Available online at www.sciencedirect.com

SCIENCE DIRECT°

ELSEVIER

# Has egg location influenced the radiation of *Diplolepis* (Hymenoptera: Cynipidae) gall wasps on wild roses?

Joseph D. Shorthouse*, Jonathan J. Leggo[1], Monica D. Sliva, Robert G. Lalonde[2]

*Department of Biology, Laurentian University, Sudbury, Ont., Canada P3E 2C6*

## Summary

The oviposition sites of 6 species of gall wasps of the genus *Diplolepis* on their host roses in Ontario, Canada were examined using plant histological techniques. A leaf galler, *Diplolepis polita*, and a stem galler, *D. triforma*, gall *Rosa acicularis*; whereas the leaf galler *D. bicolor* and the stem gallers *D. nodulosa*, *D. spinosa* and *D. fusiformans* gall *R. blanda*. *D. polita* and *D. bicolor* deposit their eggs on developing leaflets within leaf buds. *D. nodulosa*, *D. triforma*, and *D. spinosa* deposit their eggs between the leaf primordia near the apical meristem of leaf buds. *D. fusiformans* deposits eggs on the surface of current-year stems. We consider leaf buds as adaptive zones. The precise deposition of eggs within these organs contributes to species-specific differences in gall structure, and may have influenced radiation of the genus. Inquilines and parasitoids are not deterred by differences in gall structure, and thus it appears that enemies are not driving forces in the radiation of these cynipids.
© 2005 Gesellschaft für Ökologie. Published by Elsevier Gmbh. All rights reserved.

## Zusammenfassung

Es wurden die Eiablageorte von sechs Arten von Gallwespen der Gattung *Diplolepis* auf ihren Wirtsrosen mit pflanzenhistologischen Techniken in Ontario, Kanada, untersucht. Ein Blattgallbildner, *D. polita*, und ein Stammgallbildner, *D. triforma*, befallen *Rosa acicularis*, während ein Blattgallbildner, *D. bicolor*, und die Stammgallbildner *D. nodulosa*, *D. spinosa* und *D. fusiformans R. blanda* befallen. *D. polita* und *D. bicolor* legen ihre Eier auf sich entwickelnde Blättchen in Blattknospen. *D. nodulosa*,

*Corresponding author. Tel.: 705 675 1151x 2287; fax: 705 675 4859.
  *E-mail address:* roses@cyberbeach.net (J.D. Shorthouse).
[1]Current address: Population Ecology Group, Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6C2.
[2]Current address: Biology Department, Okanagan University College, Kelowna, BC, Canada V1V 1V7.

*D. triforma* und *D. spinosa* legen ihre Eier zwischen die Blattprimordien nahe dem Apikalmeristem der Blattknospen. *D. fusiformans* legt die Eier auf die Oberfäche diesjähriger Stängel. Wir betrachten die Blattknospen als adaptive Zonen. Die präzise Ablage der Eier in diesen Organen trägt zu artspezifischen Differenzen in der Gallenstruktur bei und kann die Radiation der Gattung beeinflusst haben. Inquilinen und Parasitoide werden durch Unterschiede in der Gallstruktur nicht abgeschreckt und deshalb scheint es so, dass Feinde keine treibende Kraft für die Radiation dieser Cypnipiden sind.

## Introduction

There has been much speculation on the factors responsible for the radiation of gall-inducing insects (Price, Fernandes, & Waring, 1987; Cook, Stone, & Rowe, 1998; Price, Roininen, & Zinovjev, 1998; Nyman, Widmer, & Roininen, 2000). Despite two centuries of studying galls and the insects associated with them (see reviews in Meyer, 1987, Shorthouse & Rohfritsch, 1992; Williams, 1994; Stone, Schönrogge, Atkinson, Bellido, & Pujade-Villars, 2002), little is known about selective pressures responsible for interspecific variation in gall morphology and the evolution of inducer diversity. It is still not clear whether gall size and shape are selected for by the activities of parasitoids and inquilines (Cornell, 1983; Stern, 1995; Price, & Clancy, 1986; Stone, & Schönrogge, 2003) or by aspects of their life history strategies such as oviposition behaviour, host and organ specificity, or the effects of selectively neutral variation in oviposition strategy (Waring & Price, 1989).

Our contributions to the knowledge of gall-inducing insects have concentrated on cynipids of the genus *Diplolepis*, tribe Diplolepidini. Members of the Diplolepidini form a monophyletic group (Plantard, Shorthouse, & Rasplus, 1998). All species of the group induce galls on shrubs of the genus *Rosa*. The genus *Diplolepis* is Holarctic, but most of the approximately 42 species are found in North America.

Although the gross external appearance of cynipid galls varies extensively, the inner organization of gall tissues for most species is similar (Rohfritsch, 1992). Most maturing cynipid galls have a layer of nutritive cells encircling the larval chamber, followed by layers of sclerenchyma and cortical parenchyma. Finding mature galls in the wild for morphological study is usually easy, but locating the eggs and recently initiated galls is much more difficult and usually requires culturing the galls under controlled conditions.

As part of a comparative anatomical study of cynipid galls, we were successful at culturing six species of wasps and their galls in the laboratory; *Diplolepis polita* (Ashmead) and *D. bicolor* (Harris) on leaves and *D. nodulosa* (Beutenmuller), *D. triforma* Shorthouse and Ritchie, *D. spinosa* (Ashmead) and *D. fusiformans* (Ashmead) on stems. In this paper, we describe oviposition sites for these six species and speculate on how differences may have contributed to the radiation of cynipid gallers. We speculate that slight differences in the location of the eggs would partially explain how galls of different species become structurally distinct. Finally, we suggest that differences in the structures of these six galls influence the composition of associated component communities of inquilines and parasitoids.

## Natural history of *Diplolepis* and inhabitants of their galls

*Diplolepis* are univoltine. Larvae are found in individual gall chambers throughout the summer. They overwinter as prepupae and have a short pupal stage lasting 7–14 d the following spring (Shorthouse, 1993; Shorthouse, & Leggo, 2002). Appearance of adults, which live for 4–10 d the following spring, is synchronized with the presence of tissues at stages of development appropriate for oviposition.

