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# DEVELOPMENTAL POTENTIAL OF GALLS INDUCED BY *DIPLOLEPIS ROSAEFOLII* (HYMENOPTERA: CYNIPIDAE) ON THE LEAVES OF *ROSA VIRGINIANA* AND THE INFLUENCE OF *PERICLISTUS* SPECIES ON THE *DIPLOLEPIS ROSAEFOLII* GALLS

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The larval stages of the cynipid wasp *Diplolepis rosaefolii* induce the formation of single-chambered, lenticular galls on the leaves of the wild shrub rose, *Rosa virginiana*. The development of galls induced by *D. rosaefolii* was studied using light and scanning electron microscopy. The gall consists of four tissue layers, which surround a centrally located larval chamber. These include an outermost dermal layer, underlying parenchyma, sclerenchyma, and nutritive tissue. Development in the *D. rosaefolii* galls involves a number of characteristics that are unique to this gall and differ markedly from development in other *Diplolepis* galls studied. These characteristics include the presence of double sclerenchymal layers and vascularization embedded between the sclerenchyma. *Periclistus* is an inquiline in the galls induced by *D. rosaefolii*. Under the influence of *Periclistus* larvae, a number of morphological changes, including an increase in the number of larval chambers, are observed in the galls. Changes in tissue type, proportion, and overall morphology exhibited by *Periclistus*-modified galls are also studied using conventional light microscopy techniques.

*Keywords:* lenticular gall development, inquiline, *Periclistus*, *Diplolepis*, nutritive tissue, gall tissues.

## Introduction

### *Galls Induced by Cynipid Wasps*

Insect galls are atypical growths induced in plants. They arise as the result of an interspecific association between a plant and an insect (Rohfritsch 1992). Gall morphology is influenced by two genotypes: that of the insect, which provides the stimulus, and that of the plant, which determines the growth response (Weis and Abrahamson 1986). Gall inducers actively manipulate the host plant through mechanical and/or chemical stimuli to form a structure that provides the inducer with both nutrition and shelter (Askew 1984; Shorthouse 1993).

Cynipid wasps (Hymenoptera: Cynipidae) of the genus *Diplolepis* Geoffroy (formerly the genus *Rhodites* Hartig) are widely distributed, with most species being found in North America (Shorthouse and Ritchie 1984). All *Diplolepis* species restricted to North America induce galls on rose (*Rosa*) species. Galls induced by *Diplolepis* wasps may be found on the leaves, stems, buds, or roots of various rose species. The site of induction is specific to the wasp species. Many wasps have the potential to induce galls on more than one species of rose (Stille 1984; Shorthouse and Brooks 1998). With few exceptions, the galls have a characteristic shape and structure, which is unique to the species of gall inducer.

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### *Phases of Development*

Although it varies in many respects, the development of most cynipid galls that have been investigated can be divided into three phases: initiation, growth, and maturation ( Rohfritsch 1992). Initiation begins with oviposition of eggs on or within a specific organ of the host plant (Mani 1964; Shorthouse 1975). During initiation, the newly hatched larva exerts control over the development of the plant cells in its vicinity, resulting in changes in the level of physiological activity of the affected plant cells and in their growth patterns.

The growth phase of the gall involves an increase in cellular mass of the plant tissue surrounding the larva (Cosens 1912; Shorthouse 1975). During this phase, cells lining the larval chamber differentiate into nutritive cells, which are important both in feeding the larva and in the morphogenetic development of the gall (Rohfritsch 1992). The orientation of the larva while feeding is responsible for the direction of growth of the cells in the gall and causes the definitive shape of the gall (Rohfritsch and Shorthouse 1982).

The maturation phase marks the end of gall growth and corresponds to the active feeding stage of the larva (Lalonde and Shorthouse 1984). Differentiation of plant cells occurs, causing the development of distinct tissue layers, arranged more or less concentrically around the larval chamber.

### *Inquilines*

A large percentage of galls are host to a number of other wasps that secondarily inhabit the structures (Narendran 1984; Schönrogge et al. 1994). These secondary inhabitants are (i) inquilines, which are phytophagous and feed only on

the gall tissue, and (ii) parasitoids, which feed on any larvae they find in the gall. Inquiline species have the ability to modify the galls they inhabit, although they cannot induce gall formation (Shorthouse 1975, 1980; Brooks and Shorthouse 1997*b*).

*Periclistus* species are inquilines in *Diplolepis* galls (Ronquist 1994). All *Periclistus* females associated with *Diplolepis* galls kill the *Diplolepis* larva with their ovipositor when they oviposit into the gall (Shorthouse 1980). Eggs are deposited on the inside surface of the larval chamber, and once hatched, the larvae begin to feed on the gall tissue (Brooks and Shorthouse 1997*a*). As feeding continues, the cells of the inner wall of the gall grow to surround each larva in its own individual chamber. In many *Diplolepis* galls, this results in galls that are much larger than those containing only the larva of an inducer. *Periclistus* larvae overwinter in the gall and pupate in the spring. The adults exit from the gall, mate, and then search for immature galls in which to oviposit. Emergence of adults is synchronized with the presence of immature galls of the host wasp species (Shorthouse 1975).

To this date, no work has been done on the developmental morphology of *Diplolepis rosaefolii* galls or on the inquiline-modified galls of this species. For that matter, very few comparative studies focusing on differences between inducer-inhabited galls and inquiline-inhabited galls have been conducted (Brooks and Shorthouse 1997*b*). In addition, few studies in gall morphology (e.g., Anthony et al. 1983; Anthony and Sattler 1990) have utilized the unique perspective obtained through scanning electron microscope (SEM) (Goldstein et al. 1992).

The interaction between a specific inducer (*Diplolepis*), plant (*Rosa virginiana*), and inquiline (*Periclistus*) presents us with interesting questions. Can lenticular galls be accommodated in the general model of gall development described by Rohfritsch (1992)? How different is the arrangement of tissue types between the single-chambered gall of the inducer and the multichambered gall of the inquiline?

The aims of this study are (i) to investigate the developmental morphology of galls induced by *D. rosaefolii* on *R. virginiana* using both high-resolution light microscopy (resin-embedded tissue sectioning) and scanning electron microscopy and (ii) to investigate the changes in morphology that occur in *D. rosaefolii* galls when the inquiline *Periclistus* sp. inhabits the gall, using high-resolution light microscopy.

## Material and Methods

### *Collection of Specimens*

Galls were collected biweekly in 1995 (July to September: 12 wk) and 1996 (June to October: 14 wk) from *Rosa virginiana* bushes in Brackley Beach, Prince Edward Island (63°12′12″W, 46°25′20″N). Leaflets with eggs on them were found by careful examination of newly opened leaves of *R. virginiana* in areas of the rosebush where galls had been found previously. Confirmation of egg presence was carried out in the laboratory using a stereoscope. Random samples of visible galls were hand-collected by examining each section of the rosebush and taking the first galled leaf encountered. Each sampling consisted of ca. 150–200 galls, and a total of 3500 galls were collected over the 2-yr study period. Collected galls were cut from the leaf tissue with a razor blade. Following removal from the leaf, newly initiated galls, those barely visible to the naked eye, were placed directly in glutaraldehyde, while older galls were first dissected under a stereoscope to determine the inhabitants present. Only galls that contained larvae of *Diplolepis rosaefolii* or *Periclistus* sp. were used in further processing. Initially, the approximate stages of gall development were determined by visual observation of size changes in gall diameter. The galls were sectioned to study the development of the various tissue layers and to determine the developmental series. The developmental stages were based on changes in the tissues and are not necessarily synonymous with the phases referred to in some gall papers (e.g., Shorthouse 1975; Rohfritsch 1992).

Voucher specimens of adult *D. rosaefolii* and *Periclistus* species were identified by J. D. Shorthouse, Laurentian University, and are deposited in the insect collection at the Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada. Leaves and stems of *R. virginiana* were also collected, and their identification was confirmed by C. A. Lacroix at the Herbarium of the University of Guelph, Guelph, Ontario.

### *Tissue Fixation and Processing*

All tissue fixation and dehydration were carried out at room temperature following the procedures of Hayat (1981) and O'Brien and McCully (1981). Chemicals used for processing of all samples for light and scanning electron microscopy were purchased from J. B. EM Services (Montreal, Canada) unless otherwise stated. Gall tissues were fixed in 5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) for 12 h. Following primary fixation, samples were rinsed three times in 0.1 M phosphate buffer (pH 7.2) for 30-min intervals. Samples were postfixed in 2% osmium tetroxide in 0.2 M phosphate buffer (pH 7.2) for 2 h and rinsed three times in 0.2 M phosphate buffer (pH 7.2) for three 30-min intervals.

### *Light Microscopy*

Following fixation, gall samples were dehydrated in a graded ethanol series. Samples were embedded in Spurr (1969) resin (medium mixture) and serial sections of ca. 0.30 $\mu$m thick were cut using a Reichert-Jung Ultracut or Porter-Blum MT2-B ultramicrotome. Sections were mounted on glass slides and stained with 1% toluidine blue in 1% sodium borate (after Kramer and Windrum 1955). Additional sections were mounted on slides and contrasted with Sudan black B to reveal starches and lipids in the cells (after Bronner 1975). Since a dark precipitate, which could not be removed, was present on all sections even after repeated rinsing, photographs of these sections are not included. Photographs of all other sections were taken on an Olympus BH2 microscope fitted with an SC35 camera using Kodak Tmax film and printed on Kodak polycontrast RC III paper.

