

## Modification of Flowerheads of Diffuse Knapweed by the Gall-inducers *Urophora affinis* and *Urophora quadrifasciata* (Diptera: Tephritidae)

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### Abstract

*Urophora affinis* and *Urophora quadrifasciata* are gall insects used in the biological control of *Centaurea diffusa* and *Centaurea maculosa* in Western Canada. The larvae of both species induce galls within unopened flowerheads with a resulting loss of seed production and host vigour. *U. affinis* induces a complex gall from tissues of the receptacle and ovary, whereas *U. quadrifasciata* induces a simple gall from tissues of the ovary wall. Gall induction by *U. affinis* in immature flowerheads and the appearance of thick layers of nutritive cells explains why this species does more host damage than *U. quadrifasciata* which attacks older flowerheads and induces few nutritive cells in its gall.

### Introduction

Diffuse and spotted knapweed (*Centaurea diffusa* Lam. and *C. maculosa* Lam.; Asteraceae) are herbaceous plants of European origin that have become major weeds of dry rangeland in British Columbia, Canada, where they displace native herbaceous vegetation and reduce productivity to the detriment of both ranching and wildlife (Harris and Cranston 1979). Two gall-inducing flies, *Urophora affinis* Frauenfeld and *U. quadrifasciata* (Meigen) (Diptera: Tephritidae) were introduced into British Columbia in 1970 and 1971 for biological control of these weeds. Both insects induce galls within the flowerheads and much has been written on the ecology of the flies and galls since their release (Berube 1980, Harris 1980a,b, Myers and Harris 1980). However, little is known about how these insects form their galls and structurally damage their hosts. The purpose of this paper is to describe the developmental morphology of the galls induced by *U. affinis* and *U. quadrifasciata* on *C. diffusa* and illustrate how they damage their hosts.

### Biology of *Urophora affinis* and *U. quadrifasciata*

*U. affinis* and *U. quadrifasciata*, like all gall-inducing tephritids, are highly host- and organ-specific. They are amongst 15 of the 60 Palearctic species known to be gall-inducers, nearly all of which attack plants of the family Compositae (Freidberg 1984). Most *Urophora* spp. attack flowerheads on plants of the tribe Cynareae; an exception being *U. cardui* (L.) which induces galls on stems (Lalonde and Shorthouse 1984).

The life cycles of *U. affinis* and *U. quadrifasciata* are similar to other gall-inducing tephritids (Freidberg 1984). It is not difficult to synchronize ovipositing adults of both species with susceptible flowerheads in the laboratory and galls at all stages of development can be obtained. Galls from laboratory cultures are identical to those from the field.

*U. affinis*, a native of western Europe, was first released in Canada in 1970 with stock from France, and was later supplemented with stock from the U.S.S.R. Adults appear in British Columbia in early July as the flowerheads of diffuse knapweed start to form (Berube 1980). Adults in both the field and laboratory prefer ovipositing in flowerheads 5.0 to 7.5 mm long, which is about half the length reached before flowering. Eggs are laid in groups of 1 to 5 within the closed flowerheads. The same flowerhead may be attacked again by the same or

another female. Eggs hatch in 3 - 4 d both in the field and laboratory, and larvae tunnel to the receptacle where galls are induced. Only one larva is found per gall. Larvae mature in about 28 d, and pupate by the 33<sup>rd</sup> day. Galls of *U. affinis* have thick, hard walls. Between 10 to 25% of the population in British Columbia emerges in August for a second generation; all others overwinter as mature larvae in their galls.