*Diplolepis* galls in a rose patch commonly have an associated component community of inquilines and parasitoids. The composition of this community is usually consistent throughout the range of the inducer (Shorthouse, 1993). Inquilines of the genus *Periclistus* inhabit and modify galls of several *Diplolepis* species and kill the inducers (Brooks & Shorthouse, 1998; Shorthouse, 1998). Many species of parasitoids of the genera *Eurytoma* (Eurytomidae), *Torymus* (Torymidae), *Pteromalus* (Pteromalidae), *Aprostocetus* (Eulophidae), *Eupelmus* (Eupelmidae), *Ormyrus* (Ormyridae) and *Orthopelma* (Ichneumonidae) are associated with *Diplolepis* galls.

## Materials and methods

*Diplolepis* oviposited on potted roses in growth cabinets or on excised branches brought to the laboratory. Ovipositions were also obtained by placing recently emerged adults on host roses in the field. Tissues in which *Diplolepis* had oviposited were excised from the host plants and fixed via vacuum-infiltration in formalin-acetic acid-alcohol (FAA; 90 parts 70% alcohol, 5 parts 37% formalin and 5 parts glacial acetic acid) according to O'Brien and McCully (1981). Tissues were washed with tap water for 12 h, dehydrated in an ethanol-tertiary butyl alcohol series, and embedded in paraffin. Tissues were sectioned at 8 μm using a Jung rotary microtome, affixed to slides, and stained with 1% aqueous safranin and 0.5% fast green (O'Brien & McCully, 1981).

Information on the composition of gall communities was obtained by collecting mature galls throughout central Ontario, Canada, and allowing the adults to emerge from them in the laboratory.

## Results

Oviposition behaviour of all six species is similar. Females position themselves with the head directed towards the proximal end of the bud or stem. Those laying in buds probe the buds with their ovipositor prior to oviposition. Pictures of galls of the six species and details on the locations of their eggs are presented in three plates (Figs. 1–3) below. Mature galls are shown in Fig. 1, dissected galls in Fig. 2 and egg location in Fig. 3. Within each plate, the six *Diplolepis* species are arranged in the same order, A–F.

(A) *Diplolepis polita* induces spherical galls on the adaxial surface of leaflets of *Rosa acicularis*. Mature galls average 3.4–4.5 mm in diameter and are clothed with weak spines (Fig. 1A). Galls have a single, thin-walled, spherical chamber (Fig. 2A). Galled leaves usually abscise by the end of July.

*D. polita* is one of the first species to appear in the spring (Fig. 4). It lays its eggs in the upper part of buds (Fig. 5). Eggs are attached to one or two epidermal cells on unfolded leaflets (Fig. 3A). Within 24 h, cells beneath the egg are stimulated to enlarge. Some eggs are laid a few mm apart on the same leaflet; others are laid on different leaflets. Eggs rest on a dark-staining deposit left by the female (Fig. 3A).

*Gall community*: Commonly, less than 5% of mature galls are inhabited by *D. polita* by the end of the season, making the gall inducer among the less frequent inhabitants in the community (Fig. 6). *Periclistus* inquilines, the most abundant inhabitants (65.5%), commonly kill 70% of all inducers. Parasitoids from eight genera comprise 29.8% of gall inhabitants (the majority, 24.6%, are *Eurytoma* sp.).

(B) *Diplolepis bicolor* induces clusters of spherical galls on the adaxial surface of immature leaflets of *R. blanda*. Mature galls average 7.5 mm in diameter and are clothed with dense, sharp, thorn-like spines (Fig. 1B). Galls have a single, thick-walled, spherical chamber (Fig. 2B). Galled leaves are usually difficult to remove from the plant and often remain on the host plant throughout the winter.

Like *D. polita*, adults of *D. bicolor* also appear early in the spring (Fig. 4) laying their eggs on the surface of undifferentiated leaflets near the apical meristem (Fig. 5). Eggs are usually laid in clusters with each egg attached to one or two epidermal cells (Fig. 3B).

*Gall community*: Commonly less than 5% of mature galls are inhabited by an inducer (Fig. 6): *Periclistus* kill 80% of all inducers. Most galls are inhabited and modified by *Periclistus*, the most abundant inhabitant (66.9%) of the galls in the spring. Parasitoids from eight genera are found in most gall populations. Only about 1% of the inhabitants are *Eurytoma* sp.

(C) *Diplolepis nodulosa* induces stem galls that arise from the base of early spring buds of *R. blanda*. Mature galls are fusiform, spineless swellings of the stem (Fig. 1C) and often circumscribed by a series of stunted leaflets at the proximal end. Mature galls average 3.1 mm in diameter at their widest point and all growth of the stem is terminated beyond the gall. The single-chambered pith galls are thin-walled with the elliptical larval chamber comprising a major portion of the gall (Fig. 2C). Galls remain on the host plant throughout the winter.

Adults appear later than *D. polita* and *D. bicolor* (Fig. 4) and lay one egg per leaf bud at the proximal end of buds, in leaf internodes at the base of maturing leaves (Fig. 5). A small channel is cut down to the vascular cylinder into which an egg is deposited. A dark-staining plug deposited by the female surrounds the proximal end of the eggs (Fig. 3C). The base of each *D. nodulosa* egg is placed in contact with the procambium (Fig. 5). The eggs and freshly-hatched larvae sink into the pith and the gall is initiated from tissues within the pith.

