STRUCTURE OF INSECT GALLS ON TWO SYMPATRIC SUBSPECIES OF *CHRYSOTHAMNUS NAUSEOSUS* (PALL. EX PURSH) BRITTON (ASTERACEAE)[1]

JANE ELIZABETH KRAUS*, ROSY MARY DOS SANTOS ISAIAS**, CLAUDIA VECCHI* & GERALDO WILSON FERNANDES***

*Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo. Caixa Postal 11461, 05422-970, São Paulo, SP, Brasil.
**Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 486, 31270-901, Belo Horizonte, MG, Brasil.
***Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 486, 31270-901, Belo Horizonte, MG, Brasil.

Abstract – (Structure of insect galls on two sympatric subspecies of *Chrysothamnus nauseosus* (Asteraceae). The structure of eight insect galls was studied on two sympatric rabbitbrush, *Chrysothamnus nauseosus* subsp. *consimilis* and *C. nauseosus* subsp. *hololeucus* (Asteraceae). In this system 3 galls are specific to one of the host subspecies, 3 are specific to the other subspecies, and 2 are common to both subspecies. Galls are caused by Cecidomyiidae and Tephritidae, and are formed in shoot apical meristem, axillary buds, nodes and internodes of the host plants. Trichomes, secretory structures, and sclerenchyma occurred in several of the studied galls. These structures may represent protective and/or defensive strategies against the natural enemies of the gall maker. Seven of the studied galls presented nutritive tissue whose cells were similar to meristematic or parenchymatic cells. Nutritive tissue was absent in one ambrosia gall. Analyses of galls developed in these phylogenetically closely related plants led to the conclusion that cecidogenous responses are influenced by a specific parasitic action and directed by the stage of plant cell differentiation by the time of induction.
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Resumo - (Estrutura de galhas causadas por insetos em duas subespécies simpátricas de *Chrysothamnus nauseosus* (Asteraceae). Foi estudada a estrutura de oito galhas causadas por insetos em duas subespécies simpátricas, *Chrysotamnus nauseosus* subsp. *consimilis* e *C. nauseosus* subsp. *hololeucus* (Asteraceae). Neste sistema, 3 galhas são específicas para uma das subespécies, 3 para a outra e 2 são comuns a ambas as subespécies. As galhas são causadas por Cecidomyiidae e Tephritidae, ocorrendo no ápice caulinar, nas gemas axilares, nos nós e entrenós das plantas hospedeiras. Tricomas, estruturas secretoras e esclerênquima foram observados em várias das galhas estudadas e podem representar estratégias protetoras e/ou defensivas contra os inimigos naturais do galhador. Sete das galhas estudadas apresentaram tecido nutritivo, cujas células podem ser semelhantes às meristemáticas ou então mostrar características de células parenquimáticas. Tecido nutritivo não ocorreu na única galha de ambrosia. A análise de galhas desenvolvidas em plantas filogeneticamente relacionadas possibilitou concluir que as respostas cecidogênicas estão sob influência da ação específica do parasita e são direcionadas pelo estágio de diferenciação da célula vegetal no momento da indução.

Key words: Insect galls, *Chrysothamnus nauseosus*, Cecidomyiidae, Tephritidae, Asteraceae

Introduction

Specific interactions between animals and plants are very common in nature. Among these interactions, those of galling insects and their host plants are believed to be the most intimate (Mani 1964, 1992, Mattson *et al.* 1988, Fernandes 1990). Most gall-forming insects are highly host and organ specific, that is, they induce galls on only one species or a closely related group of host species (Dreger-Jauffret & Shorthouse 1992). Many galling insects are also capable of discriminating among several hosts where morphology and genotypes are only slightly distinguishable. The specificity of some species is so strong that researchers can benefit from the knowledge of abundance and frequency of gall types to separate hybrids from their parents (McArthur *et al.* 1979, Dodson 1991, Floate & Whitham 1995, Floate *et al.* 1996, Abrahamson *et al.* 1998). Floate *et al.* (1996) described three groups of sympatric host plants that included *Chrysothamnus nauseosus* (Pall. *ex* Pursh) Britton subsp. *consimilis* (Greene) H.M. Hall & Clem., *C. nauseosus* subsp. *hololeucus* (A. Gray) H.M. Hall & Clem., and their putative offspring by gall specificity and abundance in the field. In this study, the plant

genotypes were identified by several plant traits (architecture, leaf and stem color, plant length and width, stem length and diameter, leaf width and length) as well as gall abundance and richness of morphotypes. Some galls in this group of plants, are specific to one of the host subspecies, some are specific to the other subspecies, some are common to both species, and a fourth group is primarily found on the offspring of these two subspecies.

Entomogenous galls are pathological structures originated from neoformed tissues, as a result of mechanical and/or chemical insect stimuli. During gall development structural modifications occur with formation of cecidogenetic tissues. There is generally a nutritive tissue that lines the larval chamber whose cells show characteristics of high physiological activity suitable to the parasite's nutrition (Bronner 1992).

The intimate association between gall morphology and galling insect taxa has also been reported many times in the literature (Mani 1964, Meyer & Maresquelle 1983, Meyer 1987, Mattson *et al.* 1988, Fernandes 1990, Dreger-Jauffret & Shorthouse 1992, Fernandes *et al.* 2000). It is known that in some systems gall morphology and structure varies enormously according to the galling taxa, while in other

systems most of the morphogenetic responses to the galling insect are directed by the host plants (Price et al. 1987, Dodson 1991, Dreger-Jauffret & Shorthouse 1992, Isaias 1998).

Therefore, the understanding of the variation in gall morphology within and among host taxa is very important. The present study comparatively describes galls on Chrysothamnus nauseosus subsp. consimilis and C. nauseosus subsp. hololeucus, and investigates whether or not the cecidogenous responses are directed by the host plants.

Materials and methods

Galled and ungalled shoots of Chrysothamnus nauseosus subsp. consimilis and C. nauseosus subsp. hololeucus were collected in Coconino National Forest, Schultz Pass (U.S. Forest Service Road 420, 2.4 km from Highway I-89), north of Flagstaff, Arizona at an elevation of 2.400 m above sea level.

Samples of galled and ungalled shoots were fixed in FAA (37% formaldehyde: acetic acid: 50% ethanol, 1:1:18, v/v) (Johansen 1940) for anatomical studies. Ungalled leaves and stems were freehand sectioned and the histological sections were stained with 0,5% astra blue and 0,1% basic fuchsin (Kraus et al. 1998). Galled shoots were dehydrated through ethanol series, embedded in historesin (Reichert-Jung) and, sectioned at 5-10 μm with a rotary microtome. The sections were stained with 0,5% toluidine blue O containing 0,1% carbonate calcium, pH 11 (O'Brien & McCully 1981).

Some samples were prepared for scanning electron microscopy (SEM) analysis. They were dehydrated through cetonic series, CO_2 critical point dried, and coated with gold (Silveira 1989).

Results

Anatomy of the ungalled leaf and stem

Ungalled leaves of Chrysothamnus nauseosus subsp. consimilis and C. nauseosus subsp. hololeucus were isobilateral (Fig. 1A, B, E-G) and amphistomatic (Fig. 1B, G) in transverse section. Stomata were anomocytic (Fig. 1C). The epidermis was uniseriate (Fig. 1A, B, E-G) bearing different trichome types (for detail see Fig. 2). The cuticle of C. nauseosus subsp. consimilis (Fig. 1A, B, D) was thicker than that of C. nauseosus subsp. hololeucus (Fig. 1E, G). The spongy parenchyma of C. nauseosus subsp. consimilis (Fig. 1A, B) had 4-6 layers of isodiametric cells with few intercellular spaces. This tissue was surrounded by 3-4 layers of palisade parenchyma cells in both faces. The mesophyll of C. nauseosus subsp. hololeucus (Fig. 1E, G) was more homogenous than that of C. nauseosus subsp. consimilis (Fig. 1A, B), because the palisade parenchyma cells are longer and more conspicuous. Spongy parenchyma was almost completely restricted to the bundle sheaths in C. nauseosus subsp. hololeucus (Fig. 1E, G).