*U. quadrifasciata* is common from western Europe to North Africa and was released in Canada in 1972 in the same sites already inhabited by *U. affinis*. The life cycle is similar to that of *U. affinis* except that in British Columbia it has a complete second generation. The peak emergence in British Columbia occurs in the 3<sup>rd</sup> week of July and the flies lay into slightly more mature flowerheads than does *U. affinis*. *U. quadrifasciata* prefers flowerheads that are 7.0 to 9.5 mm long. Eggs are laid singly among developing florets, and hatch in 3 to 4 d. Larvae chew down to the ovary, where they induce a gall. Only one larva is found per galled ovary, but several galls are commonly found per flowerhead. They also can develop in the same flowerheads as *U. affinis*. Feeding is finished about the 24<sup>th</sup> day and pupation occurs soon after. Offspring of the second generation overwinter inside the gall, and the adults emerge the following July. Mature galls of *U. quadrifasciata* are thin-walled and do not resemble the galls of other tephritids; the fly previously has been referred to as a seed feeder (Zwölfer 1975).

### Materials and Methods

*C. diffusa* was cultured in a greenhouse at the Agriculture Canada Research Station in Regina, Saskatchewan. Once the plants had developed flowerheads susceptible to oviposition by either *U. affinis* or *U. quadrifasciata*, individual plants were placed in cages. The cages were placed under a bank of fluorescent and incandescent lights in a quarantine room with a 16L:8D photoperiod and temperatures that varied along a daily cycle from 20 to 28°C.

Six gravid females of either *U. affinis* or *U. quadrifasciata* were placed on the caged plants and flowerheads tagged in which ovipositions were observed. The flies were removed after 24 h. Flowerheads with eggs were considered one-day-old at the end of 24 h, two-days-old at the end of 48 h, etc. A least four flowerheads were harvested every three days from day one to the time when adults emerged, for both species.

All harvested flowerheads were fixed in formalin-acetic acid-alcohol (FAA). Immature flowerheads were fixed whole, whereas a slice was removed from one side of larger flowerheads to facilitate infiltration of the fixative. Uninhabited flowerheads at all stages of development also were fixed. All fixed tissues were dehydrated in a tertiary butyl alcohol (TBA) series, infiltrated with paraffin (Jensen 1962) and sectioned on a rotary microtome at 8  $\mu$ . Sections were then affixed to slides with Haupt's adhesive and stained with Safranin-Fast Green (Jensen 1962).

### Results

#### Galls of *Urophora affinis*

Flowerheads chosen by *U. affinis* for oviposition (Fig. 1), have immature florets that are tightly enclosed by unopened bracts. The immature ovules occupy less than one-quarter of the ovary volume. The petals of each floret are united at the base to form a tube within which is found the stigma, style and the large anthers, which are fused laterally. The immature ovules occupy less than one-quarter of the ovary volume and pollen grains are just beginning to form in the anthers.

Gravid females probe extensively the flowerheads before ovipositing, presumably to determine the size and stage of development of the florets and ovaries. They then insert their ovipositors between the inner and outer layers of bracts and deposit eggs singly or in groups of 2 to 5 on the developing florets (Fig. 2). The ovipositor often damages the florets (Fig. 2)

and in some cases the damage is so severe the floret is aborted. Many of the eggs are enveloped by growing tissues of the floral tube by the time they are ready to hatch.

Freshly hatched larvae chew into a floret and tunnel towards the ovary. They consume the ovule and then begin feeding on cells of the inner layer of the ovary wall (Fig. 3). They remain in the ovary for about four days, feeding on tissues of the ovary wall, and then about the 12<sup>th</sup> day they penetrate the base of the ovary and begin consuming adjacent tissues of the receptacle. While feeding in an inverted manner on cells of the receptacle, cells of the inner layer of the ovary wall in the lower half of the ovary begin to proliferate, such that by the 16<sup>th</sup> day, the larvae are surrounded by a thick layer of parenchymatous cells (Fig. 4). Proliferating parenchymatous cells of adjacent galls commonly coalesce. Cells of the receptacle adjacent to those eaten, also are stimulated by the larva and begin to proliferate; however, in contrast to the proliferating cells of the ovary wall, these cells develop dense cytoplasm and become nutritive cells.