*Gall community*: About 60% of galls are commonly invaded by *Periclistus,* which kill 60% of the inducers in invaded galls. Numerous *Periclistus* can develop within a single *Diplolepis* gall, making
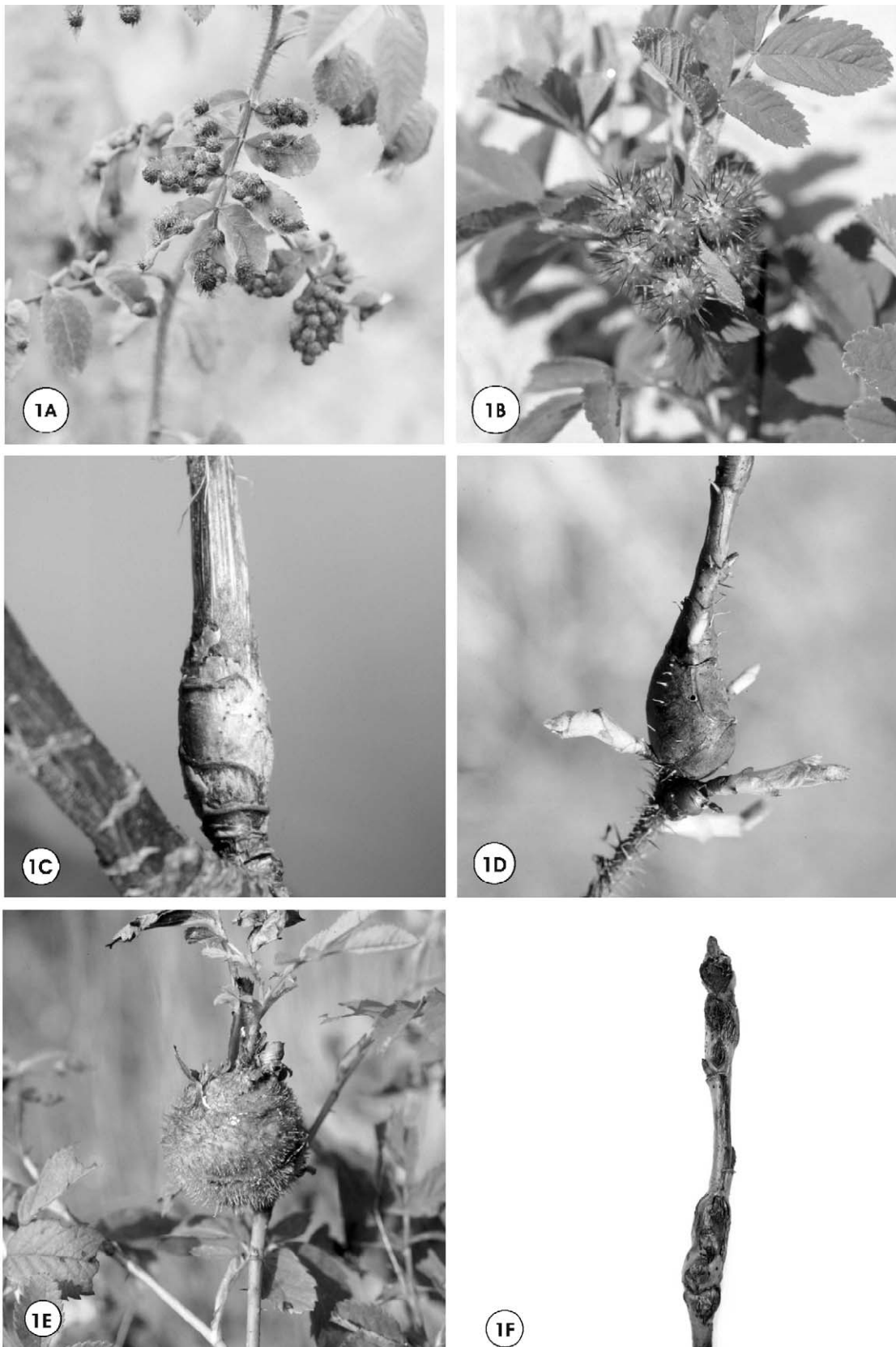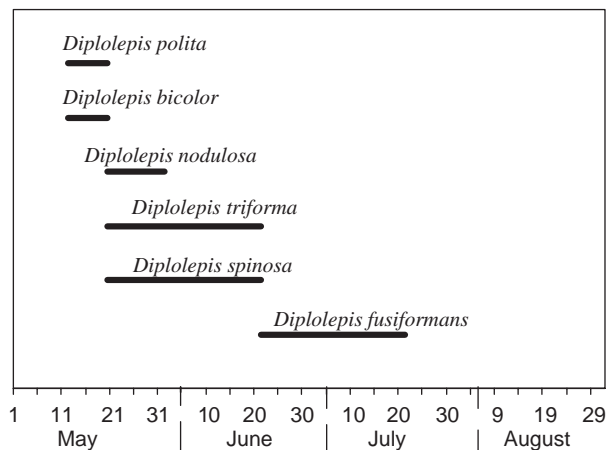
**Figure 1.** (plate) Mature galls of *Diplolepis*: (A) *D. polita*. (B) *D. bicolor.* (C) *D. nodulosa*. (D) *D. triforma*. (E) *D. spinosa*. (F) *D. fusiformans*.
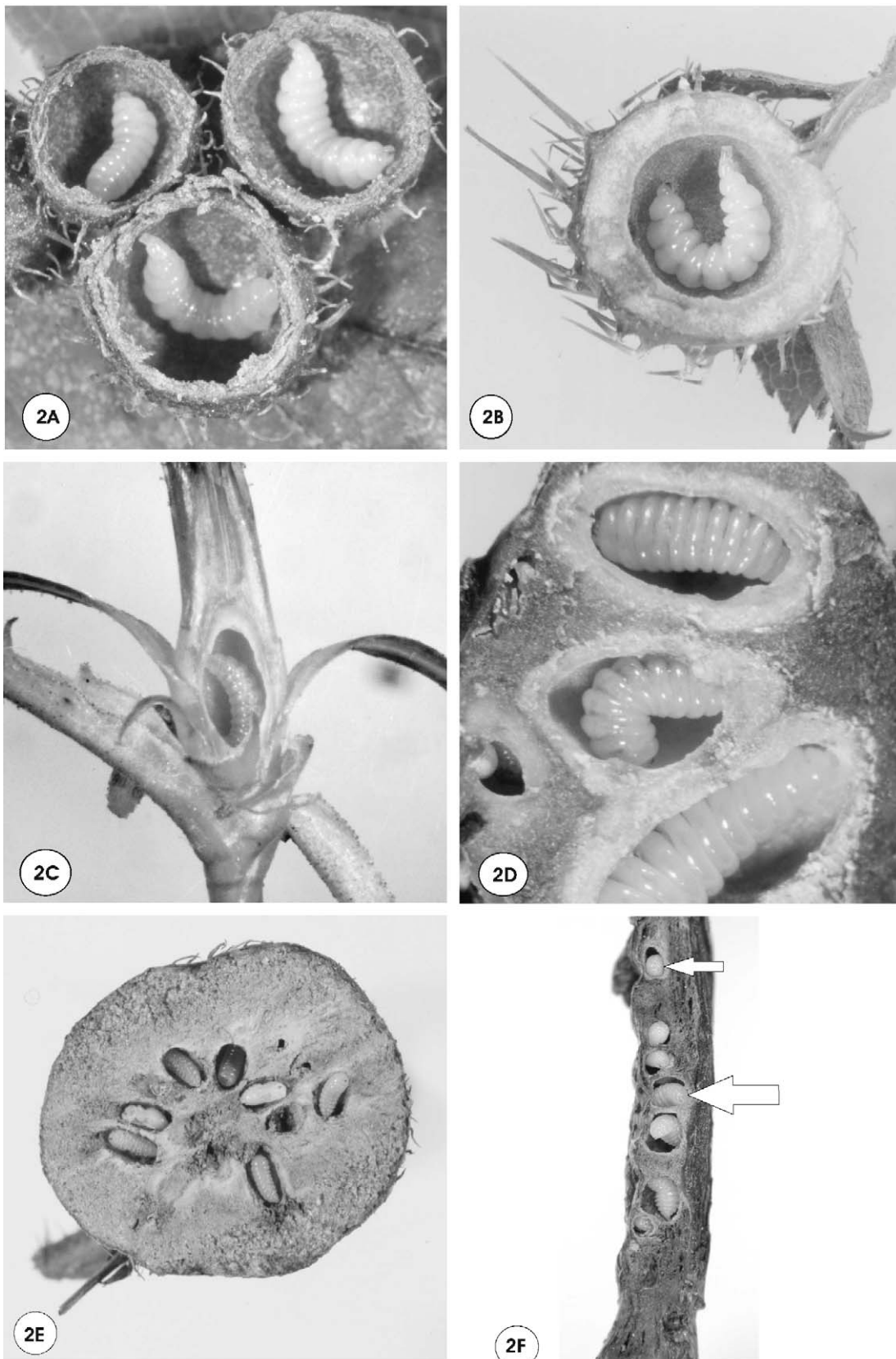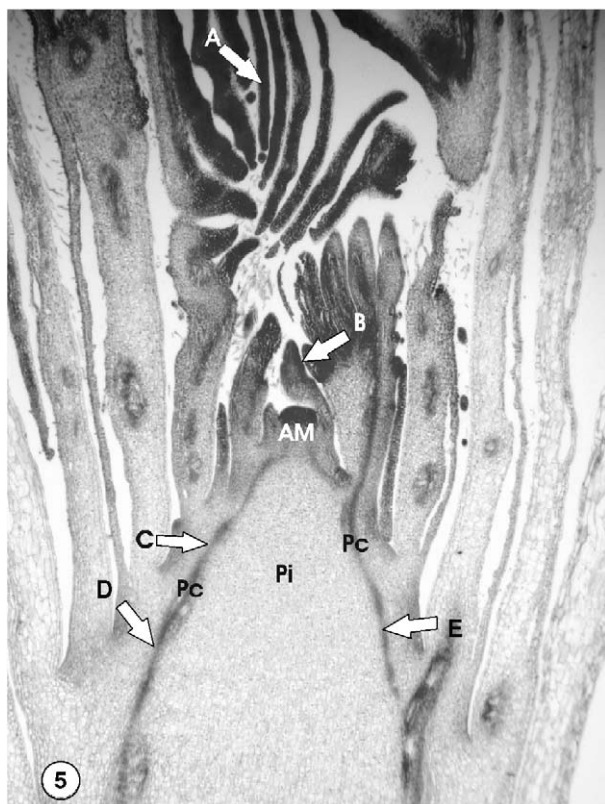
