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# Leaf and stem galls of *Schinus polygamus* (Cav.) Cabr (Anacardiaceae): Anatomical and chemical implications

Lubia M. Guedes [a], Narciso Aguilera [b], José Becerra [a], Víctor Hernández [a], Rosy M. dos Santos Isaias [c, *]

[a] Departamento de Botánica, Universidad de Concepción, Facultad de Ciencias Naturales y Oceanográficas, Casilla 160-C, CP 4030000, Concepción, Chile
[b] Departamento de Silvicultura, Facultad de Ciencias Forestales, Universidad de Concepción, Casilla 160-C, CP 4030000, Concepción, Chile
[c] Departamento de Botânica, Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Av. Antônio Carlos, 6627, Pampulha, 31270-090, Belo Horizonte, Minas Gerais, Brazil

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ABSTRACT

Galling insects commonly induce anatomical and metabolic changes in their host plant tissues, which is true for *Schinus polygamus* (Cav.) Cabr. (Anacardiaceae) in Chile. Currently, anatomical and chemical changes induced by galling insects in stems and leaves of *S. polygamus* were analyzed. Methanolic extracts of non-galled and galled tissues were analyzed by gas chromatography–mass spectrometry (GC-MS). Differences in the secondary metabolite profiles, and their relation with plant responses to gall development were evidenced. Transverse sections of non-galled host organs and galls were done, and observed under light and scanning electron microscopies. One stem gall (conical) and one leaf gall (globoid) morphotypes were identified. The globoid and conical galls have dense trichomes, large nymphal chambers, and develop mainly by tissue hyperplasia. The chemical profiles of stems, leaves and galls are distinct, except for the concomitant detection of pyrogallol in galls. The highest abundance of terpenes and phenols in gall tissues were identified, and two triterpenes were firstly reported for the non-galled tissues of *S. polygamus*. Host plant tissues are highly responsive to the Psyllidae stimuli toward the over development of a phenolic-rich parenchyma, which ends up favouring the *Callophya* sp. establishment and gall development.



## 1. Introduction

*Schinus polygamus* (Cav.) Cabr (Anacardiaceae), commonly known as hardee peppertree, is an aromatic and medicinal shrub native to Argentina, Bolivia, Uruguay, Brazil and Chile (Rodríguez, 2011). In some South American countries, such as Chile and Brazil, *S. polygamus* presents numerous phytosanitary problems caused by herbivorous and pathogenic insects (Damasceno et al., 2010). This species also have been reported as a host of a diversity of galling insects (Sáiz and Núñez, 1997) capable of inducing galls on leaves, branches, and flowers (Sáiz and Núñez, 1997; Burckhardt and Basset, 2000). Accordingly, *S. polygamus* can be defined as a super-host of galling insects (Dias et al., 2013a), which may be Diptera (Cecidomyiidae), Hemiptera (Psylloidea), and Lepidoptera (Cecidosidae) (Sáiz and Núñez, 1997; Burckhardt and Basset, 2000).

* Corresponding author.
*E-mail address:* rosy@icb.ufmg.br (R.M.S. Isaias).

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Galls are specialized plant structures induced by a parasite organism, usually an insect, which alters the normal developmental patterns of plant tissues (Felt, 1940). The galling organisms induce anatomical and metabolic alterations, probably, in response to secretions injected by the larvae during the feeding activity or by the female during oviposition (Dias et al., 2013a). The galling insects are capable of inducing morphogenetic changes in their host plants towards obtaining food or/and shelter (Rohfritsch, 1992). Cell hypertrophy, tissue hyperplasia, inhibition of some developmental programs and cytological changes occur during the development of galls (Ferreira and Isaias, 2013). Moreover, gall-inducing herbivores have the ability to manipulate plant tissues growth and development for their own benefit, and can also manipulate their chemical composition (Moura et al., 2008; Dias et al., 2013b). This chemical manipulation redirects plant cell responses towards the determination of tissues with higher nutritional quality, the inner nutritive tissues, and tissues rich in defensive compounds, the outer tissue layers, which may help in protecting the galling insects against their natural enemies (Stone and Schönrogge, 2003; Formiga et al., 2009).