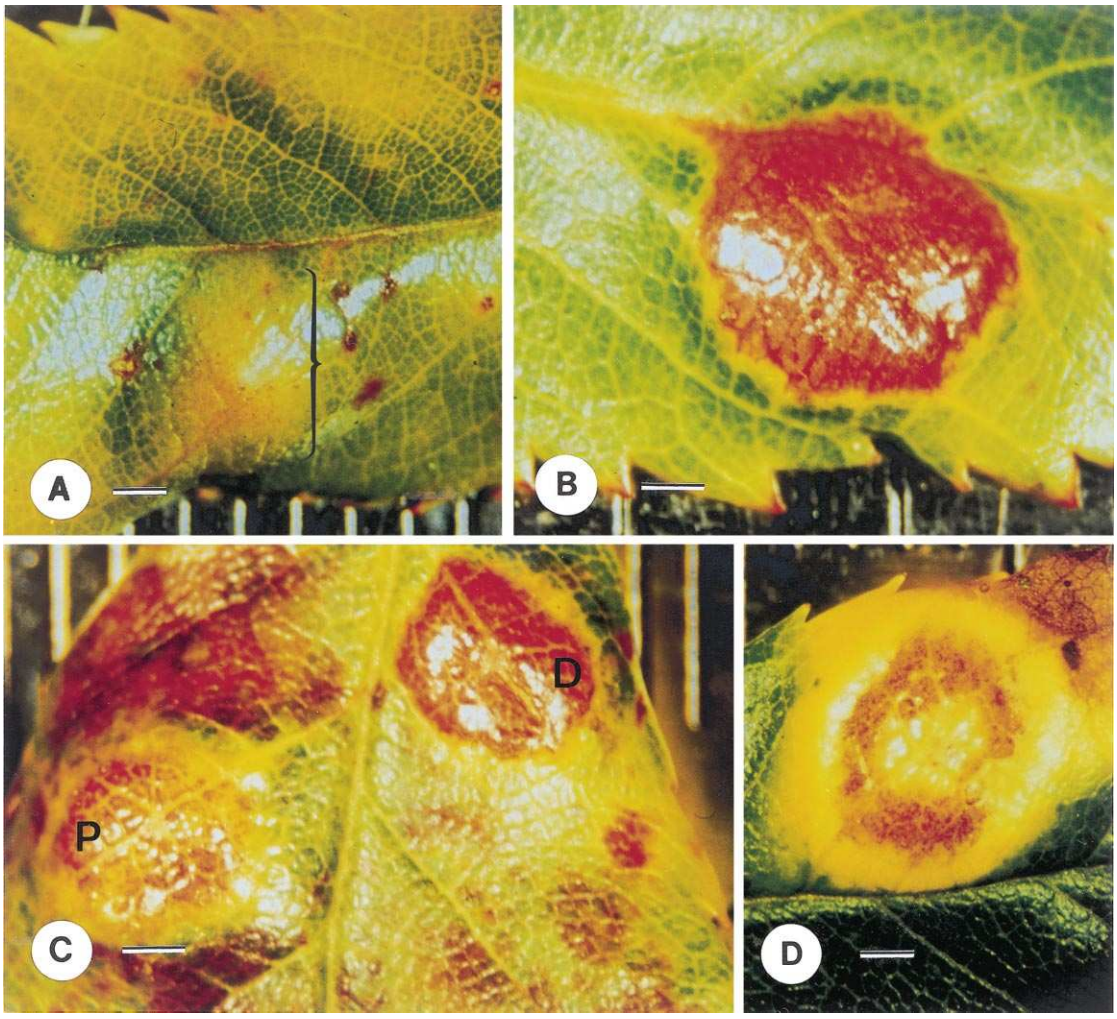



**Fig. 1** Macroscopic surface features of different stages in the development of *Diplolepis rosaefolii* galls (*A*, *B*) and *Periclistus*-inhabited galls (*C*, *D*). Bars = 1.0 mm. *A*, Adaxial view of a *D. rosaefolii* gall (bracket) prior to maturation. *B*, Adaxial view of a mature gall of *D. rosaefolii*. Note the strong red pigmentation. *C*, Galls containing *D. rosaefolii* (*D*) and *Periclistus* (*P*) larvae. The *Periclistus*-containing gall protrudes from the surface of the leaf much more than the *D. rosaefolii*–containing gall. *D*, Young *Periclistus*-inhabited gall showing lightly pigmented central area on the upraised portion of the gall.

### *Scanning Electron Microscopy*

A total of 25 galls were collected to examine surface and internal features using SEM. They were fixed and dehydrated as described above.

Galls selected for examination of internal features were dehydrated through a graded tertiary butyl alcohol (TBA) series. Specimens were then transferred to a 50-50 solution of TBA and paraffin at 61°C and left overnight, and they were then subjected to two changes in 100% paraffin at 61°C overnight. Specimens were subsequently embedded in paraffin and cut with a rotary microtome (American Optical model 820 microtome, American Optical, Buffalo, N.Y.) until the gall chamber was reached. Following the procedures of Kemp et al. (1993), xylene was used to solubilize all paraffin from the galls. In preparation for critical-point drying, galls were dehydrated once again in a graded ethanol series.

A sample of 20 leaflets with eggs on the surface was also collected. These were placed in two overnight changes in 100% ethanol.

All galls were critical-point dried in a Ladd 28,000 critical-point drier, using $CO_2$ as the transitional fluid. Dried specimens were mounted on aluminum stubs with two-sided adhesive tape, and silver paint was applied to the base of the specimens to ground them. Specimens were coated with gold palladium in a Denton Vacuum Desk II sputter-coater to a thickness of 30 nm. All specimens were viewed with a Cambridge Stereoscan 604 scanning electron microscope. Digital images were captured using Semicaps software and printed on thermal paper using a Mitsubishi P67U video copy processor.

## Results

### *External Morphology of Galls*

***Diplolepis rosaefolii.*** The galls of *D. rosaefolii* are found on the second-order veins of rose leaflets close to the midvein.

Many galls are arranged in clusters or in a line adjacent to the veins. The majority of galls collected were found on the three most distal leaflets, but occasionally galls were found on the more proximal ones. The galls are smooth and lenticular and protrude more from the adaxial surface of the leaflet than from the abaxial surface.

Newly initiated galls of *D. rosaefolii* (0.5–1 mm in diameter) are of the same color or slightly lighter than the leaflets and are therefore difficult to distinguish. As the galls begin to grow, the tissue under larval influence is a lighter green than in the remaining leaflet, and a red spot may be visible in the center of the gall on the adaxial surface of the leaflet (fig. 1*A*). Most galls turn completely red as they mature (fig. 1*B*), while others are yellow or only partially red. Although color is visible on both surfaces of the gall, the adaxial coloration is often more vivid.

***Periclistus.*** Once the *Periclistus* larvae are actively feeding in the parasitized galls, the overall size of these structures is larger than those containing *D. rosaefolii* larvae (fig. 1C). The adaxial surface of the gall is irregular and the central portion is raised, forming a semicircular projection from the leaf. In young galls, the central upraised portion of the gall is characterized by a lightly pigmented area (yellow or green) surrounded by a ring of darkly pigmented red or green tissue (fig. 1*D*). Around the darkly pigmented area, there is a flat ring of yellow tissue. At maturity, the entire gall turns red.

## Rosa virginiana*: Typical Leaf Histology*

The following is a brief description of the leaflet anatomy of *R. virginiana* for future comparison with the gall tissues. The epidermal cells of the leaf (fig. 2*A*) are compactly arranged and covered with a thin cuticle. The cells of the abaxial epidermis are visually smaller than those of the adaxial epidermis. Guard cells are usually found in the abaxial epidermis. The mesophyll consists of typical palisade and spongy parenchyma (fig. 2*A*). The vascular tissue of the leaf forms typical bundles throughout the mesophyll.

## *Developmental Morphology of Galls Induced by* D. rosaefolii

*Stage 1: initiation.* Eggs are found on immature leaflets ranging in size from 5 to 10 mm. These leaflets are usually in the process of unfolding as the compound leaf expands, and the mesophyll cells are not yet differentiated into palisade and spongy mesophyll. The eggs, which range from 150 to 260 $\mu$m in length, are laid on the abaxial side of the leaflets along secondary veins (fig. 2*B*). The ovipositional fluid surrounds the distal pole of the eggs (opposite the egg stalk) and attaches the eggs to the leaflets (fig. 2*C*, 2*D*).

The egg is attached to a single epidermal cell, although the ovipositional fluid covers a number of cells (fig. 3*A*, 3*B*). Changes are first observed in the cells adjacent to the ovipositional fluid at the point of attachment (fig. 3*B*, area delimited by arrowheads). These cells contain fragmented vacuoles, and the nucleus and nucleoli appear enlarged in relation to those in adjoining cells (fig. 3*B*, 3*D*). As development progresses, a pad of tissue forms under the egg attachment site (fig. 3*C*). Subsequently, a small chamber forms directly under the egg. The larva will enter that chamber through a narrow channel after it hatches (fig. 3*E*, 3*F*). The size of the chamber at this stage of development is relatively small compared to later stages, as shown in subsequent figures.

*Stage 2: nutritive tissue formation.* Once the larva enters the chamber, the larval pathway appears to be obstructed (fig. 4*A*, 4*B*). The egg chorion (casing) remains on the outer surface of the leaf (fig. 4*B*). As development continues, a group of densely cytoplasmic (i.e., metabolically active) cells, called "nutritive cells," are visible on one side of the larval chamber (fig. 4*A*, 4*C*). Eventually, the nutritive cells surround the entire larval chamber (fig. 4*D*). Nutritive cells have fragmented vacuoles, which are large in those cells located at the periphery of the nutritive tissue but considerably reduced or absent in cells located nearest to the larval chamber, thus forming a vacuolar gradient running toward the larval chamber (fig. 4C). A cellular gradient is also visible; the innermost cells lining the chamber are larger than adjacent cells (fig. 4*C*). The nucleus and nucleoli in these innermost cells are also much larger than those in the adjoining cells, referred to in this study as parenchymal nutritive tissue (PNT) (fig. 4*C*). Specific staining with Sudan black B reveals large amounts of lipids sequestered in the cytoplasm of the cells closest to the larval chamber. The parenchyma cells, which underlie the epidermis, form more or less radial files, suggesting some type of meristematic activity (fig. 4*D*), and contain starch granules (visible with Sudan black B staining) and few chloroplasts. The vascular tissue of the leaflet is lateral to the gall tissue (fig. 4*D*). During later stages of development, the larval chamber becomes oval in cross section (fig. 4*E*).

*Stage 3: sclerification and gall growth.* Cells underlying the epidermis on either side of the larval chamber lay down secondary walls, forming a plate of cells above and below the chamber (fig. 5*A*, 5*B*). These layers are commonly referred to as the primary sclerenchymal plates. The locus of the origin of these primary plates below the epidermis layers appears to be variable. The walls of these sclerenchyma cells thicken, and the cytoplasm in each cell is restricted to a thin ring surrounding the large central vacuole at this stage (fig. 5*C*). Formation of the primary sclerenchymal plates is polarized, differentiating first on the adaxial side of the gall chamber and then spreading to the abaxial side (fig. 5*A*, 5*B*). Differentiation on the abaxial side of the gall chamber begins before the differentiation on the adaxial plate is completed. The sclerenchymal plates extend beyond the larval chamber in the longitudinal axis of the gall and define the lateral extent of larval influence on the leaf tissue. They separate the gall tissues into those of the inner gall, containing nutritive and parenchymal tissue, and the outer gall, containing chlorenchymal and dermal tissue.

At this stage of development, nutritive cells are more abundant and the vacuolar gradient is more pronounced (fig. 5*D*) than in previous stages. Nutritive cells on the sides of the gall chamber are more plentiful than those above and below the chamber and appear cambial in nature during later stages of sclerification (fig. 5*E*). Vascular tissue is sometimes found in the lateral regions of the lower gall wall below the primary sclerenchymal plate and is present as small bundles in central regions of the wall (fig. 5*E*, 5*F*).