By the 20<sup>th</sup> day, the larva feeds both on the proliferating parenchymatous cells of the ovary (Fig. 4) and on nutritive cells at the base of the gall. The zone of nutritive cells (Fig. 5) is thickest between the days 20 to 25. However, cells of the ovary wall do not proliferate as fast as those at the base, and as a result, the walls in the upper region of the gall become reduced in thickness. Also during this period a few layers of cells around the outside periphery of the gall begin to lignify (Fig. 4) as does a layer of cells within the patch of nutritive cells at the base of the gall (Fig. 5). As a result, the larva is completely encircled with sclerenchyma by the 24<sup>th</sup> day, except for the apex and a small circular patch at the base, where the sclerenchyma layer is thin or non-existent. As the gall matures, cells of the ovary wall are completely consumed and the nutritive cells at the base become the larva's sole source of food. Vascular bundles develop within the nutritive cells (Fig. 5) and join those of the receptacle.

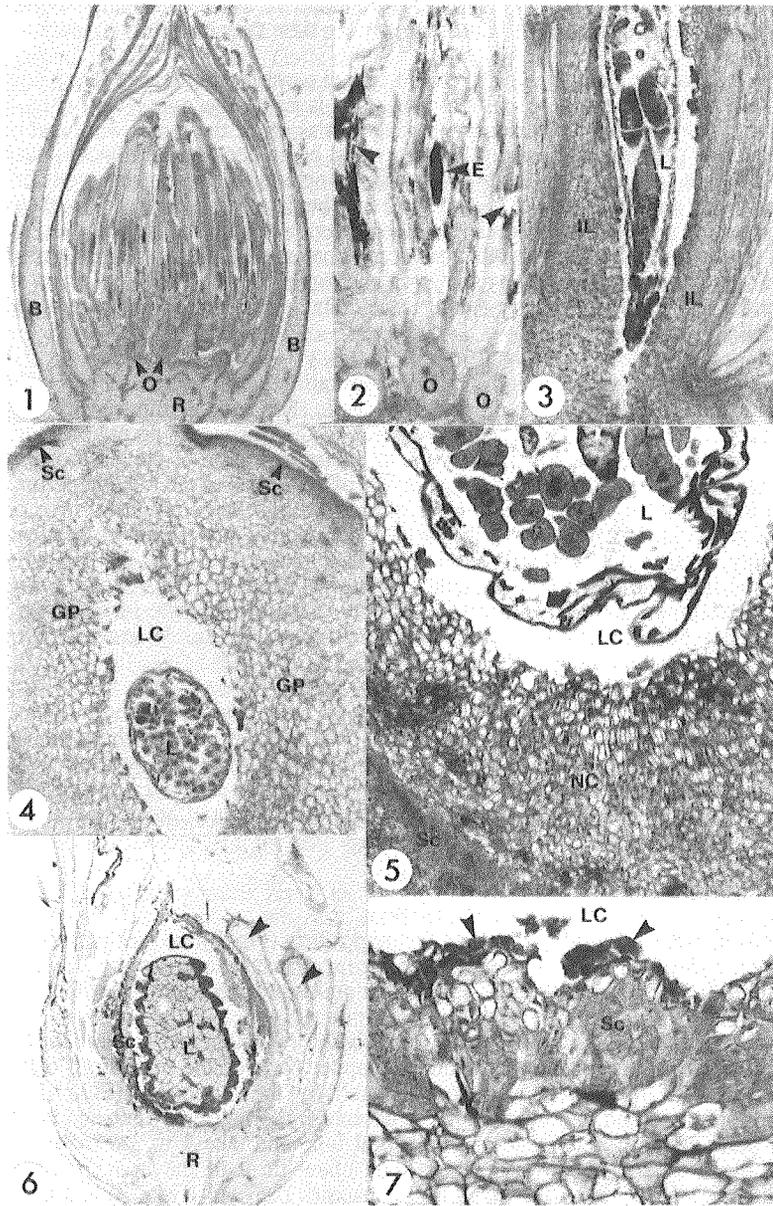
By the time the larva reaches its full size (Fig. 6), all parenchymatous and nutritive cells are consumed down to the sclerenchyma layer, leaving the larval chamber lined with sclerenchyma cells (Fig. 7). Remains of consumed parenchyma and nutritive cells also are found throughout the surface of the larval chamber (Figs. 6 and 7). As the mature larva prepares to pupate, it changes position such that the head is pointed upwards.

