plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Anatomical and phenological implications of the relationship between *Schinus polygama* (Cav.) (Cabrera) and the galling insect *Calophya rubra* (Blanchard)

L. M. Guedes[1], N. Aguilera[2], B. G. Ferreira[3], J. Becerra[1], V. Hernández[1] & R. M. S. Isaias[3]

1 Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile

2 Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile

3 Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Pampulha, Belo Horizonte, Minas Gerais, Brazil

Keywords
Conical stem gall; diapause; life cycle; mediterranean region; univoltine.

Correspondence
R. M. S. Isaias, Departamento de Botânica, Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Av. Antônio Carlos, 6627, Pampulha, 31270-090, Belo Horizonte, Minas Gerais, Brazil
E-mail: rosy@icb.ufmg.br

Editor
K. Leiss

Received: 15 October 2017; Accepted: 12 January 2018

doi:10.1111/plb.12696

ABSTRACT

- The success of galling insects could be determined by synchronisation with host plant phenology and climate conditions, ensuring suitable oviposition sites for gall induction and food resources for their survival. The anatomical, histochemical and phenological synchronisation strategies between *Calophya rubra* (Blanchard) (Hemiptera: Psylloidea) and its host, the evergreen plant *Schinus polygama* (Cav.) (Cabrera) (Anacardiaceae), in the Mediterranean climate of southern Chile was evaluated and compared to that of the congeneric *C.* cf. *duvauae* (Scott) from Brazil and closely related host plant *S. engleri* in a subtropical climate.
- Anatomical, histometric, histochemical and vegetative phenology studies of the stem and galls were conducted from June 2015 to December 2016.
- Based on the anatomical, histometric and histochemical analysis, the conical stem gall traits imply gains over the non-galled stem toward the galling insect survival, but the maintenance of phellem, secretory ducts and pith indicate conservative developmental traits that cannot be manipulated by *C. rubra*. Our results indicate that the conditions of the Mediterranean climate zone limit *C. rubra* immature activity during unfavourable periods, probably determining a diapause period and a univoltine life cycle, which are peculiarities of the *S. polygama*– *C. rubra* system.
- The synchronisation between development and seasonality confers peculiarities to the *S. polygama*– *C. rubra* system in the Mediterranean climate zone.

INTRODUCTION

Galls are abnormal plant structures developed under the stimuli of a gall-inducing animal, fungus, bacterium or virus, which govern tissue neoformation (Mani 1964; Oliveira & Isaias 2010a). The specific plant tissues differentiated at a gall development site provide shelter, protection and nutrition to the gall-inducing organisms and their descendants (Shorthouse *et al.* 2005). The ability to induce galls on plants is a specialised habit within the broad context of herbivorous insects (Raman 2011). The relationship between host plants and their galling herbivores is highly specific (Abrahamson & Weiss 1997). This specificity is strongly linked to the choice of site, time of oviposition, feeding habits of the galling herbivore, local host plant abundance (Gonçalves-Alvim & Fernandes 2001) and the systems of recognition established with the plant surface traits (Eigenbrode & Jetter 2002).

In particular, synchronisation with host plant phenology is a critical event for galling herbivores, as a time lag in synchronisation will determine quality and quantity of available food resources (Kerslake & Hartley 1997; Yukawa 2000; Oliveira *et al.* 2016). Hence, there is a strong tendency in temperate regions among galling insects towards univoltinism (Weis *et al.* 1988; Hodkinson 2009), probably due to availability of responsive tissues being restricted to specific seasons of the year (Rohfritsch & Anthony 1992). However, multivoltine life cycles can occur in parasites of evergreen plants; apparently, the availability of resources and environmental conditions determine this behaviour (Carneiro *et al.* 2013; Carneiro & Isaias 2015).

The crucial morphogenetic changes for gall development involve anatomical and metabolic changes (Rohfritsch 1992). Accordingly, cell hypertrophy, tissue hyperplasia, inhibition of some development programmes and cytological changes may occur (Mani 1964; Oliveira *et al.* 2011; Ferreira *et al.* 2017a). Even though galls are usually observed in plant vegetative organs (Araújo *et al.* 2011; Isaias *et al.* 2013; Guimarães *et al.* 2014; Mendonça *et al.* 2014), few studies, particularly into the development of stem galls and their subsequent effects on this organ, have been conducted (Ferreira & Isaias 2013), in particular for plants from Mediterranean climate regions.

Schinus polygama (Cav.) Cabrera (Anacardiaceae) is an evergreen shrub, previously considered native to Argentina, Brazil and Chile (Rodríguez 2011), which hosts gall morphotypes on its leaves and stems (Sáiz & Núñez 1997; Burckhardt & Basset 2000; Moreira *et al.* 2012; Dias *et al.* 2013a; Guedes *et al.* 2016). The galls induced by *Calophya* cf. *duvauae* (Scott) on leaves of *S. polygama* have previously described in Brazil (Dias *et al.* 2013a,b). However, the taxonomy of genus *Schinus* is confused

and is under review. Probably *S. polygama* is distributed in Chile and in adjacent Argentina and *S. engleri* (Barkley) in Brazil (D. Burckhardt, personal communication). In response to this information, in this work we consider that *C.* cf. *duvauae* induces galls in leaves of *S. engleri* but not in leaves of *S. polygama*, as suggested by Dias *et al.* (2013a,b).

Usually, plants adjust their phenological events to local climate conditions, which are followed by the associated galling organisms (Yukawa & Akimoto 2006). As *S. polygama* is a perennial species with potential responsive oviposition sites throughout the year, its association with gall-inducing insects with multivoltine life cycles is expected. The galls induced by a multivoltine insect, *C.* cf. *duvauae* on leaves of *S. engleri* have been previously been described in the transition zone between tropical and temperate climates of southern Brazil (Dias *et al.* 2013a,b). Although phenological adjustments between the host plant and the galling insect have been studied in the Neotropics (see Oliveira *et al.* 2016), the Mediterranean climate has yet to be investigated; especially the phenological events of the life cycles of calophyids and *S. polygama*.

Anatomical studies on galls induced by calophyids have been restricted to leaf galls (Oliveira & Isaias 2010a; Isaias *et al.* 2011; Dias *et al.* 2013a), and anatomical and morphological characteristics of the conical stem gall on *S. polygama* have only recently been described (Guedes *et al.* 2016). The conical gall morphotype is the most frequent in stems of *S. polygama* in the Mediterranean region of southern Chile (Guedes *et al.* 2016). It is induced by *Calophya rubra* (Blanchard) (Hemiptera: Psylloidea), which has been described as a bivoltine species in Chile (Sáiz & Núñez 1997; Burckhardt & Basset 2000).

In leaves of *S. engleri*, the feeding activity of the co-generic species, *C.* cf. *duvauae*, induces parenchyma homogenisation and the neoformation of vascular bundles and trichomes (Dias *et al.* 2013a). Assuming the gall inducer's feeding behaviour as the determining factor for the anatomical peculiarities of the galls (Stone & Schönrogge 2003), it is expected that the processes of cell differentiation and redifferentiation induced by *C. rubra* and *C.* cf. *duvauae* in the stems of *S. polygama* and leaves of *S. engleri* should be similar. Accordingly, the host organ should impose morphogenetical and phenological constraints, which might explain gall peculiarities. To assess these premises, we linked the phenological and anatomical development of the conical stem gall with the life cycle of *C. rubra*, focusing on the following questions: (i) do distinct climate zones drive distinct galling synchronisation strategies in the closely related evergreen host plant species; and (ii) what are the traits of the stem conical gall over the host stem morphogenetic potentialities? These questions should address the peculiarities of the *S. polygama–C. rubra* system in comparison to a cogeneric galling insect, *C.* cf. *duvauae* and the closely related host plant *S. engleri*.

