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Effectiveness of gall inducers in weed biological control[1]

P. HARRIS and J. D. SHORTHOUSE

Research Centre, Agriculture and Agri-Food Canada, PO Box 3000, Lethbridge, Alberta, Canada T1J 4B1; Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

Abstract:

Gall inducers are favoured as biocontrol agents of weeds because they tend to have a narrow host range. Six insect and one nematode gall inducer used in Canada are described in terms of their biology, gall morphology, gall physiology, and effectiveness in weed control. The species differ in plant organ attacked, requirement for moisture, whether the galls are induced by secretions or by severing xylem, and effectiveness, which in part relates to the ability of the gall to import nutrients. The most powerful galls divert assimilates from other sinks via a gall's vascular system joined to that of their host. One of our examples also has mechanisms to compensate for reduction of turgor during drought. Two of the gall inducers enhance their nutrient supply by severing xylem in a plant nutrient sink. One, in the short-term sink of a thistle capitulum, obtains about a quarter of its assimilates at the expense of other capitula. The other, in the long-term sink of a rosette root, approximately halves seed production. Hypotheses are presented to explain various aspects of gall development and function.

Introduction

Classical biocontrol of weeds involves establishing herbivores, usually insects, in new regions to reduce the density of an introduced weed. The technique was used over 125 years ago (Johnston and Tryon 1914) and to date nearly 400 species of insects, several pathogens, nematodes, and vertebrates have been released on over 100 weed species around the world (Julien 1992). On uncultivated land, biocontrol usually achieves the same weed reduction as a herbicide, but it is persistent and does so in a more environmentally friendly and cost-effective manner; cost:benefit ratios can exceed 1:50 (Marsden

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et al. 1980). To be successful, biocontrol agents must reduce the weed over much of its range by reducing survival, growth, or reproduction, all of which depend on the agent's fecundity, ecological range, susceptibility to parasitism, and the harm it does to the host.

Gall inducers are organisms that induce atypical plant growth. Most are host and organ specific, and live within a chamber in the gall where they feed on special tissues (Abrahamson and Weis 1987). Occasionally, the food of the gall inducer is unmodified tissue within the chamber, but usually it is proliferating parenchymatous cells or "nutritive cells" on the walls of the gall chamber (Meyer and Maresquelle 1983; Bronner 1992). These tissues are a metabolic sink for nutrients from adjacent plant tissues or other parts of the plant. It is this ability to bring nutrients to the organism that distinguishes gall inducers from non-galling organisms in terms of their effectiveness as weed biocontrol agents.

Only 2% of plant-feeding insects are gall inducers (Dreger-Jauffret and Shorthouse 1992), but 17 out of the 64 weed biocontrol agents released in Canada up to 1993 are gall inducers. This is because they tend to have a narrow host range and hence threaten few non-target plants. Some gall inducers have been highly successful in controlling the target weed, but others have either not established or had little impact. Failures are expensive, because the cost of demonstrating that on release an organism will not damage desirable plants in the new region is about two scientist-years (Harris 1979), currently about $700,000.

Our paper summarizes and reviews the literature on seven gall inducers used for weed biocontrol in Canada. It provides a compendium for those interested in the organisms and is arranged with sections under each on biology, development and structure of the gall, gall physiology, and biocontrol value. Our aim is also to improve the selection of effective agents by identifying characteristics of successful and unsuccessful species. Success may be related to mortality from parasitism or to environmental factors, but we believe that a major part of an agent's ability to damage its host relates to the power of the gall as a nutrient sink. We have, therefore, classified galls into four nutritional categories, departing from Dreger-Jauffret and Shorthouse (1992), who used location and appearance, and from Mister (1911), who used cellular organization. Doing the review suggested new ideas on gall development and function, which in several instances we have investigated with preliminary experiments. Where details of these experiments interfere with the flow of the text, we have placed them in an appendix.