#### *Galls of Urophora quadrifasciata*

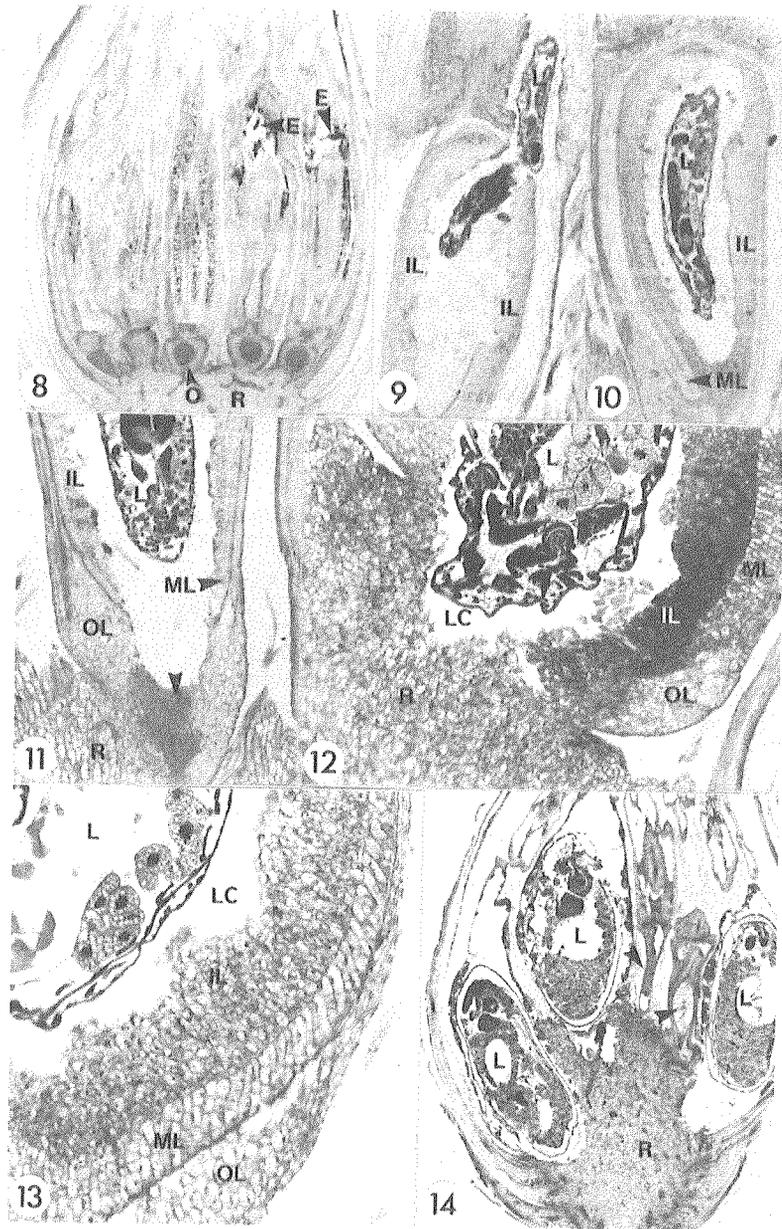
Flowerheads chosen by *U. quadrifasciata* for oviposition (Fig. 8) are more mature than those chosen by *U. affinis* (Fig. 1). The ovules also are more mature and occupy about one-half of the volume of the ovary (Fig. 8). Maturing pollen grains are present in the anthers (Fig. 8).