MATERIAL AND METHODS

Sampling and collection

The current study was carried out in a population of *S. polygama* in Chile, Biobío Region, Ñuble Province, Chillán Viejo, Chile, kilometer 4 (36°39'32"S, 72°16'43"W, 150 m a.s.l.), between July 2015 and March 2016. Plant identification was confirmed by specialists from the Department of Botany at the University of Concepcion (CONC). A voucher specimen was deposited in CONC under accession number 180330. Meteorological data were obtained from Weatherbase (http://www.weatherbase.com).

The vegetative phenology was monitored from June 2015 to December 2016. Ten individuals of *S. polygama* were randomly selected, based on the presence of axillary buds and new branches, which were visually inspected each month. Ten branches were collected from each tree, stored in plastic bags and transferred to the Laboratory of Natural Products Chemistry at the University of Concepcion. There, each conical stem gall (CSG; n = 5 for each individual, total of 50 galls) was dissected with a razor blade under a stereomicroscope, and grouped considering the insect instar. Nymphs were collected and preserved in 70% ethanol and sent to the Naturhistorisches Museum of Switzerland (NHMB) for species identification. The insect vouchers are deposited in NHMB under accession numbers NMB-PSYLL0004268–NMB-PSYLL0004274 and NMB-PSYLL0004276–NMB-PSYLL0004282.

Sample fragments of non-galled stems (NGS) and CSG, at four developmental phases, were collected from individuals of *S. polygama* (n = 5) for anatomical, histometric and histochemical analyses. Galls in four developmental phases were sorted according to the instar. Growth and development were assessed in: phase I (GDI), first instar nymphs; phase II (GDII), second, third and fourth instar nymphs; maturation phase (MP), fifth instar nymph; and senescent phase (SP), open gall without insect.

Anatomical and histometric analysis

After each sampling, fragments of NGS and CSG were fixed in 4% Karnovsky (2.5% glutaraldehyde and 4.5% formaldehyde in phosphate buffer 0.1 M, modified to pH 7.2; O'Brien & McCully 1981) for 24 h, FAA (37% formaldehyde, glacial acetic acid, 50% ethanol, 1:1:18, v/v; Johansen 1940) or 2.5% glutaraldehyde (Karnovsky 1965) and subsequently stored in 70% ethanol. Permanent slides were prepared by dehydrating tissue fragments in an n-butyl series and embedding in Paraplast® (Kraus & Arduin 1997). Sections were produced using a rotary microtome (12–18 μm), the slides were stained with 0.5% astra blue and safranin (9:1 v/v; Bukatsch 1972) and mounted in clear varnish (Paiva *et al.* 2006).

Histometric and cytometric data were obtained from photomicrographs of NGS and mature CSG (n = 5) using the AxioVision LE software (CarlZeiss MicroImaging, Jena, Germany). Five different sections were used for each organ and five measurements were taken for each section. In order to compare NGS and galls in MP, cortex thickness, height and width of cells in the NGS and in middle cortices of CSG were measured.

Histochemical analyses

Histochemical analyses were performed for the detection of polyphenols, lignins, suberin and lipids, according to Ferreira *et al.* (2017b). Gall samples in GDI were fixed in Karnovsky's solution (Karnovsky 1965) and hand-sectioned. Stem and gall samples, in GDII and MP, were fixed in Karnovsky's solution, embedded in polyethylene glicol (PEG; Ferreira *et al.* 2014, 2017b) and sectioned in rotary microtomes. Each section (20–40 μm) was tested with the following reagents: Sudan Black B and Sudan IV to detect suberin and lipids (Jensen 1962),

Maule's test for lignins, iron sulphate and 3% ferric chloride for polyphenols (Johansen 1940). Blank sections, without staining, were also mounted and analysed for comparison. Digital images were obtained with an optical photomicroscope Zeiss® Primo Star.

Statistical analysis

Student's *t*-test was used to compare NGS and CSG for each of the independent variables. Data normality was verified with the Shapiro–Wilk test. Levels of significance for all statistical analyses were carried out using InfoStat version 2016, considering $P \leq 0.05$ (Rienzo *et al.* 2013).

RESULTS

Schinus polygama and gall description

Schinus polygama (Fig. 1A) individuals grow throughout the year, with a sprouting peak during spring (September–November). Inflorescences are produced in the middle of spring and fruiting occurs during the dry season (December–January). Four gall morphotypes were observed on *S. polygama*: one bud gall and one fusiform stem gall induced by Lepidoptera: Cecidosidae (Fig. 1B, C), one globoid leaf gall induced by *Calophya mammifex* (Burckhardt & Basset; Psylloidea; Fig. 1D) and a conical stem morphotype (Fig. 1E): the main focus of this study. This conical gall is induced by the first-instar nymphs of *C. rubra* in young stems of *S. polygama*.

The CSG of *C. rubra* occur in young stems (Fig. 1E), petioles (Fig. 1F) and occasionally leaves (Fig. 1G). Galls at the beginning of GDI are small conical protuberances with a large amount of white trichomes emerging from the tip centre (Fig. 1E). Conical stem gall colour varies slightly, from green at the beginning of GDI (Fig. 1E) to brown in almost all gall development stages (Fig. 1H). Through GDII, trichomes turn coppery and disappear at the end of the MP. The GD and MP are characterised by an increase in gall size, and change in shape at the end of MP, when the gall turns from conical (Fig. 1E) to globose (Fig. 1H). At this time, the gall breaks, with an apical cross-shaped opening (Fig. 1I). Grouped galls induced by *C. rubra* can occur, and in this case, they take varied and indefinite forms, and each gall shelters one chamber with a single galling insect; insects of distinct gall chambers are often in different instars. Senescent galls remain on the stems and the cross-shaped opening can be still observed (Fig. 1I).

Gall phenology

Gall growth and development started in November and lasted until the following October. Between December and July, only the first three instars are observed in galls, indicating a period

Fig. 1. Galls on *Schinus polygama* (Anacardiaceae). (A) Host plant in natural habitat. (B) Stem branch with bud galls. (C) Stem branch with fusiform galls. (D) Mature leaf with several globoid galls. (E–I) Conical galls induced by *Calophya rubra*: (E) on a stem, at the beginning of growth and development, with white trichomes emerging from its tip centre (arrow), (F) on petioles, (G) on the leaf (arrow), (H) mature globoid galls. (I) Senescent gall with the cross-shaped opening. Scale bars: F: 2 mm; C, D, H: 5 mm; G: 0.5 cm; E: 1 cm; I: 1.5 cm; B: 10 cm.

of slow insect development due to diapause. The diapause period coincides with the driest months (December to February) and warmest (January), wettest (June) and coldest (July; Fig. 2) times, typical of the Mediterranean climate of southern Chile. From August to November, coinciding with the peak of leaf sprouting, all instars were observed, with prevalence of the fourth and fifth instars. The presence of different instars of *C. rubra* during the sampled time indicates an asynchrony in the oviposition of the female; it appears that the species is univoltine. In early October, some galls reached maturation, which lasted until December when the last senescent galls were recorded. Gall maturation occurred during the flowering stage of *S. polygama*, which begins in October and lasts until December.

