Development of stem galls induced by *Diplolepis triforma* (Hymenoptera: Cynipidae) on *Rosa acicularis* (Rosaceae)

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Abstract—The cynipid *Diplolepis triforma* Shorthouse and Ritchie induces a fusiform, multichambered stem gall from leaf buds on *Rosa acicularis* Lindl. in central and western Canada. Galls at all stages of development were fixed and sectioned using botanical histological techniques to illustrate, for the first time, the unique host-modifying abilities of gall-inducing cynipids that distinguish them from other phytophagous insects. Key events in gall ontogeny, whereby *D. triforma* gains control and redirects the development of attacked host tissues to provide larvae with shelter and food, include proliferation of cytoplasmically dense parenchymatous cells within the strands of the procambium at the point of egg contact, appearance of nutritive cells when larvae first begin to feed, formation of new xylem and phloem extending from unaffected vascular bundles to the larval chambers, formation of several layers of nutritive cells during the period of larval feeding, and formation of sclerenchyma cells around each larval chamber. The role of these tissues in galler biology is explained.

Résumé—Le cynips *Diplolepis triforma* Shorthouse et Ritchie provoque la formation de galles fusiformes à logettes multiples à partir des bourgeons foliaires sur les tiges de *Rosa acicularis* Lindl. dans le centre et l'ouest du Canada. Nous avons fixé et sectionné à l'aide de techniques histologiques botaniques des galles à tous les stades de leur développement afin d'illustrer, pour la première fois, les capacités exceptionnelles des cynips cécidogènes pour modifier leur hôte, ce qui les distingue des autres insectes phytophages. Les étapes importantes de la cécidogenèse, par lesquelles *D. triforma* prend le contrôle des tissus de l'hôte attaqué et en dévie le développement pour procurer le gîte et la nourriture à ses larves, inclut la prolifération de cellules parenchymateuses à cytoplasme dense au milieu des bandes de procambium au point de contact de l'oeuf, l'apparition de cellules nourricières au moment où les larves commencent à s'alimenter, l'élaboration de nouveaux tissus de xylème et phloème s'étendant des faisceaux vasculaires non affectés vers les logettes des larves, la formation de plusieurs couches de cellules nourricières durant la période d'alimentation des larves et l'apparition de cellules sclérenchymateuses autour de chaque logette larvaire. Nous expliquons le rôle de ces tissus dans les biologie des insectes cécidogènes.

[Traduit par la Rédaction]

Introduction

The growth and development of plant galls induced by cynipid wasps represents one of the most complex and intimate insect–plant relationships in the natural world (Shorthouse and Rohfritsch 1992). Although their small, legless, and sedentary larvae look rather primitive for a plant-consuming insect (Shorthouse and Leggo 2002), cynipids have evolved the means of stimulating host plants to surround their feeding sites with specialized plant cells that provide food, shelter, and protection from predators and inclement weather (Price *et al.* 1987; Shorthouse 1993; Csóka *et al.* 2005). Further, each of the approximately 1400 species of cynipids in the world is responsible for inducing distinct, anatomically complex galls (Shorthouse 1993;

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Stone and Cook 1998; Ronquist and Liljeblad 2001; Stone *et al.* 2002; Stone and Schönrogge 2003; Csóka *et al.* 2005), all of which develop from undifferentiated tissues of immature stems, roots, or leaves. As a result, it has been suggested that galls are phenotypic and physiological extensions of the gall inducers (Dawkins 1982; Crespi and Worobey 1998; Stone and Cook 1998; Turner 2000).

Much has been written on the biology of cynipids and their galls (see literature reviewed in Meyer and Maresquelle 1983; Askew 1984; Meyer 1987; Rohfritsch 1992; Stone *et al.* 2002; Stone and Schönrogge 2003; Csóka *et al.* 2005); however, we still know surprisingly little about how these minute wasps "hijack" the growth potential and physiology of the host plants. Most of our knowledge of the anatomy of cynipid galls comes from studies in which specific stages of a variety of galls were examined (Houard 1903; Kostoff and Kendall 1929; Fourcroy and Braun 1967; Meyer 1987; Rohfritsch 1992). Another approach is to compare in detail all stages in the development of a range of gall types induced by closely related species. This is not feasible for large groups such as the Cynipini, which induce galls on oaks (Stone and Cook 1998; Stone and Schönrogge 2003), but it is possible for smaller groups such as the genus *Diplolepis* (Hymenoptera: Cynipidae) in the tribe Diplolepidini, all of which induce galls on wild roses (Shorthouse 1993, 1998). Galls of 2 of the approximately 30 endemic species of *Diplolepis* found in North America have received detailed anatomical study: a leaf gall induced by *D. rosaefolii* (Cockerell) on *Rosa virginiana* Mill. (Rosaceae) (LeBlanc and Lacroix 2001) and a single-chambered stem gall induced by *D. nodulosa* (Beutenmüller) on *Rosa blanda* Ait. (Brooks and Shorthouse 1998). The goal of the present study is to provide details on the development and anatomy of a multi-chambered stem gall induced by *D. triforma* Shorthouse and Ritchie. We do this by using the histological techniques of plant anatomists, whereby host organs and gall tissues at all stages of development are fixed and sectioned. This allowed us to highlight the unique attributes of cynipid gallers that distinguish them from other endophytophagous insects and explain how anatomical features provide clues as to how galls function.

Natural history of the study system

All species of *Diplolepis* are univoltine and spend all but about a week of their lives within chambers of their galls (Shorthouse 1993). Adults emerge from pupae in the spring (Shorthouse and Leggo 2002) and chew a tunnel to the outside. The appearance of adults is synchronized with the presence of host tissues at a stage suitable for oviposition. Eggs are attached either to leaf tissues (Bronner 1985; Shorthouse 1993, 1998; LeBlanc and Lacroix 2001) or to stem tissues within the bud (Shorthouse 1993; Brooks and Shorthouse 1998; Shorthouse *et al.* 2005). Gall tissues arise rapidly around the freshly hatched larvae, enclosing them in individual chambers. Larvae feed from mid-May to mid-August and then enter a non-feeding, overwintering, prepupal stage with a thick cuticle (Shorthouse and Leggo 2002) and high levels of glycerol, enabling them to avoid freezing and desiccation (Williams *et al.* 2002).

Cynipid galls undergo three phases of development known as initiation, growth, and maturation (Rohfritsch 1992; Brooks and Shorthouse 1998). Initiation encompasses all events from oviposition to the formation of larval chambers. In this phase, the development and polarity of plant cells come under the control of the insect rather than the host plant (Rohfritsch 1992; Shorthouse 1993). During the growth phase, nutritive and vascular tissues differentiate and gall biomass increases owing to cell proliferation and growth (Rohfritsch and Shorthouse 1982; Rohfritsch 1992). The maturation phase is marked by the end of gall growth and the differentiation of sclerenchyma cells (Rohfritsch 1992). Most larval feeding occurs in the maturation phase (Rohfritsch 1992; Brooks and Shorthouse 1998).

Mature cynipid galls are composed of five types of tissue concentrically arranged around larval chambers: (1) cytoplasmically dense nutritive tissue (NT) surrounded by (2) vacuolate parenchymatous nutritive tissue (PNT); (3) a layer of gall parenchyma (GP) cells beyond the PNT; (4) a sclerenchyma sheath that envelopes the nutritive tissues (Fourcroy and Braun 1967; Rohfritsch 1992; Brooks and Shorthouse 1998); and (5) a layer of gall cortex beyond the sclerenchyma layer. NT lines the interior of gall chambers and accumulates lipids, sugars, amino acids, proteins, enzymes, and other organic compounds (Bronner 1977, 1992). Starch

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concentration increases away from the larval chambers while enzyme, lipid, and sugar concentrations increase towards the larval chambers (Rohfritsch 1971; Bronner 1992). PNT is composed of storage cells, which differentiate into NT cells as the latter are eaten by the cynipid larvae (Rohfritsch 1971, 1992). PNT borders the NT in concentric layers away from the larval chamber and differentiates from a cambium-like layer in the growth phase (Rohfritsch and Shorthouse 1982; Shorthouse 1993). GP also differentiates from the cambium-like layer, but it does not differentiate into PNT (Rohfritsch 1992). Sclerenchyma cells are differentiated near the GP in the maturation phase, as are several layers of gall cortex beyond the sclerenchyma layer. New vascular tissues, differentiated from gall cells in the growth phase, supply nutrients and water to the developing gall and are connected to the vascular tissues of the host organ (Meyer 1969).

Diplolepis triforma, as the name implies, induces three forms of woody, multichambered stem galls from the first vegetative buds of spring on *Rosa acicularis* Lindl. (Fig. 1). These characteristic forms are pear-shaped, with or without distal stem growth, and fusiform (see Figs. 7–9 in Shorthouse and Ritchie 1984). The first two forms are most common, although the relative abundance of all three varies from year to year. The overall size of mature galls also varies from year to year and is determined by the number of larval chambers, each of which is inhabited by a single larva (Fig. 2). The surface of mature galls is reddish brown and may be spineless or spined (Shorthouse and Ritchie 1984).

The range of host plants is the main factor restricting the distribution of *Diplolepis* spp. Galls of *D. triforma* are found only on *R. acicularis* in Ontario and on *R. woodsii* Lindl. on the grasslands of western Canada (Lalonde and Shorthouse 2000). Galls of *D. triforma* are usually abundant in central and northern Ontario; however, from 1998 to 2003, they were rare except at two sites near Sudbury, Ontario. Galls appeared to be increasing in number throughout northern Ontario beginning in 2004.

Galls induced by *D. triforma* were chosen for this anatomical study because they are relatively small for a stem gall, large populations were present at two sites near Sudbury, and we were able to induce galls under controlled and field conditions. Further, *D. triforma* and its gall have received attention in other areas (Shorthouse and Ritchie 1984; Wiebes-Rijks and Shorthouse 1992; St. John and Shorthouse 2000; Shorthouse and Leggo 2002).

Materials and methods

Adults of *D. triforma* were obtained from mature galls on *R. acicularis* collected in the fall of 1999 and the spring of 2000 from field sites located on Manitoulin Island, near Chelmsford, and at the Lake Laurentian Conservation Authority within the city limits of Sudbury, all within central Ontario. Voucher specimens of *R. acicularis* are deposited in the herbarium at Laurentian University, and adults of *D. triforma* are deposited in the Canadian National Collection of Insects in Ottawa, Ontario.

By chilling galls in an incubator, it was possible to synchronize the appearance of adults with early bud development of rose plants in growth cabinets and in the field. Females were placed on rose stems and allowed to select suitable buds for oviposition. The duration of each oviposition period was determined for galls in the laboratory. The average number of eggs deposited into each bud was estimated from the number of chambers found in the resulting galls.