Females of *U. quadrifasciata* also probe extensively the unopened flowerheads with their ovipositors before laying eggs on or within the upper parts of the maturing florets (Fig. 8). The upper parts of the florets also are damaged by the ovipositor (Fig. 8). Attacked flowerheads are often laid into by the same or another fly. The freshly hatched larvae feed on the upper parts of the florets, penetrate the floral tube and take 1 to 3 d making their way down the floral tube to the ovary, where they chew through the upper surface (Fig. 9) between the 6<sup>th</sup> and 8<sup>th</sup> day. The larva first consumes the ovule (Fig. 9) and then feeds on cells of the ovary wall.

Once permanently inside the ovary (Fig. 10), the larvae begin feeding on cells of the inner layer of the ovary wall. (The ovary wall consists of three layers: the inner layer being the thickest; a middle layer 2 to 3 cells in thickness which are perpendicular to the ovary wall; and a thin outer layer of small cells which are orientated parallel to the ovary wall.) The larvae enlarge the larval chamber by consuming cells from the inner layer of the ovary wall. Once about half the inner wall has been consumed, usually by the 12<sup>th</sup> day, the larvae penetrate the base of the ovary and feed on adjacent cells of the receptacle. Receptacle cells next to the feeding site react to the presence of the larvae by becoming cytoplasmically dense (Fig. 11). They begin to proliferate and become a small zone of nutritive tissue. Proliferating cells of this zone often push the modified ovary above the surface of the receptacle. Few vascular bundles appear within these patches of proliferating cells.



Figures 1-7. Developmental morphology of galls of *Urophora affinis* Frauenfeld within the flowerheads of *Centaurea diffusa* Lam.: 1. Section of flowerhead 5 mm in length at a stage chosen by *U. affinis* for oviposition, x 18. 2. Section of flowerhead with egg of *U. affinis* 4 d after oviposition. Note the oviposition damage (arrows) x 28. 3. Section of ovary inhabited by larva 13 d after oviposition. The larva has consumed the ovule and part of the ovary wall and has just penetrated the receptacle, x 55. 4. Section of gall on the 20<sup>th</sup> day showing the larva surrounded by thick layers of proliferating parenchyma cells. Note the layer of sclerenchyma cells forming around the periphery of the gall, x 38. 5. Section of nutritive cells near the base of gall on the 24<sup>th</sup> day showing extensive zone of proliferating nutritive cells and a layer of sclerenchyma cells, x 61. 6. Section of entire flowerhead with mature gall and larva at the 28<sup>th</sup> day. Note that all nutritive cells have been consumed down to the layer of sclerenchyma and that sclerenchyma is absent at the base of the gall. Also note the two aborted ovaries to the right of the gall (arrows), x 9. 7. Section of region near the base of a mature gall showing that all nutritive cells have been consumed down to the sclerenchyma layer. Note the remains of consumed nutritive cells (arrows), x 137. B, bract; E, egg; GP, gall parenchyma; IL, inner layer of ovary wall; L, larva; LC, larval chamber; O, ovary; PC, parenchyma cells; R, receptacle; Sc, sclerenchyma.