The inducer's nutritional categories and the representative species examined are as follows: (1) nutrient supply to the gall not increased, e.g. the sow-thistle capitulum gall of *Tephritis dilacerata* Loew (Diptera: Tephritidae); (2) nutrients imported from adjacent tissue, e.g. the knapweed ovule gall of *Urophora quadrifasciata* (Meig.) (Diptera: Tephritidae); (3) nutrients supplied by callus following severance of vascular tissue, e.g. the thistle receptacle gall of *Rhinocyllus conicus* (Froel.) (Coleoptera: Curculionidae) and the diffuse knapweed rosette root gall of *Sphenoptera jugoslavica* Obenb. (Coleoptera: Buprestidae); (4) nutrients supplied via a gall vascular system, e.g. the knapweed receptacle gall of *U. affinis* (Frfld.) (Diptera: Tephritidae), the Canada thistle stem gall of *U. cardui* (L.) (Diptera: Tephritidae), and Russian knapweed stem-bud or leaf gall of *Subanguina picridis* (Kirj.) (Nematoda: Tylenchidae).

Nutrient supply to the gall not increased

***Tephritis dilacerata* Loew (Diptera: Tephritidae).** *Tephritis dilacerata is* a tephritid fly that transforms the capitula of *Sonchus arvensis* L. (perennial sow-thistle) into a button-like gall. The fly is native to central and northern Europe (Hendel 1927); it has been released, but has not become established in Canada.

Sonchus arvensis is a herbaceous perennial of European origin that is found in all Canadian provinces. It spreads by seeds and easily broken horizontal roots. Flowering occurs from June to September and many wind-borne seeds are produced. The plant is a particular problem in broad-leaved herbicide-sensitive crops such as canola. Peschken *et al*. (1983) estimated that sow-thistle causes annual losses in canola of $6.7 million on the Canadian prairies.

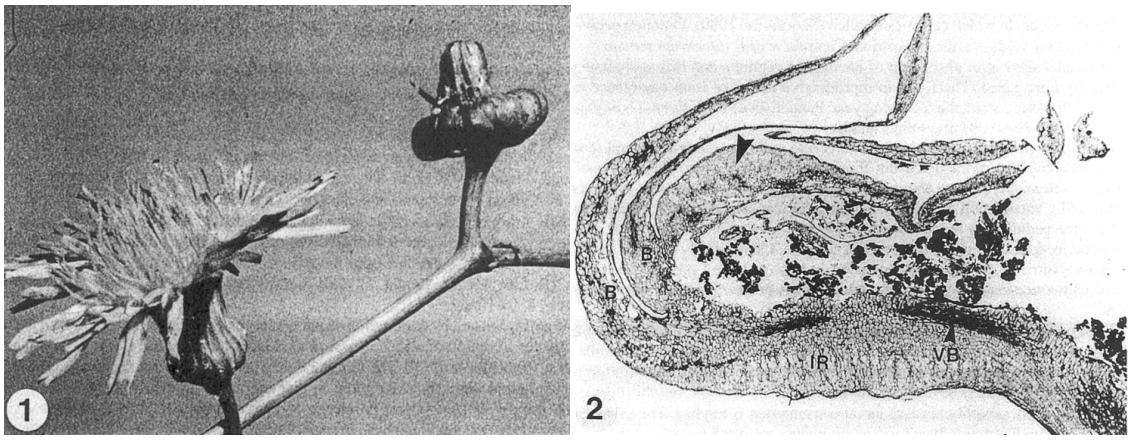
Biology. The biology and host specificity of *T. dilacerata* were studied by Berube (1978*a*, 1978*b*). The fly lays an average of seven eggs into a capitulum of *S. arvensis* with an average diameter of 3.1 mm. The capitulum size, which reflects its developmental stage, is critical as younger buds abort and older ones cannot be galled. Consequently, the perennial sow-thistle strain of *T. dilacerata* cannot develop in annual *Sonchus* spp. which have smaller capitula and faster development. Females oviposit between the bracts without piercing them, which would release latex. The eggs hatch in 4-5 days when the capitulum is elongating rapidly (Berube 1978*b*), about 12 days before flowering. The three larval instars are completed in 9-10 days.