One hundred ovipositions were obtained in growth chambers and an additional 105 in the field. Buds into which *D. triforma* were observed ovipositing were tagged and a harvest schedule was established in order to obtain various stages of gall development post-oviposition. The period within 24 h of oviposition was referred to as Day 0, that between 24 and 48 h as Day 1, and so on. In both the growth cabinets and the field, at least eight buds were harvested every day from Day 0 to Day 15 and again on Days 20 and 25. No morphological differences were observed between buds harvested the same number of days post-oviposition from field and control conditions, so these results were pooled.

Rosa acicularis plants used for ovipositions in the growth chambers were obtained from commercial bare rootstock purchased from Hortico, Inc. (Hamilton, Ontario). They were planted singly in a 1:2 sand–Promix® mixture and fertilized once in the spring. Potted roses were grown in Conviron® E15 growth chambers with a 15L:9D photoperiod at 17 °C, 90% RH, and low light intensity to approximate spring conditions in *R. acicularis* habitats.

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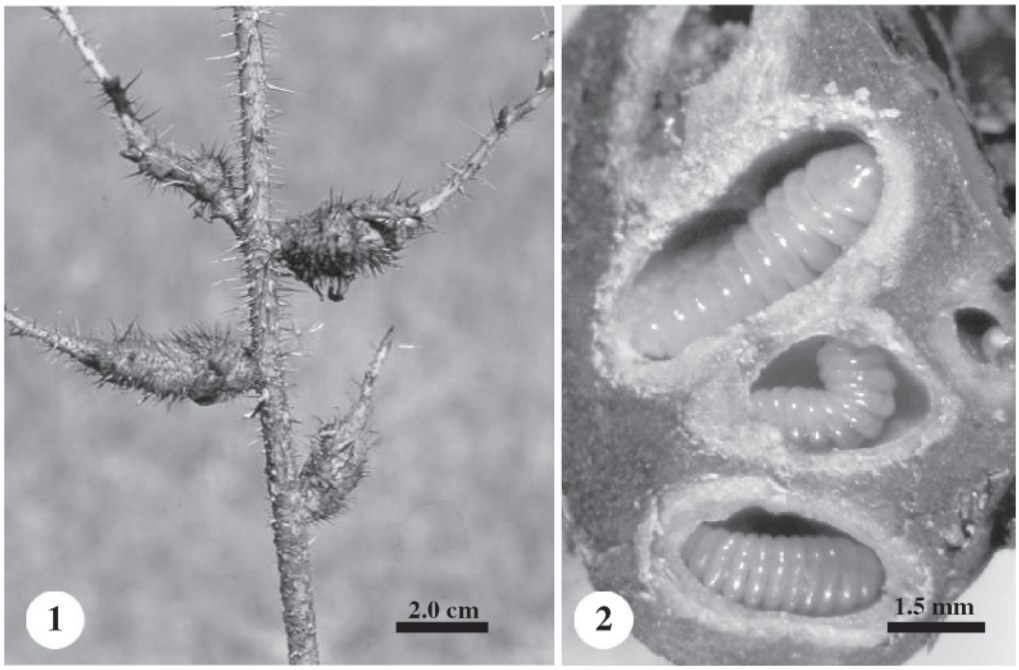
Figs. 1–2. Mature galls induced by *Diplolepis triforma*. 1, Habitus of a mature gall. 2, Dissection of a mature gall showing larvae in their chambers.



Naturally occurring galls from the field site at the Conservation Authority were collected twice-weekly from the time they became visible in mid-June (approximately 45 days after the release of *D. triforma*) until they attained maturity at the end of September. Galls were dissected to identify the larvae within each chamber and blocks of gall tissue containing individual chambers with inducer larvae were fixed. Approximately 360 galls were collected and dissected, from which 150 chambers were selected for fixation. Portions of both young and mature stems and buds into which no ovipositions had occurred were also harvested for anatomical study.

All tissues were vacuum-infiltrated with a formalin – acetic acid – alcohol fixative solution (90 parts 70% ethanol, 5 parts 37% formalin, and 5 parts glacial acetic acid), dehydrated in an ethanol – tertiary butyl alcohol series, and embedded in paraffin. Tissues were then sectioned at 10–12 µm using a Leica® Jung Biocut 2035 rotary microtome, fixed to microscope slides using Haupt's adhesive (Jensen 1962), and stained using safranin – fast green (Sass 1958). This technique stains lignified cell walls, cytoplasm of provascular and procambial tissues, and nucleoli red, the non-lignified cell walls of phloem and parenchyma cells green, and the dense cytoplasm of nutritive cells and nuclei purple.

Results

Ungalled buds and stems of *R. acicularis*

The shoot apical meristem of *R. acicularis* leaf buds at the time of *D. triforma* oviposition is surrounded by several immature leaves (Fig. 3) and is composed of two distinct zones of cells, called the tunica and corpus, which give rise to other plant tissues. The tunica is the surface layer of cells that sits like a dome over a spherical zone of cells, the corpus, in which the planes of cell division are anticlinal and periclinal. Most organs arise in the outer two or three cell layers and always include the outermost tunica layer. The procambium occurs in a sheath that circles the pith (Fig. 3) and, as the stem matures, vascular tissues are differentiated from the procambium. Individual vascular bundles of the maturing stem form a discontinuous ring separating the cortex from the pith (Fig. 4). The bases of successive leaf primordia periodically appear as bulges of cells near the apex of the meristem and develop into new leaf tissue. Procambial tissues extend into each immature

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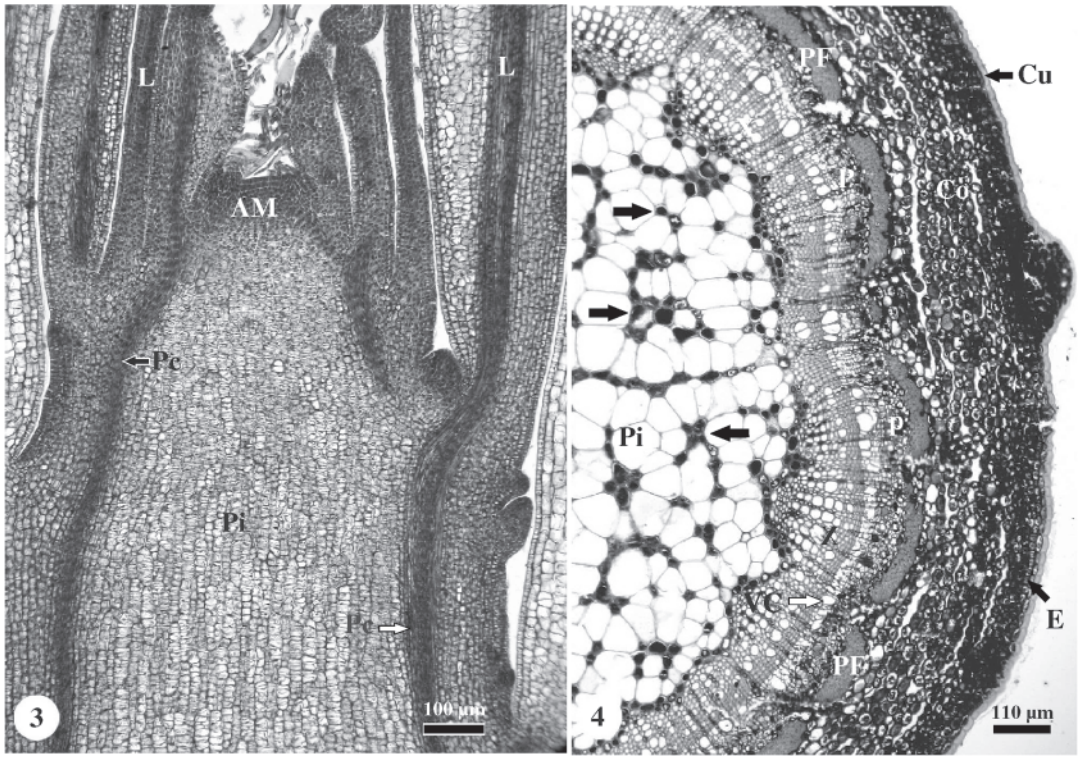
Figs. 3–4. Anatomy of the stems of *Rosa acicularis*. 3, Longitudinal section of a vegetative bud at the stage oviposition occurs. Tightly clustered immature leaves surround the apical meristem. Note the conical shape of the shoot tip with the dome-shaped apical meristem, leaves arising from beneath the meristem, and the circular ring of procambium surrounding the pith. 4, Cross section of a mature stem at the end of the first year's growth. The vascular tissues form a continuous ring about the stem. Note the accumulation of phenolic compounds in the smaller pith cells (arrows) and in the cortical cells. AM, apical meristem; Co, cortex; Cu, cuticle; E, epidermis; L, leaf; p, phloem; Pc, procambium; PF, phloem fibres; Pi, pith; VC, vascular cambium; X, xylem.



leaf, giving rise to foliar xylem and phloem (Fig. 3). The place on the stem where a leaf is attached is the node, and the space between leaves is the internode.

By August, maturing stems have a thick layer of cuticle covering the epidermis and the 7–18 layers of cortical cells are filled with dark-staining phenolic compounds (Fig. 4). The vascular cambium forms a continuous cylinder circumscribing the stem and produces phloem towards the outside of the stem and xylem towards the inside (Fig. 4). The pith forms the center region of the stem (Figs. 3, 4) and is made up of undifferentiated parenchyma cells. By the end of summer, the cell walls of the pith are lignified; smaller cells are filled with phenolic compounds, while the lumen of larger cells is empty (Fig. 4).

Gall initiation (Days 0–25)

Once a host has been located, adult wasps walk along branches in search of leaf buds suitable for oviposition. They are able to oviposit into buds in a wide range of developmental stages, from those with unforced leaves to those with unfolding leaves; however, they prefer buds in which the leaves are expanding but have yet to unfold. Upon selecting a site, the female places herself in an inverted position near the middle of the bud with her head towards its proximal end and her antennae angled ventrally, touching the surface of the bud. The hypopygium is lowered to a 90° angle and the ovipositor is forced through folded leaf tissues with rocking motions. The probing ovipositor damages immature leaflets and occasionally tissues of the apical meristem.

