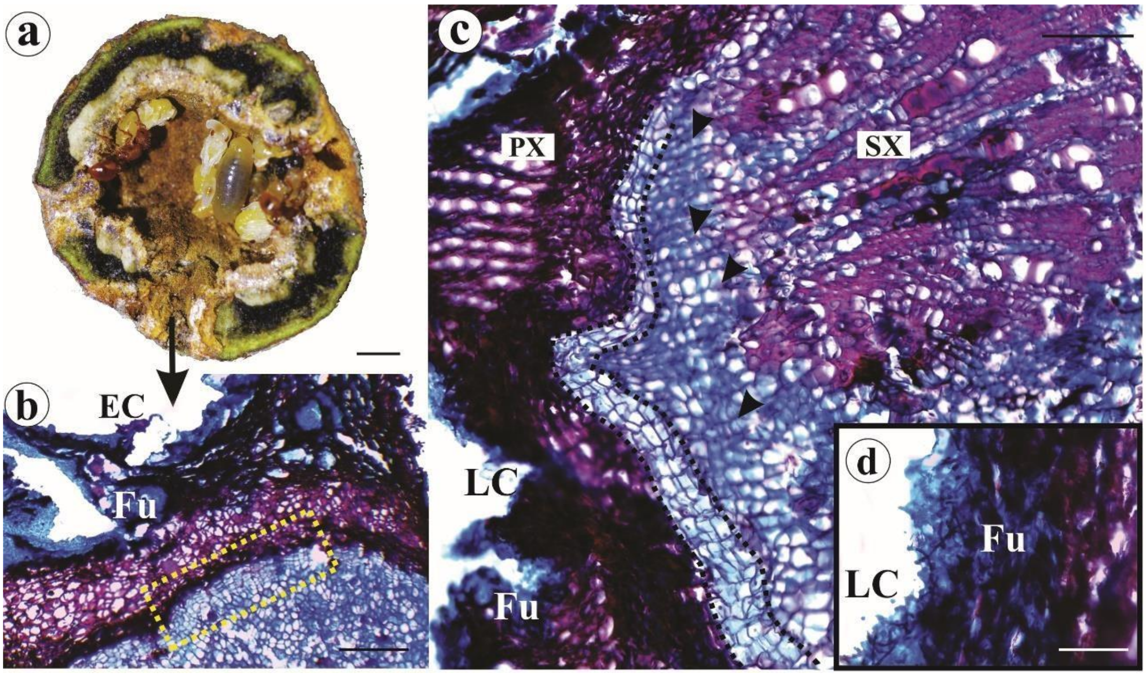
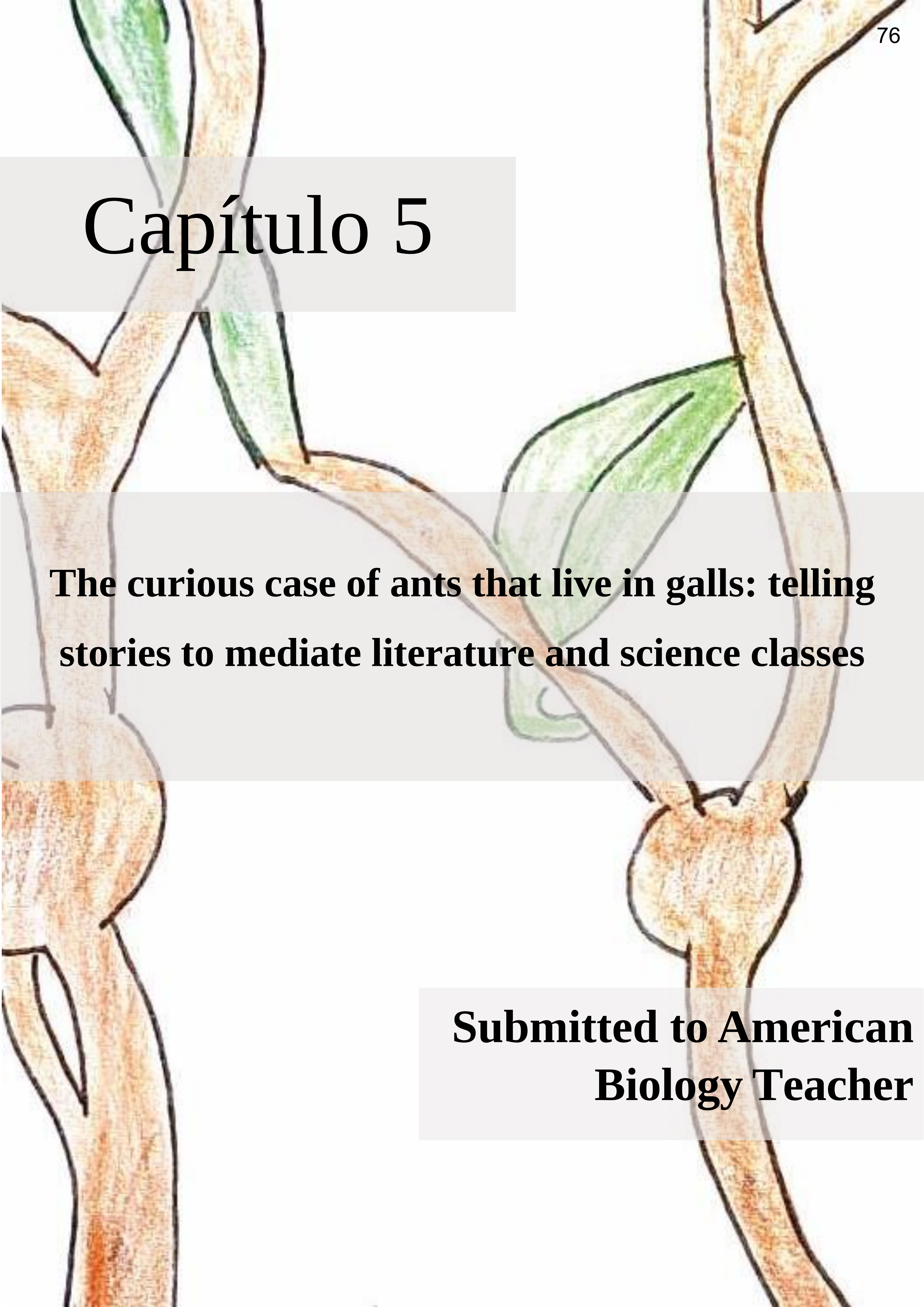


Fig. 3 *Eremanthus erythropappus* ant-occupied galls. **(a)** Hemi-section of a gall evidencing an ant nest. **(b-d)** Gall anatomy. **(b)** Exit channel lined by fungi and phelloderm; traumatic phellogen activity producing suber and phelloderm (dashed box). **(C)** Detail of a gall with ectopic phellogen activity (Dashed line) and an overproduction of parenchymatic non-lignified cells. **(d)** Detail of fungi hyphae in larval chamber. **Arrowhead:** radial pattern of non-lignified secondary xylem. **EC:** exit channel; **Fu**: fungi; **LC:** larval chamber; **PX:** primary xylem; **SX:** secondary xylem. **Scale bar:** **(a)** 0,5 cm **(b)** 200 µm **(c)** 500µm.

Table 1. Ant genera found in the galls of *Eremanthus erythropappus* and the castes present. Numbers represent the number of plant individuals

	Ant caste found in the galls			
Ant genera	Queen, workers and brood	Queen only	Workers only or workers and brood	Total
Camponotus	1	0	2	3
Crematogaster	1	0	1	2
Myrmelachista	0	2	5	7
Nesomymrmex	1	1	1	3
Procryptocerus	0	2	0	2
Pseudomyrmex	0	0	1	1
Solenopsis	0	1	0	1
Total	3	6	10	19



Capítulo 5

The curious case of ants that live in galls: telling stories to mediate literature and science classes

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The curious case of ants that live in galls: telling stories to mediate literature and science classes

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Abstract

The scientific community all over the world has been investing time and energy on the promotion of science communication from the academy toward the elementary and high schools. Different tools for communicating science discoveries have been created, e.g., videos, social media, podcasts, and texts with accessible language. Among this variety of tools, our choice relied on the production of a para-didactic book. The book is entitled "The curious case of ants that live in galls" (ISBN 978-65-00-13341-7) and has versions in English, Portuguese, and Spanish. It is directed to 8 to 12 years-old children, and its use is suggested for literature and science classes. From a methodological perspective, we propose either the reading of the text together with the students or the individual reading, followed by a debate and an exercise of re-textualization. This exercise focuses on the students' ability to go beyond the basic scientific concepts, toward practicing the construction of narratives, intertextuality, description of characters, identification of implicit and explicit information, title-text relationship, and plot structure. For science classes, it is possible to explore the biological concepts that support the understanding of the interaction between plants and insects, as well as the way the students perceive the interdependence of life forms and establish coevolution parameters. The teacher can use the story as a motivation to address the programmed content on ecology, zoology, and botany, and to discuss the importance of the scientific method, presenting the scientific research toward a dynamic and attractive learning. Our para-didactic text can enrich the science teaching practice by presenting a plant-insect interaction which results in gall development. We observed the emergence of new questions, teaching methodologies, and didactic resources capable of amplifying the scientific context, and consequently the motivation of the students.