Both subspecies leaves had secretory ducts adjacent to the vascular bundles (Fig. 1A, B, D-G). In C. nauseosus subsp.

consimilis these structures were all below their bundles. There was a large variation in number of secretory ducts, mainly near the leaf midrib (Fig. 1A, D). C. nauseosus subsp. hololeucus showed the same structures but at lower frequency (Fig. 1E-G). The epithelium of these secretory structures in both subspecies was formed by a varied number of round or rectangular cells in cross section.

The leaf midrib region was half-moon shaped in C. nauseosus subsp. consimilis and the vascular system was formed by primary xylem and phloem; some pericyclic fibres were isolated or positioned in small groups under the phloem elements (Fig. 1A, D). C. nauseosus subsp. hololeucus had a "v"-shaped leaf midrib region, and the phloem was more conspicuous (Fig. 1E, F). In C. nauseosus subsp. consimilis midrib region, a continuous adaxial palisade parenchyma was present with few collenchyma cells below it; the collenchyma near the abaxial epidermis was constituted of several cell layers (Fig. 1A, D). In C. nauseosus subsp. hololeucus, the palisade parenchyma was interrupted in the midrib region, and the collenchyma was more reduced on ad- and abaxial surface (Fig. 1E, F).

The leaf margin in both subspecies showed a single-layered epidermis with stomata and trichomes; a continuous palisade parenchyma composed of 2-3 layers was present (shown in C. nauseosus subsp. hololeucus, Fig. 1G) .

Two different types of trichomes occurred in both leaves. The non-glandular trichomes were ribbon-like (Fig. 2A-D). These trichomes were uniseriate and presented distinguishable basal, intermediate and apical cells (Fig. 2D). The apical cell was ribbon-like and thin walled (Fig. 2B, D); the basal and intermediate cell walls were thicker than those of the apical cell (Fig. 2C-D). Glandular trichomes were also present. They were biseriate (Fig. 2E, F), and each roll was constituted by 2 basal cells which was thick-walled and the 4 subsequent cells were thin walled. This trichome type was more common in C. nauseosus subsp. consimilis. More detailed studies of those trichome types are necessary.

The ungalled stem of Chrysothamnus nauseosus subsp. consimilis and C. nauseosus subsp. hololeucus was round in transverse section. On both subspecies the epidermis was uniseriate (Fig. 3A, B) and hairy (Fig. 3A-D). The trichomes were similar to those of the leaf. The cortex was constituted of 3-4 layers of palisade parenchyma cells and 5-7 layers of elliptical cells disposed periclinally direction (Fig. 3A-D). Secretory ducts (Fig. 3C, D) presented long, thin-walled epithelium cells.

Primary and secondary phloem fibres (Fig. 3D) had thick walls and small cell lumen. In both subspecies, the xylem presented two distinct regions. The external region was formed by multiple aggregated vessels, tending to form straight and oblique lines, while the internal one presented isolated or aggregated vessels with a diffuse distribution (Fig. 3D). The parenchyma rays were more frequently 2-3 layers wide. The pith was formed by parenchyma, whose cells were round and larger than those of the cortex (Fig. 3C, D).
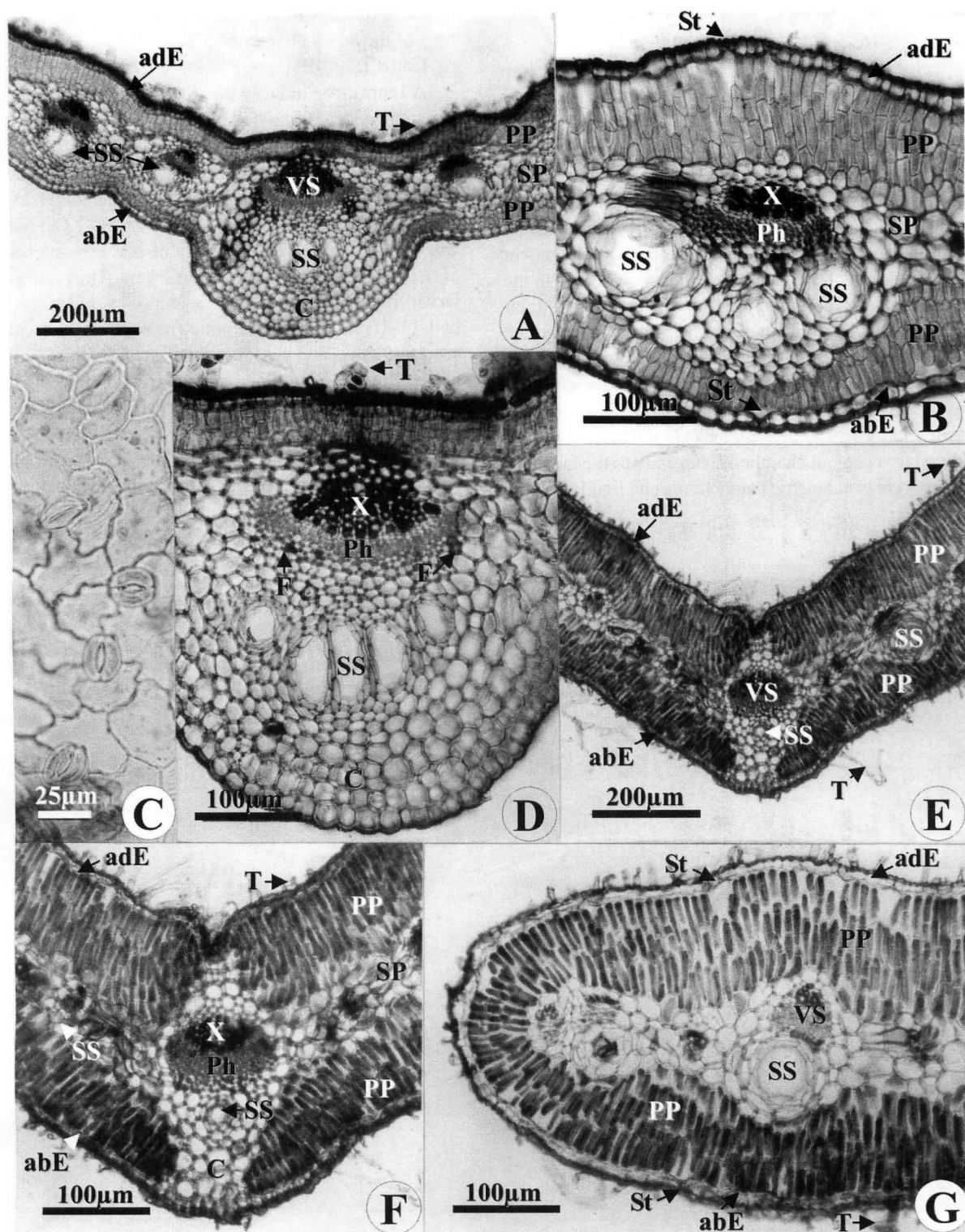
Fig. 1. A-G. *Chrysothamnus nauseosus* leaf. A-B, D-G: Transverse sections. C: Frontal view. A-D: *C. nauseosus* subsp. *consimilis*. E-G: *C. nauseosus* subsp. *hololeucus*. A. Part of the leaf lamina with the midrib region. Note the palisade parenchyma on both faces, and the secretory structures (ducts) below the vascular system. B. Secondary vein region. C. Adaxial epidermis with anomocytic stomata. D. Detail of the midrib region. E. Part of the leaf lamina with the midrib region. Note the palisade parenchyma on both faces, and the secretory structure (duct) below the vascular system. F. Detail of the midrib region. G. Leaf margin. adE = Adaxial Epidermis; abE = Abaxial Epidermis; C = Collenchyma; F= Fibre; SP = Spongy Parenchyma; PP = Palisade Parenchyma; Ph = Phloem; X = Xylem; St = Stoma; SS = Secretory Structure; T = Trichome: VS = Vascular System.