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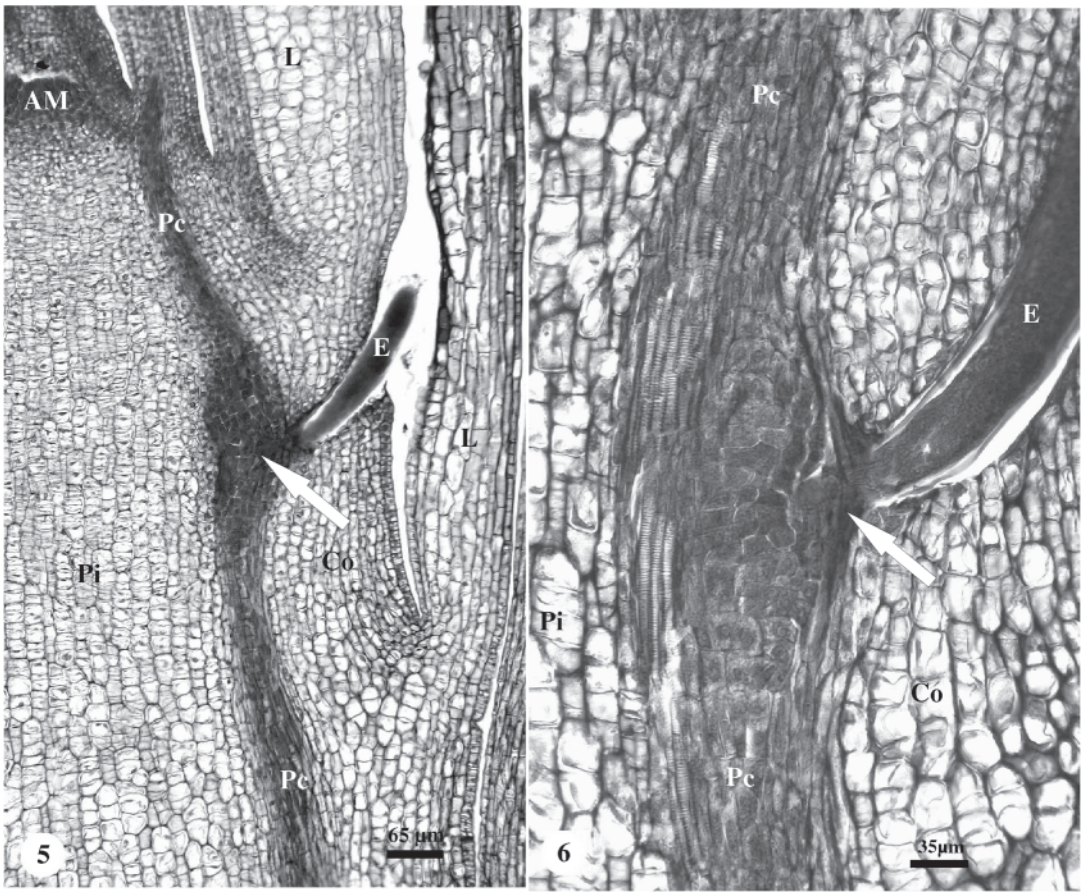
Figs. 5–6. Eggs at Day 10 deposited in shoot tips. 5, Longitudinal section of shoot tip with an egg inserted into the procambium, showing the proliferation of cells (arrow) before egg hatch. Note that the procambial strand is expanded by the proliferating cells nearest the egg. 6, Enlargement of egg showing proliferation of cytoplasmically dense cells within the procambium. Note the oviposition fluid at the tip of the egg (arrow). AM, apical meristem; Co, cortex; E, egg; L, leaf; Pc, procambium; Pi, pith.



Ovipositions take 3 to 85 min (mean = 23.08 min; n = 37) under controlled conditions, during which 1–33 eggs (mean = 7.33; n = 404) are deposited within the bud tissues. Thus, on average, adults of *D. triforma* require about 3.15 min for each egg deposited into leaf buds. Eggs are translucent, white, and slightly curved and have a long stalk emerging from the anterior end, distal to the plant. With each egg-depositing event, a channel is cut by the ovipositor in the internode region between primordial leaves and a single egg is inserted such that the base contacts the procambium (Figs. 5, 6). About one half of the egg is embedded in plant tissues and the proximal end is covered by an amorphous, dark-staining substance (Fig. 6). A small cluster of cytoplasmically dense cells with enlarged nuclei appears at the base of each egg (Figs. 5, 6) within the procambium within 24 h of oviposition. These cells continue to proliferate over the next 2–3 days and, although vascular differentiation from nearby procambium continues normally, cells of the procambium near the embedded eggs do not differentiate at this stage. By Day 4, this patch of insect-induced gall cells beneath the eggs is 2–4 cells thick and appears to separate the procambial strand (Fig. 6).

The individual cells of this mass of insect-induced gall cells are about half the size of adjoining pith cells as the developing gall starts to expand into the pith (Fig. 7). The larvae begin hatching at Day 12 and the first cells they contact are either chewed into or broken down through lytic action. The remains of these cells form a layer around the chamber surface (Fig. 7). Larvae imbibe fluids exuding from the

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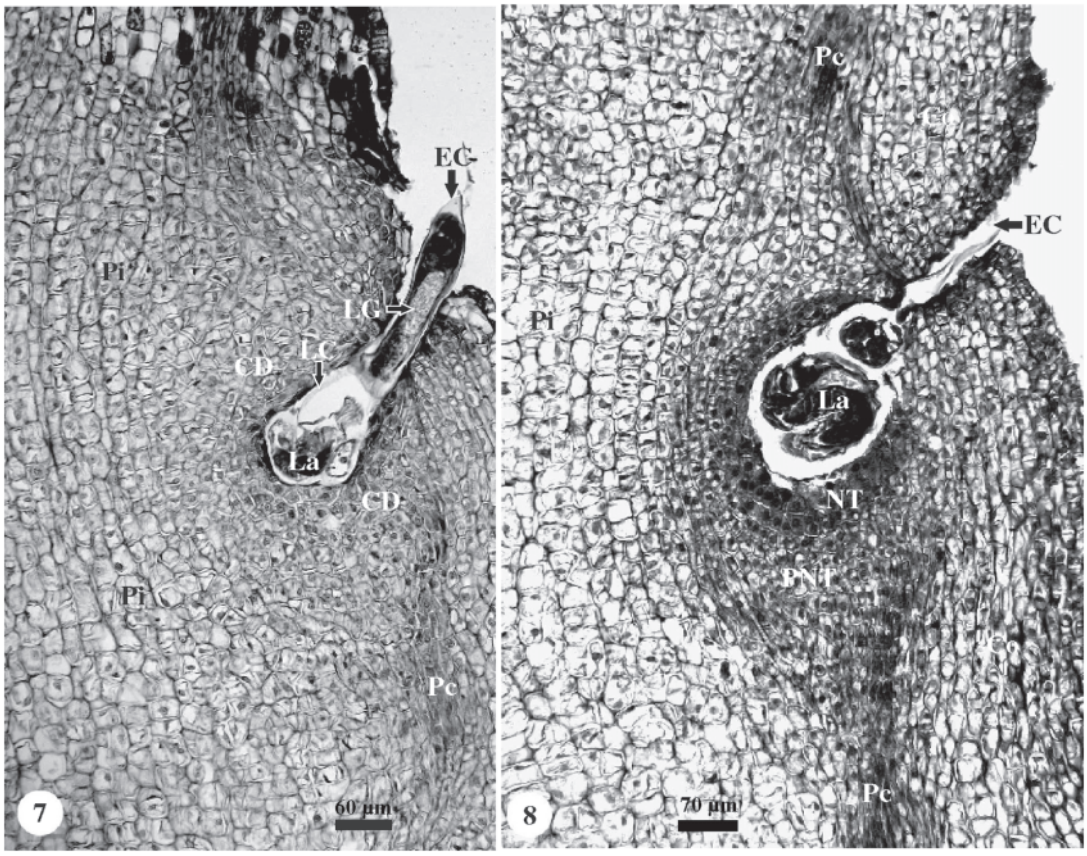
Figs. 7–8. Galls in the early initiation phase. 7, Longitudinal section of shoot tip showing a recently hatched larva at Day 20 surrounded by cytoplasmically dense gall cells that began proliferating before the larva hatched. 8, Longitudinal section of shoot tip at Day 25 showing curled larva in a spherical chamber surrounded by nutritive cells and parenchymatous nutritive cells. Note that the larval chamber is within the strand of procambium and that the zone of egg and larval influence, indicated by columns of cells, extends into the pith. CD, cytoplasmically dense cells; Co, cortex; EC, egg chorion; La, larva; LC, larval chamber; LG, larval gut; NT, nutritive tissue; Pc, procambium; Pi, pith; PNT, parenchymatous nutritive tissue.



remains of these cells while they are still partially within the egg shell. Cytoplasmic contents of consumed cells can be seen within the gut of larvae still partially within their eggs (Fig. 7). The breakdown of these cells causes the formation of a larval chamber into which the larva moves as it completely exits its egg.

Soon after the larva is free of its egg and curls within its chamber, it begins to actively chew adjoining cells. This chewing causes the differentiation of cytoplasmically dense nutritive cells, which first appear along the chamber surface furthest from the egg channel. By Day 20, the larvae are almost completely surrounded by NT (Fig. 8). Because the diameter of the chamber is smaller than the length of the larva, the larva curls its posterior end over the dorsal-anterior portion of its body, allowing it to feed on all cells lining the chamber surface. Cell proliferation closes the oviposition channel and the entire chamber surface becomes lined with one or two layers of NT. Part of the original procambial strand can still be observed towards the outside of the stem (Fig. 8). It is assumed that one of the columns of cells on the pith side of the larval chamber is a procambial layer from which insect-controlled cells are differentiated. Cells proliferating from the procambial layer that surrounds each larval chamber in concentric layers become the PNT. With the closing of the oviposition channel at the end of the initiation phase, cells proliferating from the procambium inwards become the inner layer, composed of both PNT and NT. Cells of NT

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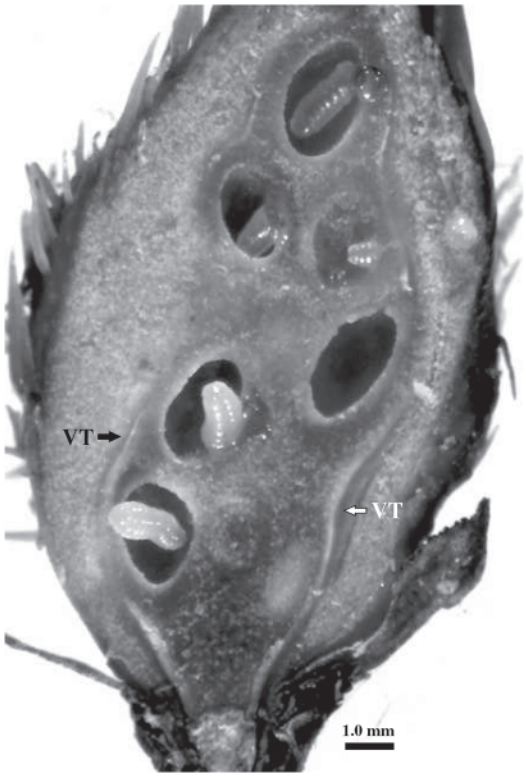
Fig. 9. Dissected gall in mid growth phase showing chambers within the cylinder of vascular bundles. VT; vascular tissue.



and PNT are smaller than the adjacent pith cells but have larger nuclei (Fig. 8). As the nutritive cells are consumed by the larva, adjoining parenchymatous nutritive cells are converted to NT and consumed. As this is happening, the cambial layer continues to differentiate into GP and PNT. Larvae do not feed extensively on NT during the initiation phase.