Keywords: plant-insect interactions, para-didactic book; scientific spreading, socio-emotional skills

Introduction

The scientific community has been interested in promoting scientific communication from the academy toward the elementary and high schools (Terrazzan, 2000; Melo & Hosoume 2003). Different tools for communicating science discoveries have been created, e.g., videos, social media, podcasts, and texts with accessible language. These tools can stimulate the creation of teaching methodologies, by placing the scientific concepts in a broader context and consequently motivating students to think and propose new questions (Salém & Kawamura, 1996; Queiroz et al., 2012). In addition, the ability to communicate scientifically in its most diverse languages provides the students with access to a greater diversity of information; and aids the development of reading skills and the mastery of concepts, which in turn enable new forms of argument and elements of scientific terminology (Martins, Cassab & Rocha, 2001).

Among the variety of tools to present the plant-insect interaction which culminates in gall development and to mediate the teaching methodology, our choice was the production of a para-didactic book using part of the doctoral project of Nina de Castro Jorge entitled

"Stem galls in *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae): anatomical changes and ecophysiological implications" developed in the *Universidade Federal de Minas Gerais*, Brazil. The book is entitled "The curious case of ants that live in galls" (ISBN 978-65-00-13341-7), and has versions in English, Portuguese, and Spanish, all of which are available in the Neotropical Gall Group web site (www.neotropicalgallgroup.com). It is directed to 8-12 years old children, and it is suggested for science and literature classes.

The use of the book was tested with students of the 6th grade (11-12 years old students) of *SEB Unimaster* elementary school, located in Belo Horizonte city, Minas Gerais state – Brazil. The activity was monitored by researchers of the Plant Anatomy Laboratory of the *Universidade Federal de Minas Gerais* (UFMG), and by the school teachers of literature and sciences. Our dynamics focused on presenting concepts of botany and ecology by means of an interdisciplinary plant-insect interaction topic. We aimed to evaluate how the students received the concepts by registering: (a) the students' ability to go beyond the text basic concepts, and explore the construction of narrative, intertextuality, description of characters, identification of implicit and explicit information, title-text relationship, and plot structure, aiming at the possibility of re-textualization; (b) the curiosities arising from the students that can serve as basis for other strategies of scientific divulgation and activities programmed by the teachers; and (c) the concepts that have been learned or that can be explored by the teachers. All the steps focus on the constructing of knowledge supported by the scientific method (Fig. 1).

The use of the para-didactic book in the classroom was divided into four steps, namely: (1) reading and analyzing the text in the literature classes; (2) construction by students of comic books about the story; (3) discussion of the story in science classes paralleling with the topics presented in the textbook; and (4) construction of science projects exploring galls as models of study.

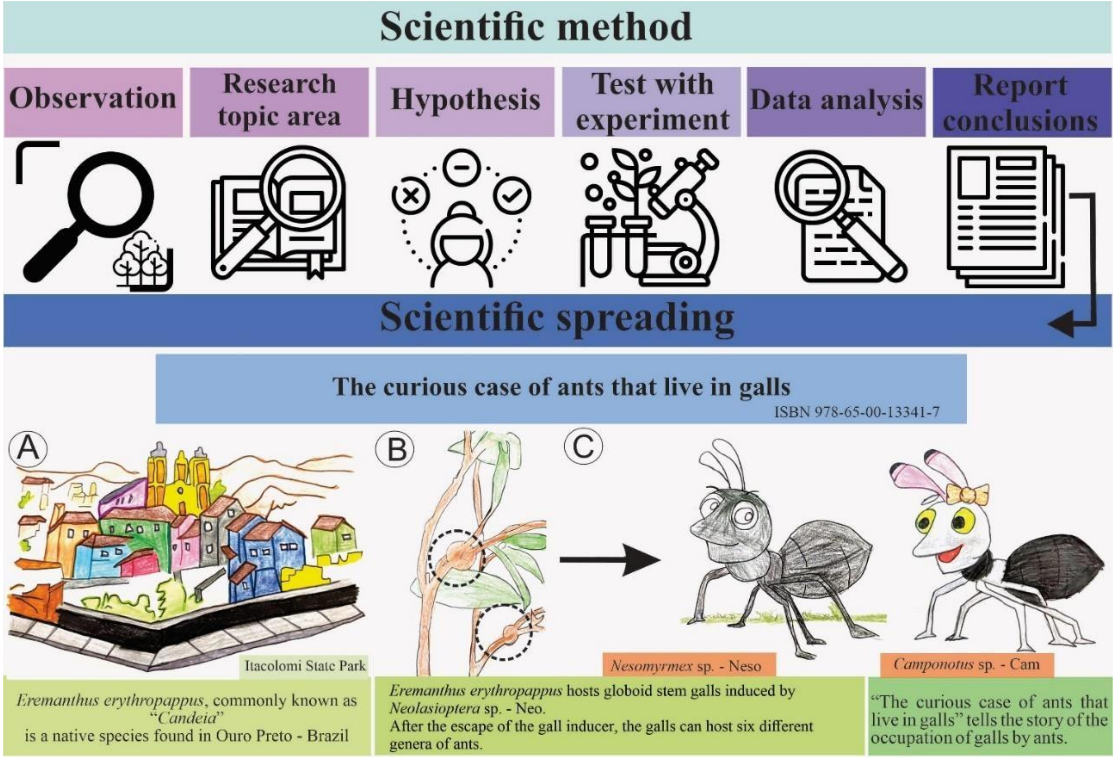


Figure 1. Graphical abstract. Scientific method theory and practice. (A-C) illustrations from the pra-didatic book "The curious case of ants that live in galls" (ISBN 978-65-00-13341-7). (A) Representation of the city of Ouro Preto, Minas Gerais states – Brazil. (B) Representation of the *Eremanthus erythropappus* globoid stem galls. (C) Representation of the main character of the book is "Neso", an ant of the genus *Nesomyrmex* and the secondary characters, "Cam", a *Camponotus* another genus of ant. Image: Flaticon.com'. This cover has been designed using resources from Flaticon.com.

How the book was created

The field experiments of the doctoral project "Stem galls in *Eremanthus erythropappus* (DC.) McLeisch (Asteraceae): anatomical changes and ecophysiological implications" were performed in the *Itacolomi* State Park - Ouro Preto, Minas Gerais – Brazil (Fig. 1A) and focus on *E. erythropappus* globoid stem galls (Fig. 1B). Galls can be defined as structures derived from the abnormal development of host plant tissues (Shorthouse, Wool, & Raman, 2005; Raman 2007). They result from the interaction between galling organisms and their host plants and demand a highly complex and intimate interaction between the associated individuals (Shorthouse, Wool, & Raman, 2005; Raman 2007).

Eremanthus erythropappus is a super host of galling herbivores, with six associated gall morphotypes: two induced in the apical buds, one in the leaves, and three in the stems (Carneiro et al., 2009). One of these stem galls is induced by *Neolasioptera* sp. (Diptera: Cecidomyiidae) (Prado & Vieira, 1999). After gall senescence, characterized by the escape of the adult *Neolasioptera* sp. from the larval chamber, we can observe two conditions: (1) empty-galls, and (2) ant-occupied galls, when the gall chamber is inhabited by ants and fungi (Jorge et al., 2022).

The para-didactic book “The curious case of ants that live in galls” tells in a ludic way the story of how the ants start living inside the galls. The main character of the book is “Neso”, an ant of the genus *Nesomyrmex* (Fig. 1C), who is the first ant to discover the gall. As secondary characters, the book presents “Cam”, a *Camponotus* (Fig. 1C), another genus of ant that helps Neso to discover what galls are; and “Neo”, the *Neolasioptera*, that is the galling insect that tells the ants the natural history of the galls. The book also brings the fungus as Cam’s partner in the occupation of the galls.

The para-didactic book was constructed with a free indirect narrative, in which the narrator is omniscient. The narrator increases what the characters are thinking and feeling in the story, which proves decisive for the understanding of the plot, and highlights the importance of curiosity to guide the scientific discoveries. Here are highlighted excerpts from the book that illustrates the role of the narrator in presenting the feelings and decisions of the characters (Jorge & Isaias, 2020):

> “Neso, who is very curious, decided to stroll around to look for more of those strange balls.”
>
> “Neso did not expect the answer and was paralyzed, when he heard a voice saying: (…).”
>
> “Neso was astonished, and speechless. He did not know what to say, indeed. Where was that voice coming from? And why did it think he would attack?”

The psychological features of the characters, such as the curiosity and fear of Neso, and the cooperation among Neso, Neo, and Cam were fundamental for the development of the plot and for the understanding of the students on the importance of the description of the characters.

Classroom methods

The book "The curious case of ants that live in galls" was presented, analyzed, and evaluated in four (4) steps.

