The Gall of Some Hitchhikers

The life history strategies of three cynipid gall wasps that migrated to the Americas on garden roses

By Joseph D. Shorthouse

Several hundred years ago, cuttings of European roses were often part of the cargo in ocean vessels and the covered wagons of pioneer settlers who had migrated to the United States and Canada. The roses were brought as a reminder of the pioneer's homeland. Many family histories include stories of the dog rose (*Rosa canina*) and the sweet briar, or eglantine, rose (*Rosa rubiginosa*, synonymous with *Rosa eglanteria*) growing proudly in their homestead gardens. If the homesteaders abandoned their property, however, the roses not only survived, but they often spread well beyond the gardens after birds and mammals ate the hips and deposited the seeds (achenes) in their droppings. In other cases, these two species of roses were introduced by the horticultural industry and botanic gardens, and some subsequently escaped cultivation. Likewise, dog rose was introduced by European colonizers into Australia, New Zealand, and South America, where it also escaped cultivation, naturalized, and now grows wild.

Both the dog rose and the sweet briar rose are structurally different from the endemic wild species of Nearctic roses. They are vase-shaped, dense, suckering shrubs with arching canes that typically grow two to three meters tall. They have large, firm prickles and small five-petalled flowers that distinguish them from endemic and garden roses in North America. In addition, the sweet briar's glandular leaves sometimes release a sweetly fragrant perfume (suggestive of apples) after a rainstorm. Flowers are followed by abundant orange-red hips that ripen in the fall and usually persist well into winter. After growing in North America for two centuries, changes in the characteristics of these two species make them difficult to distinguish.

Hitching a ride on these two rose species that crossed the Atlantic were three highly specialized species of cynipid gall wasps—*Diplolepis rosae* induces galls on leaves and hips, *D. eglanteriae* on leaves, and *D. mayri* on leaves and stems. The two rose species and the three cynipid gall wasps now appear firmly established in North American ecosystems. Fortunately, none of the three cynipid species have moved onto endemic species of roses, nor have endemic species of *Diplolepis* become established on the introduced roses. However, their presence here, along with several centuries of study in their homeland, provides the opportunity to compare their attributes with those of Nearctic species of *Diplolepis* and helps to unravel the unique life history strategies of these insects.

Diplolepis are small, short-lived, inconspicuous wasps averaging three to seven millimeters (mm) in length, depending on the species. If it wasn't for their distinctive galls, the adults would be unknown to all except a few hymenopteran specialists. About forty-eight species are known to induce galls on wild roses in the north temperate regions of the world. Six species occur in Europe, thirty-one in North America and about eleven species in Eastern Asia. Several undescribed species are known to occur in Asia.

Each species of *Diplolepis* induces structurally unique galls that come in such a wide range of species-specific sizes and shapes that the wasps' presence can be inferred in the field without seeing the adults, especially the large galls of *D. rosae*.

Of the three hitchhikers, the best-known gall in both Europe and North America is *D. rosae*. Adults were described by Carolus Linnaeus (1707–1778) in 1758. From ancient times, the gall has been known as the mossy gall, bedeguar, or Robin's Pincushion. Pliny the Elder (23–79 CE), in his treatise, *Historia Naturalis XXV*, wrote that ashes of the gall when mixed with honey and applied to the scalp cured baldness. Physician and biologist Marcello Malpighi (1628–1694) in his book *De Gallis*, published in 1679, illustrated galls of *D. rosae* and was one of the first to understand that cynipids were responsible for the galls. Folklore in the mid-1800s suggested that galls of *D. rosae* be hung around the neck to cure people with whooping cough or placed under a pillow to cure insomnia.

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Bedeguar gall initiated by the mossy gall wasp Diplolepis rosae

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Galls of all three species of Diplolepis *on one shrub*

Cynipid gall wasps are among the most sophisticated of all herbivorous insects, because they control and redirect growth of their host plants for their own benefit. Stimuli from the eggs and larvae direct nearby host cells into a new developmental trajectory. This results in the development of galls—organ-like structures within which each legless larva will spend fifty weeks of the year encased and that have individual chambers that protect them from the elements (*see "Confessions of a Gall Hunter" by Ron Russo,* Natural History*, December 2009/January 2010*).