Growth phase (Days 25–60)

The growth phase begins around Day 25, when the proliferation of PNT around each larval chamber intensifies and the gall begins to rapidly enlarge. Up until this age, galls are barely perceptible swellings of the shoot, but once the growth phase begins, the combined effects of the larvae cause the galls to expand in width and length and are more noticeable. The chambers are spherical at the beginning of the growth phase, but they gradually become more elongate as the larvae and galls grow. Dissected galls in the early growth phase show chambers gradually expanding into the pith (Fig. 9), which is replaced as the gall grows. The procambium of the stem matures into vascular bundles with phloem and xylem in the unaffected stem below the gall, encircling the developing gall, and extending into unaffected parts of the stem beyond the gall. Most chambers are near the surface of the gall and are associated with these vascular bundles (Fig. 9).

The chambers of galls in the early growth phase, up to about Day 45, are lined with an even layer of NT about 2–3 cells thick; these cells are cytoplasmically dense, have enlarged nuclei, and vary in size and shape, though they are more or less spherical (Figs. 10, 11). The PNT layer averages 6–10 cells thick and circumscribes the NT (Fig. 10). As in the initiation phase, PNT continually differentiates into NT, which is consumed by the larvae. Larvae feed equally over the entire chamber surface during this period, although they never consume all NT down to the PNT. The PNT is surrounded by a thin layer (1–3 cells thick) of undifferentiated GP; these cells do not exhibit any intracellular changes, nor do they increase in size.

Vascular tissues begin to differentiate within tissues of the gall at the beginning of the growth phase and surround the larval chambers (Fig. 10). These new bundles extend from the vascular cylinder of the host into the masses of gall cells. Strands of procambial cells extend from these vascular bundles through the GP and the PNT towards the NT (Fig. 11).

From Day 45 to Day 60, gall growth occurs from proliferation of the GP and the PNT and the expansion of individual cells. Larval chambers elongate as the larvae grow larger, eventually becoming ellipsoidal. The size and shape of the larvae begin to restrict their movement within the chambers, limiting their ability to feed on the lateral surfaces of the chambers. NT at both ends of the chamber and on the lateral surfaces of the chamber is 3–4 cells thick, and walls of collapsed cells along the chamber surface indicate feeding even though larvae do not feed extensively on NT during the growth phase. Even so, NT cells are consumed on the lateral surfaces of the chamber until the larvae can no longer reach them, at which point they dedifferentiate to GP. Towards the end of the growth phase, NT cells become much larger than PNT, GP, and other cells in the stem. The PNT becomes 6–12 cells thick and is surrounded by a thin layer (3–5 cells thick) of GP

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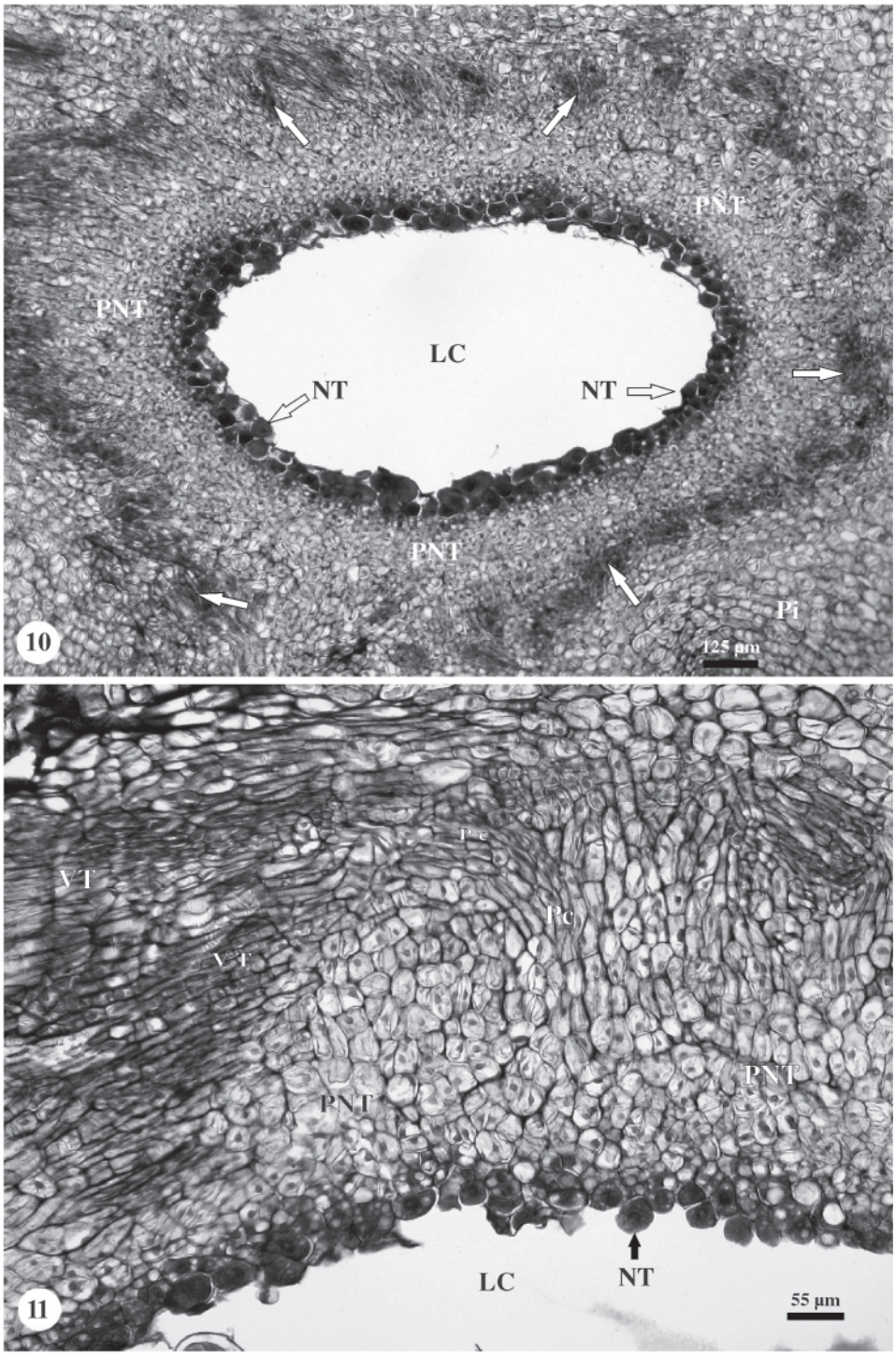
Figs. 10–11. Sections of larval chambers from galls in the early growth phase. 10, Longitudinal section of larval chamber at approximately Day 45. Note the numerous vascular bundles, some of which are highlighted with arrows, encompassing the larval chamber. 11, Enlargement of a section of a larval chamber at approximately Day 45 showing procambium extending from a vascular bundle through the parenchymatous nutritive tissues and towards the nutritive cells. LC, larval chamber; NT, nutritive tissue; Pc, procambium; Pi, pith; PNT, parenchymatous nutritive tissue; VT, vascular tissue.



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cells, which separates the PNT from the stem vasculature.

Maturation phase (Day 60 – fall)

At the beginning of the maturation phase, nutritive cells at the end of the chamber near the larval head occur in a layer 3–5 cells thick (Fig. 12). NT cells at this stage are elongate or rectangular (Fig. 12) rather than irregular or spherical as they were in the growth phase (Fig. 11). Larvae now feed much more actively and consume the contents of more cells than they did previously. Note that the larva in Figure 12 was killed and fixed with a piece of a nutritive cell in its mouth. PNT is now 4–10 cells thick and the remaining GP begins to lignify. During the early part of the maturation phase, the larvae can easily feed on both the lateral sides and the ends of the chambers. Larval feeding on the chamber sides eventually depletes the layers of NT and PNT (Fig. 13); however, NT and PNT continue to proliferate at the ends of the chambers.

A layer of small, elongate sclerenchyma cells with pitted cell walls differentiates from the GP at the beginning of the maturation phase (Fig. 14), encapsulating each larval chamber. The sclerenchyma sheath is formed by the thickening of cell walls in a layer 2–5 cells thick and divides the vascular bundles from the GP, and the GP of adjacent chambers. Vascular tissues originating from the stem form a web of vasculature that surrounds each larval chamber, with the ends of each vascular bundle extending to the PNT.

Larvae nearly fill their chambers by Day 86 and likely find it difficult to reach NT remaining on the lateral surfaces of the chamber. NT is still 3–5 cells thick at the ends of the chamber, where the larvae are able to feed (Fig. 15), whereas any NT remaining elsewhere in the chamber dedifferentiates to GP. All PNT cells at the ends of the chamber have differentiated into NT at this stage. Undifferentiated GP is 3–8 cells thick and surrounds the chambers (Fig. 16). The sclerenchyma layer is now an average of 6 cells thick between the nutritive and vascular tissues and 2–5 cells thick between larval chambers.

Galls are desiccated by Day 120 (approximately mid-September), becoming hard and reddish brown. Following consumption of the last NT, larvae defecate just before they develop into prepupae. Meconium is deposited in a thin layer at the tail end of the chamber (Figs. 17, 18) and the larval midgut is empty. The GP is 2–4 cells thick at the head end (Fig. 16) and up to 12 cells thick at the opposite end (Fig. 18) and on the lateral surfaces of the chamber. GP cells at the anterior and posterior ends of the chamber fill with dark-staining phenolic compounds (Figs. 16, 18). The sclerenchyma sheath is 5–8 cells thick between the vascular bundles and the GP (Figs. 16, 18).

Discussion

Most of the research today on cynipid galls is ecological in nature (Stone *et al.* 2002; Stone and Schönrogge 2003; Csóka *et al.* 2005), yet in the past, particularly from the late 1880s to the mid 1900s, there was much interest in the anatomical aspect of galls (Meyer and Maresquelle 1983). Key among these early works are those of Adler (1877), Beyerinck (1882), Magnus (1914), Houard (1903), Küster (1911), Weidel (1911), and Kostoff and Kendall (1929), who described many important aspects of gall anatomy and development. Most of these early workers compared the structures of mature galls induced by cynipids from a variety of genera and apparently did not have the opportunity to examine galls from initiation to maturity, as we have done here.