Literature classes

Step 1 – Reading and analyzing the text

The students individually read the book within a week, which was followed by a literary discussion about the structural and discursive aspects of the text in classroom. The aspects that the guided the discussion were: the narrative, the construction of the characters (anthropomorphizing process and its effects in meaning), the structure of the plot, the purpose of the work, the vocabulary and the implications of its choice, the association between verbal and visual resources, the inference of explicit elements in the book, the title-text relationship, and aspects of global reading. In addition, the discursive intentionality behind the linguistic-discursive resources was discussed.

Step 2 - Multisemiotic narratives

After the discussion in classroom, the students did an exercise of re-textualization and intertextuality, aiming to the reflection, creation of questions, and personal positioning based on their reading and reflections. Then, the students were invited to produce multisemiotic narratives in comic stories using the free web site "storytelling", whose structure combines verbal and imagery resources. In the narratives, the students recreated the original story from different narrative focuses, choosing one of the characters, and recounting the plot based on her/his character's point of view.

Science classes

Step 3 – Analyzing scientific nomenclature and ecological concepts

After analyzing the para-didactic book in the literature classes, the students were invited to talk about the scientific nomenclature, ecological relationships in nature, and the interdependence of animals and plants. The relationship between curiosity and application of the scientific method toward the construction of knowledge was addressed with the use of a science textbook (*AZ. Ensino Fundamental, Anos Finais. Ciência da Natureza e Humana*, ISBN: 978-65-5880-047-7). This science textbook divided the scientific method in six steps: (1) observation/question; (2) research topic area; (3) hypothesis; (4) test with experiment; (5) data analysis, and (6) report conclusion. After the discussion of the steps in science classes and associating them with the story in the para-didactic book, the students were invited to write science projects (Fig 1).

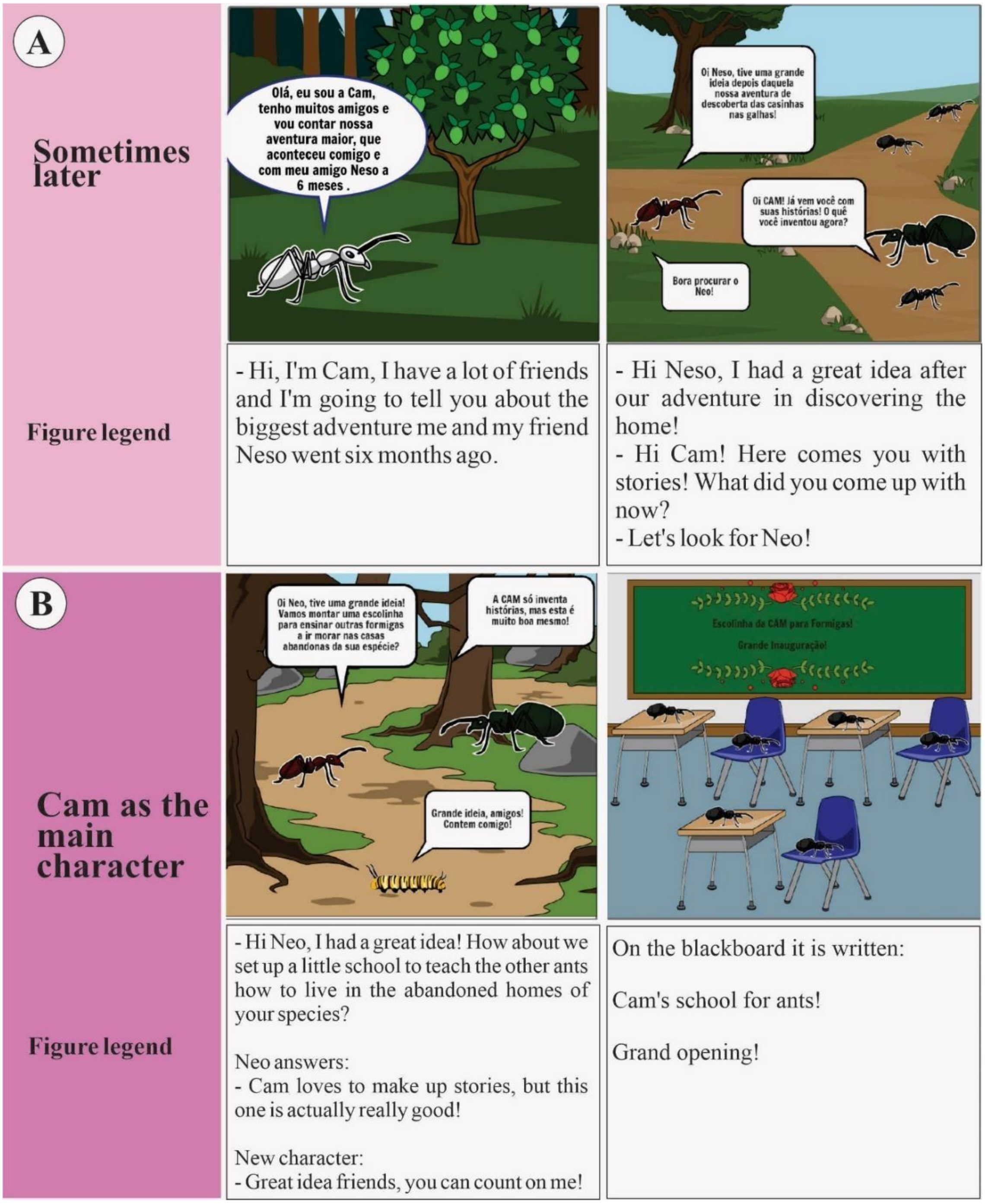
Step 4 – Science Projects

After reading and analyzing the story of the para-didactic book, each student proposed a research project that involved the study of galls using the scientific method steps learned during the science classes. In their projects, the students had to describe the stages of the scientific method and associate them with the story in the para-didactic book

Results and discussion

Multisemiotic narratives

The recreation of “Neso” story in a comic book dealt with a time later than the time narrated in “The curious case the ants that live in galls” para-didactic book (Fig. 2A). Within the school context, the production of the comic stories allowed the process of re-textualization by the students. By the transformation of the original text genre into the comic book, learning of language was more active and consistent (Marcuschi, 2001; Dell’Isola, 2007; Dikson,, 2017). Some students chose the secondary character "Cam" as the main character, sometimes assuming the role of a teacher and inviting other ants to live inside the galls (Fig. 2B). Such reconstructions of the main story emphasized the students’ ability to assign new roles to the characters in the para-didactic book, which encouraged them to capture key features and structures of the original text into those of the new content, as proposed by Marcuschi (2004) and Dikson (2017).



- Hi, I'm Cam, I have a lot of friends and I'm going to tell you about the biggest adventure me and my friend Neso went six months ago.

- Hi Neso, I had a great idea after our adventure in discovering the home!
- Hi Cam! Here comes you with stories! What did you come up with now?
- Let's look for Neo!

- Hi Neo, I had a great idea! How about we set up a little school to teach the other ants how to live in the abandoned homes of your species?

Neo answers:
- Cam loves to make up stories, but this one is actually really good!

New character:
- Great idea friends, you can count on me!

On the blackboard it is written:

Cam's school for ants!

Grand opening!

Figure 2. Re-textualization in comic book type. (A) The recreation by the students of “The curious case the ants that live in galls” (ISBN 978-65-00-13341-7) para-didactic book in a time later than the time narrated. (B) The recreation by the students of “The curious case the ants that live in galls” (ISBN 978-65-00-13341-7) para-didactic book, with the secondary character "Cam" as the main character. The comic book was written in Portuguese, the native language of the students. In the boxes under each image there is a translation.

The re-textualization demands the understanding of the original text and the observation of its main topics (Dikson, 2017). The production of the comic stories was based on the consolidated knowledge, respected the text narrative, and used the meaning that the narrator attributes to the characters. Most students continued to represent “Neso” as a fearful ant and "Cam" as a brave and intelligent ant capable of convincing all other ants to live inside the galls (Fig. 3A). This perception came from the meaning effects that the narrator's voice attributed to the characters. The more in-depth the debate on text formatting, the better the learning to read and write, increasing considerably the issues related to critical reflection, reading comprehension, and the ability to write texts. Therefore, the re-textualization appears as the most relevant axis when it comes to teaching and learning reading and writing (Dikson, 2017).

This activity demonstrated that the students understood the story of the base-text and managed to produce other texts with a narrative character, through invented stories, based on the para-didactic book. In addition to the understanding of the base text, the students used numerous other strategies in their productions, such as additions, substitutions, and insertions in the story. Then, the activity was not restricted to understanding the base text, but also to redoing it in a different type of genre, adding new features whilst maintaining the original ideas (Fig. 3B).