*Stage 4: maturation.* During this stage of development, the walls of the cells of the primary sclerenchymal plate

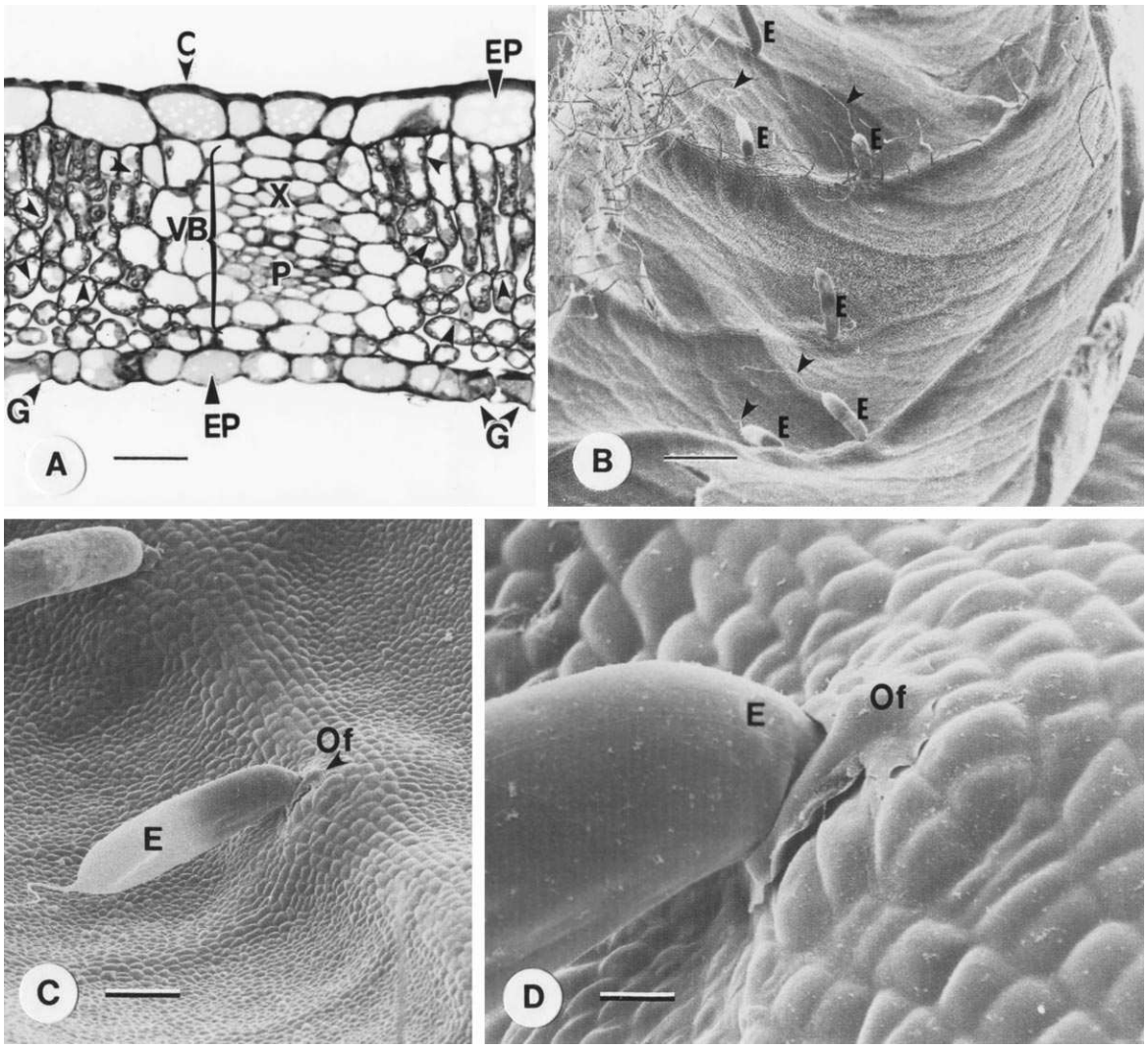



**Fig. 2** Leaf anatomy of *Rosa virginiana* (*A*) and arrangement of *Diplolepis rosaefolii* eggs on the abaxial surface of leaflets (*B–D*). *A*, Cross section of a mature leaf of *R. virginiana*. Note the cuticle (*C*) on the adaxial epidermis, the anatomy of the vascular bundle (*VB*), the arrangement of the chloroplast-containing mesophyll (arrowheads), and the presence of guard cells (*G*) in the abaxial epidermis. Bar = 25 μm. *EP* = epidermis; *P* = phloem; *X* = xylem. *B*, Scanning electron micrograph of *D. rosaefolii* eggs showing pedicels (arrowheads) of the eggs (*E*). Bar = 200 μm. *C*, Scanning electron micrograph showing the site of egg attachment (arrowhead) on the secondary vein. Bar = 50 μm. *D*, Higher magnification of *C* showing ovipositional fluid (*Of*) at the site of attachment of the egg (*E*) to the leaflet. Bar = 23 μm.

appear thicker, and the size of the cell lumen is reduced when compared to cells in the previous stage (cf. fig. 6*B*, 6*C* with fig. 5*B*, 5*E*, 5*F*). A new layer of sclerenchyma cells is visible adjacent to the primary plates toward the outer surface of the gall (fig. 6*C*). These sclerenchyma cells form the secondary sclerenchymal plates. The secondary plate in the adaxial gall wall (fig. 6*B*) appears to have more cell layers than the plate in the abaxial gall wall (fig. 6*C*). A slight undulating pattern of the secondary plate is visible in the adaxial wall (fig. 6*A*, 6*B*). Parenchyma cells are present between the primary and secondary plates of both walls and are also found adjacent to the epidermis in the adaxial gall wall (fig. 6*B*, 6*C*). Vascular tissue may be present in the lateral and central portions of the lower gall wall between the primary and secondary plates (fig. 6*C*).

During the early part of this stage, the nutritive tissue already appears to be more highly vacuolated than in previous stages (cf. fig. 6*D* with figs. 4*C*, 5*D*). Secondary wall thickenings differentiate in the parenchyma at the lateral extremes of the gall wall and eventually meet with the sclerenchyma of the primary plates to form a ring of sclerenchyma around the chamber. A portion of this ring is shown in figure 6*E*. As maturation continues, the nutritive tissue and parenchyma surrounding the larval chamber are consumed by the larva until the chamber is nearly surrounded by the lateral and primary plate sclerenchyma (fig. 6*E*, 6*F*). A summary of changes in the

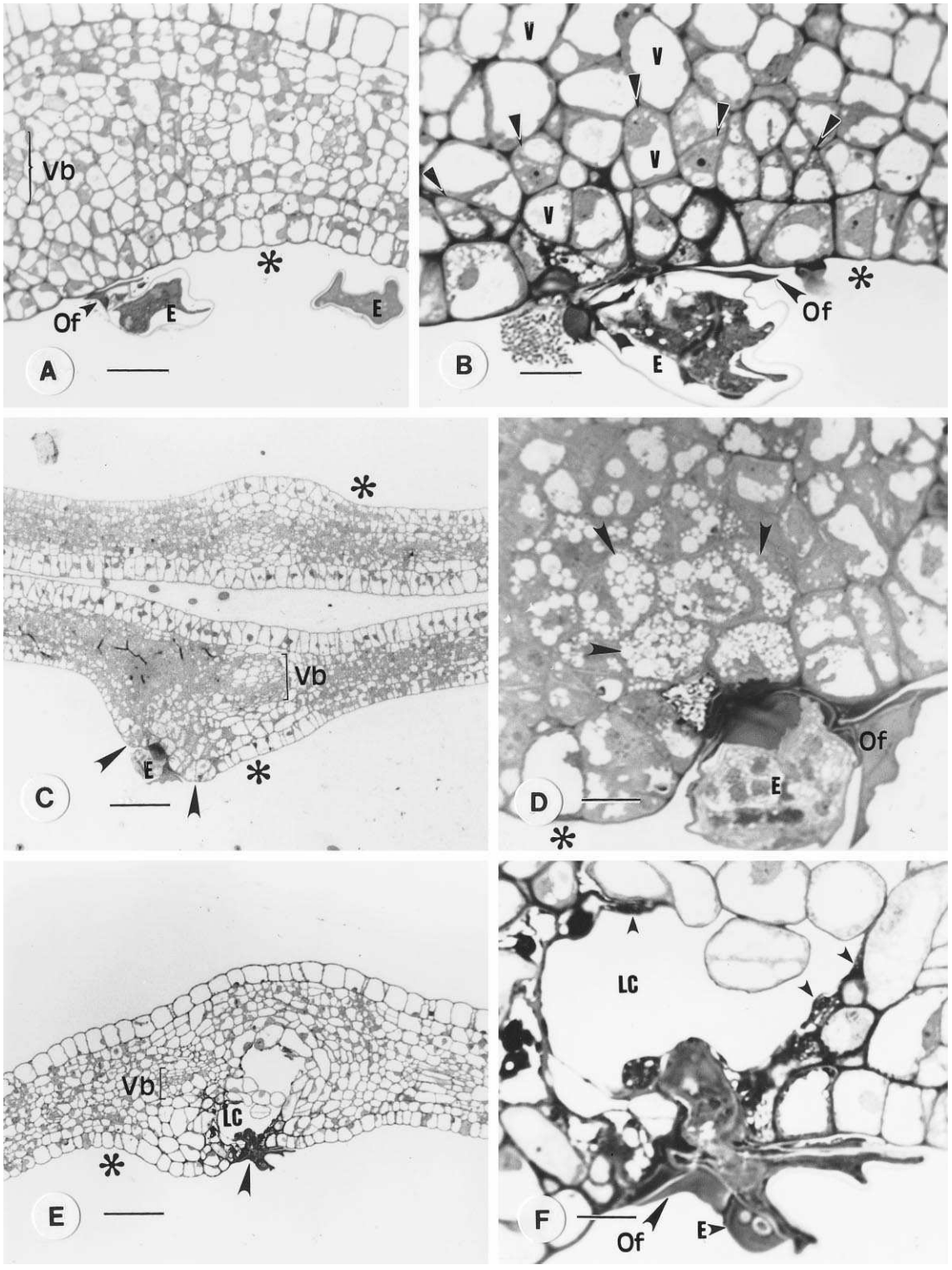



**Fig. 3** Stage 1: initiation of *Diplolepis rosaefolii* galls. *A*, Cross section of a leaflet showing the location of the point of attachment of an egg (*E*) on the abaxial epidermal layer. Bar = 25 $\mu$m. *B*, Higher magnification of a stage similar to that in *A* showing cellular changes in the vicinity of the point of attachment of the egg (*E*) to the leaflet (area delimited by arrowheads). Note the dense cytoplasm and reduced size of the vacuole (*V*) in these cells in relation to surrounding tissue. Bar = 10 $\mu$m. *C*, Low magnification of cross section of a folded leaflet blade showing the pad of tissue (arrowheads) that forms on either side of the attachment site of the egg (*E*). Bar = 0.2 mm. *D*, Detail of the site of attachment of the egg (*E*) as depicted in *C* showing fragmented vacuolation (arrowheads) of the adjacent cells. Bar = 10 $\mu$m. *E*, Low magnification of the final phase in the initiation stage. Note the presence of a chamber (*LC*) adjacent to the site of larval entry (arrowhead). Bar = 50 $\mu$m. *F*, Detail of *E* showing the presence of lysed cells (arrowheads) surrounding the chamber (*LC*). Bar = 10 $\mu$m. *Of* = ovipositional fluid; *Vb* = vascular bundle; asterisk = abaxial surface of leaflet.