Anatomical features of the host stem

The host stems can be divided into cortex, vascular system and pith (Fig. 3A), from the outer to the inner regions. Externally, the bark of *S. polygama* is flaky and grey, but in an anatomical cut, the phellogen and phelloderm could not be clearly discerned (Fig. 3B). The phellem consists of two or more layers of polygonal cells (Fig. 3B, C). The cortex is seven- to 22-layered (Fig. 3C), with elongated and narrow cells that containing lipids and polyphenols (Fig. 3D, E). Secretory ducts and pericyclic fibres are distributed within the cortical parenchyma (Fig. 3C). The secondary xylem has vessel elements and numerous fibres (Fig. 3F). Vessel elements are interspaced with radial parenchyma cells and xylem fibres with lignified cell walls (Fig. 3F, G). Both secondary phloem and xylem parenchyma cells contain polyphenols (Fig. 3G). The pith is cylindrical, with polygonal to globose cells and lignified walls (Fig. 3H).

Anatomical and histometrical features along gall development

Oviposition takes place through leaf gaps (parenchymatic space in the vascular cylinders of primary stems, occurring in regions of leaf trace divergence), in the stem cortex or in parenchyma of the axillary bud (Fig. 4A, B). The start of the first instar nymph feeding stimulates hyperplasia and hypertrophy of cortical cells (Fig. 4C). The enveloping cortical emergences then curve, covering the nymph, which becomes enclosed inside the gall chamber. There is no tissue welding in the contact of the emergences, but only the overlapping with trichomes, which originate from modified epidermal cells (Fig. 4C). In GDI phase, three tissue layers – the inner, middle, and outer cortex – are observed in the gall wall, formed from the covering tissues (Fig. 4C). The epidermis can still be observed during this phase (Fig. 4D); however, its replacement by phellem with deposition of suberin was detected (Fig. 4E). The outer cortex is continuous to the stem cortex (Fig. 4F). The middle cortex is formed from parenchyma with large polygonal cells without phenols (Fig. 4G). New vascular cells redifferentiate within the gall middle parenchyma and connect to those of the host organ through radial parenchyma (Fig. 4H). Few neoformed vascular units are observed at the GDI, which formed from vascular bundles that develop into vascular cambium. Neoformed vascular units are interspersed with cortical parenchyma and surround the stem secondary vascular system and nymphal chamber, to which it is oriented (Fig. 4H). The inner cortex surrounds the nymphal chamber (4I) and has periclinally elongated cells containing phenols and lipids (Fig. 4J, K). Secretory ducts are rarely observed in gall development sites. The apical portion of the gall is not vascularised and has isodiametric parenchyma cells.

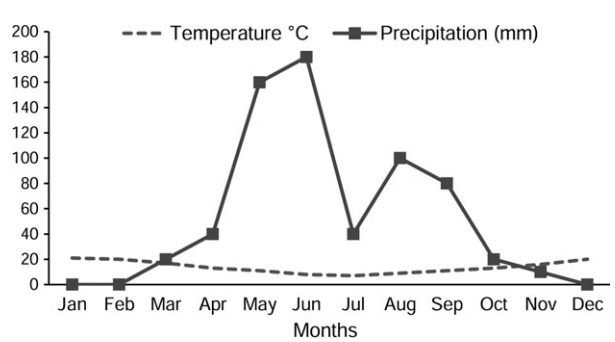

Fig. 2. Monthly record of temperature and precipitation average of Chillán, Chile, in the period 2010 to 2016 (Data from http://www.weatherbase.com).

The most relevant changes observed at the end of GDII are an increase in gall size and number of neoformed vascular units, particularly phloematic units. The deposition of phenols increases in the inner cortex, and these accumulate in some cells of the middle cortex (Fig. 4L). Lipids are present throughout the gall parenchyma (Fig. 4M). An increase in vascular units is accompanied by the development of large vascular parenchyma cells in the middle cortex (Fig. 4N). The epidermis is totally replaced by the periderm, and deposition of suberin (phellem) is observed (Fig. 4O).

In the MP, phenolics accumulate in all three layers of the gall cortex (Fig. 4P). At this phase, large vascular units with lipid-rich parenchyma cells, without phenolics, are observed (Fig. 4Q, R). At the end of MP, the nymph of *C. rubra* completely occupies the nymphal chamber, and its terminalia are arranged towards the ostiolar opening. Presumably, the pressure exerted by the terminalia on the ostiolar opening causes the cross-breaking of the gall. Then, the fully mature *C. rubra* adult emerges and the galls enter in the SP, when suberisation occurs in the ostiolar opening, inner and middle cortex (Fig. 4S). Finally, senescent galls become totally wilted and dry, but remain attached to the stem.

The thickness of cortical layers significantly increases throughout gall development in comparison to the NGS (Fig. 5A). In addition, there is hypertrophy of cells of the middle cortex, which increase in diameter and height with regard to cortex cells of the NGS (Fig. 5B, C).

DISCUSSION

Phenological adjustments favouring the univoltine gall life cycle

Current analyses could diagnose phenological adjustment between *S. polygama* and *C. rubra* in the population herein evaluated. This means that there is a synchrony: the insect reproductive maturation must match the most relevant host plant phenological stages for its development (Yukawa & Akimoto 2006; Van Asch *et al.* 2007). Currently, *C. rubra* time for oviposition coincides with *S. polygama* leaf flushing, which seems to be a successful strategy for completion of its life cycle

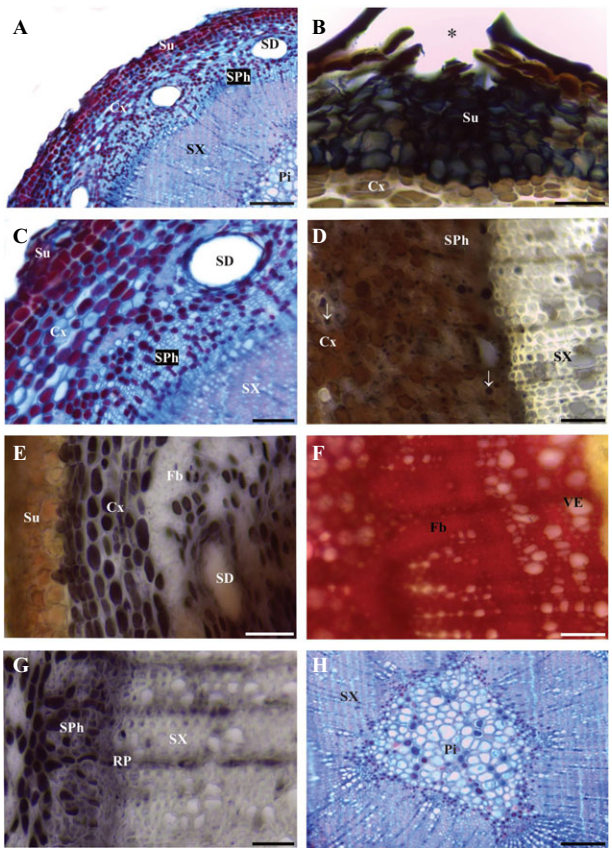

Fig. 3. Stem anatomy of *Schinus polygama* (Anacardiaceae). (A) Cross-section of young stem with secondary growth. (B) Phellem layers with suberin stained black. (C) Multilayered cortex and phellem. (D, E) Cortex cells: (D) lipid droplets (arrows) stained black, (E) phenolics stained black. (F) Fibres on secondary xylem stained red. (G) Phenolics in secondary phloem and xylem parenchyma cells stained black. (H) Rounded pith. Staining: (A), (C), (H) astra blue and safanin; (B), (D) Sudan black B; (E), (G) iron(II) sulphate; (F) Maule's reagent. Abbreviations: Cx: cortex, Fb: fibres, Pi: pith, RP: radial parenchyma, SD: secretory ducts, SPh: secondary phloem, Su: suberin, SX: secondary xylem, VE: vessel elements, (*) lenticel. Scale bars: B–E, G: 50 μm; A, F, H: 200 μm.