Galls usually contain a large amount of nutrients and low concentrations of defensive compounds (Hartley, 1999; Isaias et al., 2014). Nonetheless, some variations in this general pattern may occur, and galls may contain higher concentrations of defensive compounds in comparison to the non-galled tissues of their host plants (Hartley, 1999). The chemical profile of *S. polygamus* tissues revealed leaf volatile compounds synthesized in response to the attack of herbivores (Valladares et al., 2002; Damasceno et al., 2010). The composition of such volatiles varies between non-galled leaves and galls (Damasceno et al., 2010), mostly in relation to mono and sesquiterpenes. However, there is a lack of information about non-volatile secondary metabolites (SM), as well as their functions in galled tissues of *S. polygamus*. Also, studies on the structural profile due to galling stimuli on *Schinus* species are restricted to *Callophya duvauae* Scott-*S. polygamus* system (Dias et al., 2013a), and a phytochemical profile of this species in galled and non-galled conditions has yet to be determined. Herein, we compare the structural and chemical potential of two distinct host plant organs, the stems and leaves of a single species, *S. polygamus*, to respond to two different galling herbivores stimuli, and focus on the following questions: (i) Are there structural traits of the host organs potentiated towards the development and survival of the galls? (ii) Do the chemical profiles indicate investment in chemical defensive strategies in galls? (iii) Are the galling insects associated to stems and leaves capable of inducing convergent responses on their host organs?

## 2. Materials and methods

### 2.1. Study area and processing of plant material

Branches of *S. polygamus* were sampled at the town of Chillán Viejo, kilometer 4, Ñuble Province, Biobío Region (36°39′32″S 72°16′43″W), Chile. Plant species identification was confirmed by specialists from the Department of Botany of the University of Concepción (CONC). A voucher specimen was deposited in CONC under the accession number 180330.

The branches were visually inspected, with a magnifying glass to detect the presence of galls. Gall morphotypes were classified and described according to Isaias et al. (2013).

### 2.2. Structural analyses

For studies in scanning electron microscopy (SEM), samples of leaf and stem galls were fixed in 2.5% glutaraldehyde in sodium phosphate buffer, pH 7.2, at 4 °C, for 24 h. The samples were washed twice in 0.1 M phosphate-buffered saline (PBS) for 10 min, post-fixed in 1% osmium tetroxide in 0.1 M PBS, at 4 °C, for 2 h, and washed with the same buffer twice for 10 min. Fragments were dehydrated in 30–100% ethanol series and were dehydrated a second time in liquid $CO_2$ by a critical point dryer (Balzers® Union FL-9496, Holland) (O'Brien and Mccully, 1981). The sections were mounted on aluminum stubs with carbon film, and metalized in gold (approximately 400 Å) in a Sputter Coater (Edwards® S 150, U.S.) for 3 min at 30 mA. Observations were done under a scanning electron microscopy (JEOL® JSM - 6380 LV, Japan).

For the studies in light microscopy (LM), leaf and stem galls were fixed in 4% Karnovsky in 0.1 mM phosphate buffer for 24 h (O'Brien and Mccully, 1981; modified to pH 7.2) and stored in 70% ethanol. For the preparation of permanent slides, fragments were dehydrated in a 50–100 *n*-butyl series, and embedded in Paraplast® (Kraus and Arduin, 1997). Serial sections (12–18 μm) were obtained in a rotary microtome and stained in 0.5% safranin-astra blue (9:1) (Kraus and Arduin, 1997). The slides were observed and photographed under a light microscope (Leica® ICC50 HP).

### 2.3. Methanolic extraction and identification of chemical compounds

Non-galled stems and leaves, and stem and leaf galls were isolated and macerated with 100% methanol for 7 days, at room temperature. The non-galled stem bark was removed prior to methanol extraction. Methanol extracts were filtered through Whatman® No 1 filter paper, and undergo the identification process of secondary metabolites (SM).