**Figure 2.** (plate) Dissections of mature galls of *Diplolepis*. (A) *D. polita*. (B) *D. bicolor*. (C) *D. nodulosa*. (D) D. *triforma*. (E) *D. spinosa*. (F) *D. fusiformans*. Three larvae at the bottom (large arrow) are those of the inducer. Three larvae at the top (small arrow) are *Periclistus*.
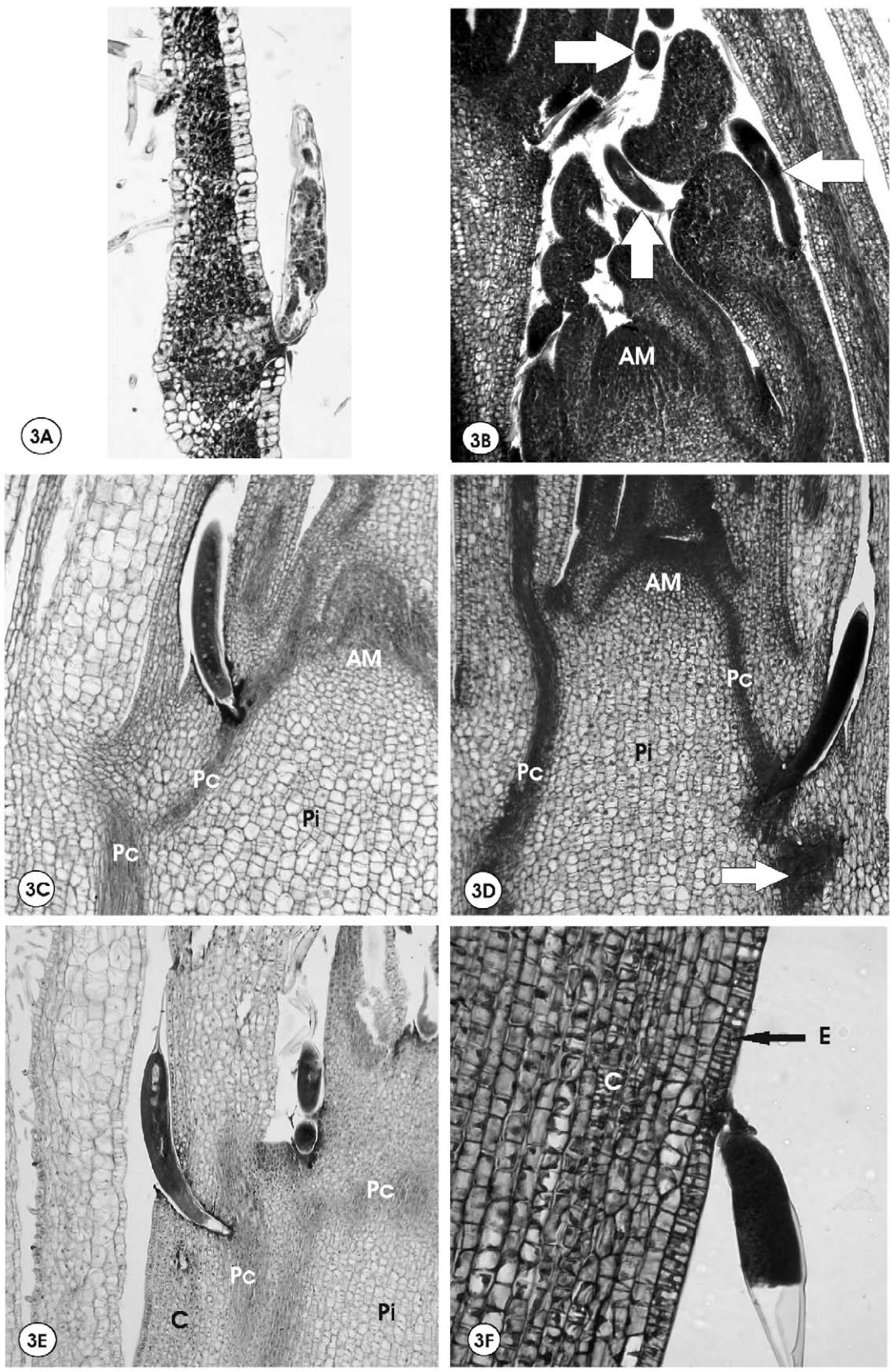
**Figure 4.** Oviposition periods of the six species of *Diplolepis*.