(Hodkinson 2009). Such synchrony has been previously observed for other Neotropical gall systems, such as *Copaifera langsdorffii* (Jacq.) (L.)–Cecidomyiidae (Oliveira *et al.* 2013), *Aspidosperma macrocarpon* (Mart)– *Pseudophacopteron longicaudatum* (Malenovský, Burckhardt, Queiroz, Isaias & Oliveira) (Phacopteronidae) (Castro *et al.* 2013), *Aspidosperma australe* (Müll.Arg.)– *Pseudophacopteron aspidospermii* (Malenovský, Burckhardt, Queiroz, Isaias & Oliveira) (Phacopteronidae) and *Aspidosperma spruceanum* (Müll.Arg.)–Cecidomyiidae (Campos *et al.* 2010). The period of leaf flushing corresponds to the preferred time for gall induction, because young tissues are more responsive to the galling insects' stimuli (Rohfritsch & Anthony 1992).

As sessile organisms, it is expected that plants adjust their phenological events to local climate conditions, which are followed by the associated galling organisms (Yukawa & Akimoto 2006). For Psylloidea in the Neotropics, the availability of resources and environmental conditions favour multivoltine life cycles (Weis *et al.* 1988; Lima 2008; Dias *et al.* 2013a,b). In southern Brazil, *C.* cf. *duvauae* establishes a multivoltine life cycle associated with *S. engleri* (Dias *et al.* 2013b). Nevertheless, the Mediterranean climate seems to impose a distinct phenological strategy for *C. rubra*, which has established a univoltine life cycle with gall induction restricted to spring. Based on the fact that diapause provides the timing and synchronisation mechanisms through which psyllids are able to survive unfavourable periods (Canard 2005; Hodkinson 2009), we propose that the climate conditions of the Mediterranean region limit *C. rubra* nymph activity during unfavourable periods, and could determine a diapause period and the univoltine life cycle.

For *C. rubra*, once diapause is broken, feeding and development resume in spring, with adult emergence soon afterwards, during the flowering period of *S. polygama*. This behaviour has been observed among insects associated with temperate evergreen plants, such as *Strophingia ericae* (Curtis) on *Calluna vulgaris* (L.) (Hodkinson *et al.* 1999) and *Psylla buxi* (L.) on *Buxus* (Hodkinson 2009), and could imply a period of adequate availability of host plant resources (Yukawa & Akimoto 2006). In general, at the onset of reproduction, plants increase the allocation of resources to reproductive stems, resulting in an increase in the quality of phloem sap in flowering/fruiting parts (Salisbury & Ross 1992; Quental *et al.* 2005), which might indirectly determine maturation of the CSG and the hatching of *C. rubra*.

Traits of stem conical gall throughout its development

The site of CSG establishment, the cortical parenchyma, has the most plastic plant cells, which retain the capacity to reassume meristematic activity and redifferentiate (Lev-Yadun 2003). Due to the parenchyma cells potential large pool of cell responses to galling stimuli (Oliveira & Isaias 2010a; Ferreira & Isaias 2013), leading to cell hypertrophy and hyperplasia, feeding sites for the nymphae and the major parts of the gall wall are produced. The gall wall is a consequence of curvature of the tissues around the nymphae, characterising a covering gall (Shorthouse & Rohfritsch 1992), which develops through proliferation of the epidermal and outer cortical cells after gall induction in the stem surface (Shorthouse & Rohfritsch 1992; Isaias *et al.* 2014a), and may also have trichomes in the ostiolar opening.

Typical gall-inducing phloem feeders are able to modify the organisation of their main feeding site, the plant vascular tissues, toward an increment in food supply (Wool 2004; Álvarez 2011; Álvarez *et al.* 2014, 2016; Muñoz-Viveros *et al.* 2014; Ferreira *et al.* 2017a). The vascular units differentiated within the gall cortex, mostly phloem elements and vascular parenchyma cells, guarantee the requirements for water and nutrients (Guedes *et al.* 2016) of *C. rubra*, since phloem is their food source (Wool *et al.* 1999). The increase in number of vascular units during MP is a functional response to increased trophic activity of the inducer during this phase (Isaias *et al.* 2014a), when it actively feeds on phloem cells (Kraus 2009). Stem galls have a well-developed vascular parenchyma, probably because gall-inducing Psylloidea can also feed on non-vascular tissue, such as vascular parenchyma (Shorthouse & Rohfritsch 1992; Raman 2012). Also, the role of the vascular parenchyma in the loading and unloading of sieve tubes is responsible for photoasimilate transport and favours insect nutrition. Therefore, neoformed phloem cells are essential for inducer nourishment and for the maintenance of gall cellular machinery (Dias *et al.* 2013a).

The morphological changes in *C. rubra* galls on *S. polygama* are followed by alterations in colour, shape and