The gall is formed by contraction of the bracts at the apex to form a button shape (Fig. 1) following consumption of all the ovaries and most of the receptacle, which requires at least five larvae (Shorthouse 1980). The larvae are then contained in the expanded capitulum base, which just before anthesis is 10.2 mm in diameter compared with 7.7 mm for uninfested capitula (Berube 1978*a*). There is intense competition between larvae and those in small capitula produce few, small puparia. In some cases, apparently as a result of crowding, larvae tunnel into the peduncle which becomes swollen (Shorthouse 1980). Pupation occurs in the gall, approximately 15 days after oviposition. Capitula with *T. dilacerata* remain green and turgid for about 7 days after abscission of unattacked capitula, and then dehydrate within hours after fly emergence. The fly is in reproductive diapause from emergence in midsummer until flowering starts the following year. Parasitism of *T. dilacerata* is negligible in Europe (Schroeder 1974) and absent in Canada.

Development and structure of the *T. dilacerata* gall. The outer involucral receptacle cells (the area at the base of the bracts but above the peduncle) extend after 6 days of larval feeding and reach maximum size about 18 days after egg hatch (Shorthouse 1980). Growth is accentuated by vacuolation and increase of intercellular space (Fig. 2). Vascular bundles extend from the peduncle to the bracts, as in normal capitula (Philipson 1942), and divide the involucral receptacle from the rest of the receptacle. Larvae rarely sever or feed beyond these strands. Nutritive cells are not produced and the only cell proliferation is a small amount of callus associated with larval feeding in the peduncle and on the receptacle at pupation.

Gall physiology. The stomatal openings in the bracts are 0.3 ± 0.08 (SE) μm in both galled and ungalled capitula, but galling suppresses corolla development. Corolla tissue typically has a high water loss so its absence likely helps maintain capituluin turgor. The receptacle's vascular tissue is severed by the larvae of *T. dilacerata*, but this does not lead to callus proliferation, possibly because larval consumption is too fast. The tissue eaten by the larvae is not modified and their food requirements are often so great that they starve, suggesting that it would be advantageous for the fly to distribute its eggs more evenly among capitula; but egg distribution is apparently limited by the need to have at least five larvae to modify the involucre. The lack of normal involucral senescence indicates that the presence of larvae and pupae stimulate the production of cytokinin.

Biocontrol value. The larvae of *T. dilacerata* reduce seed production in sow-thistle, but only in the attacked capitula. Hence, the effect is similar to that of non-galling insects that web bracts together. In Europe, *T. dilacerata* occurs in over 20% of *S. arvensis* stands, but galls only 2.5-6.0% of the capitula (Schroeder 1974).



Figs. 1-2. Capitula of *Sonchus arvensis* modified by *Tephritis dilacerata*: 1, normal and galled capitulum (without florets), ×2; 2, mature gall with laterally expanded involucral bracts (arrows), Safranin-fast green, ×12. Abbreviations: B, bract; IR, involucral receptacle; VB, vascular bundle.

A total of 19,336 flies was released across Canada between 1979 and 1980 without recovery (Peschken 1984), although *T. dilacerata* has survived in field cages provided with deep litter near Edmonton, AB (A. McClay, pers. com. 1993). From a biocontrol perspective, *T. dilacerata is* a poor candidate because its galls are not nutrient sinks and even if the insect could be established in Canada, seed reduction would probably be small.

Nutrients imported from adjacent tissue

***Urophora quadrifasclata* (Meig.) (Diptera: Tephritidae).** *Urophora quadrifasciata,* which breeds on *Serratula* and several subgenera of *Centaurea*, is one of the most common tephritid flies in Europe and Turkey (White and Korneyev 1989). The fly was introduced from the Ukraine to Canada in 1972 where its hosts are *C. diffusa* Lam., *C. maculosa* Lam., and *C. debeauxii* Gren and Godron. It is established in British Columbia, Alberta, Quebec, and possibly Ontario.