Structure of the galls on *C. nauseosus* subsp. *consimilis*

Gall c_1 (Fig. 4A)

A species of Cecidomyiidae induced a 1.0 x 3.0 mm cylindrical gall at the internodes with a dense tuft of trichomes in its apical portion.

The gall was formed by tissue folding, presenting 7-10 layers of parenchyma and 2-3 discontinuous layers of sclerified cells (not shown). The epidermis was uniseriate. There were few glandular and non-glandular trichomes similar to those of the unaffected leaf in the epidermis which recover the external portion of the gall. The epidermis located in the internal gall surface presented long modified non-glandular trichomes, formed by division of the basal and intermediate cells (Fig. 5A, B). The unique central chamber was located in the basal portion of the gall, and it was lined by a nutritive tissue formed by 10-12 layers of isodiametric cells with dense cytoplasm and hypertrophied nucleus (Fig. 5C). There was one galling larva per gall chamber. Externally to the nutritive tissue, there were isodiametric parenchyma and lignified cells.

Vascular bundles linked the stem with the gall, reaching the base of the larval chamber.

Gall c_2 (Fig. 4B)

A Tephritidae induced this 1.0 x 4.0 mm ellipsoidal gall in the stem node. It was formed by modified leaves and was connected to the stem by a long peduncle.

The apical portion of the gall was less modified. It presented a uniseriate epidermis with glandular trichomes similar to those of unaffected leaf (Fig. 5D). The mesophyll was constituted by 7 to 9 layers of parenchyma cells (Fig. 5D). Ribbon-like trichomes were absent. The region near the larval chamber also presented a single-layered epidermis and had 11-15 layers of isodiametric parenchyma cells with secretory ducts and vascular bundles. One central larval with one galling larva was lined by 1-2 layers of thick-walled cells (periclinal ones, facing the chamber) (Fig. 5E). At the base of the larval chamber, there was a large amount of secretory structures and vascular bundles. The vascular tissues were connected with those of the gall peduncle.
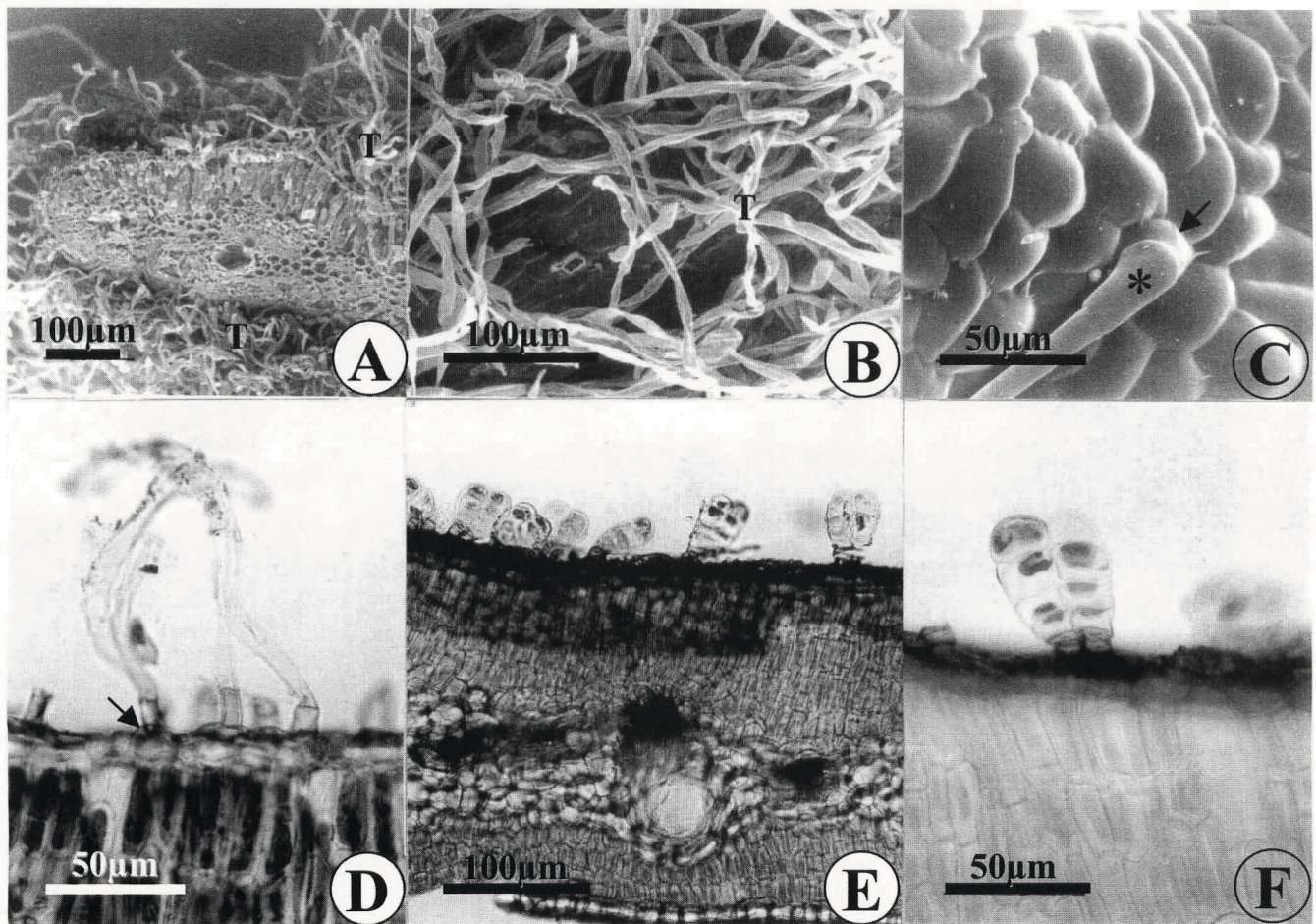