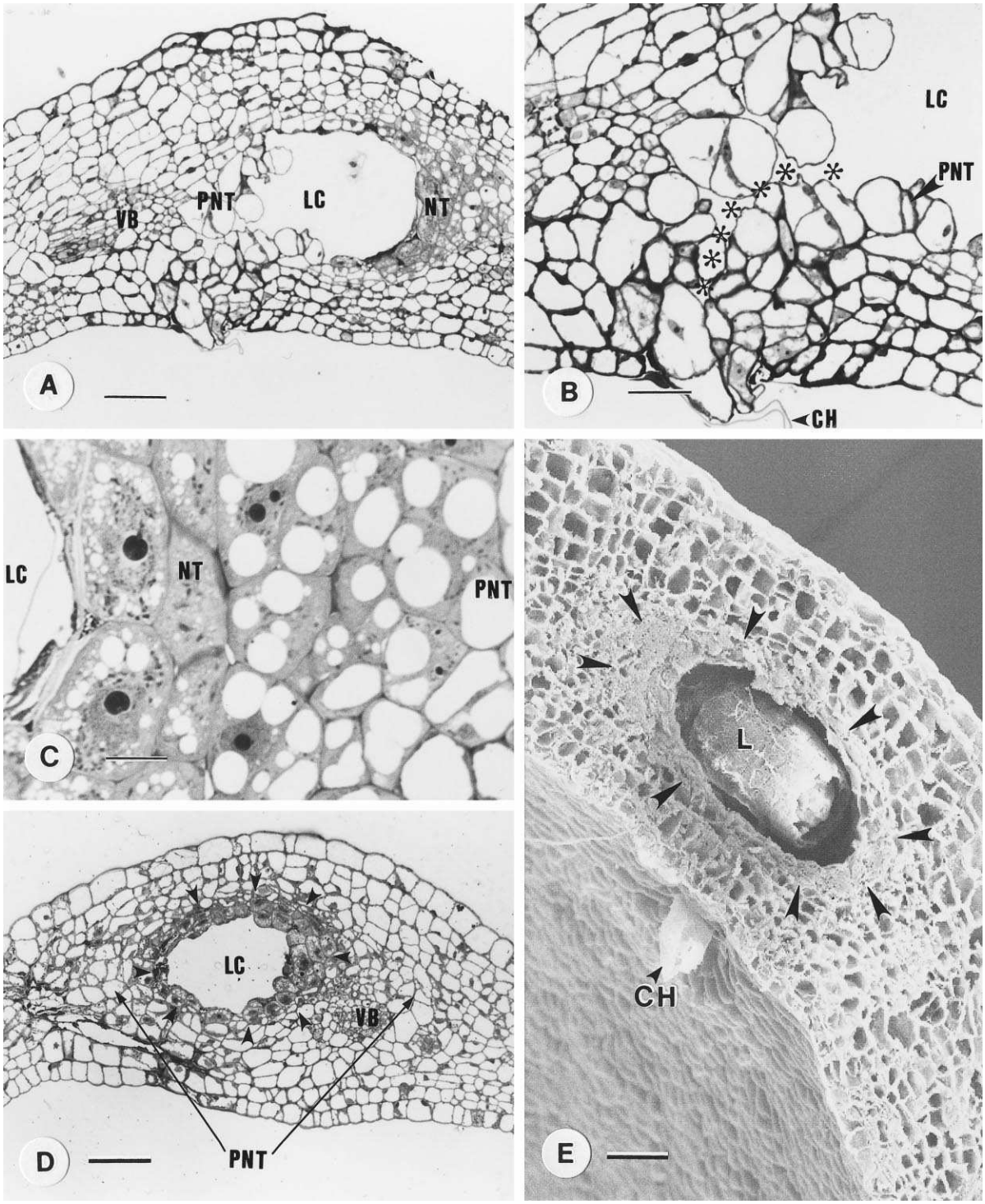



**Fig. 4** Stage 2: development of nutritive tissue in *Diplolepis rosaefolii* galls after the larva has entered the chamber. *A*, Cross section of a gall showing an early stage of nutritive tissue (*NT*) development restricted to one side of the larval chamber (*LC*). Bar = 50 $\mu$m. *B*, Higher magnification of *A* showing the occlusion of the pathway of entry (asterisk) of the larva. Bar = 25 $\mu$m. *C*, Detail of the nutritive tissue showing the vacuolar gradient and variations in cell size from the larval chamber (*LC*) toward the periphery of the gall. Bar = 10 $\mu$m. *D*, Cross section of a young gall in which the nutritive tissue surrounds the larval chamber (arrowheads). Note the vascular bundle (*VB*) on one side of the gall. Bar = 50 $\mu$m. *E*, Scanning electron micrograph of a cross section through a gall. Dense cells (arrowheads) of the nutritive tissue surround the larval chamber. Bar = 22 $\mu$m. *CH* = egg chorion; *L* = larva; *PNT* = parenchymal nutritive tissue.

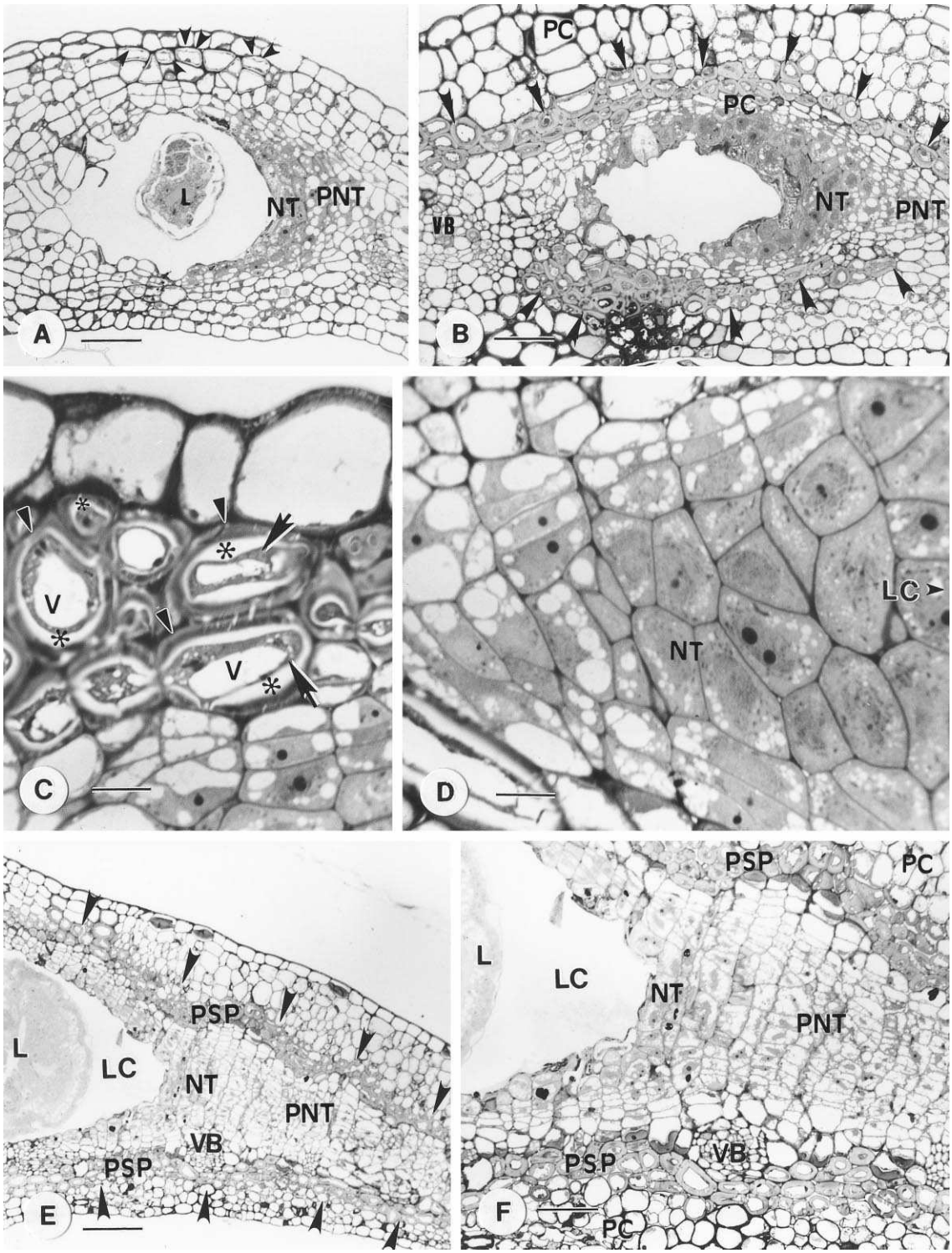



**Fig. 5** Stage 3: sclerification in *Diplolepis rosaefolii* galls and gall growth. *A*, Early stage of sclerification highlighted by the presence of secondary wall thickenings (arrowheads) in cells underlying the adaxial epidermis of the leaflet. Bar = 50 $\mu$m. *B*, Later stage of sclerification showing primary sclerencymal plates (arrowheads) above and below the larval chamber. Bar = 50 $\mu$m. *C*, High magnification of sclerenchyma cells showing the secondary wall (arrowheads) and the ring of cytoplasm (large arrows) surrounding the large central vacuole (*V*). The separation of the cytoplasmic ring from the secondary wall (asterisk) is an artifact. Bar = 10 $\mu$m. *D*, Detail of nutritive tissue showing pronounced vacuolar gradient and variation in cell size from the larval chamber (*LC*) to the periphery of the gall. Bar = 10 $\mu$m. *E*, Cross section through a gall chamber (*LC*) showing extension of the primary sclerenchymal plates (*PSP*; arrowheads) to the periphery of the gall. Bar = 0.1 mm. *F*, Higher magnification of *E* showing details of the nutritive tissue. Note the nutritive tissue (*NT*) and parenchymal nutritive tissue (*PNT*) between the primary sclerenchymal plates (*PSP*). Bar = 50 $\mu$m. *L* = larva; *PC* = parenchyma cells; *VB* = vascular bundle.