**Figure 5.** Section of leaf bud of wild rose illustrating the sites where leaf and bud gallers deposit their eggs. (1) *D. polita*; (2) *D. bicolor*; (3) *D. nodulosa*; (4) *D. triforma*; (5) *D. spinosa*.

them by far the most abundant inhabitants by the end of spring: 81.7% of the inhabitants are *Periclistus* and less than 2.8% are inducers. Parasitoids from six genera attack inducer larvae; parasitoids from seven genera attack the inhabitants of *Periclistus*-modified galls. Parasitoids comprise 15.5% of the inhabitants in the spring (9.6% are *Eurytoma* sp.) (Fig. 6)

(D) *Diplolepis triforma* induces woody stem galls on *Rosa acicularis*. Mature galls are prominent, fusiform, reddish-brown swellings (Fig. 1D) and may be either smooth or spiny. They vary in length from 1 to 10 cm and average about 15 mm in diameter. They remain on the host plants throughout the winter. Larval chambers in this multi-chambered gall (Fig. 2D) are packed closely together and are usually within 5 mm of the surface.

Adults appear from mid-May to late June (Fig. 4) and lay an average of seven eggs in clusters in the leaf internodes (Fig. 5). The ovipositor cuts a small channel down to the vascular cylinder into which the individual eggs are deposited (Fig. 3D). Galls are initiated from the procambium and larval chambers form within the region that would be occupied by pith in the ungalled plant.

*Gall community*: Inducers comprise 24.6% of gall inhabitants in the spring. Galls of *D. triforma* are not inhabited by *Periclistus*. Parasitoids from 6 genera attack galls of *D. triforma*. Parasitoids comprise about 75.4% of inhabitants in the spring (49.6% are *Eurytoma* sp.) (Fig. 6).

(E) *Diplolepis spinosa* induces one of the largest rose galls in North America on the stems of *R. blanda*. Mature galls are hard and woody, spherical, prominent swellings (Fig. 1E), covered with sharp, stout spines and are often found in clusters, several galls per branch. Galls in central Ontario average 2.3 cm in diameter. Larval chambers in this multi-chambered gall are elliptical and are dispersed throughout the gall, but often are clustered near the centre (Fig. 2E). An average of 16 larvae are found per gall with some galls containing as many as 35. Galls remain on the host plants throughout the winter.

Adults appear from mid-May to late June (Fig. 4) and deposit a cluster of eggs between the immature leaves. Each egg is deposited in an oviposition channel so that the base is in contact

**Figure 3.** (plate) Freshly deposited eggs of *Diplolepis*. (A) Egg of *D. polita* attached to the adaxial surface of leaflets in an unfolded leaf bud. (B) Eggs of *D. bicolor* (arrows) attached to leaflets in an unfolded leaf bud. (C) Egg of *D. nodulosa* below the apical meristem. (D) Egg of *D. triforma* below the apical meristem. Arrow indicates the site of another egg. (E) Eggs of *D. spinosa* below the apical meristem. (F) Egg of *D. fusiformans* attached to the surface of current year stem.
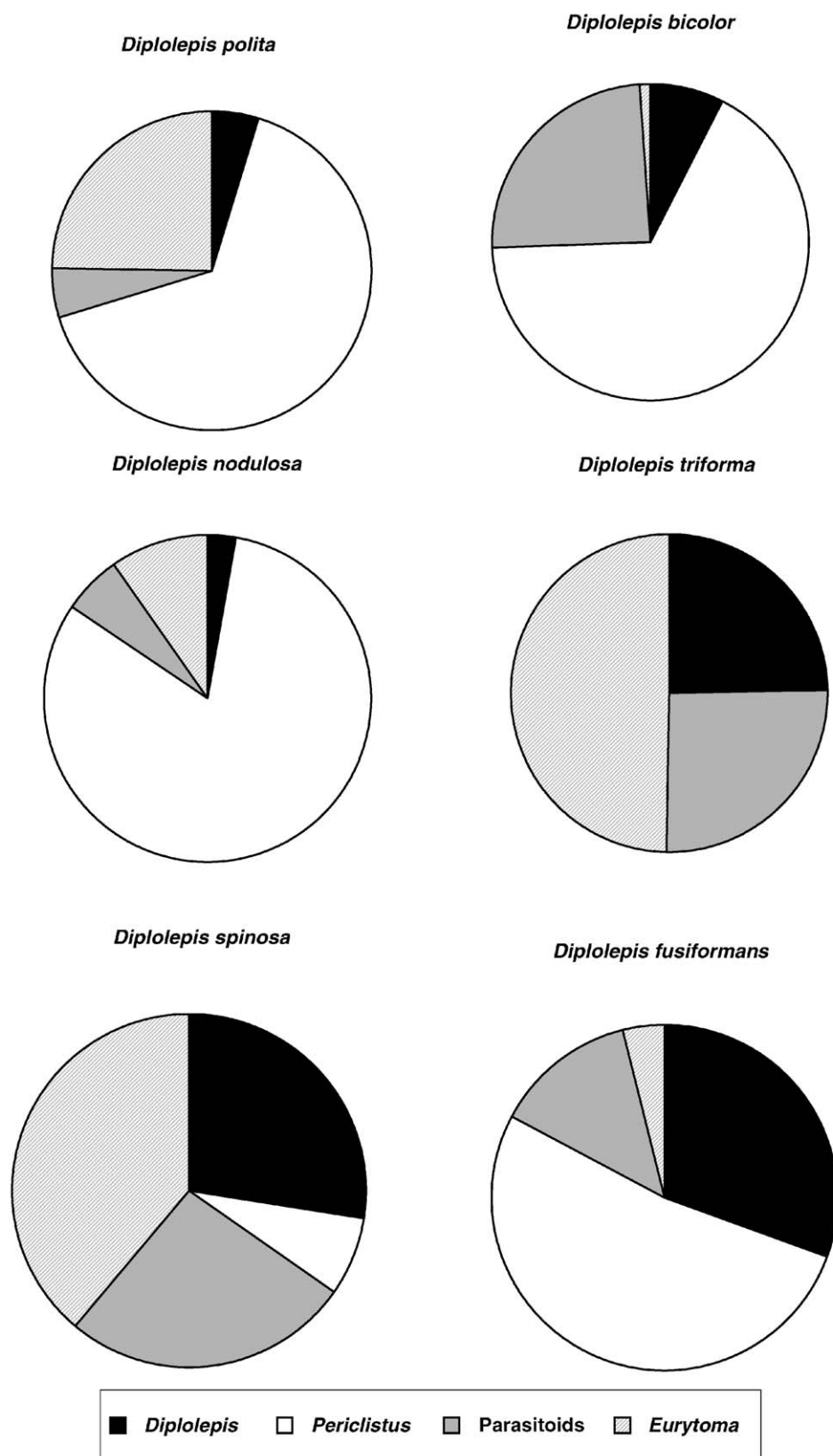
**Figure 6.** Gall community composition: relative proportion of the inhabitants of exiting mature galls in the spring of the year following gall induction.