Fig. 2. A-F. *Chrysothamnus nauseosus* leaf. A, D-F: Transverse sections. B, C: Frontal view. A-C, E, F: *C. nauseosus* subsp. *consimilis*. D: *C. nauseosus* subsp. *hololeucus*. A. Note the numerous trichomes (SEM). B. Detail of the non-glandular trichome showing the ribbon-like cells (SEM). C. Detail of the basal (arrow) and intermediate (*) cells of the ribbon-like trichome (SEM). D. Ribbon-like trichomes. Note the basal cell (arrow). E. Glandular trichomes. F. Detail of the glandular trichome. T = Trichome
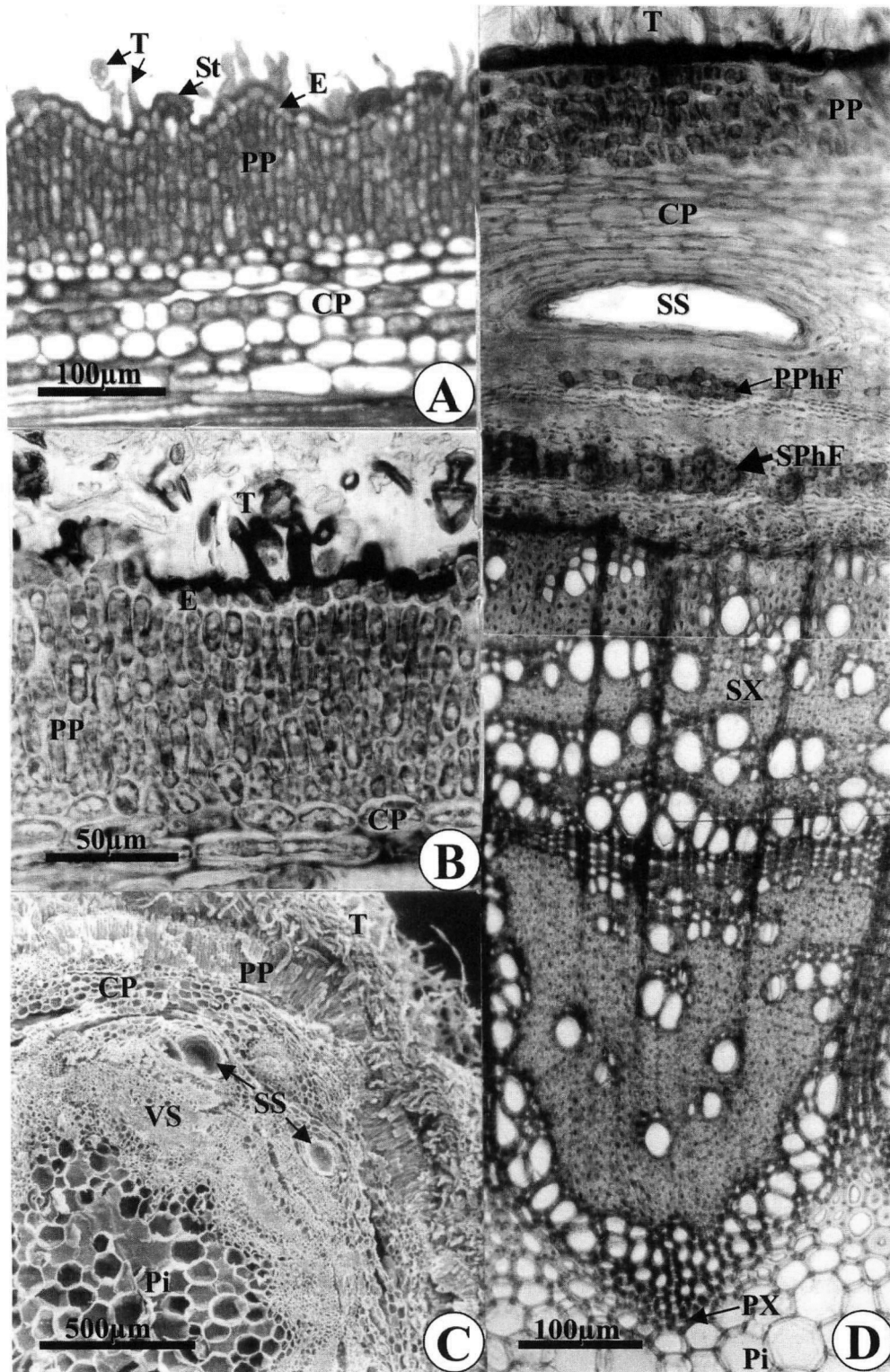
Fig. 3. A-D. *Chrysothamnus nauseosus* stem. A, B: Longitudinal sections. C, D: Transverse sections. A, D: *C. nauseosus* subsp. *consimilis*. B, C: *C. nauseosus* subsp. *hololeucus*. A, B. External portion of the stem showing the uniseriate epidermis with trichomes, and the palisade and cortical parenchyma. C. Part of the stem. Note the trichomes recovering the epidermis and the ducts in the cortex (SEM). D. Part of the stem showing the epidermis, cortex, vascular system and pith. Secretory structure (duct) is present in the cortical region. Note the primary and secondary phloem fibres, and primary and secondary xylem. CP = Cortical Parenchyma; E = Epidermis; PP = Palisade Parenchyma; Pi = Pith; PPhF = Primary Phloem Fibre; PX = Primary Xylem; SPhF = Secondary Phloem Fibre; SS = Secretory Structure; St = Stoma; SX = Secondary Xylem; T = Trichome; VS = Vascular System.
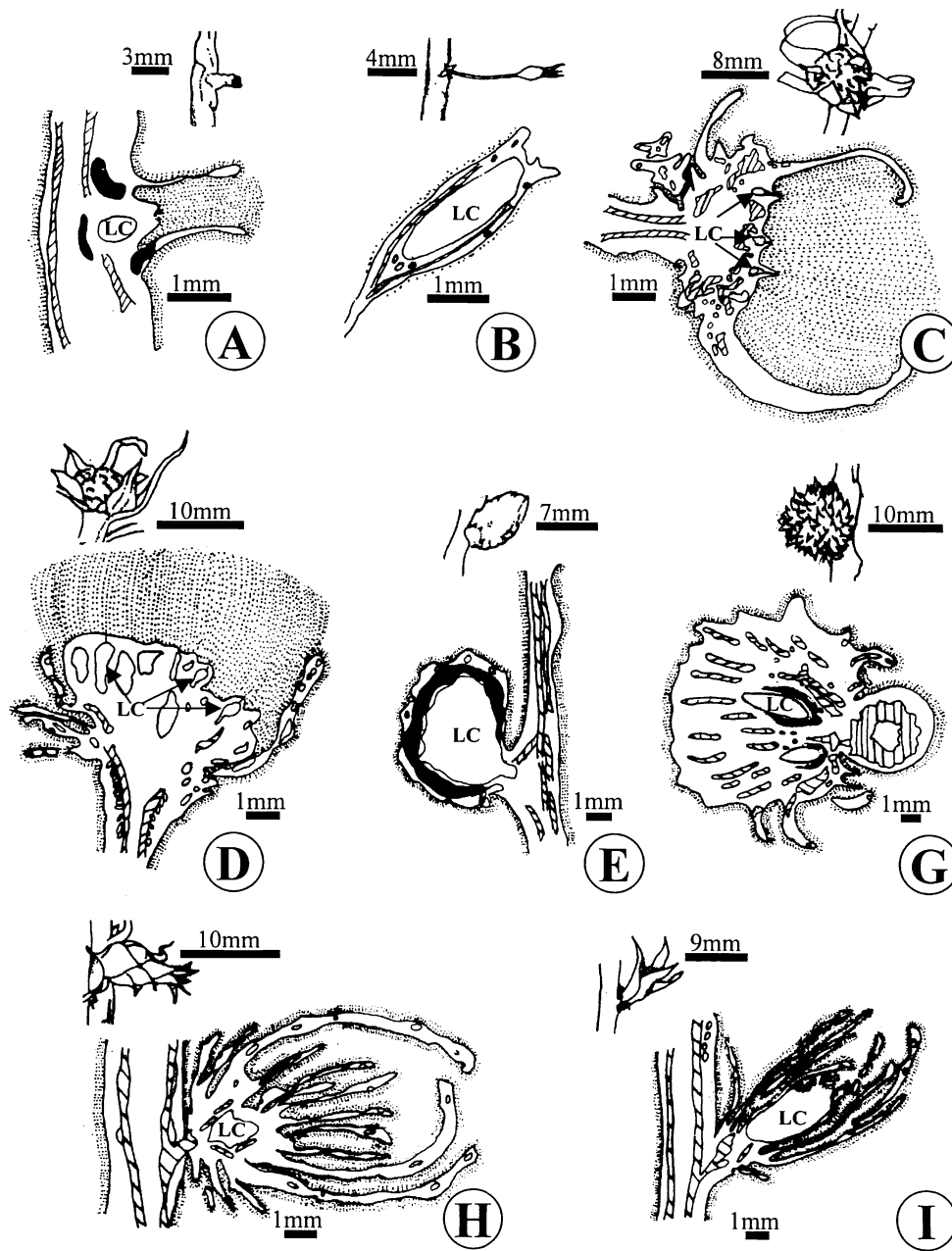
Fig. 4. *Chrysothamnus nauseosus* galls: general view, and diagram of the longitudinal section. A-C: Galls that occur on *C. nauseosus* subsp. *consimilis* (c_1, c_2 and c_3, respectively). D-F: Galls that occur on *C. nauseosus* subsp. *hololeucus* (h_1, h_2 and h_3, respectively). G-H: Galls that co-occur on *C. nauseosus* subsp. *consimilis* and *C. nauseosus* subsp. *hololeucus* (ch_1 and ch_2, respectively).