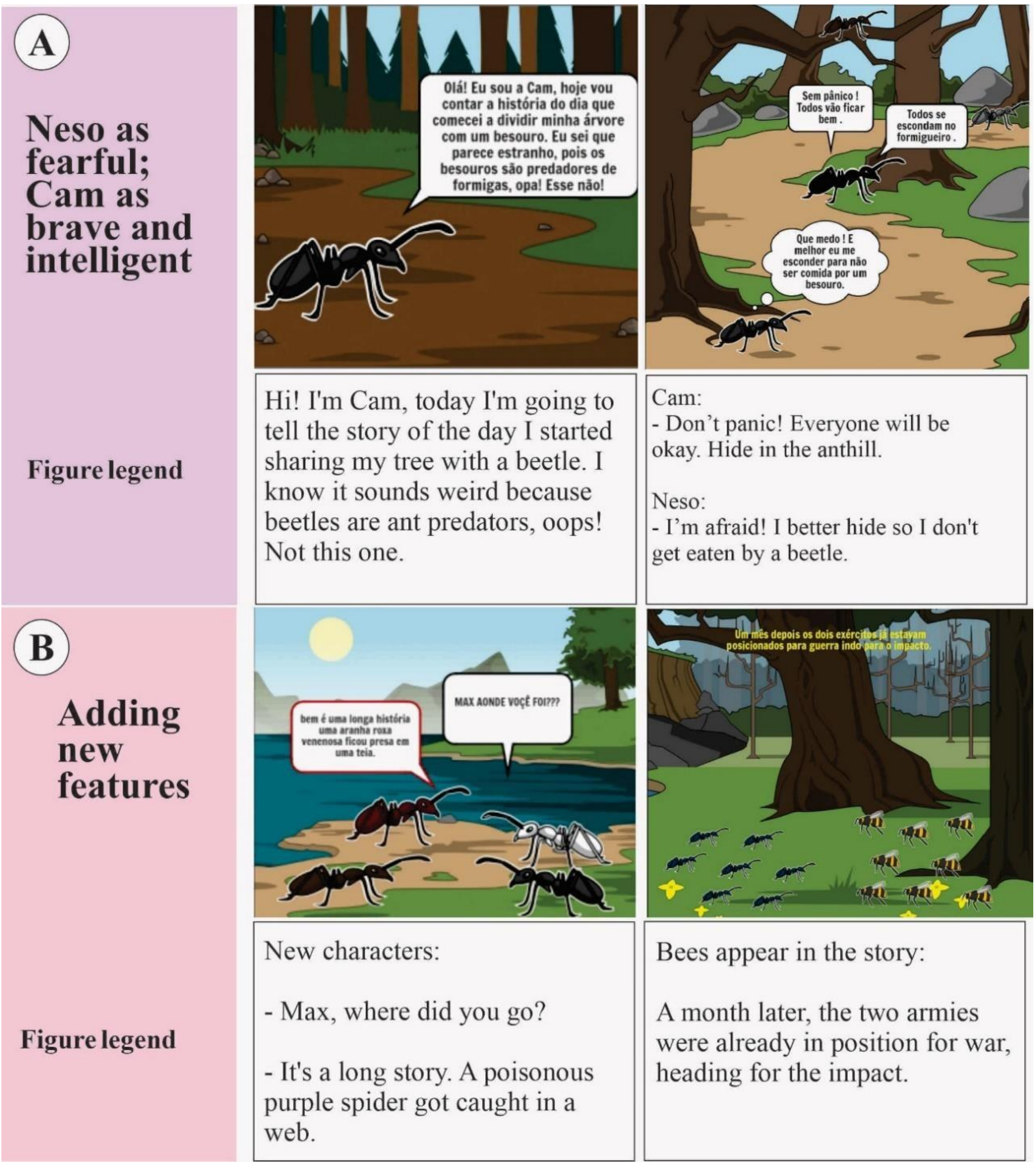


Figure 3. Re-textualization in comic book type. (A) The recreation by the students of "The curious case the ants that live in galls" (ISBN 978-65-00-13341-7) para-didactic book, with maintenance of characters representation described in the book. (B) The recreation by the students of "The curious case the ants that live in galls" (ISBN 978-65-00-13341-7) para-didactic book, with new features and characters created by the students. The comic book was written in Portuguese, the native language of the students. In the boxes under each image there is a translation.

Science Project

After the literature activities, the students were invited to write a project focused on the study of galls exploring the scientific method. In most of the projects, the first characteristic necessary for scientific research was the curiosity; a characteristic of Neso, the main ant character of the para-didactic book, who was curious and could discover what galls were. The students concluded that all scientists must be curious to perform new discoveries. Some of the reports of the students indicate such perception:

> "I realized that scientists do what Neso did: observation, questions, and experiments."
>
> "Yes, I am very curious just like Neso. I, as a scientist, would ask questions to find out who live there, examine the plant, do everything possible to find out what the galls were, who was in the galls, and how they were."

Another important aspect of being a scientist highlighted by the students was the use of the scientific method. For them, "Neso", the ant, used the steps of the scientific method when he observed a new phenomenon, proposed hypotheses about this phenomenon, and discussed it with close partners (Fig. 4). Along the discussion, the idea of the use of the scientific method is perceived:

> "When I finished my schoolwork, I was very happy to have understood the purpose of the science class, when we discussed the stages of the scientific method. I could apply the content and my curiosity, to discover something new."
>
> "Like Neso, I would apply the steps of the scientific method to find out what was inside the gall."

> “Comparing with Neso's story, I looked for support from those who know science to organize my ideas. So, I made use of the information from those who knew the place where the plant lived.”

> “In order to carry out a research methodology, it is very important that all stages of the process are respected and that the observations are performed very carefully, in order to achieve good results.”

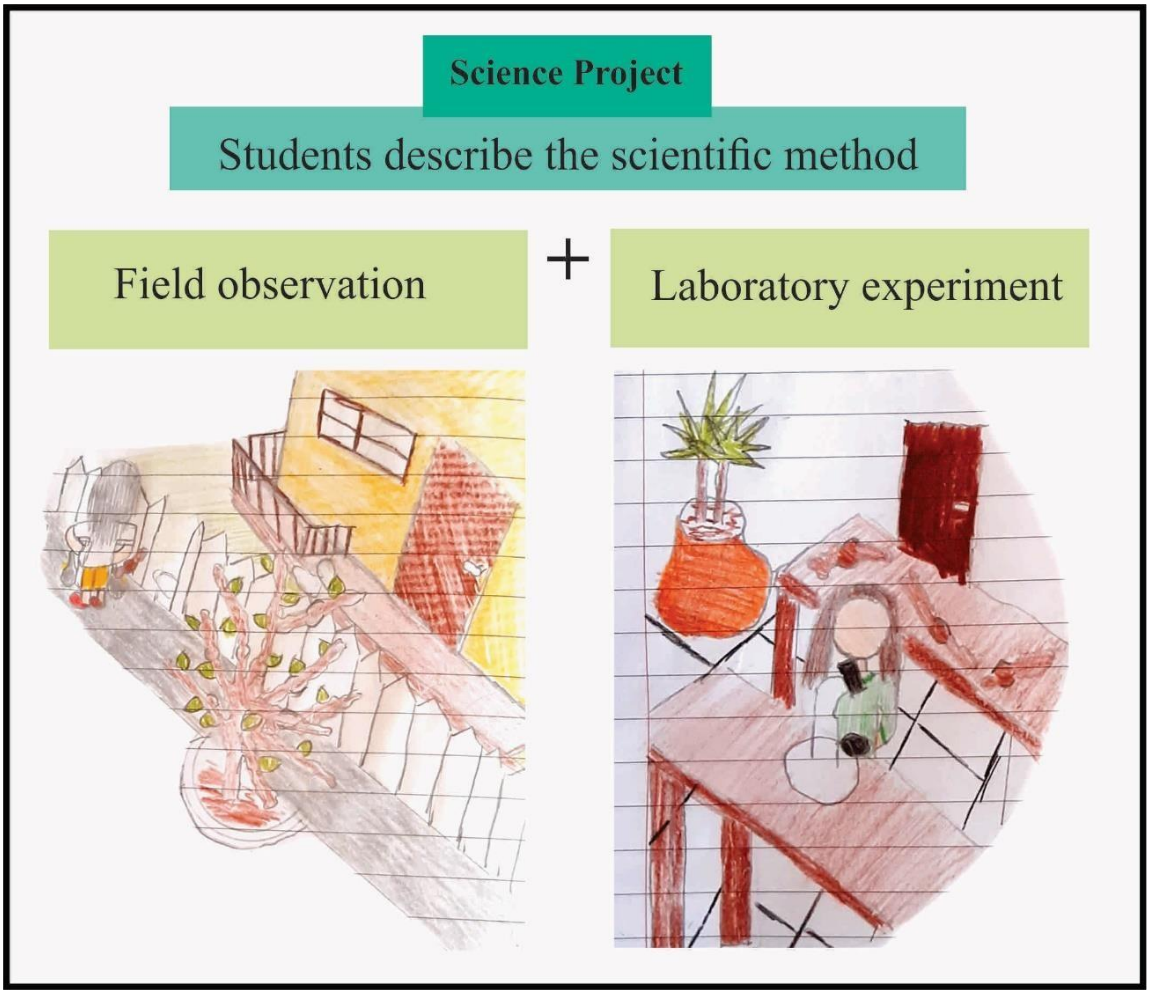


Figure 4. Scientific method draws by student. It is possible to observe the representation of steps of scientific method draw by students.