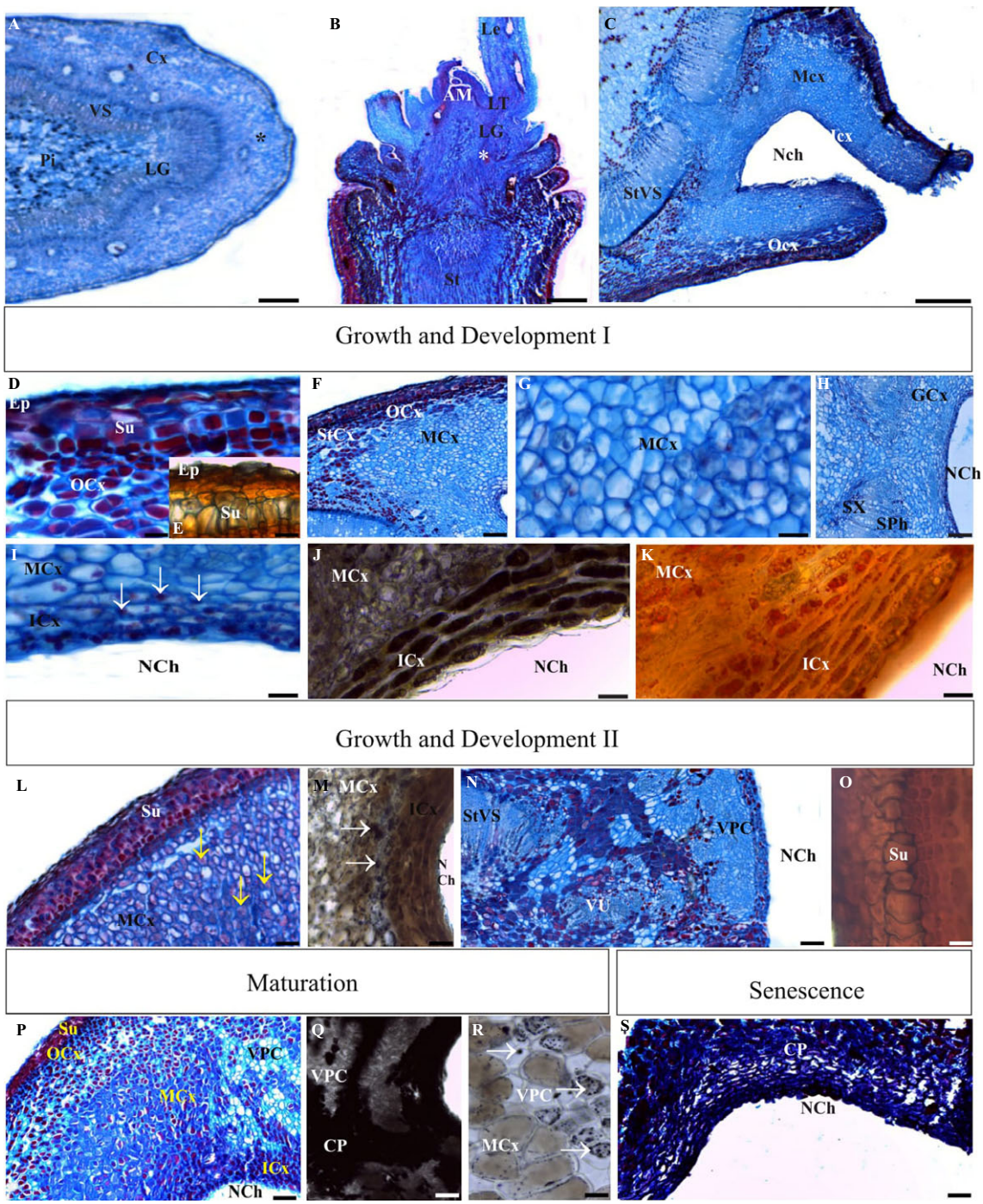

Fig. 4. Development of galls of *Calophya rubra* (Psylloidea) on the stem of *Schinus polygama* (Anacardiaceae). (A) Cross-section of a young stem, showing establishment site of *C. rubra* (*). (B) Longitudinal section of axillary bud, showing establishment site of *C. rubra* (*). (C) Cross-section of young conical stem gall with tissue hyperplasia and cell hypertrophy. (D–K) Cross-section of galls in GDI: (D) phellem formation pushed the epidermis, (E) detail of phellem detected with suberin in black, (F) continuum between the gall outer cortex and the stem cortex, (G) middle cortex with large polymorphic parenchyma cells, (H) neoformed vascular unit joining those of the stem. (I–K) Inner cortex: (I) periclinally elongated cells, (J) phenolics stained black, (K) lipid droplets stained red. (L–O) Cross-section of galls in GDII: (L) middle cortex cells containing phenols (reddish), (M) lipid droplets in gall parenchyma stained black (arrows), (N) basal region of the gall, highly vascularised and with vascular parenchyma cells, (O) suberin deposition detected in black. (P–R) Cross-section of mature galls: (P) apical region of gall with parenchyma cells containing phenolics (reddish), (Q–R) vascular parenchyma cells: (Q) negative reaction to polyphenols using iron(III) chloride reagent, (R) lipids detected in black. (S) Phellem in the inner and middle cortex of a senescent gall. Staining: A–D, F–I, L, N, P, S astra blue and safranin; E, M, O, R Sudan black B; J, Q iron(III) chloride; K Sudan red B. Abbreviations: AM: apical meristem, CP: cortical parenchyma, Cx: cortex, Ep: epidermis, GCX: gall cortex, ICx: inner cortex, Le: leaf, LG: leaf gap, LT: leaf trace, MCx: middle cortex, NCh: nymphal chamber, OCx: outer cortex, Pi: pith, SPh: secondary phloem, StCx: stem cortex, StVS: stem vascular system, Su: suberin, SX: secondary xylem, StVS: stem vascular system, VPC: vascular parenchyma cells VS: vascular system, VU: vascular unit. Scale bars: D, E, G, I–K, M: 50 μm; F, H, L, N–P, Q–S: 200 μm; A–C: 500 μm.

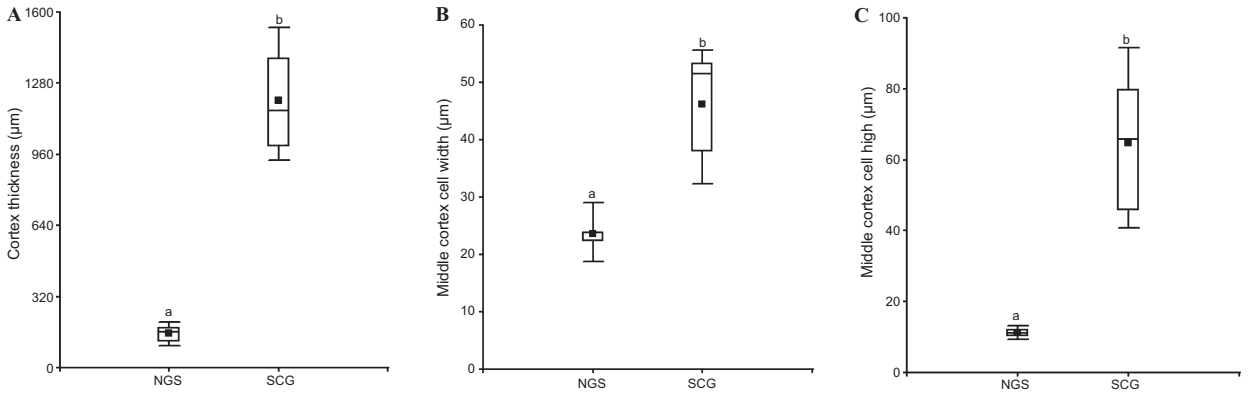

Fig. 5. Cytological and histometric analyses of cross-sections of non-galled stem (NGS) of *Schinus polygama* (Anacardiaceae) and mature conical stem galls (CSG) of *Calophya rubra* (Psylloidea). Bars with different letters are significantly different (Student's *t*-test, $P \leq 0.05$).

increases in size, which have been associated with the developmental stage of the gall and the age of the host organ at the time of oviposition, among other features (Isaias *et al.* 2014b). For CSG, the transition from conical to globoid shape has been associated with changes in water status of the host plants (Sáiz & Núñez 2000). Currently, changes in shape have also been associated with the degree of gall maturation and development of the galling insect, which reaches the fifth instar inside the gall.

Change in colour is a natural process linked to stem aging, and it similarly occurs in galls – from green to brown. Along stem and during gall maturation, there is a gradual decay of photosynthetic pigments in deeper tissues (Pilarski *et al.* 2007), which explains the colour changes. Accordingly, colour alteration in the CSG on *S. polygama* could be stimulated by variations in the balance between photosynthetic pigments and anthocyanin, as observed during aging in other lignified plant tissues and species (Pilarski *et al.* 2007).

During late MP and SP, all tissues surrounding the nymphal chamber degrade as a result of the end of the cell cycles. The peculiar trichomes of the gall aperture are detached either by the pressure exerted by the insect terminalia on the ostiolar opening, which could facilitate the exit of the adult of *C. rubra*, or by the end of gall-inducing insect feeding stimuli, as described for *S. engleri*–*C.* cf. *duvauae* (Dias *et al.* 2013b) and *Lantara camara* (L.)–*Aceria lantanae* (Cook) (Moura *et al.* 2009) systems.

Gall structural peculiarities regarding stem morphogenetical potential

Galling psyllids, such as *C. rubra*, feed by inserting their stylets into parenchyma or phloem cells (Hodkinson 2009), and induce minor modifications in their host plant tissues, thereby producing simple galls with high anatomical similarity to the host organs (Oliveira & Isaias 2010a). Nevertheless, on a quantitative basis, the CSG traits imply gains over the NGS towards *C. rubra* success.