☐	Gall Parenchyma
■	Sclerenchyma
◻	Secretory Structure

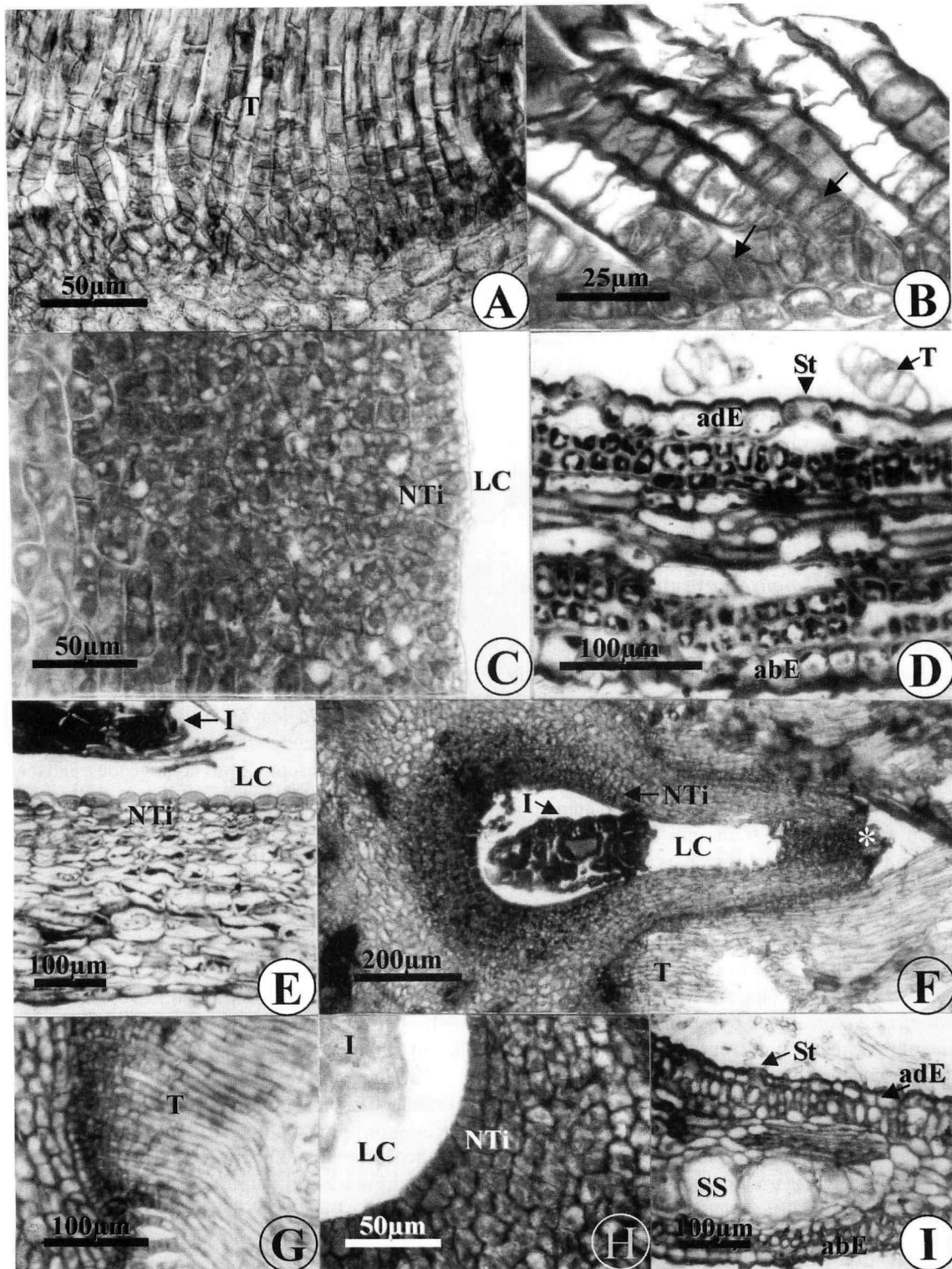|	
☐	Trichome
▦	Vascular System

LC = Larval Chamber

Fig. 5. A-I. *C. nauseosus* subsp. *consimilis* galls. A-I: Longitudinal sections. A-C: Gall c_1. D, E: Gall c_2. F-I: Gall c_3. A. Part of the internal epidermis with the modified non-glandular trichomes. B. Detail of the modified non-glandular trichomes showing the numerous basal and intermediate cells. Note divided cells (arrows). C. Nutritive tissue which cells present dense cytoplasm and conspicuous nucleus. D. Note the epidermis with stomata and glandular trichomes, and the modified mesophyll. E. Nutritive tissue with thick walled cells. F. Larval chamber with one inducer. Note that the tissues are not fussed in the apical portion of the chamber ($_*$). G. Detail of the modified non-glandular trichomes. H. Detail of the nutritive tissue showing cells with dense contents. I. Gall leaf showing the secretory structures. adE = Adaxial Epidermis; abE = Abaxial Epidermis; LC = Larval Chamber; I = Inducer; NTi = Nutritive Tissue; SS = Secretory Structure; St = Stoma; T = Trichome.

Gall c₃ (Fig. 4C)

Gall c_3 (Fig. 4C)

This gall was induced by a Tephritidae in the axillary buds of the shoot. The gall was 8.0 x 9.0 mm, cup-like form, hairy (woolly), and with numerous modified leaves at its base (rosette aspect). Several larval chambers were located in the apical region of the gall.

The epidermis of the woolly portion was uniseriate and presented numerous long, modified non-glandular trichomes (Fig. 5F, G), giving the woolly aspect. The larval chambers were superficial, and formed by tissue projections whose apical fusion was incomplete. Each larval chamber contained one galling larva (Fig. 5F). The nutritive tissue lined the larval chambers and its cells had dense contents (Fig. 5F, H). The secretory ducts were numerous, and the gall vascular system was well-developed and connected to the stem vascular tissues.

The external gall leaves had slight modifications (Fig. 5I). The leaves located internally presented alterations in their basal portion; the palisade and spongy parenchyma cells are hypertrophied (not shown).

Structure of galls on *C. nauseosus* subsp. *hololeucus*

Gall h₁ (Fig. 4D)

Gall h_1 (Fig. 4D)

An unidentified species of Cecidomyiidae induced this gall in the apical stem buds. This gall is 10.0 x 7.0 mm, hairy (woolly), and cup-like form with modified leaves at its base (rosette aspect). Several larval chambers were present in the apical region of the gall.

The epidermis of the woolly portion of the gall was uniseriate. It presents few glandular trichomes and numerous long modified non-glandular trichomes similar to those describe in c_1 and c_3 galls. Larval chambers were superficial and formed by tissue projections whose apical fusion was not complete similar to c_3 gall. Each chamber had one inducer. The nutritive tissue (Fig. 6A) was formed by several parenchyma layers of thin-walled cells with dense contents at the apical portion. Basal cells contents were less dense. Gall parenchyma was constituted by isodiametric cells. The basal portion of the gall had numerous secretory ducts (Fig. 6B). The stem vascular bundles reached the base of the larval chambers.