Using histological techniques to study galls reveals the unique attributes and specializations of cynipid gallers compared with other endophytophagous insects. Whereas leaf miners and stem borers tunnel through plant organs, consuming tissues as they go, cynipid gallers are sessile and feed only on plant cells lining their larval chambers. Miners and borers feed on typical plant cells as they tunnel (DeClerck and Shorthouse 1985), whereas gallers stimulate the formation of specialized nutritive cells that are not found elsewhere in the host plant. Gallers have also evolved the ability to control the plant's sources and sinks and to redirect nutrients and assimilates from other parts of the plant to the NT (Harris and Shorthouse 1996; St. John and Shorthouse 2000). As nutritive cells are consumed, others are quickly differentiated from adjoining PNT to take their place and are packed with nutrients prior to being consumed (Bronner 1992). Among the key attributes of cynipid wasps and their galls illustrated by this study of *D. triforma* are the accuracy in depositing eggs, the proliferation of cells near the eggs prior to hatching, the importance of cells within the procambium in gall

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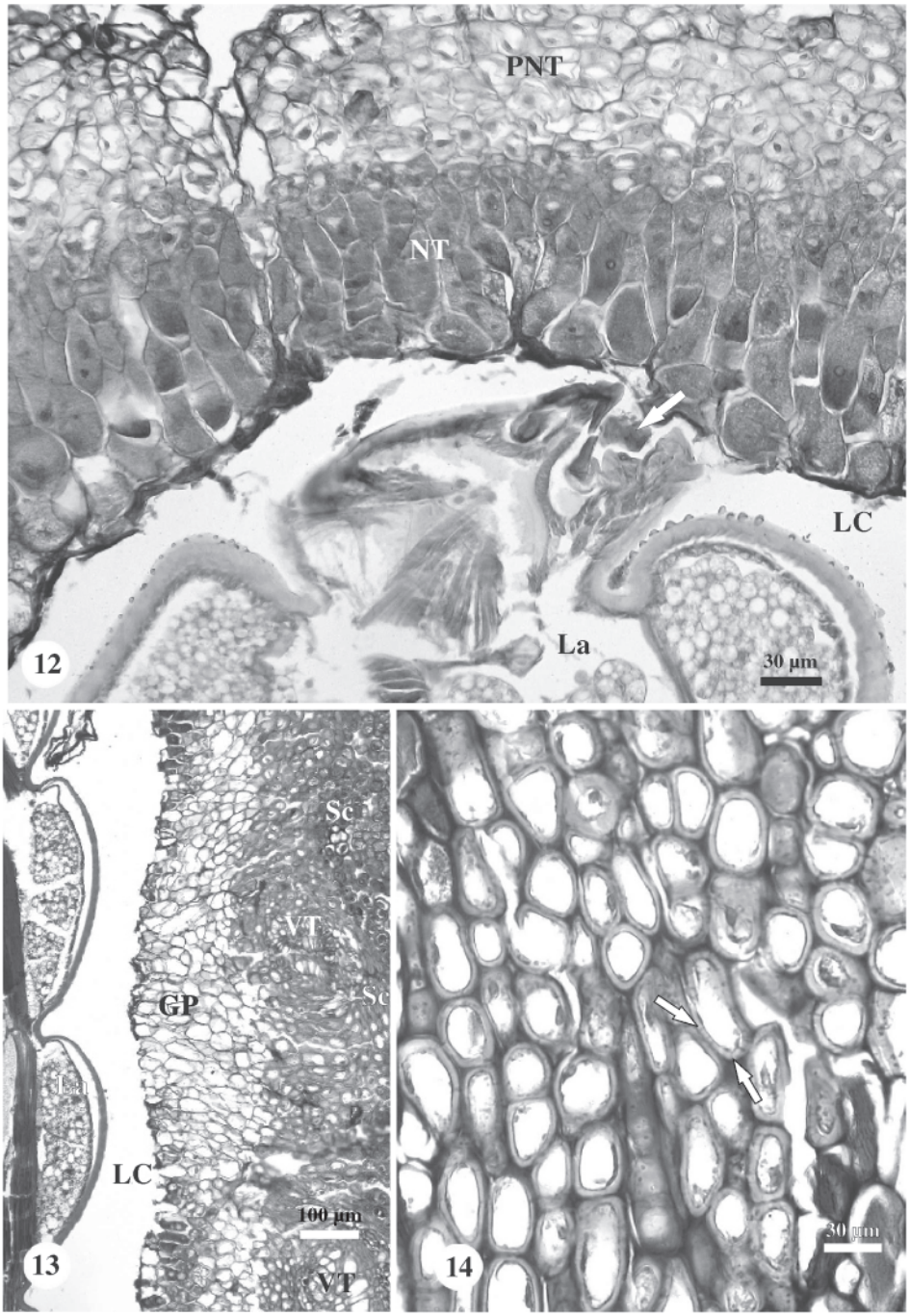
Figs. 12–14. Sections of larval chambers from galls in the mid maturation phase at approximately Day 80. 12, Longitudinal section of the end of a chamber near the larval head, and section of head and first thoracic segment of a larva. Note that this larva died with pieces of a nutritive cell (arrow) in its mouth. Note also the thick cuticle of the thorax covered with small spines. 13, Longitudinal section of a lateral region of a chamber lined by gall parenchyma. All nutritive cells have been consumed. Note that vascular bundles are separated interfascicularly by sclerenchyma. 14, Newly formed layer of sclerenchyma cells. Note the pores in the walls of the sclerenchyma cells (arrows). GP, gall parenchyma; La, larva; LC, larval chamber; NT, nutritive tissue; PNT, parenchymatous nutritive tissue; Sc, sclerenchyma; VT, vascular tissue.



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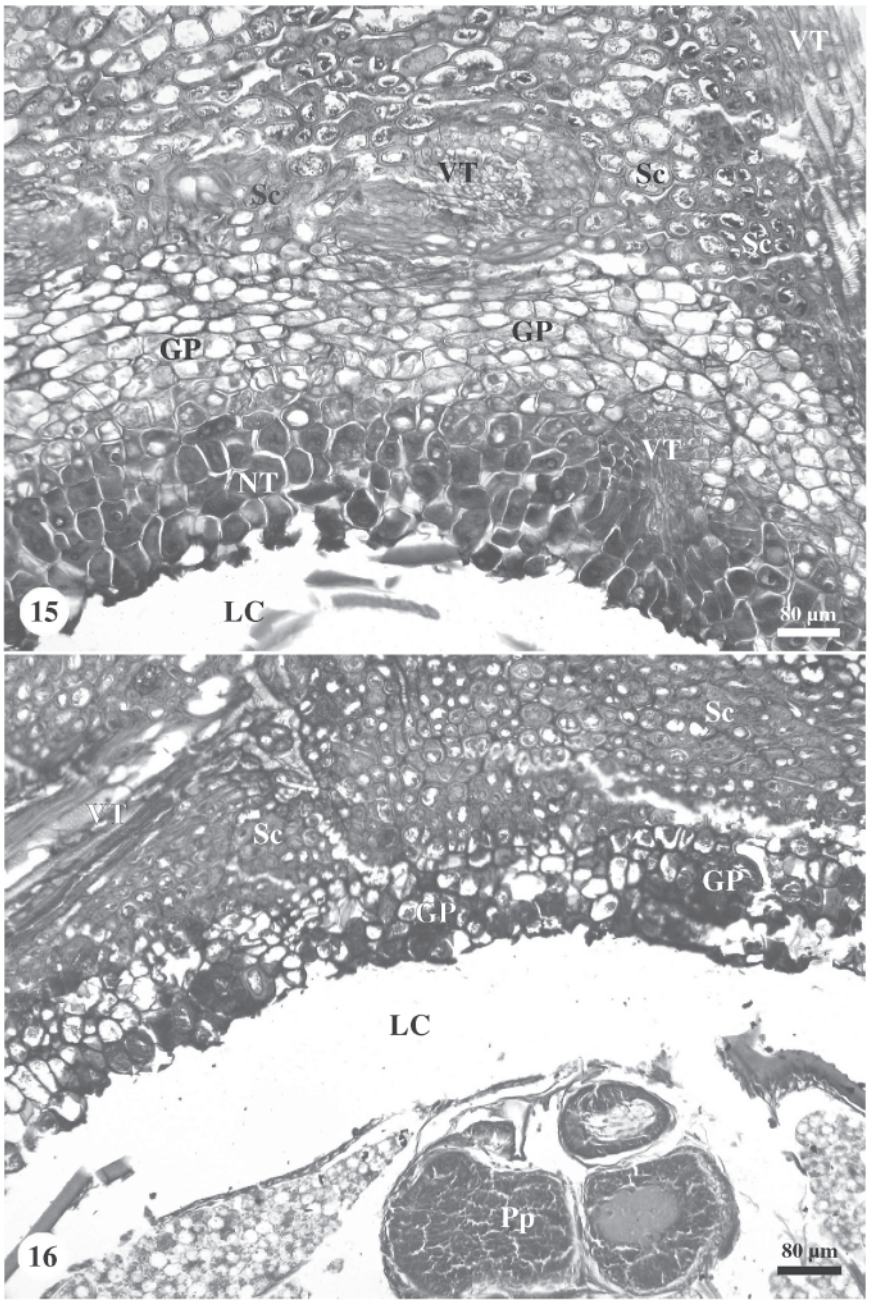
Figs. 15–16. Sections of the head end of larval chambers of galls in the mid to late maturation phase between Days 90 and 120. 15, Chamber of maturing gall at Day 90 showing layers of nutritive cells, gall parenchyma, and sclerenchyma. 16, Chamber of mature gall at Day 120 showing gall parenchyma lining the chamber wall. GP, gall parenchyma; LC, larval chamber; NT, nutritive tissues; Pp, prepupa; Sc, sclerenchyma; VT, vascular tissue.



initiation and growth, the differentiation of NT and PNT, the proliferation of vascular tissues within the gall, and the formation of sclerenchyma cells.

Galls of *D. triforma* are substantially different from the single-chambered galls of *D. nodulosa* (Brooks and Shorthouse 1998). Although the single eggs of *D. nodulosa* are deposited in the