The extracts were concentrated at a reduced pressure with a rotary evaporator until completely dry. Subsequently, the crude extract was sequentially extracted with *n*-hexane, ethyl acetate, and distilled water. The hexanic and ethyl acetate extracts were concentrated again at a reduced pressure in a rotary evaporator, diluted in ethyl acetate, and monitored by thin-layer chromatography (TLC). For the identification of chemical compounds in each fraction, these extracts were subjected to gas chromatography coupled with mass spectrometry (GC-MS) in the Agilent® 7890A equipment, with the Agilent® 5975C

mass detector, using a capillary column of fused silica type HP5-MS, 30 m, 0.25 mm internal diameter, and 0.25 μm thick, 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. Flow of carrier gas (electronic grade helium) at 1 mL/min. The characterization was carried out by means of comparison with the NIST® database.

## 3. Results

### 3.1. Features of stem and leaf galls

Leaves and branches of *S. polygamus* are infested by gall-inducing insects (Fig. 1A), which can be recognized by two gall morphotypes, a stem and a leaf gall. Stem galls are conical, dark brown, and have a bunch of trichomes towards the apical portion (Fig. 1B). Sometimes several galls may coalesce, but each gall has one larval chamber. Leaf galls are globoid, red, with the aperture located at the tip of an abaxial projection surrounded by abundant trichomes (Fig. 1C). There is a depression on the adaxial surface of leaf lamina, which is green (Fig. 1D). The globoid galls are isolated, and may vary from a single to many galls on the same leaf lamina.

The nymphal chambers, both of stem conical and of leaf globoid galls, are large, surrounded by concentric layers of cells (hyperplasia) (Fig. 2A–D). The shape of the nymphal chamber is different in the two gall morphotypes, round-shaped in leaf galls (Fig. 2A–B); and elongated in stem galls in a funnel shape that narrows towards the opening area of the gall and is wider towards the gall inner portion (Fig. 2C–D). Three tissue layers are observed in stem galls (Fig. 2D–E), while in leaf gall a homogeneous tissue is found (Fig. 2B). Moreover, the presence of trichomes was revealed in the opening area of stem galls,