with the procambium of the vascular cylinder or a leaf trace within the stem (Fig. 5). Eggs do not penetrate the procambium (Fig. 3E) and galls are initiated in the cortical region.

*Gall community*: Inducers comprise 27.4% of the inhabitants in the spring. *Periclistus* are able to attack galls of *D. spinosa,* but they are not common, comprising only 7.3% of the inhabitants. It is not known if ovipositing *Periclistus* kill the inducers in attacked chambers. Parasitoids from seven genera attack galls of *D. spinosa* comprising 65.3% of all inhabitants (38.9% are *Eurytoma* sp.) (Fig. 6).

(F) *Diplolepis fusiformans* induces small, gentle-swelling galls along the distal stem tissues of current year's growth of *R. blanda*. Mature galls are smooth to lumpy cylindrical thickenings that occur along one side of the stem (Fig. 1F). Tissues above the galls usually die and break off during the winter. Galls appear multichambered as the chambers occur in rows along one side of the stem (Fig. 2F).

Adults appear from late June to late July (Fig. 4). Eggs are laid in rows along one side of a stem. Each egg is deposited with only its proximal tip embedded in an epidermal cell (Fig. 3F). Galls are initiated from tissues of the cortex.

*Gall community*: About half of all chambers contain an inducer by fall (Fig. 2F). In the spring, inducers comprise 30.5% whereas *Periclistus* comprise 52.4% of inhabitants (Fig. 6). Chambers occupied by *Periclistus* are also found in rows along the length of the galls (Fig. 2F). Parasitoids from six genera attack galls of *D. fusiformans*. Parasitoids comprise 17.1% of the inhabitants by spring with (with 4% being *Eurytoma* sp.).

## Discussion

Galls induced by *Diplolepis* on wild roses, with their varying sizes, shapes, and number of chambers, are as diverse and complex as those of any other group of cynipids (Stone & Cook, 1998). Our histological analysis of oviposition sites provides some useful clues to understanding how this radiation has occurred within the *Diplolepis* complex. We show here that *Diplolepis* deposit their eggs with great precision. We speculate that the exact location and number of eggs laid at a site influences the structure of the resulting gall.

Galls of each of the first five species (Figs. 1A–E) arise from eggs deposited only a few mm apart inside terminal or lateral buds (Fig. 5), whereas galls of *D. fusiformans* are induced from the surface of new stem tissues. *D. polita* and *D. bicolor* lay their eggs on immature leaflets (Fig. 5), and yet these closely related species choose tissues at slightly different stages of development. *D. polita* lays clusters of eggs with the result that each gall is dispersed over the adaxial surface of the leaf (Fig. 1A) whereas *D. bicolor* lays on more immature leaflets resulting in spherical clusters of galls (Fig. 1B) that incorporate the entire leaf. Galls of *D. polita* are smaller and have thinner walls (Fig. 2A) than those of *D. bicolor* (Fig. 2B). Galls of *D. polita* have weak spines and abscise by mid-August, whereas galls of *D. bicolor* have thicker walls and stout thorn-like spines and often remain on their hosts over the winter (Fig. 2B). The spines might deter small mammals. *D. nodulosa, D. triforma* and *D. spinosa* all insert their eggs into the base of the conical meristem (Fig. 5). The single egg of *D. nodulosa* results in a single-chambered gall (Figs. 1C and 2C). The clusters of *D. triforma* and *D. spinosa* eggs result in multiple-chambered galls (Figs. 1D, 2D and 1D and 2E). Both *D. nodulosa* and *D. triforma* deposit their eggs in the centre of procambial strands of the stem (Figs. 3C and D) and their galls end up in the pith. In contrast, *D. spinosa* lays its eggs so that they contact the procambium of the vascular cylinder or a leaf trace (Fig. 3E) or just touch the outside margin of the stem procambium, but never penetrate it as do *D. nodulosa* and *D. triforma*. As a result, galls of *D. spinosa* form in cortex tissues surrounding the procambial column. Larvae of *D. triforma* are much closer to the surface of the gall (Fig. 2D) than the larvae of *D. spinosa* (Fig. 2E). *D. fusiformans* has a different strategy: it oviposits later in the season (Fig. 4) on the surface of current year stems. Its larvae sink into the narrow stems and cause a slight swelling (Fig. 1F). The chambers occur in rows and each chamber is close to the surface (Fig. 2F).