During the reading process, the students were able to recognize the stages of a scientific research inserted in the para-didactic book, as well as apply it in the classroom. According to them, being curious, making use of the scientific method, and establishing dialogue with people close to them are the most important tools for a scientific discovery. Something that is noteworthy is that through the spontaneous speeches of the students, it was possible to perceive how the work provided, indirectly, the development of socio-emotional skills (*cf*. IAS, 2020). In the sentences presented above the students' enthusiasm can be noted. It is a socio-emotional skill, linked to the engagement macro-competence, a flexible student characteristic, which has been viewed as students’ active commitment to learning activities (Eccles & Wang, 2012; Reeve, 2012), and means that the student is willing to be actively involved in their learning. Learning motivation, as expressed in the form of engagement, is higher the more the students enjoy learning (Ainley & Ainley, 2011) and this enjoyment supports students in the achievement of better academic results (Singh, Granville, & Dika 2000). However, good results are not the only aims of engaged learning, the learning motivation and engagement developed in classroom are carried over to activities at home or in hobbies and can motivate the study of science (Newton & Newton, 2011; Osborne, Simon, & Collins, 2003).

The main characteristic described by the students necessary to be a scientist, after reading the book, is the curiosity, an innate quality of many animal species, including the *Homo sapiens* (Tostes, 2006), which can be a significant motivating factor in student learning (Borowske, 2005). The students created new questions about the new system, such as: (1) do all plants have galls? And (2) what are the changes that take place in the plants to give rise to galls? In addition, the students could discuss ecological concepts, such as parasitism and protocooperation – which, in Brazil, are usually developed in high school classes – in a ludic and interdisciplinary way. In this sense, our para-didatic book does not present a simple transmission of ready and finished scientific knowledge. It is a tool for a ludic way to understand the paths taken by science in the processes of producing knowledge and all the issues involved in scientific practice (Mendonça, 2010). Thus, our para-didatic book tells part of a research project using informal language, which brings students closer to science.

The sharing of scientific contents that are not easily visible for non-academic people, in this case, the galls and the plant-insect interactions, promotes and expands scientific dissemination, a key factor for the development of student criticality, which, therefore, establishes solid parameters for respecting scientific development and knowledge. There are several types of scientific dissemination, and all these types have the same objectives, which are to educate, inform, and captivate the public with scientific discoveries. These common goals must be considered for the preparation and use of scientific dissemination materials (Tostes, 2006). For the construction of the para-didactic book presented here, the authors used methods that aimed to anthropomorphize the ants and make the galling insect tells his own story. The choice of these methods aimed to captivate children with the new discoveries that were being made by the ants, in addition to bringing them closer to small insects that are little perceived as interesting animals for urban children.

Scientific spreading is, in this sense, an efficient way to disseminate knowledge about science and technology. Moreover, critical reading, followed by the literary discussion here reported, promotes reflection, transformation, and action (Cervetti, Pardales & Damico 2001). We have shown how the students were able to use the content learned in classroom, in a new approach, as well as to retell and add to the story. The reports given by the students evidenced their ability to abstract from the text basic concepts and perform the construction of narrative, intertextuality, description of characters, identification of implicit and explicit information and scientific contents, such as scientific names and scientific methods.

Conclusion

The para-didactic book “The curios case of ants that live in galls” contributed to enrich teaching practices by presenting plant-insect interactions that are not easily perceived for the common people. Stimulated by the reading experience and the teachers, the students created new questions, learned concepts on botany, zoology and ecology in a ludic and interdisciplinary way. The activities opened the students’s and professor's perspective about science and the natural environment. The use of a para-didactic text supported teaching methodologies as a didactic resource toward introducing the scientific method in elementary classes, and consequently promoting the engagement of the students.

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CONSIDERAÇÃO FINAIS

A indução das galhas caulinares em *Eremanthus erythropappus* por *Neolasioptera* sp. promove o desenvolvimento de tecidos vasculares neoformados em resposta às necessidades crescentes da galha em desenvolvimento. No entanto, as células neodiferenciadas seguem um padrão diferente: há um aumento na proporção das células do parênquima em detrimento dos elementos de vasos e células lignificadas no xilema secundário, além de alterações no número e espessura dos elementos de vasos abaixo das galhas. Estas alterações citométricas nos elementos de vasos e a predominância de células parenquimáticas nas galhas globoides caulinares resultam do desequilíbrio de citocininas e auxinas que culminam em efeitos diretos na atividade do câmbio vascular. Ainda, em se tratando das alterações promovidas pela indução da galha, foi observada a síntese e acúmulo de novos compostos químicos, tais como dois triterpenos (ɤ-taraxasterol e β-amyrone) e um alqueno (tricosane), e a inibição da síntese de compostos anteriormente relacionados ao caule de *E. erythropappus*, como por exemplo dois triterpenos (A-neooleana-3(5),12-diene e lupenone) e uma quetona (2(1H) naphthalenone). As propriedades fitotóxicas originais do ramo da planta hospedeira e a neossíntese de compostos após a indução da galha podem ser direcionadas de forma indireta para a defesa do inseto galhador.

A comparação das galhas vazias com as ocupadas por formigas revelou que as hifas dos fungos em contato com células nutritivas remanescentes desencadeiam o estímulo para a superprodução de xilema secundário não lignificado, mesmo após o processo de senescência ter sido iniciado. Nesse sentido, a ativação de um felogênio ectópico em direção a câmara larval proporciona um micro-habitat adequado para as colônias de formigas. Essa curiosa relação entre as formigas e as galhas senescentes gerou um livro paradidático “O curioso caso de formigas que vivem em galhas” que contribuiu para enriquecer as práticas de ensino, colocando os alunos frente ao método científico, e a interações planta-inseto que não são comumente reportadas nos livros didáticos.

O sistema *E. erythroappus-Neolasioptera* sp. e, posteriormente, formigas arbóreas e fungos se mostrou um interessante micro-labotatório de estudos do ponto de vista anatômico e ecofisiológico. As alterações anatômicas observadas revelam o potencial de respostas do câmbio vascular e do felogênio como sendo responsáveis pelos atributos anatômicos típicos do sistema *E. erythropappus-Neolasioptera* sp.. As respostas anatômicas diagnosticadas ao longo do desenvolvimento das galhas e até mesmo em estágio de pós-senescência mostraram-se altamente complexas, uma vez que, mesmo após a saída do galhador o câmbio respondeu aos estímulos mecânicos da entrada e saída das formigas e ao estímulo químico gerado pelos fungos. O estágio de pós-senescência, aqui caracterizado de modo inédito, denota que a relação ecológica das galhas nos ambientes que fazem parte merece mais atenção.

O estudo desse sistema, ainda, se mostrou interessante pois apresentou respostas diferentes das descritas anteriormente para galhas caulinares, por exemplo, não confirmando a hipótese de constrição vascular. As galhas nos neotrópicos comumente apresentam padrões de desenvolvimento que divergem do que foi descrito anteriormente para galhas em regiões temperadas, demonstrando que o conhecimento da anatomia e fisiologia de galhas neotropicais pode apontar lacunas do conhecimento no que concerne às vias de desenvolvimento e adaptabilidade do hábito galhador. Entender as alterações que acontecem para o desenvolvimento das galhas é crucial para passos posteriores que envolvem, por exemplo, biologia molecular.

A anatomia vegetal, descrita como ciência básica e, por vezes “deixada de lado” na “ciência moderna” é o berço de estudo para o entendimento dos sistemas planta hospedeira-galhador, das alterações celulares e do próprio desenvolvimento da galha. Ainda que se saibam quais genes estão sendo expressos e como eles modulam as respostas das galhas, ainda precisaremos saber como se dá a expressão a níveis de células e tecidos e, para isso, somente a anatomia vegetal poderá trazer a luz.

Nesta tese, descrevemos um sistema complexo de galhas caulinares, e trazemos diversos aspectos de seu desenvolvimento, partindo de um aspecto micro, descrevendo células e tecidos para aspectos macro, mostrando relações ecológicas envolvidas no sistema. O estudo de galhas, como já dito, é um micro-laboratório com diversas potencialidades e possibilidades, algumas das quais foram aqui exploradas. O sistema *E. erythropappus*-*Neolasioptera* sp. ainda guarda um arsenal de perguntas e respostas que podem se apresentar à olhares atentos. Para tanto, é necessário sim, experimentar novas técnicas e explorar outras áreas do conhecimento, mas sem nunca esquecer das ferramentas “básicas” que construíram – e constroem – o conhecimento da cecidologia até o momento.

Anexos

O CURIOSO CASO DE FORMIGAS QUE MORAM EM GALHAS

NINA DE CASTRO JORGE
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Jorge, Nina de Castro
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DEDICATÓRIA

Dedico a Alice que me lembra constantemente da curiosidade infantil do fazer ciência.