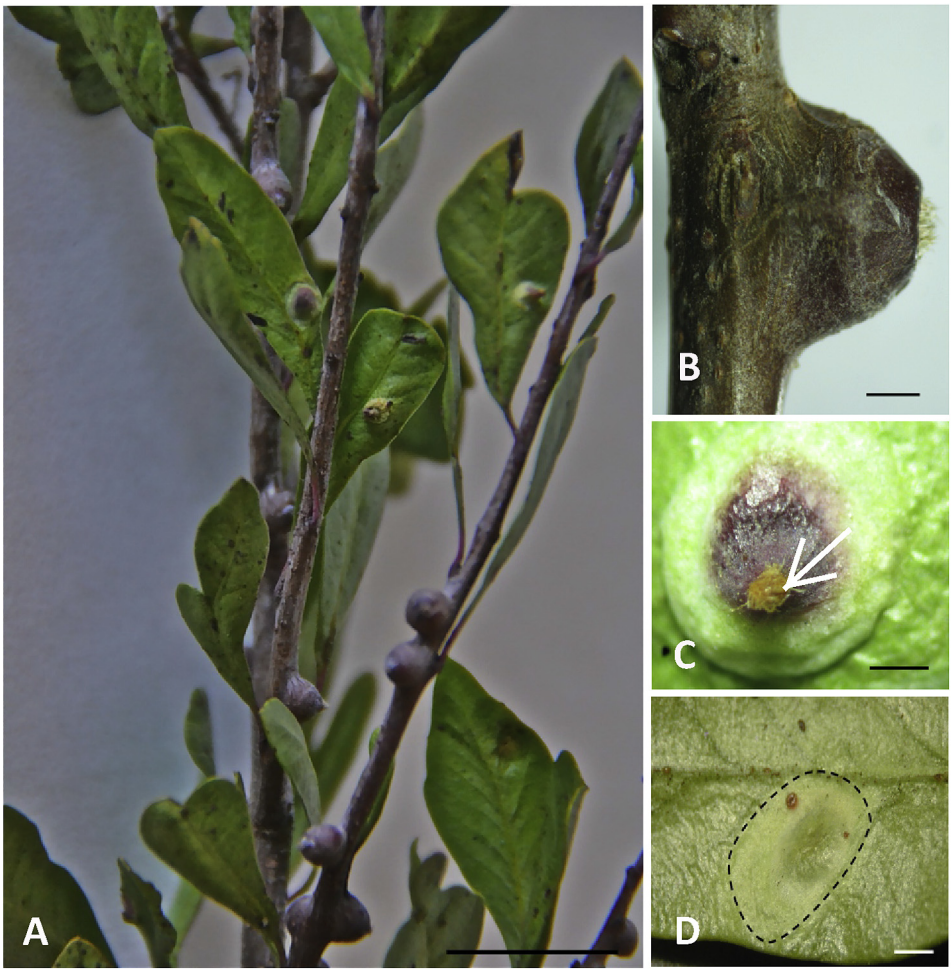


**Fig. 1.** Galls of Hemiptera (Psyllidae: Calophyidae) on *Schinus polygamus* (Cav.) Cabr. (Anacardiaceae). A: Branches with leaf and stem galls. B: Detail of a conical stem gall. C–D: Details of leaf globoid galls, C: Abaxial surface view evidencing the projection with trichomes on gall aperture (arrow), D: Adaxial view (dotted area). Bars: B, C, D = 1 mm; A = 1 cm.