Figures. 8-14. Developmental morphology of galls of *Urophora quadrifasciata* (Melgen) within the flowerheads of *Centaurea diffusa* Lam.: 8. Section of flowerhead 8 mm in length at a stage chosen by *U. quadrifasciata* for oviposition. Note the eggs and oviposition damage, x 17. 9. Section of ovary being penetrated by larva 6 d after oviposition, x 36. 10. Section of ovary with larva 10 d after oviposition. note that the ovule has been consumed and the larva has begun feeding on walls of the ovary, x 41. 11. Section of ovary with larva 12 d after oviposition. Most tissues of the inner layer of the ovary wall have been consumed. The larva also has penetrated the base of the ovary and has stimulated adjacent receptacle cells (arrow) into becoming a small zone of nutritive tissue, x 48. 12. Section of the base of an ovary with larva 16 d after oviposition feeding on proliferating cells of the receptacle. Note that cells of the three layers of the ovary wall at the base of the ovary also have begun to proliferate (arrow), x 67. 13. Section of galled ovary 20 d after oviposition. Proliferating cells of the inner layer of the ovary wall are now the larva's sole source of food, x 81. 14. Section of galled flowerhead 24 d after oviposition with three mature larvae ready to pupate. Note that the surface of the receptacle is irregular due to gall formation. Also note the two aborted ovaries (arrows) to the right, x 13. B, bract; E, egg; IL, inner layer of ovary wall; L, larva; LC, larval chamber; ML, middle layer of ovary wall; O, ovary; OL, outer layer of ovary wall; R, receptacle.

While the larvae are feeding on receptacle and nutritive tissues at the base, cells of the inner layer of the ovary wall continue to proliferate and those nearest the base become cytoplasmically dense (Fig. 12), taking on the characteristics of nutritive cells. In some cases the cytoplasmically dense cells of the inner layer extend three-quarters up the sides of the ovary wall. The larvae then move up and down the inner surface of the ovary consuming these cells until at least the 16<sup>th</sup> day, as evidenced by the presence of cellular frass. However, as the larvae mature around the 18<sup>th</sup> day, they consume cells faster than they are replaced and the inner layer of the wall begins to decrease in thickness. Feeding is complete around the 22<sup>nd</sup> day and the larva turns around so that its head is towards the gall apex. By day 21 to 29, all nutritive cells formed in the receptacle are eaten and no new cytoplasmically dense cells appear. The inner layer of the ovary wall has become substantially thinner and the remaining cells are again vacuolate (Fig. 13). By the time the larvae are ready to pupate, the entire inner layer about the ovary surface has been consumed leaving the mature larvae and pupae surrounded by a papery-thin layer of cells (Fig. 14). No sclerenchyma cells appear in galls of *U. quadrifasciata*. Disruption of the receptacle surface due to gall formation is most noticeable when more than one larva is found per flowerhead (Fig. 14) and is likely due to the depth to which the larvae had previously penetrated. All unattacked florets in such galls are aborted (Fig. 14).

### Discussion

Gall-inducing insects, with their ability to control the growth and development of plant tissues, constitute one of the most complex guilds of all phytophagous insects. Most of the advantages in the relationship go to the insect, which receives high-quality food and shelter, whereas the plant suffers loss of photosynthates and in some cases, structural damage. In all galls, there exists a specific nutritional relationship between the insect and plant which is usually established by the presence of specialized nutritive cells (Shorthouse 1986). These cells signify an active reaction by the plant to the insect's presence and they continue to proliferate as long as the insect is feeding (Rohfritsch and Shorthouse 1982).

It is of interest that the galls of *U. affinis* are more complex than those of *U. quadrifasciata* even though both species attack the same flowerheads within a few days of each other. Both species induce galls some distance from the site of oviposition; however, only *U. affinis* induces large amounts of nutritive cells and a layer of sclerenchyma, two cell types typical of complex insect galls (Rohfritsch and Shorthouse 1982). Both the nutritive and sclerenchyma cells found in the *U. affinis* galls are similar to those found in the galls of *U. cardui* (Lalonde and Shorthouse 1982).

The reason why *U. affinis* induces more structurally complex galls than *U. quadrifasciata* may be related to the stage of maturity of the host organ at the time of gall initiation. Immature flowerheads are likely drawing insufficient nutrients to sustain the larvae of *U. affinis* and therefore the nutrient flow to the flowerhead is increased. This is done by inducing nutritive cells and accompanying vascular bundles. In contrast, *U. quadrifasciata* attacks at a time when the plant is pulling sufficient nutrients to the flowerhead to sustain its larvae and there is no need to induce nutritive cells.