APRESENTAÇÃO

Este livro é um fragmento da tese de doutorado de Nina de Castro Jorge, orientada pela professora doutora Rosy Mary dos Santos Isaias, no Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Minas Gerais, intitulado Galhas lenhosas em *Eremanthus erythropappus* **(DC.) McLeisch: alterações anatômicas e implicações ecofisiológicas .**

Há uma formiguinha chamada *Nesomyrmex*, Neso para os íntimos.

Neso e sua família vivem lá no Parque do
Itacolimi na cidade de Ouro Preto
em Minas Gerais.

Eles estão sempre passeando pelo Candeial
perto da Capela, buscando alimentos e um
lugar seguro para morar.

Neso adora o Candeial, ele acha as árvores
de Candeia muito bonitas com suas flores em
bolinhas brancas,
e quando elas estão todas juntas formando um
Candeial é um show à parte.

Um dia, Neso que é bem corajoso, resolveu subir em uma das árvores de Candeia.

Ele ouviu falar que lá em cima havia um líquido docinho que sai da planta, chamado néctar,
e que muitas formigas gostam.
E lá foi ele, atrás do tão sonhado néctar.

Mas Neso não estava preparado para o que encontraria e sua surpresa foi grande quando em um dos galhos da árvore ele se deparou com

uma bolinha que parecia ser parte da planta, mas que ele nunca tinha visto antes.

Ele ficou sem entender o que era aquilo.
Será que a planta estava doente?
Será que ele tinha descoberto algo novo?
O que seria aquilo?

Neso, que é muito curioso, resolveu percorrer outros ramos e ver se encontrava mais daquelas estranhas bolinhas.

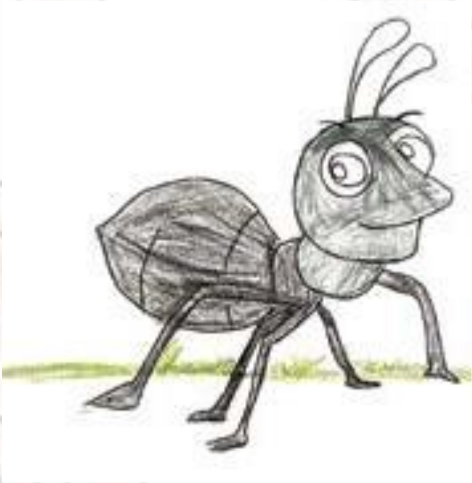
E ele não pôde crer no que via.
Quanto mais andava pela planta, mais bolinhas ele encontrava.

Ele percebeu que elas são diferentes entre si. Umas são ainda pequenas e macias, outras, por sua vez, eram maiores e bem mais duras.

Neso percebeu que de dentro das menorzinhas vinha um barulho, parece que alguém está morando lá dentro.

Ele resolveu bater com suas patinhas
em cima da bolinha e disse:
- Ei, eu consigo te ouvir. Quem é você?
Como você foi parar aí dentro?

Neso não esperava uma resposta e por isso ele ficou paralisado quando de lá de dentro veio uma voz:
- Olá. Eu sou o *Neolasiopitera*. E você pode ir embora. Não conseguirá me alcançar aqui dentro. Estou protegido.



Neso ficou assustado. Não sabia o que dizer. De onde vinha aquela voz? E por que ela achava que ele iria atacá-lo?
Neso preferiu descer da árvore e chamar sua amiga *Camponotus*, para essa aventura.
Cam era muito sábia e Neso sabia que ela teria uma resposta para essa loucura toda.

Neso e Cam então voltaram para a árvore e foram novamente conversar com o morador da bolinha estranha. Cam começou:

-Olá senhor Neolasiooo, não sei direito.
Posso te chamar de Neo? Eu e meu amigo estamos muito curiosos sobre a sua casa.

Nós nunca tínhamos visto nada assim.
O senhor nos ajudaria a entender como que o senhor conseguiu fazer essa casinha na planta?

- Ora essa minha filha, vocês não são inimigos? Tem certeza de que não querem me tirar daqui?

- Claro que não senhor Neo. Somos formigas e estamos muito curiosas sobre a sua casa, queremos entender.

- Tudo bem então, eu conto para vocês. - disse.

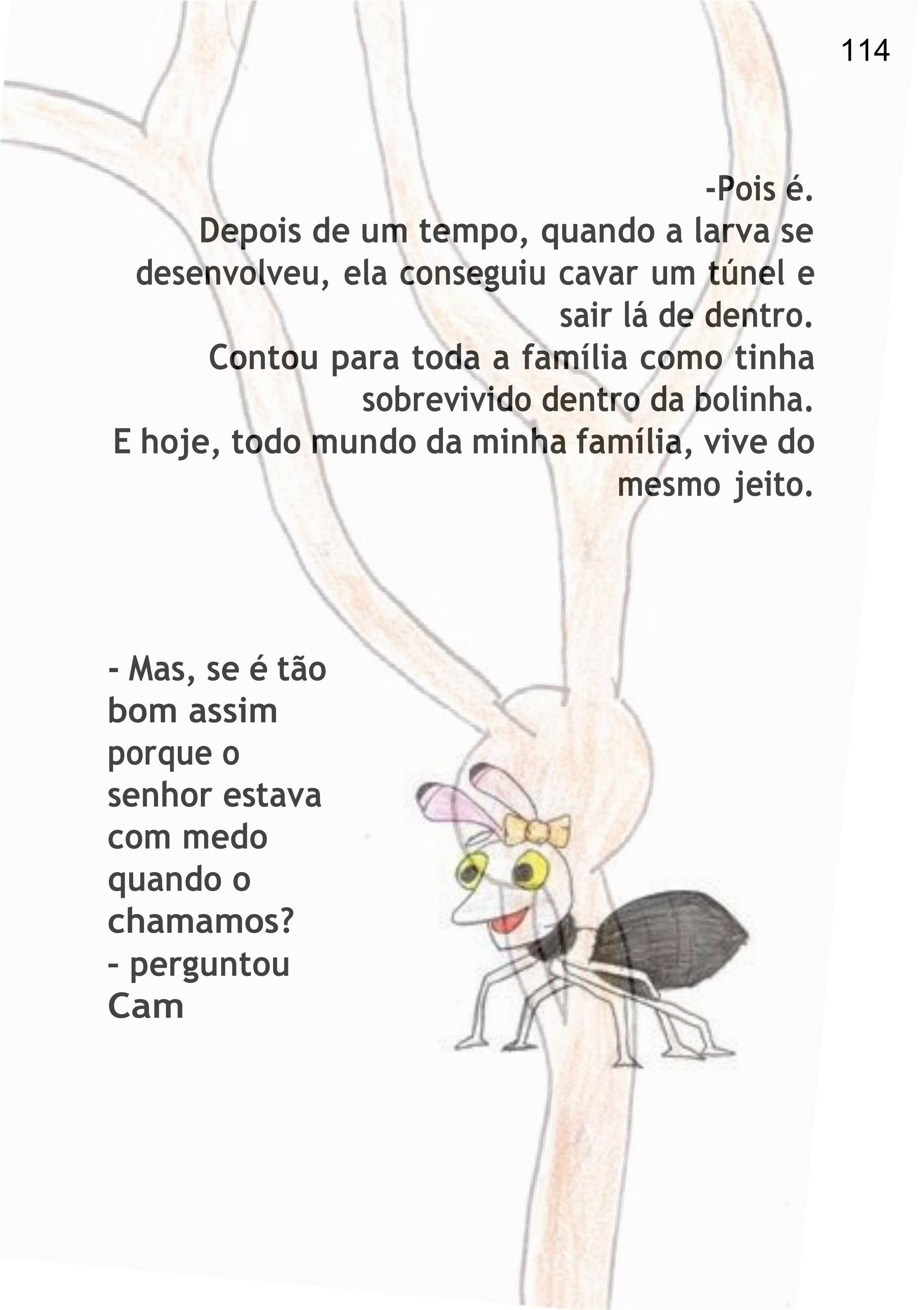
Essa história vem de muitos e
muitos anos...
Minhas trisavós, um belo dia,
resolveram colocar seus ovos em
cima dos galhos das plantas.
Elas achavam que os ovos
ficariam protegidos aqui no alto.

Elas só não esperavam que a planta não gostaria do ovo ali nos seus galhos e reagiria.
E vocês não imaginam como foi difícil a construção dessa bolinha.
A planta reagindo, minha trisavó contra atacando e dessa batalha de ataque e contra ataque se formou essa bolinha, onde hoje nós moramos.

-Óh! Que coisa terrível senhor Neo, uma batalha! O que aconteceu com os ovos das suas trisavós?

Essa é a parte mais legal da história. E acho que nem a planta esperava por isso. As larvas saíram dos ovos e conseguiram sobreviver lá dentro da bolinha e além disso, conseguiram se alimentar da própria planta.

- UAAAU, isso é incrível!



-Pois é.
Depois de um tempo, quando a larva se desenvolveu, ela conseguiu cavar um túnel e sair lá de dentro.
Contou para toda a família como tinha sobrevivido dentro da bolinha.
E hoje, todo mundo da minha família, vive do mesmo jeito.