Soon after the galls start growing, the walls of their chambers become lined with enlarged nutritive cells that provide the larvae with nutrient-packed food throughout their immature stages. Nutritive cells bulge into the chambers like grapes throughout gall growth. When the cells are punctured by the larvae and their contents consumed, other nearby cells are turned into nutritive cells to replace the ones consumed. These cells are unique to cynipid galls—there are no similar cells elsewhere in rose shrubs; they are a type of cell derived solely under insect control and not the plant.

Adult rose cynipids require host plant tissues to be in a specific stage of development for oviposition and gall induction to occur. Depending on the species, some galls are induced among the unfolding tissues of the first leaf buds of spring, whereas others induce galls later in the season on new leaves and stem tissues. Galls are initiated by stimuli applied on immature, meristematic tissues in different periods of the summer months and on tissues at slightly different stages of development.

The life cycles of all species of *Diplolepis* are identical. They have one generation per year. Most species have life cycles adapted to surviving cold winter temperatures, with adult wasps and galls developing rapidly from mid-spring to early fall. Likewise, most of their host roses are north temperate, having evolved to survive in habitats with four distinct seasons. Rapid development of leaf and flower buds in the spring is one of many attributes of roses that have made them an ideal platform for the radiation of the *Diplolepis* complex.

A new generation of gall wasps begins in the spring, after overwintering in the prepupal stage. As the temperature increases, they turn into pupae, which about a week later change into adults. Adults chew an exit tunnel from their chamber to the outside, this being the only time adults use their mandibles as they do not eat. Once on the gall surface, they clean themselves of gall dust, and, if the weather conditions are suitable, immediately begin searching for suitable oviposition sites. Emergence of each species is synchronized with the presence of host tissues at the required stage of development. Adults live for only three to five days, so quickly finding oviposition sites is crucial.

Females don't waste time finding the opposite sex and mating, since females comprise about 96 percent of the population. They not only exhibit parthenogenesis, whereby females produce offspring without fertilization, they undergo a process called thelytokous parthenogenesis, whereby virgin females give rise to females. Another form of reproduction called deuterotoky sometimes occurs when unfertilized females give rise to both males and females.

If females do not find oviposition sites on their natal plants, they launch themselves into the air. This stage is likely the most perilous of their lives because they are not strong fliers. If they fly into rising air currents on a windy day, they will be dispersed many meters and possibly ki-

lometers away. How they manage directional flight or descend onto a suitable rose bush is unknown; however, the presence of galls on widely spaced bushes tells us that at least some are successful.

After landing on and identifying the correct host leaf bud or young stem, they walk over the surface tapping with their antennae. When a suitable spot is located, they take an inverted position, spread apart a protective plowshare-shaped structure called a hypopygium at the tip of their abdomen, and insert the ovipositor. They remain motionless in this position for up to two hours, moving the tip from cell to cell inside the bud, depositing up to sixty eggs, depending on the species.

Galls begin to grow soon after the freshly hatched larvae start feeding. Although the external appearance of rose galls varies depending on the species of inducer, the inner regions of maturing galls are similar with well-defined cell types in concentric layers around the larval chamber. A common pattern of host cell responses is triggered once the inducers make contact and start feeding on carefully chosen plant cells.

In contrast to other herbivorous insects that move around their host plants to feed, *Diplolepis* larvae compel nutrients to come to them through a complex network of vascular bundles that connects to the vascular bundles of the host organ. Each gall becomes a physiological sink; that is, assimilates produced some distance from the galls move via the vascular bundles to tissues of the gall. As the assimilates enter the galls, their chemical composition is changed as they pass through the various layers of gall tissues lining the larval chambers.