Qualitative analysis has elucidated gall developmental steps under the low plasticity of host stems (Formiga *et al.* 2015). As stems have low plasticity when compared to leaves, galls must develop under major morphogenetic constraints to deviate from the ordinary pattern of the host organ (NGS; Formiga *et al.* 2015). The absence of alterations in the phellem, secretory ducts and pith are indicative traits of the *S. polygama* stem that probably cannot be maniputated by *C. rubra*. In fact, the development of secretory ducts is usually conservative in gall development on distinct host plant species (Arduin *et al.* 2005; Dias *et al.* 2013a; Amorim *et al.* 2017; Ferreira *et al.* 2017a). Regarding the outer protective cell layers, the suberised cell walls of the phellem in CSG on *S. polygama* is a pre-existing ordinary development pathway of plant secondary stems (Esau & de Morretes 1974), which may be positive for plant survival in the hot and dry summers of the Mediterranean climate of Chile (Giorgi & Lionello 2008). Moreover, suberisation and lignification confer physiologically important plant–environment interfaces, as suberin and lignin may act as barriers that limit water and nutrient transport and protect plants from invasion of pathogens (Franke & Schreiber 2007). Likewise, the presence of lignins in perivascular fibres, secondary xylem and pith parenchyma of NGS is important for plant support as well as for waterproofing (Boerjan *et al.* 2003), as suberisation and lignification in cell walls can affect radial water transport (Franke & Schreiber 2007). Another peculiarity of the CSG cells is the non-lignification of the perivascular fibres and secondary xylem, which may favour the feeding of *C. rubra*. Accordingly, the stem gall on *S. polygama* combines the stimuli of *C. rubra* and the morphogenetic constraints of the host stems, which result in the CSG.

In the Neotropics, psyllid galls are generally parenchymatic (Isaias *et al.* 2011, 2014b; Dias *et al.* 2013a; Formiga *et al.* 2015). In particular, the feeding activity of *C.* cf. *duvuaue* induces parenchyma homogenisation and the neoformation of few vascular bundles and trichomes in leaves of *S. engleri* (Dias *et al.* 2013a). As a peculiarity, the CSG induced by *C. rubra* on *S. polygama* seem to be structurally more complex than the leaf galls induced by *C.* cf. *duvuaue*. Non-homogenous and highly vascularised hyperplasic parenchyma, neoformation of vascular units and vascular parenchyma cells are the major differences to the leaf galls induced by *C.* cf. *duvuaue.*

The accumulation patterns of primary and secondary metabolites in gall tissue compartments are commonly related to both gall-inducing taxa and feeding behaviour (Bragança *et al.* 2017). In the CSG, polyphenol accumulation in the outer compartment seems to vary during gall development, due to the influence of the biotic stress of gall induction over their biosynthesis (Trabelsi *et al.* 2012). The major detected polyphenol in the CSG on *S. polygama*, pyrogallol, is thought to act in

chemical defence against natural enemies (Guedes *et al.* 2016). Nevertheless, polyphenols may also play important roles in developmental, physiological and structural processes (Hattenschwiler & Vitousek 2000; Lattanzio *et al.* 2006).

In the inner compartment of CSG, lipids accumulate, which implies similarity to other galls induced by phloem-sucking insects (Oliveira *et al.* 2006; Oliveira & Isaias 2010b). However, while for these systems lipid accumulation has been related to the maintenance of gall structure, we here assume an indirect participation in *C. rubra* nutrition, since lipid accumulation was observed in phloem cells. Fatty acid precursors related to the increment in nutritional value have been detected in the CSG tissues (Guedes *et al.* 2016), which is in accordance with histochemical detection of lipids in the outer and inner tissue compartments in a centrifugal gradient throughout CSG development. These high-energy metabolites cannot be directly used by *C. rubra*, but their location should be related to loading and unloading of sieve tubes, the actual feeding site of *C. rubra*. Current results of phenol and lipid accumulation in both NGS and CSG could indicate that *C. rubra* is favoured by the presence of these metabolites in the host stems of *S. polygama*. Thus, *C. rubra* can over-stimulate lipid and phenol synthesis pathways to favour gall nutrition, maintenance and/or protection.

ACKNOWLEDGEMENTS

This research was supported by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT)/National PhD/2014-fellowship (no. 63140050) awarded to LMG, the Research and Development Vice-Rectory of the Universidad de Concepción (UdeC), Projects VRID-215.142.034-1.0IN, MEC80170028 and REDI170025. RMSI and BGF thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support. The authors are grateful to Dra. Katia Lorena Saez Carillo (University of Concepcion) for her contribution to statistical analysis, Dr. Daniel Burckhardt (Naturhistorisches Museum, Basel, Switzerland) for identifying insects and commenting on a earlier manuscript version and Mr. Wagner A. Rocha and Miss Daniele R. Alvarenga for help with the anatomical techniques.

CONFLICT OF INTEREST

The authors declare they have no competing interests.

REFERENCES

Abrahamson W.G., Weiss A.E. (1997) *Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies*. Princeton University Press, Princeton, NJ, USA, p 458.

Álvarez R. (2011) Initial stages in the formation of galls induced by *Geoicau tricularia* in *Pistacia terebinthus* leaflets: origin of the two vascular bundles which characterize the wall of the galls. *American Journal of Plant Sciences*, **2**, 175–179.

Álvarez R., Molist P., González-Sierra S., Martinez J.J.I., Nieto-Nafría J.M. (2014) The histostructure of galls induced by aphids as a useful taxonomic character: the case of Rectinasus (Hemiptera, Aphididae, Eriosomatinae). *Zootaxa*, **3861**, 487–492.

Álvarez R., Martinez J.J.I., Muñoz-Viveros A.L., Molist P., Abad-González J., Nieto-Nafría J.M. (2016) Contribution of gall microscopic structure to taxonomy of gallicolous aphids. *Plant Biology*, **8**, 868–875.

Amorim D.O., Ferreira B.G., Fleury G. (2017) Plant potentialities determine anatomical and histochemical diversity in *Mikania glomerata* Spreng. galls. *Brazilian Journal of Botany*, **4**, 517–527.

Araújo W.S., Julião G.R., Ribeiro B.A., Silva I.P.A., Santos B.B. (2011) Diversity of galling insects in *Styrax pohlii* (Styracaceae): edge effect and use as bioindicators. *Revista de Biología Tropical*, **29**, 1589–1597.

Arduin M., Fernandes G.W., Kraus J.E. (2005) Morphogenesis of galls induced by *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) on *Baccharis dracunculifolia* (Asteraceae) leaves. *Brazilian Jorunal of Biology*, **65**, 559–571.

Boerjan W., Ralph J., Baucher M. (2003) Lignin biosynthesis. *Annual Review of Plant Biology*, **54**, 519–546.

Bragança G., Oliveira D., Isaias R.M.S. (2017) Compartmentalization of metabolites and enzymatic mediation in nutritive cells of Cecidomyiidae galls on *Piper arboreum* Aubl. (Piperaceae). *Journal of Plant Studies*, **6**, 11–22.

Bukatsch F. (1972) Bermerkungen zur doppelfárbung astrablau-safranin. *Mikrokosmos*, **61**, 255.

Burckhardt D., Basset Y. (2000) The jumping plant-lice (Hemiptera, Psylloidea) associated with *Schinus* (Anacardiaceae): systematics, biogeography and host plant relationships. *Journal of Natural History*, **34**, 57–155.

Campos P.T., Costa M.C.D., Isaias R.M.S., Moreira A.S.F.P., Oliveira D.C.D., Lemos-Filho J.P.D. (2010) Phenological relationships between two insect galls and their host plants: *Aspidosperma australe* and *A. spruceanum* (Apocynaceae). *Acta Botanica Brasilica*, **24**, 727–733.