- Mas, se é tão bom assim porque o senhor estava com medo quando o chamamos?
- perguntou Cam

Antigamente, quando a gente não vivia dentro das plantas, era muito difícil.

Não tinha comida, nós passávamos frio ou calor, e a chuva levava nossos ovos para longe e muitos morriam.
E o pior de tudo, era que sempre tinha uns bichos tentando nos comer.
Aqui dentro é tudo muito bom. Sempre tem comida, a temperatura é ótima e a gente pode crescer tranquilo.

É verdade que algumas vezes, alguns inimigos tentam nos alcançar aqui dentro.
Alguns até conseguem e eu estava com medo de que vocês fossem um deles

- Uau senhor Neo, sua história é muito interessante.
Como chama essa casa onde o senhor vive?

- Bom, a gente chama de casa mesmo, mas eu já escutei algumas pessoas curiosas que vem aqui e ficam tentando entender como eu vivo aqui dentro, chamarem as nossas casas de Galhas.

- Ah! Galha! Gostei, um nome legal.
E o que acontece com a galha quando o senhor sai?

- Infelizmente ela morre.
É a nossa presença aqui dentro que faz com que a galha continue viva e crescendo. Quando vamos embora a galha para de crescer e morre.

Cam, nesse momento, teve uma ideia brilhante. Ela estava cansada de viver passeando pelo Candeial e não ter uma casinha para chamar de sua.

E se pôs a pensar o que fazer para conseguir morar lá dentro da galha quando o senhor Neo saísse de lá.

E tinha que ser rápido.

Pelo tom sábio que o senhor Neo falava, ele certamente já estava quase pronto para sair.

Cam conhecia um senhor muito inteligente, que morava ali por perto nos ramos da Candeia, o senhor Fungo.
O senhor Fungo deveria saber como ela poderia morar dentro da galha.

O senhor Fungo ficou encantado com a ideia.
Um lugar protegido, com temperatura boa e comida a disposição? Parecia muito impressionante.

Quando o senhor Neo saiu de dentro da galha, o senhor Fungo foi até lá e achou que realmente que era um bom lugar para se viver.
Cam e o senhor Fungo combinaram de morar juntos dentro da galha.

Com o tempo, Cam fez da galha, além de sua casa, um ninho. E em pouco tempo, Cam, operárias, larvas e ovos, que em breve virariam novas formigas, começaram a habitar lá dentro.

Da mesma maneira que a presença do senhor Neo estimulava a galha a continuar viva, o que contribuía para a sobrevivência dele lá dentro, a presença do senhor Fungo e da família de Cam fazia o mesmo papel.

E desse jeito, mesmo depois que o senhor Neo saiu a galha ficou viva.
Ao contrário do que acontecia quando o senhor Neo saía e não havia ninguém para aproveitar a casinha.

Neso, que não teve coragem de morar na
galha inicialmente,
contou esta novidade para várias famílias
de formigas.

IMAGEM REAL DA GALHA OCUPADA POR FUNGOS E FORMIGAS

Essas famílias passearam pelo Candeial para ver essa novidade e encontraram suas próprias casinhas. Desde então se você olha com cuidado as galhas com buraquinhos nas árvores de Candeia, você pode encontrar formigas e fungos lá dentro, vivendo bem e protegidos dos perigos do lado de fora.

Curiosidades Científicas:

Você sabia que todos os seres vivos têm um nome que chamamos de nome científico? Estes nomes são dados por pessoas curiosas, os cientistas, que estudam, descrevem e nomeiam cada um dos seres vivos.

Os personagens da nossa história são reais e também têm um nome científico, vamos descobrir?

Neso no meio científico é uma formiga chamada *Nesomyrmex* e Cam entre os estudiosos é uma formiga chamada *Camponotus*.

O sábio senhor Neo, foi descrito pelos cientistas como *Neolasioptera*.

E a Candeia que é a árvore onde toda essa história se passa é chamada de *Eremanthus erythropappus*.

THE CURIOUS CASE OF ANTS THAT LIVE IN GALLS

NINA DE CASTRO JORGE
ROSY MARY DOS SANTOS ISAIAS

Translate into english:
Rosy Mary dos Santos Isaias

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(Câmara Brasileira do Livro, SP, Brasil)

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DEDICATION

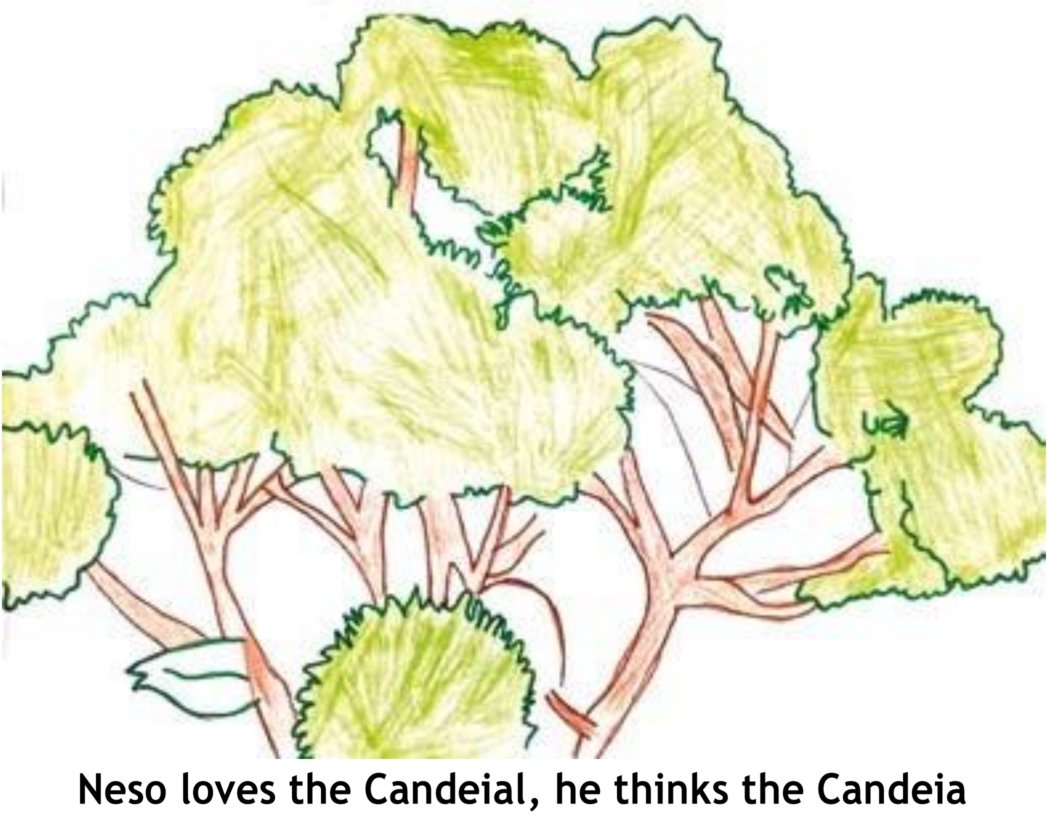
To Alice who brings out the
pure curiosity of making science.

PRESENTATION

This book is part of the PhD thesis of Nina de Castro Jorge, supervised by Professor Rosy Mary dos Santos Isaias, in the Graduation Program in Plant Biology of the Universidade Federal de Minas Gerais, entitled Woody galls in *Eremanthus erythropappus* **(DC.) McLeisch: anatomical alterations and ecophysiological implications.**

Once upon a time, there was an ant named *Nesomyrmex*, Neso for his close friends.

Neso and his family live at Itacolomi Park in Ouro Preto city in Minas Gerais state, Brazil. They are always walking around the Candeial next to the Capel, looking for food and a safe place to live.



**Neso loves the Candeial, he thinks the Candeia trees are very pretty with their flowers grouped as small white balls,
and many trees are all together forming the Candeial, is an spectacular show.**

One day, Neso, that is very corageous, decided to climb the candeia trees.

He has heard that there was a sweet juice, named nectar, coming from the plant up there, and that many ants like it. And there he goes, looking for the dreamy nectar.

But Neso was not prepared to what he would find there, and he was greatly surprised when in one of the branches, he found

a small ball that looked like part of the plant, but that he had never seen before

He became astonished, and did not understand what the small ball was. Was the plant sick?
May he have discovered something new?
What was that?

Neso, that is very curious, decided to stroll around to look for more of those strange balls.

And he could not believe in what he saw.
The more he walked, the more numerous the balls were.

He realized that there were different balls. Some were small and soft, while others were large and hard.

Neso also realized that there was a noise coming from inside the small balls, it looks like someone was living inside it.

He tapped with his legs on top of the ball, and called:
- Hey, I can hear you. Who are you?
How did you get inside this ball?

Neso did not expect the answer and got paralized when he heard a voice saying:
- Hi. I am the *Neolasiopitera*, you can get away from here. You cannot touch me, I am protected inside here.