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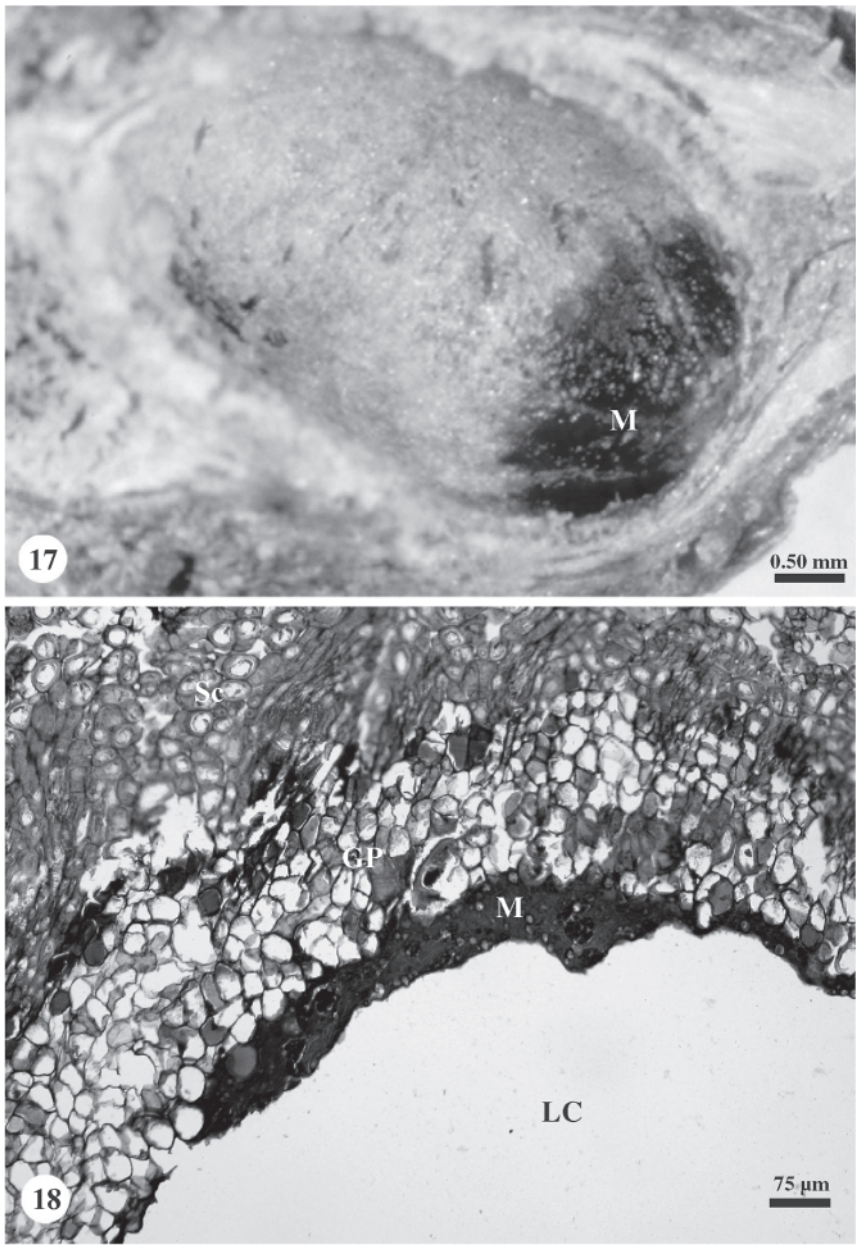
Figs. 17–18. Surface near posterior end of a chamber from a gall in the late maturation phase at Day 130. 17, Section of chamber showing dark meconium at one end of chamber. 18, Dissection of chamber showing gall parenchyma cells covered by a layer of meconium. GP, gall parenchyma; LC, larval chamber; M, meconium; Sc, sclerenchyma.



internodal region of the buds of *R. blanda*, the larval chamber of the gall occupies a central position within the stem and lies longitudinally along the stem axis. In contrast, chambers of *D. triforma* lie in all directions within the pith of galled stems. No new vascular tissues are initiated within the *D. nodulosa* gall tissues; instead, the larvae take their water and nutriment from nearby vascular tissues of the host stem. Galls of *D. triforma*, in contrast, have new vascular bundles within their tissues to adequately supply the larva within each chamber. There is also more sclerenchyma tissue around each chamber in the *D. triforma* gall than in the

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D. nodulosa gall. Further, mature chambers of *D. nodulosa* galls are lined with sclerenchyma, whereas mature chambers of *D. triforma* galls are lined with deteriorating GP.

None of the galls of *D. triforma* examined as part of this study, nor any of the hundreds collected throughout central and northern Ontario since 1975, were inhabited by *Periclistus* (Hymenoptera: Cynipidae) inquilines. This is significant because a high proportion of the galls induced by each leaf-galling species of *Diplolepis* are inhabited and modified by *Periclistus* spp. (Shorthouse 1998), as are the stem galls of *D. nodulosa* (Brooks and Shorthouse 1997) and some galls of *D. spinosa* (Ashmead) (Offman 2004). The single-chambered galls of *D. nodulosa* are grossly modified by *Periclistus* spp., whereas these inquilines do not alter the morphology of *D. spinosa* galls and instead form chambers in one region of the gall. That *Periclistus* spp. do not attack galls of *D. triforma* is perplexing, especially because *R. acicularis* and *R. blanda* grow together and *R. blanda* is galled by several species of *Diplolepis*, the galls of which are frequently attacked by species of *Periclistus*. Perhaps *D. triforma* galls are harder than galls of *D. nodulosa* and *D. spinosa*, thus preventing the ovipositors of *Periclistus* spp. from penetrating down to the inducer chambers.

Each new generation of *D. triforma* begins in the early spring, when the overwintering prepupae pupate and adults emerge within their chambers and chew an exit channel to the outside (Shorthouse and Leggo 2002). Adult cynipids are unusual among insects in that they possess active mandibles for chewing plant tissues but do not eat. The oviposition period of *D. triforma* is much longer than that of other species of *Diplolepis*, extending from mid-May to late June, even though each adult lives for about 5 days. As a result, adults are available to oviposit into buds that appear throughout the spring. Further, the ability of *D. triforma* to oviposit into buds at several stages of development likely explains why galls of this species occur in varying sizes and shapes (Shorthouse and Ritchie 1984). In contrast, species such as *D. polita* (Ashmead) (Shorthouse 1973) and *D. nodulosa* (Brooks and Shorthouse 1998) have narrower emergence periods and oviposit into the first unforced leaf buds of spring, and their galls do not vary in size and shape.

Eggs of all *Diplolepis* spp. studied to date are known to be deposited with great accuracy (Beyerinck 1882; Magnus 1914; Bronner 1985). Beyerinck (1882) illustrated eggs of an unknown *Diplolepis* sp. on the outside surface of unfolded leaflets along with details of eggs with their tips attached to individual epidermal cells. Bronner (1985) showed eggs of *D. rosae* (L.) also attached to individual epidermal cells. In contrast, eggs of *D. triforma* (Figs. 5, 6) and *D. nodulosa* (Brooks and Shorthouse 1998) are embedded within channels cut by the ovipositor in the internodes of leaves, and the non-stalked end is in contact with the procambium. Eggs deposited in such a manner are likely better protected from desiccation and predators than those deposited on the surface of leaves and exposed to the elements for a period after the leaflets unfold.

The initiation phase of the *D. triforma* gall starts with wounding by the ovipositor as channels are cut down to the procambium and a single egg is deposited into each. An amorphous substance is deposited by the female at oviposition and the egg is placed upon it (Fig. 6). This substance has been observed in galls of other cynipids (Kostoff and Kendall 1929; Bronner 1985), but its identity and role remain unknown. Although wounding of cells within the procambium by the ovipositors of *D. triforma* must have occurred, we did not observe it.

Oviposition by *D. triforma* provides a good example for the "insect egg hypothesis" of Zeh *et al.* (1989), which argues that insect ovipositors and eggs are of critical importance in insect evolution. In the case of *D. triforma* and other *Diplolepis* spp., accurate deposition of eggs within the procambial tissues of leaf buds or near vascular bundles of immature leaflets was likely an important step in the evolution of gall induction (Shorthouse *et al.* 2005).

Eggs of cynipids inducing leaf galls cause the formation of new gall cells well before the larvae hatch and begin to feed (Rohfritsch 1992). This mass of cells, called the "plastem" by Beyerinck (1882) and the "lysenchym" by Magnus (1914), forms a pad beneath the eggs on the surface of leaves (Hough 1953; Rohfritsch 1992). Cells directly beneath the eggs of leaf gallers begin to break down by lytic action and when the larva hatches, it moves into a small cavity with fluid from these cells (Rohfritsch 1992). The first larval food comes from these fluids rather than chewed cells. Eggs of *D. nodulosa* (Brooks and Shorthouse 1998) and *D. triforma* are in contact with a similar pad of cells (Fig. 6), but they are not supported

by it. We did not observe a similar lytic action at the time *D. triforma* eggs were hatching.

The first nutritive cells in galls of *D. triforma* differentiate from the layer of gall cells along the surface of the chamber furthest from the freshly hatched larva. How the larvae cause the differentiation of nutritive cells is not known; however, it has been suggested that the stimulation is provided by wounding with the mandibles and the presence of salivary enzymes and various plant cell substances that are liberated into the larval chamber (Rohfritsch 1992). These substances, along with fragments of cell walls, constitute what Rohfritsch (1992) called a "cocktail" on which the larva feeds. As the first nutritive cells are being stimulated to differentiate, adjacent cells differentiate into PNT (Fig. 8). It is assumed that once the larva is free from its egg and surrounded by NT, new rows of PNT proliferate from a cambium-like zone that surrounds each chamber.

Nutritive cells lining the chambers throughout cynipid larval growth are characterized by an abundance of cytoplasm, reduced and fragmented vacuoles, a hypertrophied nucleus and nucleoli (Figs. 11, 15), and an abundance of cytoplasmic organelles (Bronner 1992). Bronner (1992) suggested that numerous plastids and mitochondria in the nutritive cells of cynipid galls were a sign of high metabolic activity. Larvae of cynipid gallers are the only phytophagous insects capable of altering the physiology and improving the nutritive value of plant cells before consuming them.

The larval chambers of most cynipid leaf galls are spherical, allowing the larvae to feed evenly over the chamber surface (Kostoff and Kendall 1929; Shorthouse 1998). In contrast, chambers in *D. triforma* galls are spherical only when the galls are immature and become ellipsoidal as the galls mature (Fig. 2). Although it is likely difficult for full-grown larvae to feed over the entire chamber surface, the presence of NT at both ends of chambers in maturing galls (Fig. 15) indicates that the larvae are able to switch positions. Of interest, little NT is found along the sides of chambers in maturing galls (Fig. 13). Brooks and Shorthouse (1998) found NT along the lateral surfaces of maturing, ellipsoidal chambers of galls of *D. nodulosa*, where most larvae are positioned vertically with the head end down, and suggested that cells here are torn open by ventral spines on the larvae. Fluids thus released would flow downwards towards the head end. Similar spines are found on the larvae of *D. triforma* (Fig. 12) and may have the same role, though GP found on the lateral surfaces does not appear to diminish.

Most of the increase in gall biomass occurs in the growth phase as a result of the proliferation and expansion of GP, PNT, and gall cortex. PNT cells are initially smaller than adjacent GP and pith cells (Fig. 10) as a result of rapid anticlinal and periclinal divisions but gradually enlarge as growth progresses. As the volume of maturing galls within the pith increases, the vascular cylinder is forced apart, but vascular tissues in fusiform galls are still contiguous with those of stem tissues below and above the gall. This allows a normal flow of water and assimilates through the galls to stem tissues protruding above, with the inducers and growing gall tissues removing what they need in the process (St. John and Shorthouse 2000).

Galls of most species of cynipids develop a distinct cambial zone between the nutritive tissues and the cortex (Bronner 1992), but this zone is not as prominent around the chambers in galls of *D. triforma*. This zone is thought to differentiate into GP, PNT, and NT towards the larval chambers, a part of the gall referred to as the inner gall by some authors (Bronner 1992; Rohfritsch 1992). Cells on the other side of the cambial zone differentiate into cortex cells, known as the outer gall. This cambium also gives rise to vascular tissues and cells destined to become the sclerenchyma layer in mature galls. The cortex contains the peripheral vascular tissue of the gall, which connects to the vascular tissue of the host organ. Assimilates, nutrients, and water available to the gall are directed towards the inner gall, which acts as a sink (Jankiewicz *et al.* 1969). If the larva is killed, the sink effect ceases and all NT and PNT cells dedifferentiate to cells resembling GP (Rohfritsch 1971).