Canard M. (2005) Seasonal adaptations of green lacewings (Neuroptera: Chrysopidae). *European Journal of Entomology*, **102**, 317–324.

Carneiro R.S.G., Isaias R.M.S. (2015) Gradients of metabolite accumulation and redifferentiation of nutritive cells associated with vascular tissues in galls induced by sucking insects. *AoB Plants*, **7**, 1–16.

Carneiro R.G.S., Burckhardt D., Isaias R.M.S. (2013) Biology and systematics of gall-inducing triozids (Hemiptera: Psylloidea) associated with *Psidium* spp. (Myrtaceae). *Zootaxa*, **3620**, 129–146.

Castro A.C., Oliveira D.C., Silvia A., Moreira F.P., Isaias R.M.S. (2013) Synchronism between *Aspidosperma macrocarpon* (Apocynaceae) resource allocation and the establishment of the gall inducer *Pseudophacopteron* sp. (Hemiptera: Psylloidea). *Revista de Biología Tropical*, **61**, 1891–1900.

Dias G.D., Ferreira B.G., Moreira G.R.P., Isaias R.M.S. (2013a) Developmental pathway from leaves to galls induced by a sap-feeding insect on *Schinus polygamus* (Cav.) Cabrera (Anacardiaceae). *Anals da Academia Brasileira de Ciencias*, **85**, 187–200.

Dias G.G., Moreira G.R.P., Ferreira B.G., Isaias R.M.S. (2013b) Why do the galls induced by *Calophya duvauae* Scott on *Schinus polygamus* (Cav.) Cabrera (Anacardiaceae) change color? *Biochemical Systematics and Ecology*, **48**, 111–122.

Eigenbrode S.D., Jetter R. (2002) Attachment to plant surface waxes by an insect predator. *Integrative and Comparative Biology*, **42**, 1091–1099.

Esau K., de Morretes B.L. (1974) *Anatomia das plantas com sementes*. Editora Edgard Blucher Ltda, Sao Paulo, Brazil, p 293.

Ferreira B.G., Isaias R.M.S. (2013) Developmental stem anatomy and tissue redifferentiation induced by a galling Lepidoptera on *Marcetia taxifolia* (Melastomataceae). *Botany-Botanique*, **91**, 752–760.

Ferreira B.G., Teixeira C.T., Isaias R.S.M. (2014) Efficiency of the polyethylene-glycol (PEG) embedding medium for plant histochemistry. *Journal of Histochemistry & Cytochemistry*, **62**, 1–7.

Ferreira B.G., Álvarez R., Avritzer S., Isaias R.M.S. (2017a) Revisiting the histological patterns of storage tissues: beyond the limits of gall-inducing taxa. *Botany-Botanique*, **95**, 173–184.

Ferreira B.G., Falcioni R., Guedes L.M., Avritzer S.C., Antunes W.C., Souza L.A., Isaias R.M.S. (2017b) Preventing false negatives for histochemical detection of phenolics and lignins in PEG-embedded plant tissues. *Journal of Histochemistry & Cytochemistry*, **65**, 1–12.

Formiga A.T., Silveira F.A.O., Fernandes G.W., Isaias R.M.S. (2015) Phenotypic plasticity and similarity among gall morphotypes on a superhost, *Baccharis reticularia* (Asteraceae). *Plant Biology*, **17**, 512–521.

Franke R., Schreiber L. (2007) Suberin – a biopolyester forming apoplastic plant interfaces. *Current Opinion in Plant Biology*, **10**, 252–259.

Giorgi F., Lionello P. (2008) Climate change projections for the Mediterranean region. *Global and Planetary Change*, **63**, 90–104.

Gonçalves-Alvim S.J., Fernandes G.W. (2001) Biodiversity of galling insects: historical, community and habitat effects in four neotropical savannas. *Biodiversity Conservation*, **10**, 79–98.

Guedes L.M., Aguilera N., Becerra J., Hernández V., Isaias R.S.M. (2016) Leaf and stem galls of *Schinus polygamus* (Cav.) Cabr (Anacardiaceae): anatomical and chemical implications. *Biochemical Systematics and Ecology*, **69**, 266–273.

Guimarães A.L.A., Cruz S.M.S., Vieira A.C.M. (2014) Structure of floral galls of *Byrsonima sericea* (Malpighiaceae) induced by *Bruggmanniella byrsonimae* (Cecidomyiidae, Diptera) and their effects on host plants. *Plant Biology*, **16**, 467–475.

Hattenschwiler S., Vitousek P.M. (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Tree*, **15**, 238–243.

Hodkinson I.D. (2009) Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. *Journal of Natural History*, **43**, 65–179.

Hodkinson I.D., Bird J.M., Miles J.E., Bale J.S., Lennon J.J. (1999) Climatic signals in the life histories of insects: the distribution and abundance of heather psyllids (Strophingia spp.) in the UK. *Functional Ecology*, **13**, 83–95.

Isaias R.M.S., Carneiro R.G.S., Oliveira D.C. (2011) Role of *Euphalerus ostreoides* (Hemiptera: Psylloidea) in manipulating leaflet ontogenesis of *Lonchocarpus muehlbergianus* (Fabaceae). *Botany-Botanique*, **89**, 581–592.

Isaias R.M.S., Carneiro R.G.S., Oliveira D.C., Santos J.C. (2013) Illustrated and annotated checklist of Brazilian gall morphotypes. *Neotropical Entomology*, **42**, 230–239.

Isaias R.M.S., Oliveira D.C., Carneiro R.G.S., Kraus J.E. (2014a) Developmental anatomy of galls in the neotropics: arthropods stimuli versus host plant constraints. In: Fernandes G. W., Santos J. C. (Eds), *Neotropical Insect Galls*. Springer, Heidelberg, Germany, pp 15–34.

Isaias R.M.S., Carneiro R.G.S., Santos J.C., Oliveira D.C. (2014b) Gall morphotypes in the neotropics and the need to standardize them. In: Fernandes G. W., Santos J. C. (Eds), *Neotropical Insect Galls*. Springer, Heidelberg, Germany, pp 51–67.

Jensen W.A. (1962) *Botanical histochemistry*. Principles and practices. Freeman and Co., San Francisco, CA, USA, p 408.

Johansen D.A. (1940) *Plant microtechnique*. McGraw-Hill, New York, USA, p 523.

Karnovsky M.J. (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*, **27**, 137–138.

Kerslake J.E., Hartley S.E. (1997) Phenology of winter moth feeding on common heather: effects on source population and experimental manipulation of hatch dates. *Journal of Animal Ecology*, **66**, 375–385.

Kraus J.E. (2009) Galhas: morfogênese, relações ecológicas e importância econômica. In: Tissot-Squalli ML (ed), *Interações Ecológicas & Biodiversidade*. Editora Inijuí, Ijuí, Brazil, 109–140.

Kraus J.E., Arduin M. (1997) *Manual básico de métodos em Morfologia Vegetal*. EDUR, Rio de Janeiro, Brasil, p 198.

Lattanzio V., Lattanzio V.M.T., Cardinali A. (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato F. (Ed), *Phytochemistry: Advances in Research*. Research Signpost, Kerala, India, pp 23–67.

Lev-Yadun S. (2003) Stem cell in plants are differentiated too. *Current Opinion in Plant Biology*, **4**, 93–100.

Lima F.S. (2008) Respostas estruturais e fisiológicas de *Richeria grandis* Vahl. (Phyllanthaceae) ao estabelecimento do Psyllidae galhador. Unpublished doctorate thesis. Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

Mani M.S. (1964) *Ecology of plant galls*. Springer, Dordrecht, the Netherlands: 434 pp.