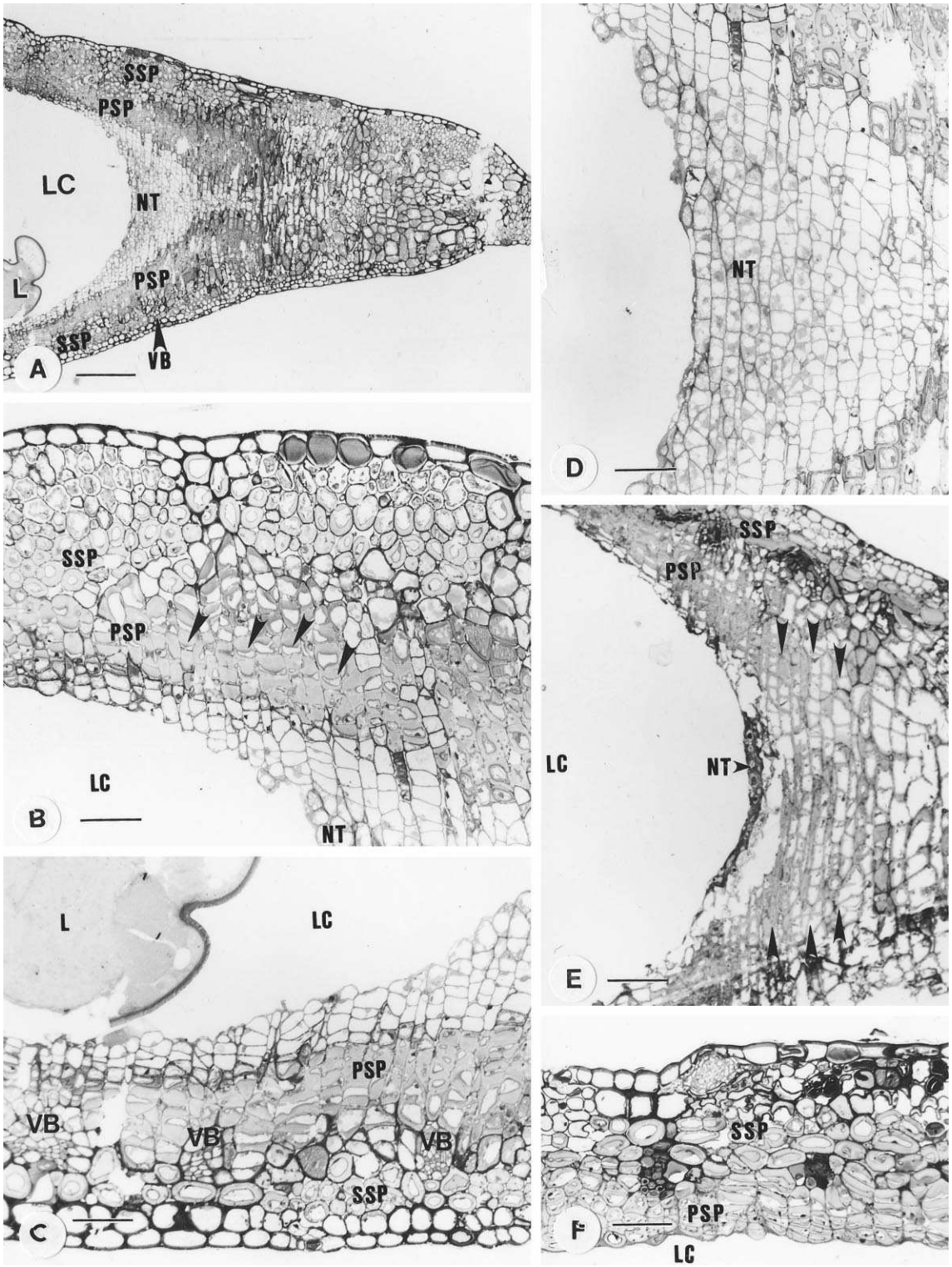



**Fig. 6** Stage 4: maturation of *Diplolepis rosaefolii* galls. *A*, Early stage in maturation showing different tissue types: *L* = larva; *LC* = larval chamber; *NT* = nutritive tissue; *PSP* = primary sclerenchymal plate; *SSP* = secondary sclerenchymal plate; *VB* = vascular bundle. Bar = 0.25 mm. *B*, Higher magnification of the adaxial gall wall in *A*. Note the partial occlusion of the lumen (arrowheads) in the sclerenchyma of the primary plates (*PSP*) and the slight undulation of the plate. Bar = 50 $\mu$m. *C*, Higher magnification of the abaxial gall wall in *A*. Note the smaller number of cell layers in the lower secondary sclerenchymal plate (*SSP*) in comparison to the plate of the adaxial wall in *B*. Bar = 50 $\mu$m. *D*, Higher magnification of the nutritive tissue (*NT*) found laterally in the gall from *A*. At this stage of development, the nutritive cells appear to be highly vacuolated. Bar = 50 $\mu$m. *E*, Detail of the lateral sclerenchyma (between the two rows of arrowheads) present in the gall wall at later stages of maturation. Bar = 0.1 mm. *F*, Mature adaxial gall wall. The larva has consumed the nutritive tissue and parenchymal nutritive tissue. Consequently, the chamber (*LC*) is lined by cells of the primary sclerenchymal plates (*PSP*). Bar = 50 $\mu$m.

**Table 1**

**Summary of Developmental Changes in Tissue Layers of *Diplolepis rosaefolii* Galls**

| Tissue layer and portion of gall | Stage 1: initiation | Stage 2: nutritive tissue formation | Stage 3: sclerification/ gall growth | Stage 4: maturation |
|---|---|---|---|---|
| Parenchymal | Cells have fragmented vacuoles; nucleus and nucleoli enlarged | Starch granules in cells; cells square in cross section; few chloroplasts | Few chloroplasts | Cells lost in later stage |
| Nutritive | Not present | Reduced vacuole; nucleus and nucleoli enlarged; large amount of lipids | More cells on sides of chamber than above and below | Cells more highly vacuolated; cells lost in later part of stage |
| Sclerenchymal: | | | | |
| Primary plate | Not present | Not present | Thin secondary walls; thin ring of cytoplasm; large central vacuole | Thick secondary walls; lumen reduced |
| Secondary plate | Not present | Not present | Not present | Thin secondary walls |
| Lateral | Not present | Not present | Not present | Forms at sides of chamber; meets primary plate |

tissue layers during development is presented in table 1 (see also top of fig. 10).

### *Developmental Morphology of Galls Containing* Periclistus

Eggs of *Periclistus* can be deposited in galls induced by *D. rosaefolii* during all but the earliest stages of development. This wide developmental window in *D. rosaefolii* gall formation, during which *Periclistus* can lay eggs in the galls, results in some variability in the changes which are subsequently induced by the *Periclistus* larvae in the galls. In general, when *Periclistus* takes over the influence of the gall at an early stage (during stages 2 or 3), more changes are evident than when the gall is inhabited at stage 4. However, the initial changes induced by *Periclistus* larvae are the same regardless of the stage at which the *Periclistus* eggs are laid in galls of *D. rosaefolii*. The following sections describe the general arrangement of tissue types in *Periclistus*-inhabited galls so that it can be compared with that of the inducer.

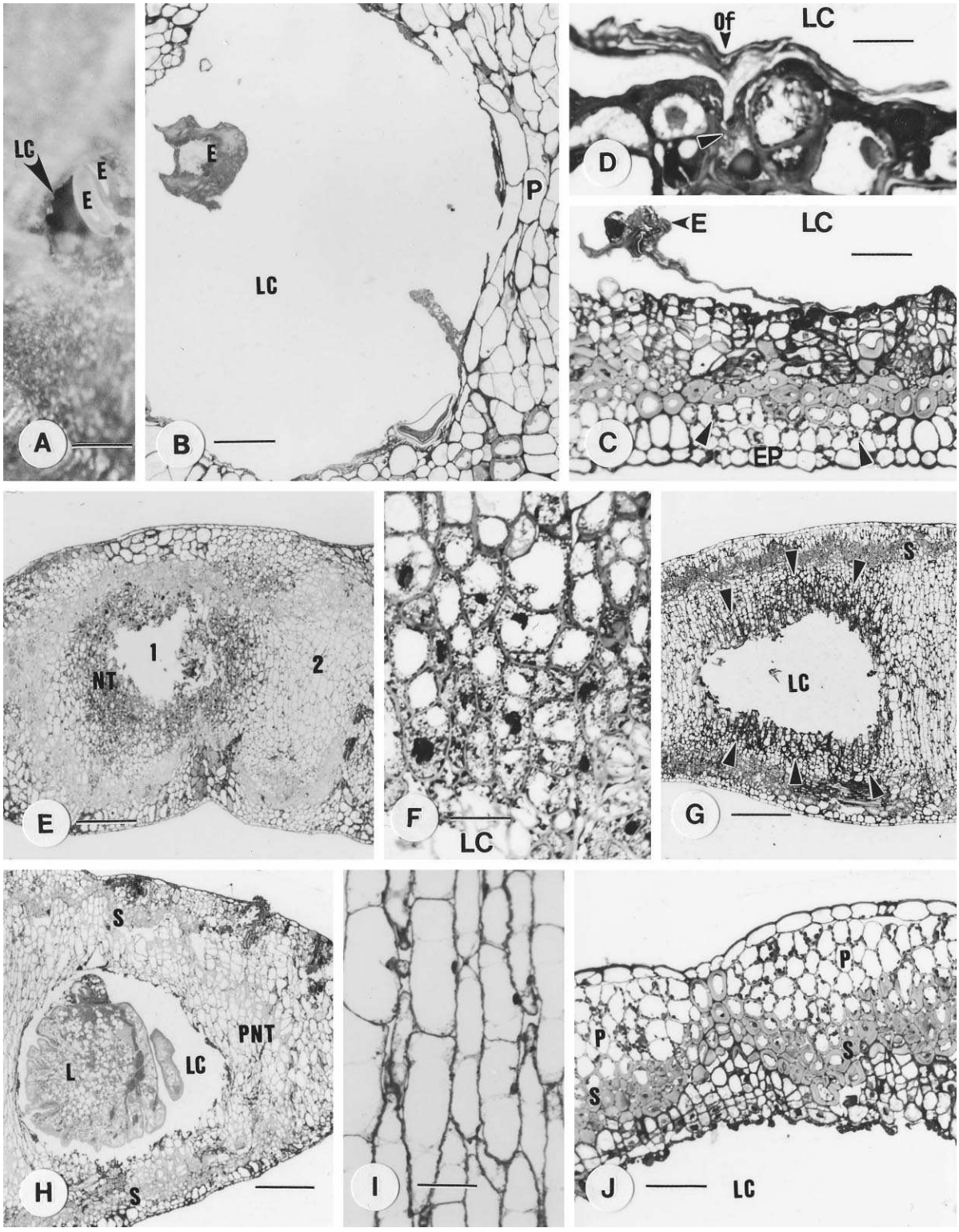
One or more eggs of *Periclistus* are laid in the *D. rosaefolii* gall chamber (fig. 7*A*–7*C*). The egg stalk is attached to a single cell of the innermost gall wall by ovipositional fluid (fig. 7*C*, 7*D*). No nutritive tissue is observed around the gall chamber when *Periclistus* eggs are in the gall (fig. 7*B*, 7*C*). However, parenchymal tissue is visible on the sides of the larval chamber (fig. 7*B*). These parenchyma cells are rectangular in cross section and are devoid of chloroplasts. Parenchyma cells containing chloroplasts are found adjacent to the epidermis (fig. 7*C*).

### *Changes in Gall Tissues Induced by* Periclistus *Larvae*

*Nutritive tissue.* When *Periclistus* larvae hatch and begin to feed, this event is correlated with the presence of nutritive tissue surrounding the larval chamber (fig. 7*E*). The nutritive tissue consists of multiple files of cells that appear evenly dispersed around the larval chamber (fig. 7*E*, 7*F*). A decreasing vacuolar gradient is present in the nutritive cells; vacuoles are large in cells abutting the parenchyma and become increasingly smaller in cells closer to the larval chamber (fig. 7*F*).