Neso got astonished, and speechless. He did not know what to say, indeed. Where were that voice coming from? And why did it think he would attack?
Neso decided to go down the tree and call his friend Camponotus, to follow this adventure.
Cam was very smart and Neso know she should have an explanation for all that madness.

Neso and Cam came back to the tree to talk to the inhabitant of the bizarre ball. Cam began the conversation:

-Hi Mr I don t know… Neolasiooo. May I call you Neo? Me and my friend are curious about your house.

We have never seen something like this before.
May you help us understand how did you construct this plant-house?

- Well sweetie, aren't you enemies? May I be sure you are not trying to take me out of here?

- Of course not, Mr Neo. We are ants and are very curious about your home, we just want to understand.

- Okay, that's all right, I will tell you, he said.

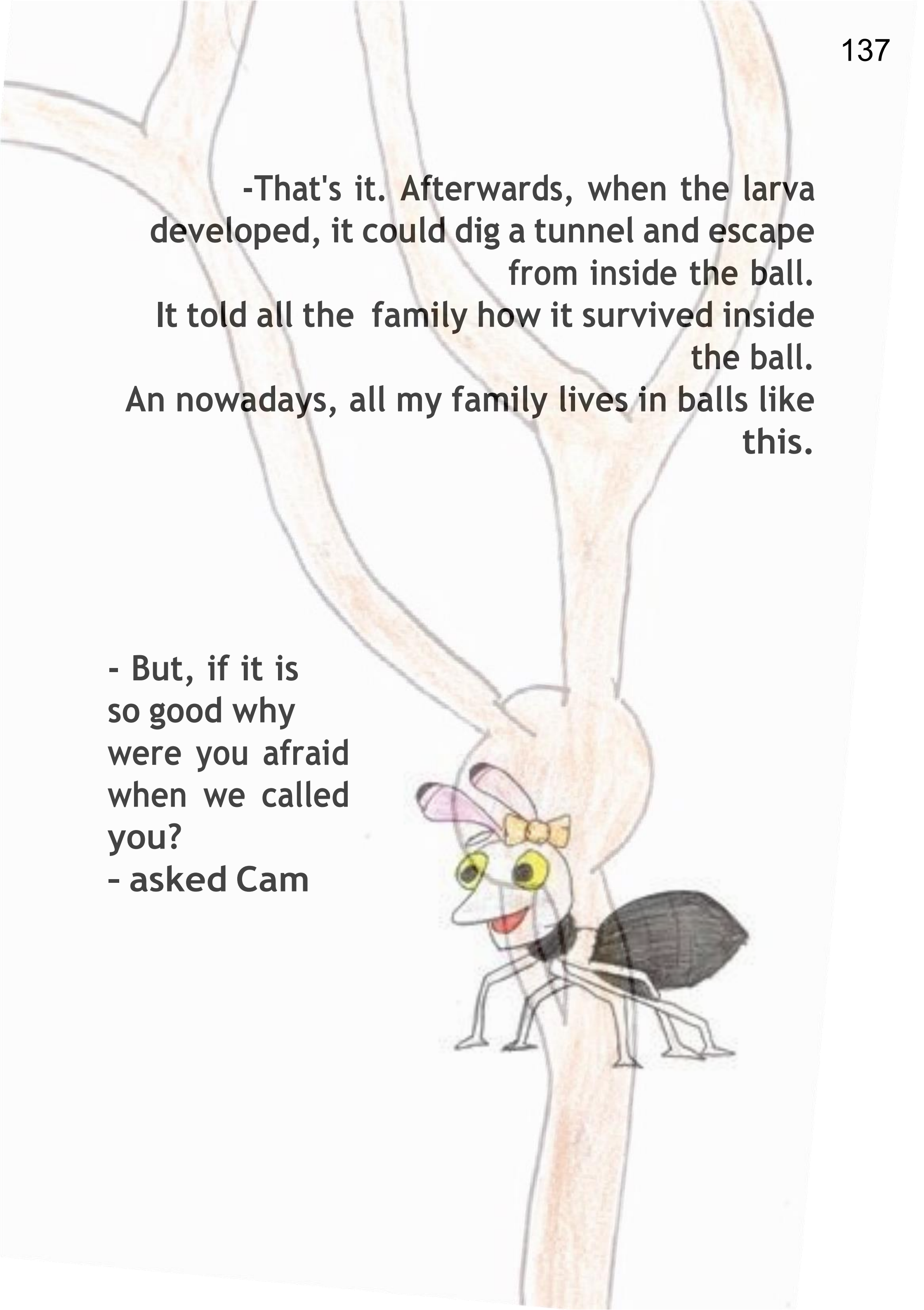
This history comes from a long long time ago...
One day, my great-grandparents, decided to put their eggs on some plant branches.
They thought the eggs would be protected up here.

They did not expect the plant to react to the eggs in its branches.
And you cannot imagine how hard it was to construct this ball.
The plant reacting, and my great-grandmother counter attacking, and from this battle comes a ball like this one, where I live today.

-Wow! What a terrible thing Mr Neo, a battle! What happened to the eggs of your great grandmother?

This is the cool part of our history.
And neither us nor the plant was waiting for that.
The larvae hatched from the eggs and could survive inside the balls, and more, they could feed from the plant cells.

- Wooow, sounds incredible!



-That's it. Afterwards, when the larva developed, it could dig a tunnel and escape from inside the ball.

It told all the family how it survived inside the ball.

An nowadays, all my family lives in balls like this.

- But, if it is so good why were you afraid when we called you?

- asked Cam

Well, in the old days, when we did not live inside the plants, life was very hard.

There was little food, we were cold or hot, the rain carried our eggs away, and lot of us died.
And what was worse, there were always some predators trying to eat us.
Inside the balls, everything is quite good. We have food, the temperature is warm and comfortable, and we can grow in peace.

It is true that sometimes, some enemies try to catch us here.
Sometimes they can do it, and I was afraid you were one of these enemies.

-Wow Mr Neo, yourhistory is very interesting.But tell me, does your house have a name?

- Well, we call it our home, but I have heard some curious people that come here and try to understand how we leave naming our homes as GALLS.

- OH! Gall! Loved it, it is a nice name.
But what happens with the gall when you leave it?

- Unfortunatelly, it dies. It is our presence inside it that makes the gall alive and growing. When we leave the gall, it stops growing and die.

Cam, at this moment, had a brilliant idea.
She was tired of walking around the Candeial
without a house to be her home.
And she started thinking what she could do to
live inside a gall when Mr Neo left it.

And she must be fast. For his smart tone of voice,
Mr Neo would certainly leave the gall soon.

Cam knew a very smart guy,
that leaved nearby in other
Candeia branches, Mr Fungo.
Mr Fungo should know what
she might do to live in the
gall.

Mr Fungo got enchanted with the idea.
A protected place, with warm climate and
available food? It sounds impressive.

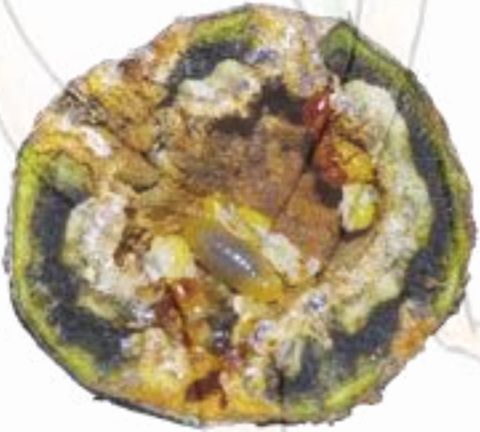
When Mr Neo left the gall,
Mr Fungo went there and
found it really a nice place to
leave in.
Cam and Mr Fungo decided
to live together inside the
gall.

As time went by, Cam turned the gall into her home, a real ant nest. And soon, Cam, the worker ants, larvae and eggs, from where new ants would soon be born, inhabited the gall.

Just like, wise Mr Neo stimulated the gall to be alive, which contributed to its own survivorship, the presence of Mr Fungo and of Cam family performed a similar role.

And then, even after Mr Neo left, the gall stayed alive. Differently from what happened when one of Mr Neo's parents went out and there was no one to leave in that house.

Neso, which was not brave enough to live inside the gall promptly, told the news to some ant families.



Real image of a gall occupied with fungi and ants.

These families strolled around the Candeial to see the novelty and found galls to be their houses. Since then, if you look carefully to the galls with a small hole in Candeia trees, you can find ants and fungi inside them, leaving protected from the enemies and dangers from the outside.

Scientifc curiosities:

Do you know that all living beings have a name, which are their scientific names? These names are created by curious people, the scientists, who study, describe, and name each one of the living beings.

The characters of our story are real and they have scientific names, as well. Let's find out?!

Neso is the scientific name of an ant named *Nesomyrmex* and Cam for the scientists is an ant named*Camponotus*.

The smart Mr Neo was described by the scientists as *Neolasioptera*.

And the Candeia, the tree where the whole story goes on is the *Eremanthus erythropappus*.