The main events in the maturation phase of *D. triforma* galls, as in galls of other cynipid species (Houard 1903; Fourcroy and Braun 1967; Rohfritsch 1992), are the increased rates of larval feeding and formation of PNT and NT and the differentiation of sclerenchyma cells in a layer midway between the larval chambers (Figs. 13, 15) and the outside of the gall. In normal plant tissues, sclerenchyma serves primarily as support, though it is often involved in protecting the plant from fungi and herbivores (Mauseth 1988). Sclerenchyma also serves as support tissue in galls but likely has other functions as well. Cynipid larvae exhibit cannibalism

when placed together (Shorthouse 1993) and therefore sclerenchyma cells may prevent larvae from entering an adjacent chamber and consuming the inhabitant. The sclerenchyma sheath may also function in transporting water and nutrients to the nutritive tissues, as it does in galls of the cynipid *Aylax glechomae* (L.) (Fourcroy and Braun 1967). Porous sclerenchyma cells (Fig. 14) are generally associated with the xylem and allow the transport of nutrients (Mauseth 1988). Sclerenchyma may also provide protection from parasitoids and vertebrate predators (Roth 1949; Cornell 1983; Askew 1984); however, if this was so in the past, parasitoids now attack galls before the sclerenchyma forms (Shorthouse 1973, 1993).

Once the galls have reached maturity and the larvae have ceased feeding, a thin layer of undifferentiated GP remains on the chamber surface (Figs. 16, 18). In the galls of other species of *Diplolepis*, the remaining GP degenerates and the larval chamber is surrounded by sclerenchyma (Shorthouse 1993, 1998; Brooks and Shorthouse 1998). Chamber surfaces left lined with sclerenchyma are thought to reduce ice nucleation (Rickards and Shorthouse 1989; Williams *et al.* 2002), which freezing-intolerant larvae must avoid to survive the winter. GP cells lining larval chambers in *D. triforma* galls accumulate lignin in their cell walls and the majority either have an occluded lumen or are filled with phenolic compounds (Figs. 16, 18), resembling pith of the typical mature stem (Fig. 4). Food materials in the gut are also subject to ice formation (Salt 1961), but *D. triforma* larvae void their gut in the fall and spread meconium in a thin layer on the wall of the chamber (Figs. 17 and 18). Meconium here may smooth the surface and reduce ice nucleation.

An important characteristic of all gall-inducing insects is their close association with the vascular system of their host plants (Meyer 1969; Meyer and Maresquelle 1983). Gallers such as aphids induce galls with extensive amounts of vascular tissue close to the inner surface of their chambers, within easy reach of their mouthparts (Wool *et al.* 1999). Vascular bundles within the host organ at the point of attachment in aphid galls are modified to increase the flow of water and assimilates to the gall (Aloni *et al.* 1989). Cynipid galls are likewise endowed with a rich vascular system that encircles the larval chambers (Fig. 10), extending from the host organ to the NT and providing the water and nutrients required for growth of both gall tissues and larvae. The appearance and physiology of vascular tissues within galls and the movement of water and assimilates to the galls from other parts of the plant are controlled by the gallers, another important attribute of gallers not shared by other endophytophagous insects.

Vascular systems in plants provide conduits for the movement of water and solutes over long distances, with water and mineral nutrients traveling from the roots to the sites of evapotranspiration in the shoots and leaves via the xylem and dissolved carbohydrates moving from tissues that are net producers of photoassimilates to tissues that are net users via the phloem (Dengler 2001). As the woody stems of roses mature, the procambium becomes the lateral meristem that produces radial cells of phloem and xylem (Fig. 4), providing continuous channels for transport throughout the plant.

Cells within the procambium are known to have a capacity for modification (Dengler 2001) and it is these cells that are first stimulated by the eggs of *D. triforma* and (or) the material deposited by the ovipositing females (Figs. 5, 6). Similar strands of procambium are differentiated within the growing gall and join those of the host stem. As the galls mature, procambial strands within tissues of the gall also differentiate into xylem and phloem (Fig. 10) and extend towards the NT (Fig. 11). The patterns of cell division within the cambium are not genetically programmed but rather depend on signals from hormones and intercellular messenger molecules (Savidge 2001). There are strict developmental relationships between the vascular tissues and other tissues and organs in plants (Aloni 1987), and cynipid larvae have somehow evolved the means for intercepting some of the water, mineral nutrients, and photoassimilates destined for other parts of the plant and redirecting them towards the tissues of their galls (St. John and Shorthouse 2000). How they do this is still unknown.

The overall morphology of shrubs such as roses is determined by developmental pathways taken within or near the terminally positioned apical meristems (Berleth and Sachs 2001), the same chosen for oviposition by *D. triforma* (Fig. 3). Plants control the procambium and the differentiation of various cells of the mature vascular system with the endogenous plant hormone indole-3-acetic acid (Aloni 2001; Savidge 2001). The vascular differentiation that occurs

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in normal plants in a gradient from the procambium near the shoot tip (Fig. 3) to the roots is determined by varying auxin concentrations (Sachs 2000). The possibility that insect-derived hormones or insect-altered plant hormones are implicated in gall formation warrants attention. It is known that agrobacteria that cause tumours disrupt all aspects of normal organization by manipulating hormone synthesis. This suggests that turning genes on and off locally could lead to the formation of new forms (Sachs 2000), perhaps even ones as complex as the organized galls of cynipids. Studies measuring minute amounts of auxins within tissues of normal plants suggest that auxins may be morphogens, substances whose precise concentrations specify different cell fates and thus patterned differentiation (Sachs 2000). Cynipid galls, with their divergent structures, may be the result of cynipid eggs, female secretions, or secretions from the larvae interfering with and controlling auxin signaling and disrupting auxin transport throughout the plant.

Our knowledge of the driving forces behind the evolutionary divergence of gall types, such as those induced by cynipid wasps on roses, is limited. Factors thought to be involved include natural enemies, competition, and behavioural constraints (Csóka *et al.* 2005). Cynipids are likely evolving towards an improved ability to manipulate plants and the development of more structurally complex galls. Species such as *D. polita*, *D. bicolor* (Harris), and *D. nebulosa* (Bassett), which induce galls on leaves (Shorthouse 1998), are considered ancestral to species such as *D. triforma*, *D. nodulosa*, and *D. spinosa* (Ashmead), which induce stem galls (Plantard *et al.* 1998). It is suspected that the greater structural complexity of stem galls compared with leaf galls provides stem gallers with an adaptive advantage, such as protection against parasitoids and inquilines.

Understanding how cynipids gain control of attacked plant organs and redirect growth into the formation of species-specific, structurally distinct galls remains the most important challenge for students of cynipid galls. Several stimuli have been suggested for gall initiation and host-plant control, including wounding of tissues with the ovipositor; ovipositional fluids; secretions from the egg; viruses and bacteria; feeding activities of the larvae; and larval secretions of mimics of plant hormones (Magnus 1914; Kostoff and Kendall 1929; Hough 1953; Cornell 1983; Bronner 1985; Rohfritsch 1992; Stone *et al.* 2002). Some progress is being made at the molecular level (Harper *et al.* 2004), but much remains to be learned. Anatomical studies of events following egg deposition for other species, along with biochemical and molecular analyses of the surface and contents of eggs, the fluid deposited by the females, fluids deposited by the larvae, and the contents of cells influenced by both the eggs and the larvae, will likely provide useful clues. Perhaps yet unidentified symbiotic relationships between gall cells and species-specific microorganisms found on or in the eggs, in the deposits made by females, or in secretions from the larvae are responsible. Whatever the stimulus, key events in gall formation start when cells within the procambium begin dividing, and NT appears once the larvae exit the egg shell and begin feeding. It would be fascinating to unravel the genetic information within these cells, for in doing so, one would for the first time gain an understanding of how cynipids control plant growth.

It is interesting to speculate how events as complex as the manipulation by *D. triforma* of the growth and development of leaf buds of roses may have evolved. Adaptive radiation in phytophagous insects is considered most common on wildly distributed plants (Strong *et al.* 1984), and roses have been ideal hosts for the adaptive radiation of *Diplolepis* spp. *Rosa acicularis* is found from the tree line in northern Canada to the mid United States and across northcentral Eurasia (Lewis 1959). Hybridization is common in *Rosa* spp. and can enhance diversification rates in host plants and, by extension, in phytophagous insects (Nuismer and Thompson 2001).The numerous buds on mature, well-branched shrubs also provide ample resources for ovipositing *Diplolepis* spp. Leaf buds were likely an underexploited resource for phytophagous insects when discovered by *Diplolepis* spp. but became an ecological opportunity for these gallers and a stage for their adaptive radiation (Shorthouse *et al.* 2005). It appears that gall diversity is at least partially the result of each species developing a slightly different means of exploiting the resources and growth potential of leaf buds. The ability to accurately deposit eggs and manipulate the development of leaf buds was a key innovation for *Diplolepis* spp., and once the novelty was added to their phenotype it triggered new ecological

opportunities and the adaptive radiation and array of species and their galls we see today.

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References

Adler, H. 1877. Beiträge zur Naturgeschichte der Cynipiden. Deutsche Entomologische Zeitschrift, **21**: 209–247.

Aloni, R. 1987. Differentiation of vascular tissues. Annual Review of Plant Physiology, **38**: 179–204.

Aloni, R. 2001. Foliar and axial aspects of vascular differentiation: Hypotheses and evidence. Journal of Plant Growth Regulation, **20**: 22–34.

Aloni, R., Katz, D.A., and Wool, D. 1989. Effect of the gall-forming aphid *Slavum wertheimae* on the differentiation of xylem in branches of *Pistacia atlantica*. Annals of Botany, **63**: 373–375.

Askew, R.R. 1984. The biology of gall wasps. *In* Biology of gall insects. *Edited by* T.N. Ananthakrishnan. Oxford and IBN, New Delhi, India. pp. 223–271.

Berleth, T., and Sachs, T. 2001. Plant morphogenesis: long-distance coordination and local patterning. Current Opinion in Plant Biology, **4**: 57–62.

Beyerinck, M.W. 1882. Beobachtungen über die ersten Entwicklungsphasen einiger Cynipidengallen. Natuurkundige Verhandelingen Koninklijke Akademie van Wetenschappen, **22**: 1–198.

Bronner, R. 1977. Contribution à l'étude histochimique des tissus nourriciers des zoocécides. Marcellia, **40**: 1–134.

Bronner, R. 1985. Anatomy of the ovipositor and oviposition behavior of the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae). The Canadian Entomologist, **117**: 849–858.