During later stages of development, the nutritive cells appear

**Fig. 7** *Periclistus* egg attachment and initial changes in *Diplolepis rosaefolii* galls (*A–D*). Cross sections of *Periclistus*-inhabited galls, highlighting the nutritive and parenchyma tissues (*E–J*). *A*, Dorsal view of *D. rosaefolii* gall chamber (*LC*) showing *Periclistus* eggs (*E*). Bar = 0.17 mm. *B*, Cross section of *D. rosaefolii* gall showing enclosed *Periclistus* egg (*E*). Note the absence of nutritive tissue surrounding the chamber (*LC*) and the presence of thin-walled enlarged parenchyma (*P*) peripheral to the chamber. Bar = 10 μm. *C*, Cross section of the abaxial gall wall showing attachment of *Periclistus* egg (*E*). Note the chloroplasts (arrowheads) in the parenchyma cells. Bar = 50 μm. *D*, Higher magnification of *C* showing details of attachment site of a *Periclistus* egg inside the gall chamber (*LC*). The pedicel of the egg is attached to a single cell (arrowhead) by the ovipositional fluid (*Of*). Bar = 10 μm. *E*, Cross section of young *Periclistus*-inhabited gall. Although this gall has two chambers, only one is in median section (*1*), while the other (*2*) is sectioned through its parenchymal layer. Nutritive tissue (*NT*) surrounds the larval chamber. Bar = 0.25 mm. *F*, Detail of nutritive tissue of gall shown in *E*. Note an increasing amount of vacuolation in cells of the nutritive tissue from the gall chamber (*LC*, bottom of photo) toward the periphery of the gall (top of photo). Bar = 10 μm. *G*, Cross section of a maturing *Periclistus*-inhabited gall at a later stage of inquiline influence. At this stage, the nutritive tissue (arrowheads) is only present at the top and bottom of the gall chamber (*LC*). Bar = 38 μm. *H*, Cross section of a mature *Periclistus*-inhabited gall. The *Periclistus* larva (*L*) is large in relation to the size of the gall chamber (*LC*). Note the lack of nutritive tissue lining the gall chamber. Bar = 0.19 mm. *I*, Detail of thin-walled and highly vacuolated parenchyma cells lateral to the gall chamber in *Periclistus*-inhabited gall. Bar = 10 μm. *J*, Cross section of the adaxial wall of a *Periclistus*-inhabited gall showing the patches of parenchyma cells (*P*) underlying the epidermal layer. Note the presence of chloroplasts (darkly stained inclusions) in these cells. Bar = 50 μm. *PNT* = parenchymal nutritive tissue; *S* = sclerenchyma.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more plentiful at the top and bottom of the larval chamber, and only a few layers of cells are present on either side (fig. 7*G*). Eventually, in nearly mature galls, nutritive tissue is absent around the chamber (fig. 7*H*).

*Parenchymal tissue.* Parenchymal tissue is found around the nutritive tissue in the inner gall wall and under the epidermis in the outer gall wall (fig. 7*E*). The amount of parenchyma in the inner gall wall is more extensive on either side of the gall chamber than at the top and bottom of the chamber (fig. 7*G*, 7*H*). In the inner gall wall, the parenchyma cells are rectangular in cross section, with a thin layer of cytoplasm surrounding a large central vacuole, and contain no chloroplasts (fig. 7*I*). Parenchyma cells in the outer gall wall are square in cross section and contain numerous chloroplasts in the ring of cytoplasm that surrounds the central vacuole (fig. 7*J*). The inner gall wall is separated from the outer gall wall by sclerenchyma cells.

*Sclerenchymal tissue.* The sclerenchymal cells in the upper gall wall form an undulating layer between the parenchymal and nutritive tissues (fig. 8*A*). At various intervals along the gall wall, the sclerenchyma cells form extensions to the epidermis, thus dividing the parenchyma of the outer gall wall into a series of pockets underlying the epidermis (fig. 8*B*). In galls where layers of both primary and secondary sclerenchyma are present (fig. 8*C*), the secondary sclerenchymal layer forms an undulating band of tissue (fig. 8*D*).

The sclerenchyma cells of the abaxial gall wall form a band parallel to the gall chamber (fig. 8*E*). Vascular tissue is present along the length of the entire gall wall and is situated between the sclerenchyma and the nutritive tissues (fig. 8*F*). Sclerenchyma extends on either side and at the top of the vascular bundles, thus enclosing each bundle (fig. 8*E*). In galls where both layers of sclerenchyma are present, the vascular bundles are found between the layers (fig. 8*F*).

Secondary wall thickenings can be seen in the cells on either side of the gall chamber along the boundary of the large rectangular gall parenchyma (fig. 9*A*). During later stages of development, sclerenchyma is present on either side of the gall chamber (fig. 9*B*, 9*C*), and this extends to the sclerenchymal plates to surround the entire gall chamber. When both primary and secondary sclerenchymal plates are present, the lateral sclerenchyma extends to the inner plate. In galls where two or more chambers are present, the lateral sclerenchyma is only present in the outermost side of the chamber.

### *Summary of Changes in* Periclistus*-Inhabited Galls*

Tissue development in these galls is largely dependent on the stage of development of the inducer gall at the time the eggs are layed and on the number of eggs layed in the gall. Single chambered galls, containing only one larva, have more or less concentric layers of tissue surrounding the larval chamber (fig. 7*G*). In multichambered galls, with more than one larva present, the nutritive tissue and the inner layer of parenchyma surround each chamber. However, the sclerenchyma does not differentiate in regions between chambers. These characteristics represent the major differences in tissue arrangement between *Periclistus*-inhabited galls and *Diplolepis*-inhabited galls. Galls in which *Periclistus* eggs are laid at stage 2 (nutritive tissue formation) or stage 3 (sclerification) of *D. rosaefolii* development do not contain secondary sclerenchymal plates. However, if eggs are laid at stage 4 of development (maturation) when secondary plates are already present in the *D. rosaefolii* gall, both plates are present and the undulations in the upper wall are much more pronounced than when the *D. rosaefolii* larva is present (cf. fig. 6*A*, 6*B* with fig. 8*A*, 8*B*). The changes induced by *Periclistus* larvae are summarized in table 2 and illustrated in figure 10.

## Discussion

When eggs of *Diplolepis rosaefolii* are laid on the leaves of *Rosa virginiana*, the mesophyll cells are still undifferentiated. Soon after the larva enters the gall chamber, the mesophyll cells peripheral to the larval chamber are differentiated into the palisade and spongy tissues characteristic of the mature leaf. However, subsequent changes in tissue patterns induced by the *D. rosaefolii* larva diverge significantly from normal leaf development. Initially, lysis of the cells in contact with the egg results in the formation of a chamber in the leaf that the larva enters upon hatching. This is followed by the dedifferentiation of the mesophyll cells immediately surrounding the chamber and results in the formation of the nutritive tissue. Brooks and Shorthouse (1997*b*) noted similar dedifferentiation in the development of galls induced by *Diplolepis nodulosa*, as did Shorthouse (1975) in galls of *Diplolepis lens*, *Diplolepis nebulosa*, *Diplolepis ignota*, and *Diplolepis gracilis*. The parenchyma surrounding the nutritive cells remains undifferentiated, and air spaces do not develop between the cells as would typically happen during the mesophyll differentiation. Formation of the sclerenchymal plates is the most notable change that takes place in the gall tissues, as sclerenchyma is not found in the normal leaf tissue except in and around the vascular bundles. Thus, development of the gall results in the formation of tissues whose type and orientation in no way resemble that of the typical leaf. Furthermore, the gall cells are larger and more abundant than the typical leaf cells, resulting in a growth that is readily discernible on the surface of the leaf.

Changes in cell contents, such as the lack of chloroplasts in the parenchyma, are also observed in gall tissues when compared to those of the normal leaf. However, the greatest change in cell contents is observed in the nutritive cells, which exhibit enlarged nuclei and nucleoli, as well as large quantities of lipids and many small, fragmented vacuoles. These cytological features are similar to those found in other cynipid galls studied and are characteristic of cells that are physiologically and metabolically active (Bronner 1977).

### Diplolepis rosaefolii *Gall Development*

The development of *D. rosaefolii* galls was similar in sequence to that described by Rohfritsch (1992) in her general model of cynipid gall development, where three stages are highlighted. These include (i) initiation, (ii) growth, and (iii) maturation. This model is based on circular galls in which tissues are arranged in concentric rings around a centrally located gall chamber. Although the orientation of the tissues in lenticular galls, such as those induced by *D. rosaefolii*, is not circumscribed as depicted in Rohfritsch's model, the sequence of dif-

ferentiation of the various tissue layers follows the general cynipid model.

Eggs of *D. rosaefolii* are attached by ovipositional fluid to a single epidermal cell on the abaxial surface of the leaf. This is consistent with other studies on *Diplolepis* species (e.g., Bronner 1985 on *Diplolepis rosae*; Shorthouse 1993 on *Diplolepis polita*) and provides further evidence of the precise manner in which the *Diplolepis* females lay their eggs. Eggs of *D. rosaefolii* are normally laid on the upper three distal leaflets, but the reason for the almost total exclusion of the lower proximal leaflets is unclear. The distal leaflets may simply be more accessible to the ovipositing female. If the leaflets are still enclosed in the stipules or have just begun emerging from the stipules when the female oviposits, these would be uppermost and therefore the first to emerge. Similarly, Declerck-Floate and Steeves (1995) found that the gall midge *Cystiphora sonchi* (Bremi) oviposits on the tips of the leaves of *Sonchus arvensis* L. because of the basipetal pattern of leaf maturation. This type of development is also a feature of leaves of *R. virginiana* (D. A. LeBlanc, unpublished data) and could explain the placement of eggs on the three distal-most leaflets.

Rohfritsch (1992) lists a number of factors that are believed to be involved in cynipid gall initiation. These include (i) tissue wounding during oviposition, (ii) influence of the ovipositional fluid, and (iii) activity of the egg and/or larva. When the *D. rosaefolii* female lays an egg on the leaf, only one epidermal cell is damaged. One could therefore question whether the subsequent response is elicited by this incident. However, the ovipositional fluid covers a number of epidermal cells, and therefore, it is more likely that this is the mediator involved in initiation. Rohfritsch (1992) reports that the ovipositional fluid and the egg chorion act as an interface between the egg and the underlying plant, allowing for the movement of substances between the two. This suggests that some influence may also be exerted by the unhatched larva through this interface. Even before the larva hatches, significant changes take place directly below the point of attachment of the egg. Bronner (1977) showed that cynipid eggs have a lytic effect *in vitro*. The initial signs of change in the leaf cells is a fragmentation of the vacuole in cells underlying the egg. This manifestation is believed to be the first stage in the autolytic process (Rohfritsch 1992). The opening that forms as a result of this lytic effect will become the chamber that the larva enters upon hatching. Although we did not directly observe chamber formation in *D. rosaefolii* galls, evidence of this autolytic process was visible as remains of lysed cells surrounding the chamber at the time of larval entry.

Hough (1952) found that the nutritive tissue in galls of *Neuroterus quercus-baccarum* L. on oak leaves differentiated as a dome surrounding the head of the horizontally oriented larva. In *D. rosaefolii* galls, nutritive tissue appeared to differentiate at one end of the chamber first and eventually spread out, surrounding the larval chamber. This may be the result of larval feeding patterns initially starting on one side of the chamber and spreading to other points as the larva turns while feeding. During these early stages of gall development, the larval chamber is small in relation to the size of the larva and restricts its movements. The fact that nutritive tissue is sparse at the top and bottom of the chamber may be due to the fact that the larva feeds less frequently in these areas, as they are not easily accessible.