*D. polita* and *D. bicolor*, two closely related species, lay their eggs in buds at the same stage of development, but with *D. polita* placing its eggs on slightly more mature leaflets (Fig. 3A) than *D. bicolor* (Fig. 3B). Further, both species insert their ovipositors between the folded leaves to deposit eggs into a single epidermal cell without damaging nearby cells. Avoiding leaflet damage likely ensures that the host leaves continue to function and serve as physiological sinks while the galls mature. Eggs of *D. polita* and *D. bicolor* are protected while the leaflets remain unexpanded, whereas eggs laid on unfolded, maturing leaves might be easily dislodged. This is avoided by quickly sinking into small depressions that develop below their point of attachment, as has been reported for other cynipids (Rohfritsch, 1992). In contrast,

*D. nodulosa*, *D. triforma* and *D. spinosa* damage leaflets while ovipositing. Damaging bud tissues often prevents the development of leaves and stem above the gall and redirects assimilates into the developing galls instead. Eggs of *D. nodulosa*, *D. triforma*, and *D. spinosa* are firmly attached as they are partially embedded in stem tissues. *D. fusiformans* are precise in attaching their eggs to individual epidermal cells (Fig. 3F). These galls likely interrupt the flow of assimilates as the stems above the galls often stop growing (Fig. 1F). It is not known how *D. fusiformans* avoids having its eggs dislodged from exposed surfaces.

The precision of ovipositing *Diplolepis* wasps when depositing their eggs supports the 'egg hypothesis' of Zeh, Zeh, and Smith (1989). These authors proposed that the egg stage of insects, combined with the ability to oviposit into sites difficult to reach, has played an important role in insect diversification. *Diplolepis* not only manage to maneuver their ovipositors around folded leaves within rose buds and deposit eggs on individual epidermal cells or within a small cavity (Figs. 3A–F), but the eggs themselves, or secretions deposited at the oviposition site, play a role in gall initiation. The deposits made by females at the point of attachment (see Figs. 3A and C) need to be investigated as possible sources of gall-inducing substances.

In Ontario, *D. polita* and *D. triforma* are always found on *R. acicularis* whereas the other four species are always found on *R. blanda*. However, in southern Alberta and British Columbia, *D. spinosa* and *D. bicolor* gall *R. woodsii*. Further, *D. spinosa* in eastern Canada also galls the introduced *R. rugosa* (Bagatto & Shorthouse, 1994). Galls of identical anatomy on different species of rose (Shorthouse, 1993; Shorthouse, unpublished observations) confirm that the host plants do not influence gall anatomy, and thus galls can be considered an extended phenotype (*sensu* Dawkins, 1982) of the galler.

The greatest cause of cynipid mortality is attack by parasitoids and inquilines which reach the inducer larvae by penetrating the gall with a drilling ovipositor (Askew, 1984; Washburn & Cornell, 1979; Shorthouse, 1998). Parasitoids commonly inflict mortalities of 40–100% on gall wasps (Shorthouse, 1998; Stone et al., 2002; Stone & Schönrogge, 2003), thus selection should favour any modifications of gall morphology that enhances galler survival. Heritable gall traits reducing gall-inducer mortality inflicted by natural enemies should spread by natural selection. Defence is the commonest function attributed to gall traits.

Authors studying the radiation of other gallers have proposed several hypotheses to explain the adaptive significance of varying gall structures (Price et al., 1987). One of these, the 'Enemy Impact Hypothesis', predicts that communities of one trophic level are organized by the trophic level above it and that gall structures have been selected to reduce mortality imposed by natural enemies on the gall inducer. Cynipid gallers are often attacked by greater numbers of parasitoid species than free-feeding forms (Hawkins, 1994) and consequently there should be strong selection for enhanced protection. It is argued that cynipid gallers avoided attack by inducing galls of differing structural complexity in different locations (Stone & Cook, 1998; Stone & Schönrogge, 2003; Stone et al., 2002).

Gall hardness and large gall diameter in cynipid galls impede attack by certain species of parasitoids (Washburn & Cornell, 1979). However, the relationship between gall morphology and protection from natural enemies remains unclear in many systems. For example, Waring and Price (1989) tested the Enemy Hypothesis using cecidomyiid gallers on creosote bush, and predicted that the species with thick-walled galls would exclude parasitoids with shorter ovipositors, and therefore should have lower levels of parasitism. Surprisingly, they found no significant differences in the number of associated parasitoids or percent parasitism in galls with different sizes, shapes, surface features or location on the plant. The authors concluded that enemy impact has not influenced gall diversity, and that variation may have arisen instead through selectively neutral mechanisms. However, it remains possible that traits once effective in defence, have now been circumvented by parasitoid coevolution (e.g. changing period of oviposition or evolution of longer ovipositors).

Galls of *Diplolepis*, like those of most cynipids (Stone et al., 2002), are inhabited by rich assemblages of parasitoids and inquilines which are important sources of mortality for *Diplolepis*, and are key members of the associated component communities (Fig. 6). More than 90% of *D. bicolor* and *D. polita* are commonly killed by inquilines or parasitoids. However, there is no evidence that the thick walls of galls of *D. bicolor* (Fig. 2B) provide better protection from parasitoids and inquilines compared to the thin-walled galls of *D. polita* (Fig. 2A). Thick-walled galls, such as those of *D. spinosa* (Fig. 2E), do not appear to protect the inducers any better from inquilines and parasitoids than the thinner-walled galls of *D. triforma* (Fig. 2D). Both *D. triforma* and *D. spinosa* have high mortality as a result of parasitoids ovipositing

soon after gall initiation (Wiebes-Rijks & Shorthouse, 1992; Bagatto & Shorthouse, 1994). Most parasitoids are koinobionts, allowing the immature inducers and inquilines to continue feeding and growing; they are killed and consumed later in the season. The relative proportion and abundance of the parasitoids associated with *Diplolepis* galls appear to be species-specific and similar throughout the range of the inducers in the province of Ontario. Some parasitoids such as *Eurytoma* sp. appear to be co-evolving with *Diplolepis* galls.