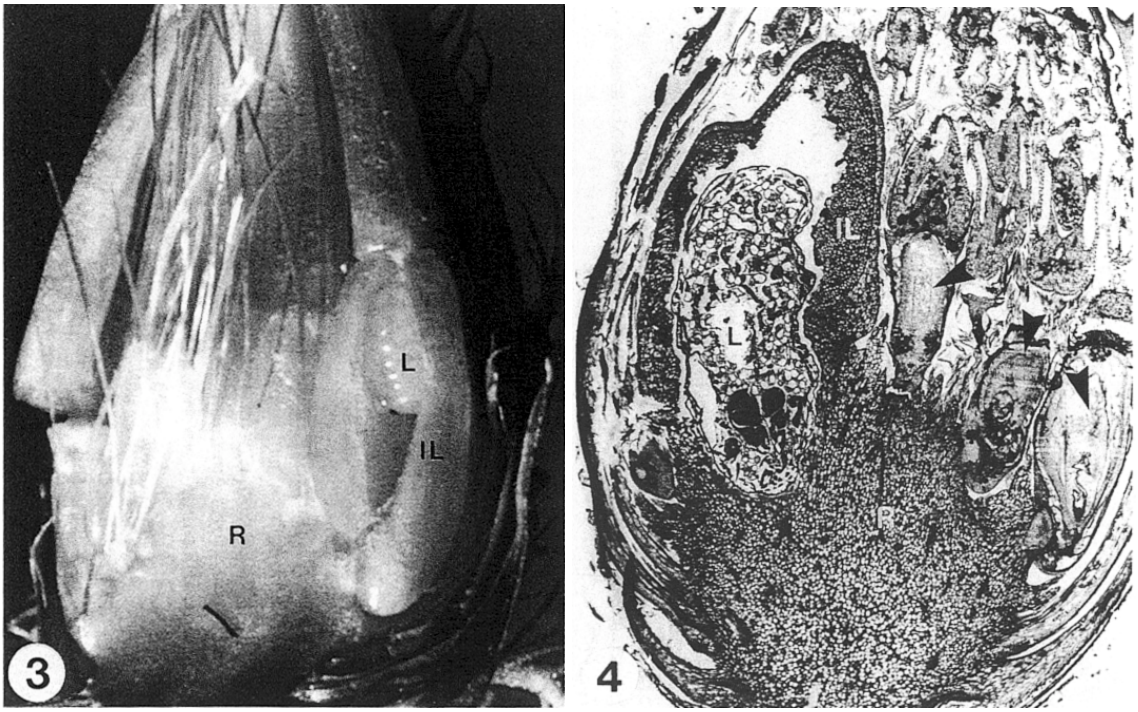
Centaurea diffusa and *C. maculosa* are herbaceous Asteraceae of Palaearctic origin, probably introduced from the Caspian Sea region with Turkestan alfalfa seed. These knapweeds thrive on the dry grasslands of southwest Canada, reducing forage production by up to 90%, to the detriment of both ranching and wildlife. In addition, *C. diffusa* has spiny capitula, which makes it undesirable in recreational areas. The two knapweeds, which infested almost 30,000 ha in British Columbia in 1972 (Watson and Renney 1974), spread to about 83,000 ha by 1984 (Jenson 1984) and threaten 8.4-10.7 million ha in western Canada (Harris and Cranston 1979). The perennial, *C. maculosa,* flowers mainly in early summer, each plant producing an average of 16 capitula with 26 seeds each, In contrast, *C. diffusa* is largely monocarpic, and on dry range, plants produce an average of 74 capitula with 12 seeds each in succession from mid-summer until frost (Watson and Renney 1974).