Mendonça M.S., Toma T.S.P., da Silva J.S. (2014) Galls and galling arthropods of southern Brazil. In: Fernandes G. W., Santos J. C. (Eds), *Neotropical Insect Galls*. Springer, Heidelberg, Germany, pp 221–256.

Moreira G., Gonçalves G.L., Eltz R.P., San Blas G., Davis D.R. (2012) Revalidation of *Oliera* Brèthes (Lepidoptera: Cecidosidae) based on a redescription of *O. argentinana* and DNA analysis of Neotropical cecidosids. *Zootaxa*, **3557**, 1–19.

Moura M.Z.D., Soares G.L.C., Isaias R.S.M. (2009) Ontogenese da folha e das galhas induzidas por *Aceria lantanae* Cook (Acarina: Eriophyidae) em *Lantana camara* L. (Verbenaceae). *Revista Brasileira da Botanica*, **32**, 271–282.

Muñoz-Viveros A.L., Martinez J.J.I., Molist P., González-Sierra S., Julián P.G., Álvarez R. (2014) Microscopic study of galls induced by three species of Geopemphigus (Hemiptera: Aphididae: Eriosomatinae) on *Pistacia mexicana*. *Arthropod-Plant Interactions*, **8**, 531–538.

O'Brien T.P., McCully M.E. (1981) *The study of plant structure principles and selected methods*. Termarcarphi, Melbourne, Australia, p 345.

Oliveira D.C., Isaias R.M.S. (2010a) Redifferentiation of leaflet tissues during midrib gall development in *Copaifera langsdorffii* (Fabaceae). *South African Journal of Botany*, **76**, 239–248.

Oliveira D.C., Isaias R.M.S. (2010b) Cytological and histochemical gradients induced by a sucking insect in gall of *Aspidosperma australe* Arg. Muell (Apocynaceae). *Plant Scince*, **178**, 350–358.

Oliveira D.C., Christiano J.C.S., Soares G.L.G., Isaias R.M.S. (2006) Reações de defesas químicas e estruturais de *Lonchocarpus muehlbergianus* Hassl. (Fabaceae) à ação do galhador *Euphalerus ostreoides* Crawf. (Hemiptera: Psyllidae). *Revista Brasileira da Botanica*, **29**, 657–667.

Oliveira D.C., Carneiro R.G.S., Magalhães T.A., Isaias R.M.S. (2011) Cytological and histochemical gradients on two *Copaifera langsdorffii* Desf. (Fabaceae) Cecidomyiidae gall systems. *Protoplasma*, **248**, 829–837.

Oliveira D.C., Mendonca M.S., Moreira A.S.F.P., Lemos-Filhoc J.P., Isaias R.M.S. (2013) Water stress and phenological synchronism between *Copaifera langsdorffii* (Fabaceae) and multiple galling insects: formation of seasonal patterns. *Journal of Plant Interactions*, **8**, 225–233.

Oliveira D.C., Isaias R.M.S., Fernandes G.W., Ferreira B.G., Carneiro R.G.S., Fuzaro L. (2016) Manipulation of host plant cells and tissues by gall-inducing insects and adaptive strategies used by different feeding guilds. *Journal of Insect Physiology*, **84**, 103–113.

Paiva J.G.A., Fank-De-Carvalho S.M., Magalhães M.P., Graciano-Ribeiro D. (2006) Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. *Acta Botanica Brasilica*, **20**, 257–264.

Pilarski J., Krzysztof T., Kocurek M. (2007) Comparison of photosynthetic pigment content in stems and leaves of fruit trees: cherry, sweet cherry, common plum, and walnut tree. *Folia Horticulturae Annales*, **19**, 53–65.

Quental T.B., Trigo J.R., Oliveira P.S. (2005) Host-plant flowering status and the concentration of sugar in phloem sap: Effects on an ant–treehopper interaction. *European Journal of Entomology*, **102**, 201–208.

Raman A. (2011) Insect–plant interactions: the gall factor. In: Seckbach J., Dubinsky Z. (Eds). *All Flesh is Grass. Plant–Animal Interrelationships*. Springer, Dordrecht, the Netherlands: 119–146 pp.

Raman A. (2012) Gall induction by hemipteroid insects. *Journal of Plant Interactions*, **7**, 29–44.

Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2013) *InfoStat versión 2013*. FCA, Universidad Nacional de Córdoba, Argentina, Grupo InfoStat.

Rodríguez R. (2011) Anacardiaceae. In: Rodríguez R., Marticorena C. (Eds), *Flora de Chile*. Ediciones Universidad de Concepción, Concepción, Chile, pp 88–103.

Rohfritsch O. (1992) Patterns in gall development. In: Shorthouse J. D., Rohfristsch O. (Eds), *Biology of insect-induced galls*. Oxford University Press, New York, USA, pp 60–86.

Rohfritsch O., Anthony M. (1992) Strategies on gall induction by two groups of homopterans. In: Shorthouse J. D., Rohfristsch O. (Eds), *Biology of insect-induced galls*. Oxford University Press, New York, USA, pp 102–123.

Sáiz F., Núñez C. (1997) Estudio ecológico de las cecidias del género *Schinus*, especialmente las de hoja y de rama de *Schinus polygamus* y *S. latifolius* (Anacardiaceae), en Chile Central. *Acta Entomológica de Chile*, **21**, 39–59.

Sáiz F., Núñez C. (2000) Cecidias de hoja y de rama de *Schinus polygamus* (Cav.) Cabrera (Anacardiaceae): doble agente formador o secuencia temporal de cecidias formadas por huéspedes diferentes? *Revista Chilena de Entomología*, **27**, 57–63.

Salisbury F.B., Ross C.W. (1992) *Plant Physiology*. Wadsworth, Belmont, CA, USA, p 682.

Shorthouse J.D., Rohfritsch O. (1992) *Biology of insect-induced galls*. Oxford University Press, New York, USA, p 285.

Shorthouse J.D., Wool D., Raman A. (2005) Gall-inducing insects – nature's most sophisticated herbivores. *Basic and Applied Ecology*, **6**, 407–411.

Stone G.N., Schönrogge K. (2003) The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution*, **18**, 512–522.

Trabelsi N., Falleh H., Jallali I., Daly A.B., Hajlaoui H., Smaoui A., Abdelly C., Ksouri R. (2012) Variation of phenolic composition and biological activities in *Limoniastrum monopetalum* L. organs. *Acta Physiologiae Plantarum*, **34**, 87–96.

Van Asch M., Tienderen V., Peter H., Holleman L.J., Visser M.E. (2007) Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*, **13**, 1596–1604.

Weis A.E., Walton R., Crego C.L. (1988) Reactive plant tissue sites and the population biology of gall makers. *Annual Review of Entomology*, **33**, 467–486.

Wool D. (2004) Galling aphids: specialization, biological complexity, and variation. *Annual Review of Entomology*, **49**, 175–179.

Wool D., Aloni R., Ben-Zvi O., Wollberg M. (1999) A galling aphid furnishes its home with a built-in pipeline to the host food supply. *Entomologia Experimentalis et Applicata*, **91**, 183–186.

Yukawa J. (2000) Synchronization of gallers with host plant phenology. *Population Ecology*, **42**, 105–113.

Yukawa J., Akimoto K. (2006) Influence of synchronization between adult emergence and host plant phenology on the population density of *Pseudasphondylia neolitseae* (Diptera: Cecidomyiidae) inducing leaf galls on *Neolitsea serícea* (Lauraceae). *Population Ecology*, **48**, 13–21.