Rose galls, like those of all cynipid galls, undergo three phases of development—initiation, growth, and maturation. The growth phase is a period of rapid proliferation of gall cells. Larvae feed only minimally during the growth phase, when the innermost nutritive cells accumulate lipids, sugars, amino acids, proteins, and other organic compounds, while cells farthest from the larval chamber accumulate starch. This process results in nutrient gradients extending across the gall tissues.

The maturation phase begins with the differentiation of a layer of thick-walled sclerenchyma cells midway between the larval chamber and the outside. Sclerenchyma cells provide stiffness and strength to plant structures. Larvae do most of their feeding during the maturation phase. Nutrients attained during the maturation phase sustain the wasps through development of their prepupal, pupal, and adult stages, along with their dispersal and oviposition. As the wasps mature, all nutritive cells are consumed down to the sclerenchyma. Besides providing structural strength for the gall, sclerenchyma provides some protection from small mammals and birds attempting to consume the inducer larvae.

The larvae undergo five instars and by late summer, or early fall, they stop feeding and turn into a prepupa, on which the developing eyes of the adult can be seen within the second segment. The prepupae develop glycerol cryoprotectants, which keeps their blood from freezing, down to about -38 C. The smooth surface of sclerenchyma also

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Top: Feral dog rose galled by Diplolepis eglanteriae *growing at the edge of a forest above the Niagara River gorge in Ontario, Canada. Seeds from the shrub came from a nearby botanical garden; Bottom left: Galls of* D. eglanteriae*; Bottom right: Dissected gall of* D. eglanteriae *showing inducer larva in its chamber*

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Galls of Diplolepis mayri

prevents the formation of ice crystals, which would trigger fatal flash-freezing of the prepupae.

The presence of plump, defenseless larvae inside galls does not go unnoticed by tiny insect-eating microhymenopterans. From the time the galls are barely noticeable, they are attacked by numerous small wasps that gain access to the larvae when the galls are immature and their ovipositors can puncture and reach through the walls of the gall. About twelve species of entomophagous insects referred to as parasitoids commonly inhabit galls of *D. rosae* in Europe, and some of these came to North America inside the introduced galls. Most feed as parasitoids rather than as parasites or predators. Parasitoids feed as a parasite on or in a living *Diplolepis* host for most of the summer and then kill and consume it, whereas a true parasite does not necessarily kill its host. It may be that other parasitoids have moved from galls of endemic species of *Diplolepis*, onto the introduced galls, but this is yet to be determined.

The story of *D. rosae* galls in Europe gets even more complicated by the presence of other cynipid wasps that live inside the galls. These wasps of the genus *Periclistus* are known as inquilines, or guests, and must live inside the galls of the *Diplolepis*. However, they are not good house guests, since the female first kills the *Diplolepis* with her ovipositor, then lays her eggs on the walls of the chambers. The gall temporarily stops growing, but when the *Periclistus* hatch, their larvae redesign structures of the gall by producing their own chambers. Although common in the European populations of *D. rosae* galls, they have not yet been found in North America. The interactions of inhabitants of galls, such as *D. rosae*, form intricate food webs and gall-specific component communities.

The common appearance of parasitoids in the introduced galls of all three species, even when the galls are sparsely distributed, illustrates that both inducers and equally small parasitoids have great powers of vagility. The inducers must be able to locate their host plants with tissues at the right stage for oviposition, whereas parasitoids must locate the same plants and then immature galls. In all cases, there is a narrow window of opportunity for oviposition. How they manage to do this is an important part of the life history strategy of gall-inhabiting insects that remains unknown.

Each of the European species of *Diplolepis* have slightly different phenologies and structurally distinct galls. *D. rosae* exit their galls in the early spring and deposit eggs on the surface of immature leaflets or the base of developing flowers that are just opening and starting to grow. Eggs are laid in a cluster, but as the galls grow, each larva becomes separated in its own chamber, resulting in multichambered galls. Eggs of *D. eglanteriae* are laid later in the spring on leaflets that are already forced open from leaf

buds and are sufficiently far apart that each results in a separate, spherical single-chambered gall. Eggs of *D. mayri* are laid in late spring on newly formed stem tissues, and sometimes on leaf tissues. As with *D. rosae*, eggs are laid in a cluster but each larvae appears in its own chamber in a multi-chambered gall.