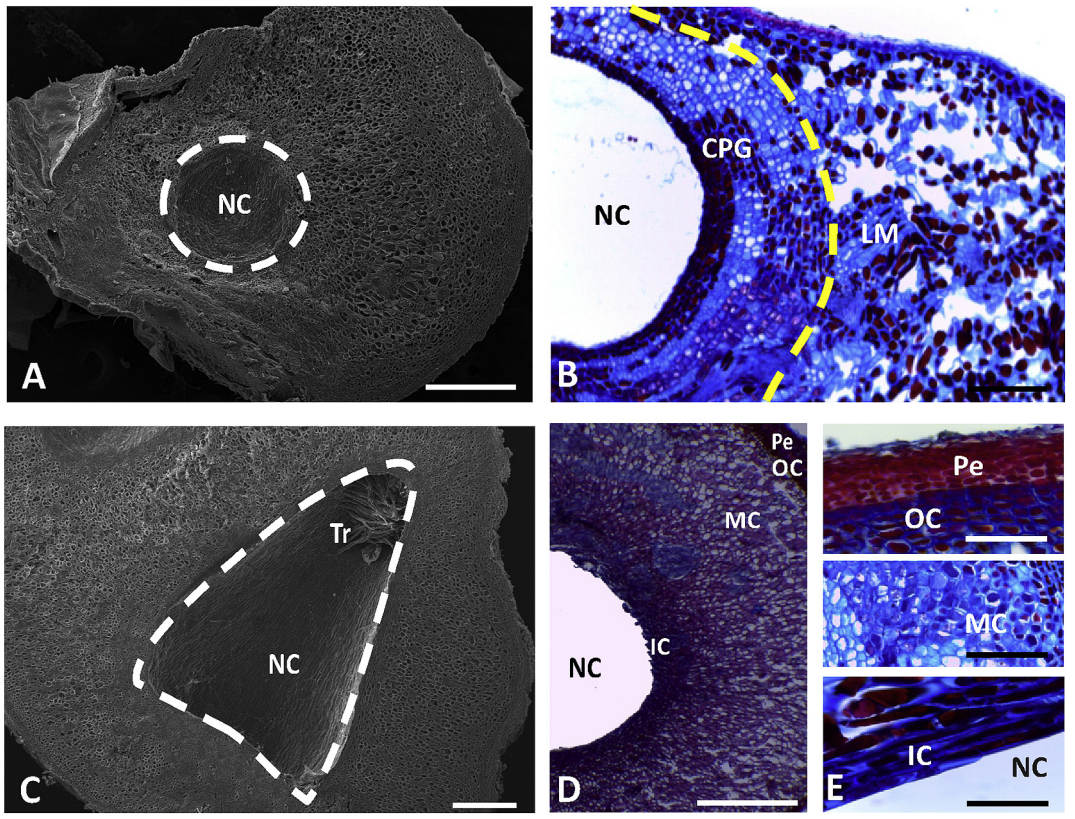


**Fig. 2.** Leaf and stem galls on *Schinus polygamus* (Cav.) Cabr. (Anacardiaceae). A–B: Leaf gall showing tissue hyperplasia and round nymphal chamber in SEM preparation (A), and LM preparation evidencing the homogeneous parenchyma (B). C–E: Stem gall with tissue hyperplasia and elongated nymphal chamber in SEM preparation (C) where trichomes in gall aperture can be seen, and LM preparation where the three tissue layers are evidenced (D) and details of each three tissue layers (E). Bars: E = 50 μm; B, D = 200 μm and A, C = 500 μm. Abbreviations: NC: nimphal chamber; CPG: cortical parenchyma of gall; LM: leaf mesophyll, Tr: trichomes; IC: internal cortex; MC: middle cortex; OC: outer cortex; Pe: periderm.

which is projected inwards (Fig. 2C). In both galls, each chamber hosts a single inducing insect, belonging to the superfamily Psyllidae (family Calophyidae), presumably to the genus *Calophya*.

### 3.2. Chemical profile of secondary metabolites in host organs and galls

The chemical profile of the non-galled host organs and both galls are distinct (Table 1), except for the concomitant detection of pyrogallol both in stem and leaf galls, which represents the unique similarity in new synthesis of molecules due to galling stimuli. The SM of the family of phenols corresponds to 50% and 25% of the total diversity of molecules in leaf and stem galls, respectively. Stem galls maintain the presence of steroids also detected in non-galled stems, but with differences in the chemical structure of such metabolites.

Generally, the predominating compounds in *S. polygamus* are terpenes (61.9%), mainly sesquiterpenes. In overall analyses, the non-galled stems and leaves have terpenes but with differences in their chemical structures. The non-galled stems have the greatest diversity of sesquiterpenes, which are not detected in non-galled leaves, and are not conservative in galls, as well. Current analyses detected α-amyrin and ursenal triterpenes for the first time in *S. polygamus*.

The uniqueness of the chemical profile of leaf galls is represented by the detection of nitrogen compounds, aromatic hydrocarbon benzofuran and quinone; while the uniqueness of the chemical profile of stem galls is represented by the detection of fatty acid esters.

## 4. Discussion

### 4.1. Structural traits and gall survival

Gall morphological and anatomical characteristics are usually related to protective mechanisms against unfavorable environmental conditions, especially desiccation (Stone and Schönrogge, 2003) and natural enemies of the galling insects (Rohfritsch, 1992; Oliveira et al., 2006; Oliveira and Isaias, 2010). For example, trichomes are related to mechanical protection

**Table 1**
Biologically important secondary metabolites identified in extracts of non-galled leaves, non-galled stems, and leaf and stem galls of *Schinus polygamus* (Cav.) Cabr. (Anacardiaceae).

| | Compound | Relative Peak Area (%) | Chemical Family |
|---|---|---|---|
| Non-galled leaves | α-Amyrin | 11.7 | Triterpene |
| Leaf galls | 1,2,3-Benzenetriol (pyrogallol) | 12.33 | Phenol (tannins) |
| | 6H-Purin-6-one, 1,7-dihydro-2-(*N*-methyl guanine or hypoxanthine) | 5.23 | Nitrogen compound |
| | Ethanone, 1,1'-(6-hydroxy-2,5-benzofurandiyl)bis (euparone) | 8.94 | Aromatic hydrocarbon benzofuran |
| | Anthraquinone, 1-(methoxyphenyl) (1-methyl phenyl -anthraquinone) | 10.66 | Phenol (Quinone) |
| Non-galled stems | α-Bergamotene | 2.43 | Sesquiterpene |
| | Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (7-epi-α cadinene) | 6.32 | Sesquiterpene |
| | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8aα)]-(eremophylene) | 5.75 | Sesquiterpene |
| | Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-(Δ cadinene) | 7.0 | Sesquiterpene |
| | Tricyclo[4.4.0.02,7]dec-3-ene-3-methanol,1-methyl-8-(1-methylethyl)-(15- copaene) | 12.91 | Sesquiterpene |
| | Pregn-4-ene-3,20-dione, 18,21-dihycroxy (4-eno-18,21-dihidroxi-3,20-diona) | 7.94 | Steroidal |
| | Urs-12-ene-28-al (ursenal) | 8.44 | Triterpene |
| | Copaene | 4.71 | Sesquiterpene |
| | Cyclohexene, 1-methyl-4-(5-methylene-4-hexenyl)-, (S) | 3.05 | Sesquiterpene |
| | Spathulenol | 6.29 | Sesquiterpene |
| | 6-isopropenyl-4-8a-dimethyl-1,2,3,5,6,7,8,8a-octohydro-napthalen-2-ol | 5.40 | Sesquiterpene |
| | 1-piperideneacetonenitrile | 3.43 | Amine |
| Stems galls | 1,2,3-Benzenetriol (pyrogallol) | 25.22 | Phenol (tannins) |
| | Tridecanoic acid, methyl ester (myristic acid methyl ester) | 24.62 | Fatty acid ester |
| | 9, 12, 15-Octadecatrienoic ac, methyl ester (methyl linoleate) | 30.80 | Fatty acid ester |
| | Ergosta-4,6,22-trien-3[a]-ol (ergosta trienol) | 3.06 | Steroidal |