The gall leaves had a wavy aspect, and they presented great alterations (Fig. 6C). The epidermis had modified glandular and non-glandular trichomes. The mesophyll did not exhibit differentiated palisade and spongy parenchyma, and the secretory ducts were common.

Gall h₂ (Fig. 4E)

Gall h_2 (Fig. 4E)

A Tephritidae induced this 7.0 x 5.5 mm ellipsoidal gall in the stem internodes. This gall was hairy (woolly aspect) and resinous.

The gall had 2 distinct regions. The external region had uniseriate and hairy epidermis with glandular and non-glandular trichomes, similar to those of the ungalled portion of the stem. Several layers of isodiametric cellulosic thin-walled cells occurred below the epidermis. They were followed by

several layers of pectic walled cells (Fig. 6D). Secretory ducts occurred within this second group of cells. The internal region was composed of sclereids (Fig. 6E), and parenchyma cells. A central larval chamber bearing one galling larva was lined by a nutritive tissue. This tissue was formed by thin-walled parenchyma cells, some of which were ruptured (Fig. 6F). The vascular system was well-developed and linked to the stem vascular tissues.

Gall h₃ (Fig. 4F)

Gall h_3 (Fig. 4F)

This 10.0 x 8mm gall was induced by a Cecidomyiidae in the axillary buds. It was hairy and pineapple-like, with numerous small modified leaves. Several larval chambers occurred in the central region of the gall.

The epidermis was uniseriate (Fig. 6G), presenting some modified glandular trichomes (Fig. 6H). The non-glandular trichomes were absent. Gall parenchyma had hypertrophied cells in various shapes, and conspicuous intercellular spaces (Fig. 6F). The internal cell layers near larval chamber showed reduction in size. The secretory ducts were numerous and located among the parenchyma cells. There was one gall inducer per larval chamber. The apical region of the chamber was lined by a nutritive tissue with 3 layers of isodiametric and hypertrophied parenchyma cells (Fig. 6I). Sclereids lined most of the chamber, mainly at the basal region. The vascular bundles were spread all over gall tissues, and were connected with those of the stem.

The modified leaves (Fig. 6J) presented uniseriate epidermis with glandular trichomes. Non-glandular trichomes were rare. The mesophyll was homogenous, and the secretory structures occurred over the vascular bundles.

Structure of galls that co-occur in both subspecies

The morphology and anatomy of the galls that co-occur in both subspecies were similar. We described the galls developed on *C. nauseosus* subsp. *hololeucus*.

Gall ch₁ (Fig. 4G)

Gall ch_1 (Fig. 4G)

A Cecidomyiidae induced a 4.5 x 10.0 mm gall in axillary buds. This gall was mainly formed by modified imbricated leaves (artichoke-like aspect).

The epidermis was uniseriate with few glandular and non-glandular trichomes (Fig. 7A, B). Sclerified cells and secretory ducts were present (Fig. 7A, B). At the basal portion there was no differentiation between the palisade and the spongy parenchyma cells, which ones exhibit conspicuous hypertrophy (Fig. 7B). The central portion of the gall had a larval chamber, where only galling larva developed. The chamber was delimited by rectangular parenchyma cells, which were perpendicular to the main axis of the gall (Fig. 7C). The adjacent parenchyma had isodiametric cells. The vascular bundles were connected with those of the host stem.

Gall ch₂ (Fig. 4H)

Gall ch_2 (Fig. 4H)

A Cecidomyiidae induced a 3.0 x 9.0 mm in the axillary buds. This gall was formed mainly by numerous modified imbricated leaves (artichoke-like aspect).

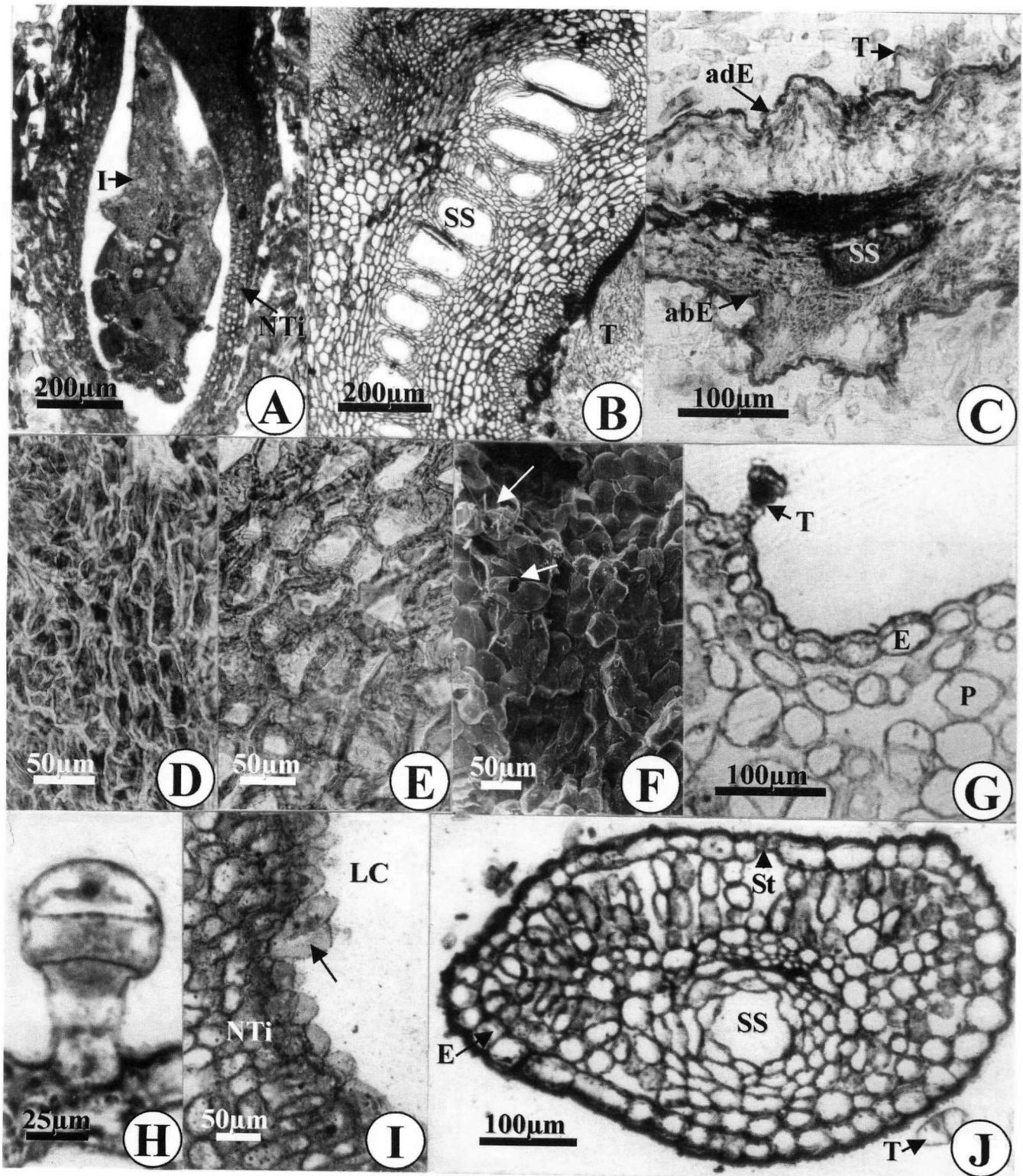The epidermis was uniseriate with numerous glandular and