Galls of *D. rosae* are the most distinctive of all *Diplolepis* galls, with a surface covered with masses of branched and resinous filaments, or fronds, which continue to lengthen throughout gall growth. The fronds give the impression of a red or green ball of moss that enlarges over the summer, becoming brown in the fall. Mature galls range in size from a large pea with one chamber to multi-chambered masses the size of an apple with up to sixty chambers and a diameter of ten centimeters. Galls remain on the host plant throughout the winter. Galls are found from St. John's, Newfoundland, in eastern Canada, to Vancouver Island in western Canada and most of the northern states in the United States.

One sweet briar rose brought from Europe to the Annapolis Royal Botanical Gardens in Nova Scotia in the 1950s must have been galled by *D. rosae*. The shrub became a dense thicket and large numbers of galls have persisted to this day. Unfortunately, both the rose and the galls of *D. rosae* are now common along roadsides and in nearby pastures, and it is likely both came from the Annapolis Royal gardens. Other large populations of *D. rosae* galls occur throughout the western states and in southern British Columbia. Here, roses and galls, both of which are abundant in pastures, were introduced by early farmers. In some cases, the roses are so dense they interfere with cattle ranching.

Open gall of Diplolepis mayri *shows two full-grown parasitoids in the upper portion, a parasitoid to the left in the lower portion, along with two full-grown larvae of the inducer to the right.*

Galls of *D. eglanteriae* are not as common in Europe and North America as those of *D. rosae*. Galls of this species are single-chambered, spherical, about five to seven mm in diameter, smooth surfaced, and usually found singly on the upper surface of leaflets of dog rose. They are often on the rachis (main axis of compound rose leaves). Sometimes, two galls coalesce during growth and their outer walls become conjoined. Galls are green when immature, turning tan or grey as they mature. Walls of the galls become thin in comparison to galls of other *Diplolepis*. Mature galls abscise along with the host leaflet in the fall and overwinter on the ground.

Galls of *D. eglanteriae* have been found from southern Ontario to Prince Edward Island in eastern Canada. I first recorded them in Ontario in 1994 on feral dog rose along a roadway near the top of the Niagara River gorge (downstream from the City of Niagara Falls, Ontario). I later learned that the Niagara School of Horticulture, across the road from the galled shrubs, had brought one specimen of dog rose from the Netherlands in 1951 for their botanic garden. Galls must have been on the plant or in the container in which it was shipped. This original shrub has since been removed, but up until 2018 it hosted galls of *D. eglanteriae*. Adult wasps would have flown from the original rose to the feral roses that had previously been dispersed by birds eating the hips on the botanic garden rose.

Galls of *D. mayri* occur on the leaves, rachis, stipules, and stems. Galls are bulbous, multi-chambered and vary in size and shape depending on where the galls are induced and the number of larval chambers. Their surfaces are either smooth or clothed with short prickles. Walls of the galls are hard and the chambers are deep within gall tissues. Mature galls of *D. mayri* overwinter on the host plant and hang like grapes. Galls are found on road– and ditch-side feral dog roses in agricultural regions of southern Ontario.

An extraordinary observation was made in 1998 about twenty kilometers south of London, Ontario, where in the center of an abandoned pasture, I found a large, single dog rose with galls of all three species of European *Diplolepis*. Perhaps this was the result of multiple introduced roses each with a different species of *Diplolepis* at nearby farms or all three species arrived on one introduced plant.

It will be interesting to see if these three species of cynipids will in time switch hosts and induce galls on endemic species of roses, or if endemic species of *Diplolepis* successfully gall either the dog or sweet briar rose. Because of the frequency by which the horticultural industry hybridizes roses and attaches shoots from one cultivar or species onto another, it is likely a matter of time before the wasps expand their host range.

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