against invading organisms (predators and parasitoids) and to stabilization of temperature and humidity inside the nymphal chamber (Oliveira et al., 2006; Álvarez et al., 2009).

Trichomes in stem and leaf galls of *S. polygamus* along with the hyperplasia of cortical parenchyma can protect the galling insect both against natural enemies and unfavorable environmental conditions. Enhance in trichomes differentiation in leaf galls can represent an overpotentialization of the ordinary morphogenetical pattern of the host plant tissues for trichomes are rarely observed in leaves of *S. polygamus* (Dias et al., 2013a). Such manipulation of host plant morphogenesis has also been induced by *C. duvauae* in leaf galls *S. polygamus* (Dias et al., 2013a), *Euphalerus ostreoides* Crawf-*Lonchocarpus muhelbergianus* Hassl (Oliveira et al., 2006), and *Baccharopelma dracunculifoliae* Burckhardt on *Baccharis dracunculifolia* DC (Arduin et al., 2005), in Brazil. The presence of trichomes was reported, too, for galls induced by *Calophya mammifex* Burckhardt & Basset on *Schinus longifolius* (Lindl.) Speg., and has also been interpreted as a mechanical defence mechanism (Agudelo et al., 2013).

For stem galls, the trichomes differentiate in the dermal system of the nymphal chamber, which originated from the folding of the stem epidermis towards the inner gall surface. Even though the mother cells of a phellogen are present close to the nymphal chamber, suber differentiation does not occur. As a consequence of the spatial replacement, trichoblasts differentiate from cells previously determined to be discharged by suberization. Moreover, the parenchyma cells, which originate the cork cambium beneath the epidermis, turn into adult stem cells (Sugimoto et al., 2011). Their differentiated state is likely to follow internal (developmental) and external signals (stress) that force such cells to redifferentiate to become competent for switching their fates (Lev-Yadun, 2003). Redifferentiation processes are uncovered by multiple phenomena in plants (Lev-Yadun, 2003), and in galls, plant cells under the influence of gall-inducing insects have their competence reprogrammed, and often assume rapid cell cycles and new cell fates for the neo-ontogenesis of plant galls (*cf.* Carneiro et al., 2014).

Both stem and leaf galls show hyperplasia of cortical parenchyma, a typical process of gall formation (Oliveira and Isaias, 2010; Moura et al., 2009a), which is one of the most common processes in various Hemiptera galls (Isaias et al., 2011; Dias et al., 2013a, b; Formiga et al., 2015). Hyperplasia is induced in young tissues, which have a greater capacity for cell division, and may respond promptly to the stimuli of the gall inducers (Rohfritsch, 1992). Such process results in increased biomass in galls, and can confer a high number of nutritive cells, as well as thickens gall wall, which ends up mechanically protecting the galling insect against unfavorable environmental conditions and natural enemies attack (Stone and Schönrogge, 2003).