Fig. 6. A-J. *C. nauseosus* subsp. *hololeucus* galls. A-G, I: Longitudinal sections. H: Transverse section of the gall leaf. A-C: Gall h_1. D-F: Gall h_2. G-J: Gall h_3. A. Larval chamber lined by nutritive tissue. Note the inducer. B. Detail of the secretory structures. C. Modified leaf. D. Parenchyma cells with pectic walls. E. Sclerenchyma. F. Nutritive tissue (SEM). Note the ruptured parenchyma cells (arrows). G. Epidermis with modified glandular trichome, and parenchyma cells with conspicuous intercellular spaces. H. Modified glandular trichome. I. Nutritive tissue lining the gall chamber. Note hypertrophied cell (arrow). J. Modified leaf gall showing the secretory structure. adE = Adaxial Epidermis; abE = Abaxial Epidermis; E = Epidermis; I = Inducer; LC = Larval Chamber; NTi = Nutritive Tissue; P = Parenchyma; SS = Secretory Structure; St = Stoma; T = Trichome.

non-glandular trichomes (Fig. 7D). The region toward the larval chamber presented sclerified epidermal cells, and 3-4 layers of lignified cells (Fig. 7D). Secretory tissues were present. The internal portion formed the larval chamber with one galling inducer. Nutritive tissue was absent, and fungus hyphae were conspicuous at the basal region of the larval chamber (Fig. 7E). Stem vascular bundles were continuous with those of the gall.

Discussion

The choice of the site of gall induction requires extreme specialization by the galling herbivore. The gall is a sessile structure and in the majority of cases must persist for a considerable length of time, until the inducer's progeny emerges from it. Thus, the viability of the offspring is influenced by the ability of the gall to protect its inhabitants from abiotic factors, predation, parasitism, pathogens, and plant resistance (Price *et al.* 1986, Weis *et al.* 1988, Fernandes 1992, Fernandes *et al.* 2000).

In *C. nauseosus* subsp. *consimilis* and *C. nauseosus* subsp. *hololeucus*, galls are located on the apical shoot meristem, axillary buds, internodes and nodes. There is a morphological convergence of the galls formed in the apical shoot and axillary buds, resulting in rosette (c_3 and h_1), artichoke (ch_1 and ch_2) or pineapple (h_3) arrangements. Rosette galls are formed due to the inhibition of internodes elongation. Artichoke arrangements are caused by increase in the stimulus for leaf formation (see Dreger-Jauffret 1977). The pineapple type is formed by fusion of modified leaves.
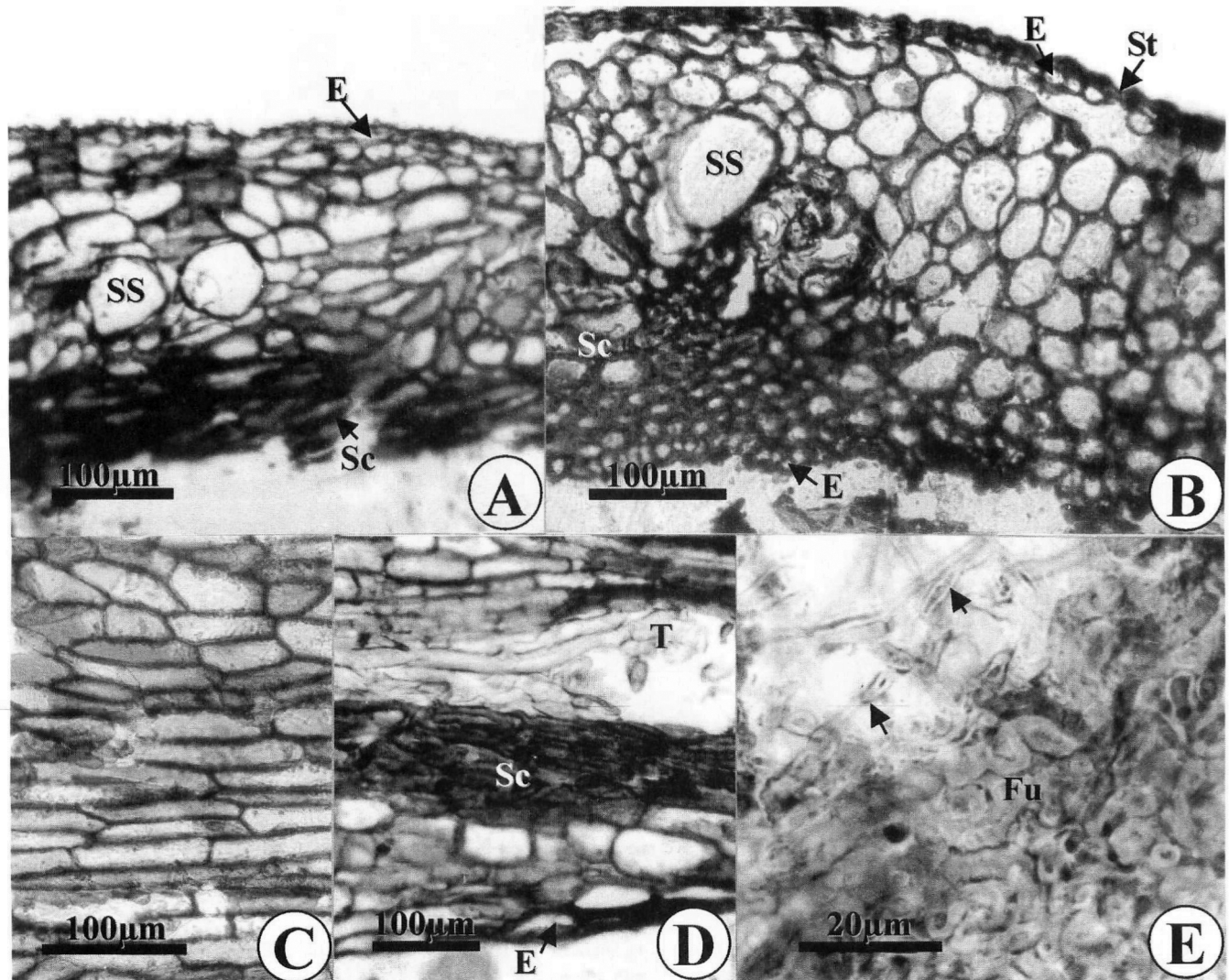

Fig. 7. Galls that co-occur in *C. nauseosus* subsp. *consimilis* and *C. nauseosus* subsp. *hololeucus*. A-F: Longitudinal sections. A-C: Gall ch_1. D, E: Gall ch_2. A. Epidermis and modified mesophyll. Note the sclerified cells. B. Epidermis and modified mesophyll. Note the hypertrophied cells. C. Nutritive tissue. D. Epidermis with trichomes, and the mesophyll with sclerified layers. E. Larval chamber with fungus hyphae (arrows). E = Epidermis; Fu = Fungus; Sc = Sclerenchyma; SS = Secretory Structure; St = Stoma; T = Trichome.

The anatomical analysis of the h_1 gall showed its origin at the tip of the shoot, while the c_3, h_3, ch_1 and ch_2 galls were formed on axillary buds. The formation of the c_3 and h_1 galls involved cells of the apical meristem. The larval chamber on both galls showed no fusion in their apical portion. The formation of the ch_1 and ch_2 galls involved leaf cells, although cells of the apical meristem participate. The larval chamber was more protected in gall ch_1 than ch_2. Gall h_3 was also formed on an axillary bud but it is more complex with great hyperplasia of the parenchyma. Furthermore, an increasing gradient of larval chamber protection exists from c_3 and h_1 to ch_2, ch_1 and h_3 galls.