Beyond the basic protective envelope provided by the new cells of plant galls, it is possible that the sizes and shapes of galls we see today have little adaptive function. Structural variation seems to be controlled by the genotype of the gall inducers. The parasitoids seem to be tracking gall populations, not regulating them. As a consequence, *Diplolepis* appear well able to tolerate the high mortality caused by parasitoids and inquilines, as there are usually sufficient numbers of inducers at most sites by spring to re-establish gall populations. Thus, although differential mortality of gall inhabitants may have been a factor in the past, it is difficult to conclude that natural enemies are the driving force responsible for the diversity of galls seen today.

*Diplolepis* wasps are largely or entirely asexual reproducers (Shorthouse, 1993). A series of mutations that changed oviposition site selection and gall induction could easily occur within a lineage, and become fixed without need for allopatry, since there is no loss of novel ovipositional traits through recombination. Speciation may thus have occurred many times in the past, generating clearly delineated gall morphologies between species of *Diplolepis*. In the absence of selection on oviposition sites and gall morphology, speciation may have occurred by genetic drift as suggested by Waring and Price (1989) for speciation among cecidomyiid gallers.

In his overview on the adaptive radiation of gall-inducing insects, Price (2005, this issue) argues that understanding adaptive radiation requires knowledge of the insect's behaviour in relation to their hosts, and their ovipositional preference for certain plant conditions and modules. He concluded that the radiation of sawfly, cynipid, aphid and cecidomyiid gallers is in a dynamic evolutionary state and that a key factor in their evolution has been ovipositional accidents on host plants that are geographically widespread and rich in species. Price argues that for galling sawflies, radiation consisted of a series of ovipositional mistakes where eggs were laid in new localities, coupled with host shifts and variation in gall-inducing

factors. We suggest that leaf buds and shoot tips of roses are examples of Price's adaptive zones, and slight variation in the sites where eggs were deposited in the past, possibly as a result of oviposition mistakes, were important events in the radiation of *Diplolepis*.

We conclude here that the observed variation in *Diplolepis* gall morphology may be simply driven by variation in the selection of oviposition sites. It is also possible that some variation in the actual gall-inducing mechanism may play a role in this process. Further, our histological study of *Diplolepis* oviposition sites again demonstrates the benefit of using such techniques to investigate the biology of cynipid gallers (Shorthouse & Leggo, 2002). Applying these botanical techniques to other genera of cynipids, as suggested by Stone and Cook (1998), will likely reveal even broader evolutionary patterns.

## Acknowledgements

## References

Askew, R. R. (1984). The biology of gall wasps. In T. N. Ananthakrishnan (Ed.), *Biology of gall insects* (pp. 223–271). New Delhi: Oxford & IBH.

Bagatto, G., & Shorthouse, J. D. (1994). Mineral nutrition of galls induced by *Diplolepis spinosa* (Hymenoptera: Cynipidae) on wild and domestic roses in central Canada. In M. A. J. Williams (Ed.), *Plant galls: organisms, interactions, populations* (pp. 405–428). Oxford: Clarendon Press.

Brooks, S. E., & 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.

Cook, J. M., Stone, G. N., & Rowe, A. (1998). Patterns in the evolution of gall structure and life cycles in oak gall wasps (Hymenoptera: Cynipidae). In G. Csóka, W. J. Mattson, G. N. Stone, & P. W. Price (Eds.), *The biology of gall-inducing arthropods* (pp. 261–279). St. Paul, MN: Technical Report NC-199, US Department of Agriculture Forest Service.

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

Dawkins, R. (1982). *The extended phenotype: The gene as a unit of selection*. Oxford: Oxford University Press.

Hawkins, B. A. (1994). *Pattern and process in host–parasitoid interactions*. Cambridge: Cambridge University Press.

Meyer, J. (1987). *Plant galls and gall inducers*. Berlin: Gebrüder Borntraeger.

Nyman, T., Widmer, A., & Roininen, H. (2000). Evolution of gall morphology and host–plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). *Evolution, 54*, 526–533.

O'Brien, T. P., & McCully, M. E. (1981). *The study of plant structure: Principles and selected methods*. Melbourne, Australia: Termacarphi Pty.

Plantard, O., Shorthouse, J. D., & Rasplus, J. Y. (1998). Molecular phylogeny of the genus *Diplolepis* (Hymenoptera: Cynipidae). In G. Csóka, W. J. Mattson, G. N. Stone, & P. W. Price (Eds.), *The biology of gall-inducing arthropods* (pp. 247–260). St.Paul, MN: Technical report NC-199, US Department of Agriculture Forest Service.

Price, P. W. (2005). Adaptive radiation of gall-inducing insects. *Basic and Applied Ecology, 6*, 413–421.

Price, P. W., & Clancy, K. L. (1986). Interactions among three trophic levels: Gall size and parasitoid attack. *Ecology, 67*, 1593–1600.

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

Price, P. W., Roininen, H., & Zinovjev, A. (1998). Adaptive radiation of gall-inducing sawflies in relation to architecture and geographic range of willow host plants. In G. Csóka, W. J. Mattson, G. N. Stone, & P. W. Price (Eds.), *The biology of gall-inducing arthropods* (pp. 196–203). St. Paul, MN: US Department of Agriculture Forest Service, Technical Report NC-199.

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

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 G. Csóka, W. J. Mattson, G. N. Stone, & P. W. Price (Eds.), *The biology of gall-inducing arthropods* (pp. 61–81). St .Paul, MN: US Department of Agriculture Forest Service. Technical Report NC-199.

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

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

Stern, D. L. (1995). Phylogenetic evidence that aphids, rather than plants, determine gall morphology. *Proceedings of the Royal Society of London, B, 260*, 85–89.

Stone, G. N., & 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, B, 265*, 979–988.

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

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

Waring, G. L., & Price, P. W. (1989). Parasitoid pressure and the radiation of a gallforming group (Cecidomyiidae: *Asphondylia* spp.) on creosote bush (*Larrea tridentate*). *Oecologia, 79*, 293–299.

Washburn, J. O., & Cornell, H. V. (1979). Chalcid parasitoid attack on a gallwasp population (*Acraspis hirta* (Hymenoptera: Cynipidae)) on *Quercus prinus* (Fagaceae). *Canadian Entomologist, 111*, 391–400.

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

Williams, M. A. J. (1994). *Plant galls: organisms, interactions, populations. Systematics Association Special Volume 49*. Oxford: Clarendon Press.

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