### 4.2. Chemical defence strategies

The chemical profile of the non-galled host plant organs is not maintained at gall developmental sites. The phenolics - pyrogallol - were the only secondary metabolite (SM) accumulated both in leaf and stem galls. The triterpene α-amyrin

detected in non-galled leaves does not accumulate in leaf galls, otherwise, accumulated metabolites which enhanced their nutritional value (nitrogen compounds), but also their anti-microbial activity, such as the benzofuran (Nascimento et al., 2004) and the quinone. The accumulation of sesquiterpenes, common in non-galled stems, is not detected in stem galls, and may indicate the use of fatty acid precursors also to increase the nutritional value of gall tissues, as proposed by Tooker and de Moraes (2009) for *Gnorimoschema gallaesolidaginis* Riley galls on *Solidago altissima* L.

The chemical accumulation of SM, especially phenolics, has been usually related to their defensive role (Nyman et al., 2000; Agudelo and Ricco, 2012). Eventually the SM have also been associated with the success of the galling insects, as observed for *E. ostreoides-L. muhelbergianus* (Oliveira et al., 2006) and for Cecidomyiidae-*Aspidosperma spruceanum* Müell. Arg. systems (Formiga et al., 2009).

The phenolic compounds were the major SM detected in leaf and stem galls of *S. polygamus*. Particularly, pyrogallol (tannins) was recognized both in globoid leaf galls and in the conical stem galls. This compound has antiseptic, antioxidant, fungicide, and insecticide properties (Balasubramanian et al., 2014), so it is also believed to enhance the galls chemical protection against natural enemies.

Another phenolic compounds detected in leaf galls belong to the quinone family. Experimental evidences suggest that sedentary insects may stimulate a defensive mechanism based on the oxidation of phenols to quinones in plant tissues (Miles and Oertli, 1993). However, herbivores that feed on tannin-rich plant material, as the leaf galls in current results, seem to possess some chemical adaptation to remove tannins from their digestive systems, as proposed by Taiz and Zeiger (2006). We infer that psyllids have developed oxidation mechanisms of quinone to non-toxic polymers, and overcome the defensive mechanism of their *S. polygamus* host plants. Also, the deterrent effect of quinones (Nyman and Julkunen-Tiitto, 2000) could improve the chemical microenvironment and its effect against natural enemies of the galling insect, which do not have the above mentioned detoxification mechanism.

The terpenes, play controversial ecological and physiological roles in plant-insect relationships (Rand et al., 2014), which are virtually poorly explored (Rostás et al., 2013). The terpenes are probably involved in the defense against pathogens and predators of galling insects (Rostás et al., 2013) or as attractants for gall natural enemies, such as the parasitoids (Tooker and Hanks, 2006).

The α-bergamotene and the spathulenol are sesquiterpenes identified exclusively in non-galled stems. The α-bergamotene was previously identified as traces (<1%) in leaves of *S. polygamus*, and may possibly attract predators of free-living herbivores, and can alternatively protect gall tissues against herbivores (Damasceno et al., 2010). Such an effect was found in the leaves of *Nicotiana attenuate* Steud under the attack of *Manduca quinquemaculata* Haworth, whose oral secretion can stimulate the production of α-bergamotene, which attracts a predator insect (Kessler and Baldwin, 2001). The spathulenol is part of the chemical composition of the essential oil in many plants, and has been detected in leaf extracts of *Melampodium divaricatum* Rich. and *Conyza albida* Willd. ex Sprengel, where it was related to repellency against ants and to antimicrobial activity, respectively (Hubert and Wiemer, 1985; Pacciaroni et al., 2000). Based on experimental evidences (Hubert and Wiemer, 1985; Pacciaroni et al., 2000; Damasceno et al., 2010), we propose that the spathulenol in the non-galled stems of *S. polygamus* can function as a chemical defense against natural enemies, as well.