The formation of the primary sclerenchymal plates also shows polarity in differentiation, with the upper plate appearing before the lower plate. Reference to a polar differentiation of sclerenchyma has not been mentioned previously in the literature. Shorthouse (1975) found that the initial differentiation of the sclerenchyma tissue in four of the *Diplolepis* galls he studied involved a disk-shaped patch of cells above and below the chamber. In galls of *D. lens*, *D. nebulosa*, and *D. ignota*, he found that the disks were gradually incorporated into the sclerenchymal layer as further differentiation occurred. In galls of *D. ignota*, two circumscribing layers of sclerenchyma are present: (i) the inner layer consisting of thin-walled cells and (ii) the outer layer containing thick-walled cells. The sclerenchymal layers in *D. rosaefolii* galls are platelike rather than disklike, as seen in *D. ignota* galls. Furthermore, the cell walls appear to be thinner in the sclerenchyma of the secondary outer layer than in the sclerenchyma of the primary layer. Mani (1964) described cynipid galls with two concentric layers of sclerenchymal tissue where the outer layer contained cells with thinner walls than those in the inner layer. These descriptions of multiple sclerenchymal layers are similar to those observed in *D. rosaefolii* galls. The types of lentil-shaped galls studied by Hough (1952) in *N. quercus-baccarum*, Schnetzler (1964) in *Neuroterus numismatis* L., and Shorthouse (1975) in *D. lens* did not have secondary sclerenchymal plates, although *N. quercus-baccarum* and *N. numismatis* galls have two disks of sclerenchyma in the upper gall wall; but they do not extend out as much as the plates in *D. rosaefolii* galls.

During the maturation stage, lateral sclerenchyma differentiated from parenchyma at the edges of the gall chamber. Hough (1952) also observed lateral sclerenchyma in galls of *N. quercus-baccarum*, but the cells of this tissue were very elongated and extended from one sclerenchymal plate to the other. The lateral sclerenchyma in *D. rosaefolii* galls consists of a series of similar cells running from the upper to the lower plates. Fourcroy and Braun (1967) proposed that the lateral sclerenchyma is a protective adaptation to avoid crushing of the galls when they drop to the ground. Because the *N. quercus-baccarum* gall is suspended from the lower surface of a leaf and drops off the leaf at maturity, it may require greater protection than the *D. rosaefolii* gall, which is incorporated in the leaf tissue and therefore falls with the leaf during normal leaf fall in the autumn. However, the sclerenchyma could aid in protecting the larva against late parasitoids, desiccation, decomposition, and excess moisture or act as an insulator while the gall overwinters in the leaf litter.

A larger amount of vascularization of the gall tissues was observed in a number of *D. rosaefolii* galls at the maturation stage. This is probably due to an increased demand on the nutritive tissue by the larva because its increasing size requires more sustenance. This increased demand on nutrients would be provided by adjacent vascular tissue. Rohfritsch (1992) found that larvae enter their most active feeding stage only after the gall tissues are differentiated.

### *Changes in Gall Morphology Induced by* Periclistus

When *D. rosaefolii* galls become inhabited by *Periclistus*, this event is correlated with the presence of a large amount of

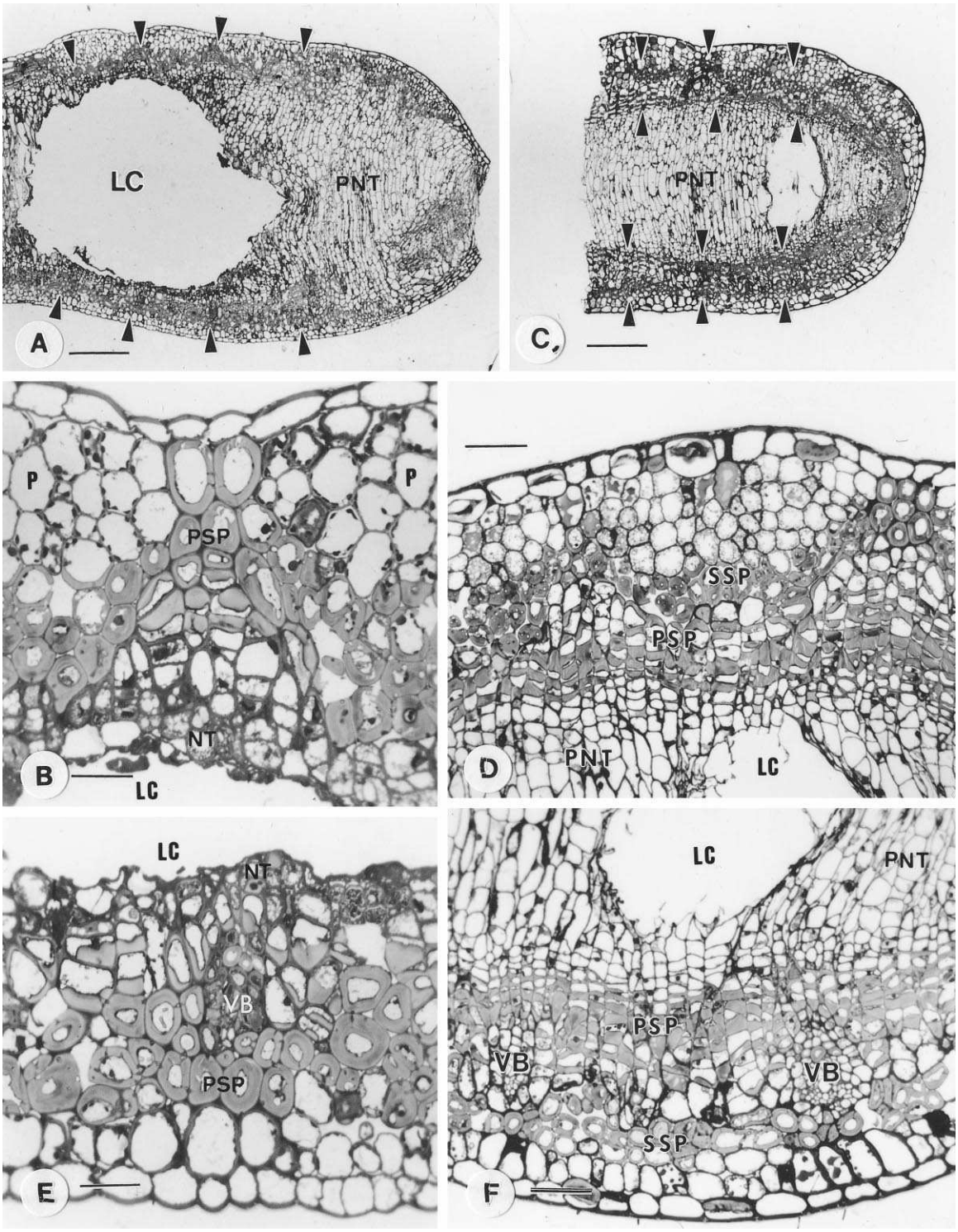


**Fig. 8** Cross sections of *Periclistus*-inhabited galls, showing arrangement of sclerenchymal layers. *A*, Cross section of *Periclistus*-inhabited gall chamber with single sclerenchymal plate (arrowheads) above and below the larval chamber (*LC*). Bar = 50 $\mu$m. *B*, Detail of an adaxial wall in *Periclistus*-inhabited gall showing undulations in the upper sclerenchymal plate (*PSP*) isolating areas of chloroplast-containing parenchyma tissue (*P*). Bar = 19 $\mu$m. *C*, Cross section of a *Periclistus*-inhabited gall with double sclerenchymal plates (arrowheads) above and below the larval chamber. Bar = 50 $\mu$m. *D*, Cross section of an adaxial wall in *Periclistus*-inhabited gall showing undulations of the secondary sclerenchymal layer (*SSP*) and the heavy deposition of secondary cell wall in the cells of the primary sclerenchymal layer (*PSP*). Bar = 38 $\mu$m. *E*, Cross section of an abaxial wall in *Periclistus*-inhabited gall with single sclerenchyma layer (*PSP*) surrounding a vascular bundle (*VB*). Bar = 19 $\mu$m. *F*, Detail of an abaxial wall in *Periclistus*-inhabited gall with a double sclerenchyma layer. Vascular bundles (*VB*) are located between the two sclerenchymal plates (*PSP* and *SSP*). Bar = 38 $\mu$m. *NT* = nutritive tissue; *PNT* = parenchymal nutritive tissue.

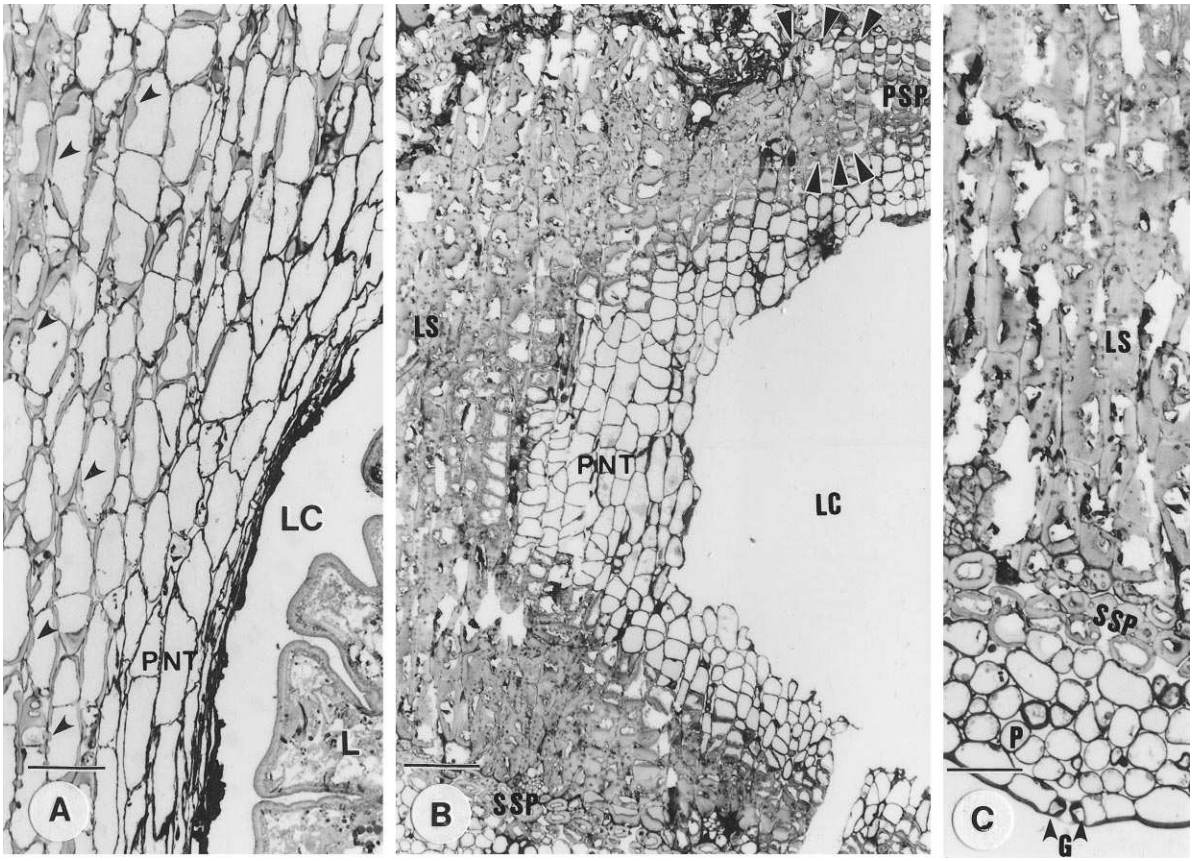


**Fig. 9** Cross sections of *Periclistus*-inhabited galls showing differentiation of lateral sclerenchyma. *A*, Initial stage of lateral sclerenchyma differentiation in *Periclistus*-inhabited gall showing secondary wall thickenings (arrowheads) in cells adjacent to the larval chamber (*LC*). Bar = 38 μm. *B*, Mature lateral sclerenchyma (*LS*) in *Periclistus*-inhabited gall. Note that the lateral sclerenchyma meets (arrowheads) the primary sclerenchymal plate (*PSP*). Bar = 38 μm. *C*, Higher magnification of lateral sclerenchyma (*LS*) shown in *B*. A large number of pits (small circular holes) are present in the secondary walls of the lateral sclerenchyma. The secondary sclerenchymal layer (*SSP*) runs adjacent to the lateral sclerenchyma and separates it from the parenchymal nutritive tissue (*P*). Bar = 19 μm. *G* = guard cell; *L* = larva; *PNT* = parenchymal nutritive tissue.