Bronner, R. 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiids. *In* Biology of insect-induced galls. *Edited by* J.D. Shorthouse and O. Rohfritsch. Oxford University Press, New York. pp. 118–140.

Brooks, S.E., and Shorthouse, J.D. 1997. Biology of the rose stem galler *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and its associated component community in central Ontario. The Canadian Entomologist, **129**: 1121–1140.

Brooks, S.E., and Shorthouse, J.D. 1998. Developmental morphology of stem galls of *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and those modified by the inquiline *Periclistus pirata* (Hymenoptera: Cynipidae) on *Rosa blanda* (Rosaceae). Canadian Journal of Botany, **76**: 365–381.

Cornell, H.V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how? The American Midland Naturalist, **110**: 225–234.

Crepsi, B.J., and Worobey, M. 1998. Comparative analysis of gall morphology in Australian gall thrips: the evolution of extended phenotypes. Evolution, **52**: 1686–1698.

Csóka, G., Stone, G.N., and Melika, G. 2005. Biology, ecology, and evolution of gall-inducing Cynipidae. *In* Biology, ecology and evolution of gall-inducing arthropods. Vol. 2. *Edited by* A. Raman, C.W. Schaefer, and T.M. Withers. Science Publishers, Inc., Enfield, New Hampshire. pp. 573–642.

Dawkins, R. 1982. The extended phenotype. Oxford University Press, Oxford.

DeClerck, R.A., and Shorthouse, J.D. 1985. Tissue preference and damage by *Fenusa pusilla* and *Messa nana* (Hymenoptera: Tenthredinidae), leaf mining sawflies on white birch (*Betula papyrifera*). The Canadian Entomologist, **117**: 351–362.

Dengler, N.G. 2001. Regulation of vascular development. Journal of Plant Growth Regulation, **20**: 1–13.

Fourcroy, M., and Braun, C. 1967. Observations sur la galle de l'*Aulax glechomae* L. sur *Glechoma hederacea* L. II. Histologie et rôle physiologique de la coque sclérifiée. Marcellia, **34**: 3–30.

Harper, L.J., Schönrogge, K., Lim, K.Y., Francis, P., and Lichtenstein, C.P. 2004. Cynipid galls: insect-induced modifications of plant development create novel plant organs. Plant, Cell and Environment, **27**: 327–335.

Harris, P., and Shorthouse, J.D. 1996. Effectiveness of gall inducers in weed biological control. The Canadian Entomologist, **128**: 1021–1055.

Houard, C. 1903. Recherches anatomiques sur les galles de tiges: Pleurocécidies. Bulletin Scientifique de France et Belgique, **38**: 140–419.

Hough, J.S. 1953. Studies on the common spangle gall of oak. I. The developmental history. New Phytologist, **52**: 149–177.

Jankiewicz, L.S., Plich, H., and Antoszewsji, R. 1969. Preliminary studies on the translocation of ^{14}C-labelled assimilates and $^{32}PO^{3-}_{4}$ towards the gall evoked by *Cynips* (*Diplolepis*) *quercus-folii* L on oak leaves. Marcellia, **36**: 163–174.

Jensen, W.A. 1962. Botanical histochemistry. W.H. Freeman, San Francisco, California.

Kostoff, D., and Kendall, J. 1929. Studies on the structure and development of certain cynipid galls. Biological Bulletin, **56**: 402–459.

Küster, E. 1911. Die gallen der pflanzen. S. Hirzel, Leipzig, Germany.

Lalonde, R.G., and Shorthouse, J.D. 2000. Using rose galls for field exercises in community ecology and island biogeography. The American Biology Teacher, **62**: 436–441.

LeBlanc, D.A., and Lacroix, C.R. 2001. 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. International Journal of Plant Sciences, **162**: 29–46.

Lewis, W.H. 1959. A monograph of the genus *Rosa* in North America. I. *R. acicularis*. Brittonia, **11**: 1–24.

Magnus, W. 1914. Die Entstehung der Pflanzengallen verursacht durch Hymenopteren. G. Fischer, Jena, Germany.

Mauseth, J.D. 1988. Plant anatomy. Benjamin/Cummings, Menlo Park, California.

Meyer, J. 1969. Irrigation vasculaire dans les galles. Memoires de la Societé Botanique de France. pp. 75–97.

Meyer, J. 1987. Plant galls and gall inducers. Gebrüder Borntraeger, Berlin, Germany.

Meyer, J., and Maresquelle, H.J. 1983. Anatomie des Galles. Gebrüder Borntraeger, Berlin, Germany.

Nuismer, S.L., and Thompson, J.N. 2001. Plant polyploidy and non-uniform effects on insect herbivores. Proceedings of the Royal Society of London, Series B: Biological Sciences, **268**: 1937–1940.

Offman, S.T. 2004. Factors influencing structure of communities associated with galls induced by *Diplolepis spinosa* (Hymenoptera: Cynipidae) in northern Ontario. M.Sc. thesis, Laurentian University, Sudbury, Ontario.

Plantard, O., Shorthouse, J.D., and Rasplu, J.-Y. 1998. Molecular phylogeny of the genus *Diplolepis* (Hymenoptera: Cynipidae). *In* The Biology of gall-inducing arthropods. US Forest Service General Technical Report NC-199. *Edited by* G. Csóka, W.J. Mattson, G.N. Stone, and P.W. Price. US Department of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota. pp. 247–260.

Price, P.W., Fernandes, G.W., and Waring, G.L. 1987. Adaptive nature of insect galls. Environmental Entomology, **16**: 15–24.

Rickards, J.C., and Shorthouse, J.D. 1989. Overwintering strategy of the stem-gall inducer *Diplolepis spinosa* (Hymenoptera: Cynipidae) in central Ontario. Canadian Journal of Zoology, **67**: 2232–2237.

Rohfritsch, O. 1971. Développement cécidien et rôle du parasite dans quelques galles d'arthropodes. Marcellia, **37**: 233–339.

Rohfritsch, O. 1992. Patterns in gall development. *In* Biology of insect-induced galls. *Edited by* J.D. Shorthouse and O. Rohfritsch. Oxford University Press, New York. pp. 60–86.

Rohfritsch, O., and Shorthouse, J.D. 1982. Insect galls. *In* Molecular biology of plant tumors. *Edited by* G. Kahl and J.S. Schell. Academic Press, New York. pp. 131–152.

Ronquist, F., and Liljeblad, J. 2001. Evolution of the gall wasp – host plant association. Evolution, **55**: 2503–2522.

Roth, P. 1949. Beitäge zur biologie der gallwespen. Naturforschende Gesellschaft, Basel, **60**: 104–178.

Sachs, R. 2000. Integrating cellular and organismic aspects of vascular differentiation. Plant Cell Physiology, **41**: 649–656.

Salt, R.W. 1961. Principles of insect cold-hardiness. Annual Review of Entomology, **6**: 55–74.

Sass, J.E. 1958. Botanical microtechnique. Iowa University Press, Ames, Iowa.

Savidge, R.A. 2001. Intrinsic regulation of cambial growth. Journal of Plant Growth Regulation, **20**: 52–77.

Shorthouse, J.D. 1973. The insect community associated with rose galls of *Diplolepis polita* (Cynipidae: Hymenoptera). *Quaestiones entomologicae*, **9**: 55–98.

Shorthouse, J.D. 1993. Adaptations of gall wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) and the role of gall anatomy in cynipid systematics. Memoirs of the Entomological Society of Canada, **165**: 139–163.

Shorthouse, J.D. 1998. Role of *Periclistus* (Hymenoptera: Cynipidae) inquilines in leaf galls of *Diplolepis* (Hymenoptera: Cynipidae) on wild roses in Canada. *In* The Biology of gall-inducing arthropods. US Forest Service General Technical Report NC-199. *Edited by* G. Csóka, W.J. Mattson, G.N. Stone, and P.W. Price. US Department of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota. pp. 61–81.

Shorthouse, J.D., and Leggo, J.J. 2002. Immature stages of the galler *Diplolepis triforma* (Hymenoptera: Cynipidae) with comments on the role of its prepupa. The Canadian Entomologist, **134**: 433–446.

Shorthouse, J.D., and Ritchie, A.J. 1984. Description and biology of a new species of *Diplolepis* Fourcroy (Hymenoptera: Cynipidae) inducing galls on the stems of *Rosa acicularis*. The Canadian Entomologist, **116**: 1623–1636.

Shorthouse, J.D., and Rohfritsch, O. 1992. Biology of insect galls. Oxford University Press, New York.

Shorthouse, J.D., Leggo, J.J., Sliva, M.D., and Lalonde, R.G. 2005. Has egg location influenced the radiation of *Diplolepis* (Hymenoptera: Cynipidae) gall wasps on wild roses? Basic and Applied Ecology, **6**: 423–434.

St. John, M.G., and Shorthouse, J.D. 2000. Allocation patterns of organic nitrogen and mineral nutrients within stem galls of *Diplolepis spinosa* and *Diplolepis triforma* (Hymenoptera: Cynipidae) on

wild roses (Rosaceae). The Canadian Entomologist, **132**: 635–648.

Stone, G.N., and Cook, J.M. 1998. The structure of cynipid oak galls: patterns in the evolution of an extended phenotype. Proceedings of the Royal Society of London, Series B: Biological Sciences, **265**: 979–988.

Stone, G.N., and Schönrogge, K. 2003. The adaptive significance of insect gall morphology. Trends in Ecology and Evolution, **18**: 512–523.

Stone, G.N., Schönrogge, K., Atkinson, R.J., Bellido, D., and Pujade-Villar, J. 2002. The population biology of oak gall wasps (Hymenoptera: Cynipidae). Annual Review of Entomology, **47**: 633–668.

Strong, D.R., Lawton, J.H., and Southwood, R. 1984. Insects on plants: community patterns and mechanisms. Blackwell Scientific Publications, Oxford.

Turner, J.S. 2000. The extended organism; the physiology of animal-built structures. Harvard University Press, Cambridge, Massachusetts.

Weidel, F. 1911. Beiträge zur entwicklungsgeschichte und vergleichenden anatomie der zynipidengallen der eiche. Flora, **102**: 279–334.

Wiebes-Rijks, A.A., and Shorthouse, J.D. 1992. Ecological relationships of insects inhabiting cynipid galls. *In* Biology of insect-induced galls. *Edited by* J.D. Shorthouse and O. Rohfritsch. Biology of insect-induced galls. Oxford University Press, New York. pp. 238–257.

Williams, J., Shorthouse, J.D., and Lee, R.E., Jr. 2002. Extreme resistance to desiccation and microclimate-related differences in cold-hardiness of gall wasps (Hymenoptera: Cynipidae) overwintering on roses in southern Canada. The Journal of Experimental Biology, **205**: 2115–2124.

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

Zeh, D.W., Zeh, J.A., and Smith, R.L. 1989. Ovipositors, amnions and eggshell architecture in the diversification of terrestrial arthropods. The Quarterly Review of Biology, **64**: 147–168.