The c_2 gall was formed on the node, involving only leaf alterations, and c_1 and h_2 galls were formed on the internode. These two latter galls presented sclerenchyma around the larval chamber, which constitutes a mechanical defense to the galling insect. Compared to galls induced on apical shoot and axillary buds (meristematic tissues), the inducer stimulus in the c_1 and h_2 galls have acted on a more differentiated tissue. Thus, the cells could not be manipulated so wide, and hence parenchymatic cells differentiated in sclerenchyma tissue. The presence of this tissue in the h_3 gall indicate that cells of the stem may be involved in the ontogenesis of this complex gall. The ch_1 and ch_2 gall also shows sclerified cells. The presence of these cells in ch_2 ambrosia gall may be the consequence of the fungus hyphae. A further role of lignin is indicated by its deposition in response to various types of injury or attack by fungus (Denny, 2002).

Sclerenchyma occurred in the c_1, h_2, h_3, ch_1 and ch_2 galls, conferring mechanical support to the gall structure. It was totally absent in node gall c_2. The sclerenchyma is quite common in prosoplasmic galls (Mani 1964, Meyer & Maresquelle 1983) and its development and arrangement in galls is related to chemical conditions present during cecidogenesis and may be involved with advantages to the gall-inducing insect.

Trichomes and secretory structures formed during gall development may also represent protective and/or defensive strategies induced by the galling insects against their natural enemies (Mani 1964, Cornell 1983, Meyer & Maresquelle 1983, Rohfritsch 1992).

The presence of trichomes are notable traits of large taxonomic significance (Metcalfe & Chalk 1983), since trichomes types are well-defined genetically (Theobald *et al.* 1979). Glandular trichomes secreting lipophilic substances occur in many plant families, including Asteraceae (Fahn 2000). This reinforces the anatomical observations on the unaffected leaves and the shoots of both *C. nauseosus* subspecies, which have the same types of trichomes, indicating their phylogenetic proximity.

Trichomes were present on all studied galls. Modified non-glandular trichomes occurred in the c_1, c_3 and h_1 galls, and they were larger and with more cells than those of unaffected organs. These modified trichomes were more numerous in the c_3 and h_1 galls. In the c_1 gall they are less

conspicuous. We argue that a similar reaction occurred on the three gall types, and the difference can be associated with the site of induction. In the c_3 and h_1 galls, the induction occurred in the meristematic tissue (protodermal cells) of the buds. In the c_1 gall it occurred in differentiated epidermal cells of the stem. The presence of numerous galling insects near the surface of c_3 and h_1 galls may result in a conspicuous cecidogenetic field. In the c_1 gall the cecidogenetic field is not so extensive, because there is only one inducer. The presence of numerous, long modified non-glandular trichomes may add protection against abiotic and biotic factors, acting as an evapotranspirative barrier against water loss and herbivory, respectively (Woodman & Fernandes 1991). Once the gall-makers are coated with only a few layers of cells, they can also be vulnerable to predators, parasitoids and parasites. Detailed studies on the ecology of these galling insects are still needed.

Some of the studied galls also showed modified glandular trichomes. This fact reinforces the view that the trichomes are genetically based but they are also plastic structures, susceptible to modifications induced by *insects.*

Secretory structures are present in numerous families and they are especially common in the Asteraceae (Metcalfe & Chalk 1983). They are defined as secretory cavities or ducts (canals) of schizogenous origin, with relevant taxonomic significance for this family (Fahn 1979, Lersten & Curtis 1987). Both subspecies of *C. nauseosus* present secretory ducts in leaves and stems which are similar to those observed by Marinho (1996) on *Baccharis pseudotenuifolia* and *B. dracunculifolia.*

According to Meyer and Maresquelle (1983), the secretory structures may be modified in the galls: some galls present several conspicuous secretory structures while others present few, or rare at all. In some galls the resinous exsudate may deter either mechanically or chemically the action of predators or cecidophagous (Meyer & Maresquele 1983). The secretory ducts present on galls (c_2, c_3, h_1, h_2, h_3, ch_1, and ch_2) showed slight structural modifications and may protect the gall inducers from attack by their natural enemies. Gall c_1 was the only exception, completely lacking any of these structures. Thus, the secretory structures are plastic structures and susceptible to modifications influenced by a biotic factor, the insect.

During gall development, there is usually a formation of nutritive tissue that involves the larval chamber and whose cells show characteristics of high physiological activity suitable to the parasite's nutrition (Bronner 1992). Even though the majority of the galls have a typical nutritive tissue, some insect galls like ambrosia and rudimentary types do not have nutritive tissue (Bronner 1977, Bronner 1992). Seven of the studied galls presented different nutritive tissues whose cells were similar to the meristematic cells with small size, large nucleus, dense cytoplasm content, and thin walls (c_1 and c_3), or to parenchymatic cells with thin walls (h_1, h_2, h_3 and ch_1). The c_2 gall presented thick wall cells. In gall h_2, some cells of

the nutritive tissue were ruptured. Only in the gall ch_2 the nutritive tissue is absent, but the presence of numerous fungus hyphae indicate that it is an ambrosia gall, similar to other studied galls (Arduin & Kraus 2001).

Several authors have suggested that there is a correlation between the mode of feeding of gall insects and the complexity of their galls (Meyer & Maresquelle 1983, Rohfritsch 1992). The insect galls of *C. nauseosus* species belong to the same order Diptera (Fernandes 1992). Otherwise the Cecidomyiidae (c_1, h_1, h_3, ch_1 and ch_2) is in the suborder Nematocera, which have reduced mouth parts, and feed by sucking fluids from cells of the nutritive tissue (c_1, h_1, h_3 and ch_1) or from fungus hyphae (ch_2). The Tephritidae is in the suborder Cyclorrhapha (c_2, c_3 and h_2), which have well developed mouth parts and feed by rasping plant tissues. The inducer of the h_2 gall is *Aciurina trixa* (Tephritidae) (Fernandes & Price 1994). According to Bronner (1992), the type and thickness of nutritive tissue (layers number) which lines the larval chamber depends on the species of gall organism. No correlation was found between insect taxa and the development of the nutritive tissue on *C. nauseosus* galls, but ontogenetic studies are necessary for a more detailed analysis.

The number of insect inducers also affect the gall structure. In polythalamous galls (c_3, h_1, and h_3), size is likely related to the number of galling larvae, consistent with the observations of Jones (1983) and Arduin *et al.* (1989) in other galls. The size of the galls may also represent a defensive strategy against natural enemies (Jones 1983).

Although galls are not typical structures of the plant body, the individual components of gall tissues may be found in affected organs or in diverse parts of the plant (Mani 1964). The presence of glandular and non-glandular trichomes, the secretory structures, and the sclerenchyma in the studied galls corroborate this view. The phenotypic alterations occur within limits imposed by the plant. Therefore, no alteration is possible if it is not defined in the plant genotype (Mani 1964. Alteration also is dependent on the stage of tissue differentiation (Rohfritsch 1992).

Diverse biotic influences may affect plant tissues in different ways. While certain factors may affect all cells of the tissue, other factors can at least preferentially act on certain target cells. The apical meristems of the shoots and axillary bud in *C. nauseosus* subspecies attacked by different insect taxa showed convergent morphogenesis. In the meristem, the cells are undifferentiated and competent to divide and form daughter cells. When stimulated by galling insects these cells become determinated and can give rise to similar gall phenotypes. On the other hand, stem galls were structurally more complex and specific because the galling insects acted over already differentiated cells, which need to dedifferentiate or redifferentiate, leading to their determination which results in distinct gall phenotypes. Our observations of galls developed in the phylogenetically closely related plants, *C. nauseosus* subsp. *consimilis* and *C. nauseosus* subsp. *hololeucus,* lead to the conclusion that cecidogenous responses are influenced by a specific insect actions and directed by the stage of plant cell differentiation by the time of induction.

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