parenchyma and a lack of nutritive tissue around the larval chamber. Rohfritsch (1992) attributes these changes to the death of the inducer larva. It is not clear whether subsequent hypertrophy of parenchyma is induced by the inquiline larva or by the female either through a substance introduced into the gall during oviposition (e.g., growth promoting substance such as an auxin) or through the ovipositional fluid. Because an inquiline gall may contain larvae and eggs at the same time, this hypertrophy could be induced by the hatched larvae. Shorthouse (1975) and Brooks and Shorthouse (1997*b*) observed similar proliferation of parenchyma in *Periclistus*-inhabited galls of *D. lens* and *D. nodulosa*, respectively. They speculated that the mode of induction must involve the inquiline eggs, ovipositional secretions, or oviposition probing because proliferation of cells occurs prior to inquiline larval feeding.

The proliferation of parenchyma, which will differentiate into nutritive tissue, extends predominantly to the upper wall as the gall develops further. This results in the appearance of a distinctive, upraised central portion on *Periclistus*-inhabited galls on the outer surface of the leaf. This gross morphological feature of the gall allows them to be easily distinguished from galls inhabited by *D. rosaefolii* larvae. In contrast, the larval chamber in *D. rosaefolii*–induced galls is lined with a relatively large amount of nutritive tissue along the sides of the gall but only a thin layer at the top and bottom. As the larvae hatch and begin to feed in *Periclistus*-inhabited galls, parenchymal cells lining the chamber (or chambers) differentiate, forming the nutritive tissue. Shorthouse (1975, 1980) described similar changes in the amount and distribution of the nutritive cells in *Periclistus*-inhabited galls.

*Periclistus*-induced nutritive cells have more extensive vacuolation and a visually perceptible smaller amount of lipids than the nutritive cells induced by *D. rosaefolii*. Furthermore, the distribution of starch is not the same in those two types of nutritive cells. In *Periclistus*-inhabited galls, the starch granules are found in all layers of the nutritive tissue, whereas in *D. rosaefolii*–induced galls, starch is absent in the nutritive cells but is found in parenchyma adjacent to the nutritive cells. Bronner (1981) found similar cytological changes in inquiline-modified galls of *N. quercus-baccarrum* L. on *Quercus pedunculata* Ehrh. and postulated that there is a relationship

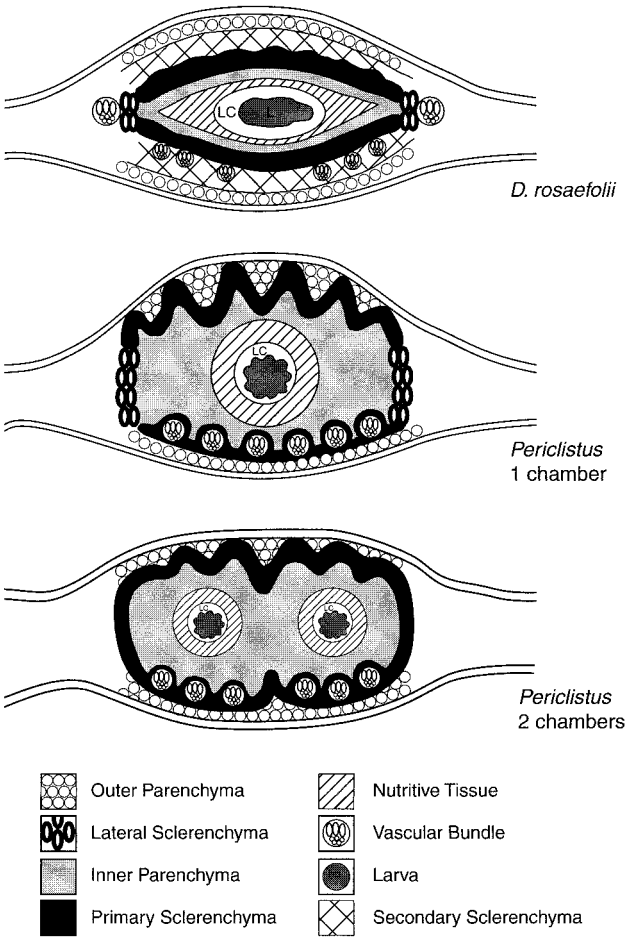


**Fig. 10** Diagrammatic representation of mature *Diplolepis rosaefolii* and *Periclistus*-modified galls. *L* = larva, *LC* = larval chamber. Drawings are not to scale.

between increased vacuolation of the inquiline cells and the change in starch deposition. Further study in this area could help to gain a more thorough understanding of the differences between these two nutritive cell types.

Changes in sclerenchymal layer patterns are dependent on the stage of gall development at which the inquiline eggs are laid in the gall chamber. Shorthouse (1975) only observed *Periclistus* eggs in galls where no sclerenchymal layer was present. This suggests that *Periclistus* species that are inquilines in *D. rosaefolii* galls are adapted to oviposit in more mature, sclerified galls than *Periclistus* species inhabiting other galls.

The *Periclistus*-induced sclerenchyma cells are similar in appearance to the newly differentiated sclerenchyma of the plates in *D. rosaefolii* galls. Irregularities in the upper sclerenchymal plate are much more pronounced and are present at a much earlier stage of development in *Periclistus* galls than in those inhabited by *D. rosaefolii*. References to the presence of an undulating sclerenchymal layer could not be found in the literature, and it is unclear what significance this pattern may have in the development of the gall. If the sclerenchymal layer serves a mechanical role, as has been suggested by a number of authors (e.g., Mani 1964; Shorthouse 1975; Rohfritsch 1992), these undulations may provide greater strength and flexibility than a similar layer with no undulations. It is interesting to note that *D. rosaefolii* galls inhabited by *Periclistus* at early stages of gall development have only one sclerenchymal plate, not two sets of plates as are found in *D. rosaefolii* galls. It may be that the single plate with undulations in the adaxial wall of the *Periclistus* gall provides a similar amount of support as the double plate in fully mature *D. rosaefolii* galls.

The large increase in cellular mass induced by *Periclistus* precludes the need for more extensive vascularization of the gall tissues to develop and maintain this increase. Bronner (1981) noted enhanced vascularization in galls of *Pediaspis aceris* on maple leaves inhabited by the inquiline *Dichatomus acerinus* (Mandl).With an increase in the number of larvae in a gall, which is often the case in inquiline-inhabited galls, the nutritional requirements of the extra larvae are believed to contribute to the need for more vascularization in order to fulfill increasing feeding requirements.

Galls of *D. rosaefolii* provide a useful model for studying the development of leaf galls. Although the development of these galls shares features with those already studied, a number of differences are observed in the types of tissues that form and their arrangement in the gall. The presence in *D. rosaefolii* galls of the double sclerenchymal layer is unique to this lenticular gall, and the lack of vascularization in the central portion of the maturing gall wall has not been reported in any cynipid galls studied to date. These differences suggest that tissue development in galls is much more dynamic than can be accommodated in a single model. A number of events in the development of lenticular galls diverge from Rohfritsch's (1992) model, which is based on developmental studies of circular galls. Therefore, as more lentil-shaped galls are studied, it may be necessary to provide a new or modified model to accommodate this specific gall shape.

Few studies contain information on galls inhabited by an inquiline that has modified the gall tissues of the inducer. A number of changes are brought about by the presence of the *Periclistus* larva in the galls induced by *D. rosaefolii*. These include changes in the abundance of nutritive tissue, the arrangement and in some cases the presence of the double sclerenchymal layers, and the shape and abundance of parenchymal tissue. Furthermore, the epidermal cells and the parenchyma cells of the outer gall wall of the inquiline-modified galls are characterized by the presence of numerous chloroplasts, a typical, common feature in ungalled leaf tissue. Interestingly, these features are not present in the gall when it is inhabited by the inducer *D. rosaefolii*.

Further study of galls induced by *D. rosaefolii* and its inquiline-modified galls is required to provide information on the exact timing of the developmental stages in the galls. This would best be carried out in a controlled laboratory setting and might provide insight into the initial inducing mechanism of gall formation in both of these galls. Mechanisms of gall formation deserve more attention because they can be used to explore the morphogenetic potential of plant organs.

**Table 2**

**Summary of Major Developmental Changes in Galls Inhabited by *Diplolepis rosaefolii* and *Periclistus* Larvae**

| Tissue | Early development | Mid-developmental stage | Late development |
|---|---|---|---|
| *Diplolepis rosaefolii*: | | | |
| Nutritive | Cells differentiate on one side of the chamber, then the other; large amount of lipids present | Cells more abundant at sides of chamber | Cells more abundant at sides; layer lost in later stages |
| Parenchyma | Cells isodiametric; starch present | Few chloroplasts in cells scattered throughout inner layer | Lost in later stages |
| Sclerenchyma | Not present | Primary plate formed | Secondary plates formed; lateral sclerenchyma present: meets primary (inner) plates |
| Vascular | Bundles at sides of chamber | Bundles at sides of chamber and in the lower wall | Bundles may be present between the sclerenchymal plates in the lower wall and at the periphery of the gall |
| *Periclistus*: | | | |
| Nutritive | Differentiates all around chamber; starch and lipids present | Cells surround larval chamber | Cells found at top and bottom of chamber only; layer lost during later stages |
| Parenchyma | Rectangular in cross section | Many chloroplasts in outer (subepidermal) layer; none present in inner layer | Inner layer lost |
| Sclerenchyma | May be none, 1, or 2 plates present; 1 plate present: undulations in upper wall; 2 plates present: undulations in outer layer of upper wall | 1 or 2 plates present | Lateral sclerenchyma present: meets inner plates |
| Vascular | Row of bundles in lower gall wall | Row of bundles in lower gall wall | Row of bundles in lower gall wall |

## Acknowledgments

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