Current analyses detected two SM in *S. polygamus* for the first time, the α-amyrin, detected just in non-galled leaves, and the ursenal, detected exclusively in non-galled stems. The α-amyrin has been reported in leaves of medicinal and oleo-resinous plants, including some species of the genus *Schinus* (Lloyd et al., 1977; Frontera and Tomas, 1994). However, the α-amyrin has not been previously detected in leaves and fruits of *S. polygamus* examined in Chile (Erazo et al., 2006), Argentina (González et al., 2004), and Brazil (Damasceno et al., 2010). Likewise, ursenal is another triterpene that has not been reported for this plant genus or species (Murray et al., 2012). Both, α-amyrin and ursenal, are related to important biological functions against various health-related conditions, as inflammation, microbial, fungal, and viral infections and cancer cells (Liu, 1995; Hernández et al., 2012). The presence of these SM in the non-galled tissues of *S. polygamus*, support the folk medicinal uses of this species, such as the antipyretic, anti-inflammatory and analgesic activities of the aerial parts of the plant (Erazo et al., 2006), but seems to be impaired by gall induction.

### *4.3. Convergent and divergent adaptive traits in the two types of galls*

The structural analyses of the stem and leaf galls of *S. polygamus* demonstrated the typical anatomical profiles of the Anacardiaceae and *Schinus* species (Álvarez et al., 2009; Agudelo and Ricco, 2012; Agudelo et al., 2013; Dias et al., 2013a). Nevertheless, the structural and chemical profile of both host organs is quite distinct, and some convergent similarities were observed in response to the stimuli of their associated galling herbivores. Trichomes, hyperplasia of cortical parenchyma, and accumulation of phenolics were convergently induced by galling stimuli.

The overdifferentiation of trichomes and the hyperplasia of cortical parenchyma could be associated with the feeding mouth apparatus of the psyllids (piercing stylets) (Burckhardt, 2005). Such morphological features are independent of the galling herbivore taxa, but presumably associated with their feeding habits. Dias et al. (2013a) described a convergence in tissue composition of *C. duvauae* galls on *S. polygamus* and those of *A. lantanae* on *L. camara*, both with piercing stylets. The mode of feeding and the number of galling herbivores per chamber should influence cell responses and consequently the generation of distinct gall morphotypes (Isaias et al., 2014).

However, not only the feeding habits of the gall-inducing insects are crucial to the establishment of plant cell responses, but the morphogenetic potentialities and constraints of the host plant tissues are also decisive (Isaias et al., 2014). The

interactive signaling both from the galling insects and from the host plants seems to cause a gradient of stimuli (Oliveira and Isaias, 2010; Carneiro et al., 2014). This gradient may trigger the differences in the external and internal morphological features of both the stem and leaf galls on *S. polygamus*, despite of the fact that they are induced by psyllids with the same feeding habits and on the same host plant species.

The psyllid induces leaf and stem galls on *S. polygamus* (Sáiz and Núñez, 1997; Burckhardt and Basset, 2000) with different morphotypes (color and shape), nymphal chamber and organization of cortical parenchyma. Phylogenetic analyses of aphids (Stern, 1995), gall wasps (Stone and Cook, 1998), thrips (Crespi et al., 1997), and sawflies (Nyman et al., 2000) report that the galling insects, and not the host plants, determine the location, size, and shape of the galls. In *Lantana camara* L., two leaf galls, induced by different galling agents: *Aceria lantanae* Cook and *Schismatodiplosis lantanae* Rubsaamen induces distinct plant tissues reorganization, and forms typical gall structures (Moura et al., 2008), which reinforces such premise. Nevertheless, even though the influence of the host plant or host plant organs potentialities is commonly neglected, stems are considered to host simpler galls than leaves (Formiga et al., 2015). Despite, in current model of study, stem and leaf galls are anatomically similar, and the differences observed could be determined by the potentialities of the host plant organs, once the mode of feeding of both galling insects is similar. Our results support the hypothesis that the inducing agent as well as the potentialities and constraints of the host plant morphogenesis are responsible for the determination of gall morphotypes.

Also, current results indicate the accumulation of steroids as the only chemical convergent feature maintained from non-galled condition toward stem galls. Plant cells synthesize a complex array of sterol mixtures, which has essential roles at the cellular level (Hartmann, 1998). On the other hand, phenolics accumulation both in leaf and stem galls is the only remarkable chemical convergence, probably linked to the influence of the psyllids on *S. polygamus*.

## Author contributions

LMGG designed the experiment, performed analyses and wrote the manuscript. NAM, JBA, VHS performed analyses and revised the manuscript. RMSI designed the experiment and wrote the manuscript.

## Competing interests

The authors declare they have no competing interests.

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