Why galls of *U. affinis* develop a layer of sclerenchyma and those of *U. quadrifasciata* do not is unknown. For many years the sclerenchyma layer in galls was thought to protect gall inducers from parasitic and predacious insects; however, many galls with sclerenchyma zones are heavily attacked by parasites. Its main role is likely one of support; however, it might also protect gall-inducers from being wetted by rain or melting snow. Perhaps its role in the gall of *U. affinis* is one of protection from birds or small mammals. Its absence in galls of *U. quadrifasciata* may be an indication that the gall-inducing ability of this species is less well-developed than that of *U. affinis*.

Although gall-inducing insects do not inflict as much damage as insects that consume foliage or entire flowerheads, they do sequester nutrients to the gall from other parts of the plant. Several authors have studied the role of insect galls as physiological sinks (Fourcroy and Braun 1967, Torii 1961); however, this aspect of gall biology has received little attention. Even so, the results of this study, along with those of Harris (1980b), clearly show that *U.*

*affinis* and *U. quadrifasciata* reduce seed production and the vigour of their host plants. Besides destroying inhabited florets and causing the abortion of those nearby (Figs. 6 and 14), Harris (1980b) found that both species attract nutrients not only from unaffected regions of attacked flowerheads, but from other parts of the plant as well. He found that each *U. affinis* reduced production by 2.4 seeds/flowerhead whereas each *U. quadrifasciata* reduced production by 1.9 seeds/flowerhead. Furthermore, entire plants infested with galls were lighter than uninfested plants. He also found that galls attract nutrients in proportion to their numbers per flowerhead and that the sink effect was powerful enough to overcome the effects of crowding. Thus the more larvae present in a flowerhead, the larger the sink and the more nutrients sequestered to it.

The results of this paper and the works of Harris show that gall-inducers vary in the amount of damage they cause their hosts. The extent of this damage can be indicated by examining the structures of galls. That is, the presence of thick layers of nutritive cells with the accompanying vascular bundles indicates that *U. affinis* is able to sequester more nutrients than is *U. quadrifasciata*. Even though galls of *U. quadrifasciata* are less of a sink than those of *U. affinis*, Meyers and Harris (1980) showed that when the two were found together, they destroyed more seed than when either was found alone. Furthermore, the value of *U. quadrifasciata* to biological control is the destruction of ovaries missed by *U. affinis*.

When rating gall insects as potential biological control agents, one would be wise to choose species that attack host organs when they are immature and have galls with a thick and long lasting nutritive layer. On the basis of the few species examined to date, the most valuable for biological control appear to be those with an aggregated attack early in the growth stage as they produce the strongest physiological sinks. One indication of the strength of the sink is when the larvae retain a constant weight regardless of the number present in the affected region.

Although gall inducers in the past have been rated low as biological control agents (Harris 1973), it is now clear that some species can play a role in certain biological control programs. The ability of gall insects to damage hosts was shown by *Phylloxera vitifoliae* (Fitch) (Hemiptera: Phylloxeridae), which devastated the European grape industry, and by *Procecidochares utilis* Stone (Diptera: Tephritidae) which was credited with the control of *Ageratina adenophora* (Sprengel) R. King & H. Robinson (= *Eupatorium adenophorum* Sprengel) (Asteraceae) in Hawaii, U.S.A. (Bess and Haramoto 1972). A major attraction of gall-inducers is their high degree of host-specificity, meaning that several gall insects from a weed can be introduced without threat to desirable plants. For this reason, approximately 25% of the agents released in Canada by the end of 1983 were gall insects (Harris 1984). Even though most gall insects will not control weeds on their own, their future role should continue as they feed in conjunction with other insects and collectively consume a substantial proportion of annual host production.

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