Biology. Emergence of *U. quadrifasciata* in British Columbia peaks in the 3rd week of July, the preferred capitula of *C. diffusa* for oviposition are 5.5-9.5 mm long (Berube 1980) and contain distinct seed embryos. The larvae develop only in pollinated capitula or those attacked by *U. affinis,* indicating a need for a pre-existing nutrient sink. The eggs are laid singly among the developing stamens (Varley 1937), but a capitulum may be attacked several times. Eggs hatch in 3-4 days and each larva bores down a floret to the ovary. The gall arises from the ovary (Fig. 3) and, except for the stretched wall around the larva, is completely consumed at maturity. Larvae complete feeding at the end of the third instar about 20 days after oviposition, when they turn so that their heads are toward the outside. In the first generation, pupation occurs at 20-25 days, about the time achene development is complete, and the adults emerge in 5-6 weeks. The larvae of the second generation, and sometimes the first in *C. maculosa,* overwinter in the capitulum, which seems to be an adaptation to the short flowering period on summer-dry sites.

Parasitism in Canada by *Hyssopus* nr. *novus* Girault and *Prototalia carlinarum* (Szelenyi and Erodös) (Hymenoptera: Eulophidae) is less than 10%. With less parasitism and greater knapweed density, *U. quadrifasciata is* about 15 times denser in Canada than in Europe. The most important mortality in North America is the approximately 70% predation in sites of *C. maculosa* by the seed-feeding moth *Metzenaria paucipunctella* Zell. (Lepidoptera: Gelechiidae) (Story *et al*. 1991), which was introduced as a biocontrol agent of *C. maculosa.*

Development and structure of the gall. Gall development begins about 8 days after hatching, when the larva has chewed down the floral tube to the ovary (Shorthouse 1989). Here, it consumes the ovule and cells of the inner layer of the ovary wall. The larva then penetrates the base of the ovary and feeds on the adjacent receptacle tissue. The recepta-

cle cells next to the feeding site proliferate and differentiate into a small zone of nutritive tissue which is supplied by a little new vascular tissue. While the larva feeds on the nutritive tissue, cells of the inner ovary wall proliferate (Fig. 4) and those near the base become cytoplasmically dense. The larva consumes the entire wall except for the papery-thin outer layer and, when there are several in a capitulum, may penetrate into the receptacle (Shorthouse 1989).



Figs. 3-4. Galls of *Urophora quadrifasciata* in *Centaurea diffussa* capitula: 3, capitulum with larva in an ovary, ×15; 4, gall with cell proliferation on inner ovary wall and aborted ovaries (arrows), Safranin-fast green, ×16. Abbreviations: IL, inner layer of ovary wall; L, larva; R, receptacle.

Gall physiology. Some of the larval nutrition for *U. quadrifasciata* is obtained from unmodified cells of the ovary wall. However, larval feeding on the receptacle tissue at the base of the gall induces the formation of nutritive tissue and increases the nutrient supply to the galled ovary. Adjacent florets abort and abortion is increased by larval penetration into the receptacle. However, presence of galls does not increase the calorific value of a capitulum (Harris 1980a), so the larvae must be nourished from the normal supply to the capitulum. Final-instar larvae of *U. quadrifasciata* contain on average 17.8 ± 0.6 (SE) kJ, which is less than a single diffuse knapweed seed of 27.2 ± 0.4 (SE) kJ. Further evidence that the gall is weak as a metabolic sink is that, in contrast to *U. affinis,* fly development was similar in capitula from a small stand growing on soil with sublethal amounts of the herbicide picloram as from outside it (Table 1).

Table 1. Number of insects in 50 capitula of *Centaurea maculosa* from adjacent picloram-treated and untreated soil at Westwold, BC.

	No. of galls	
Treatment	*Urophora affinis*	*U. quadrifasciata*
With picloram*	33 (9 larvae dead)	61
Without picloram	104 (17 larvae dead)	54

* 7.8 ± 5.2 ppb picloram in the soil at 0-10 cm; 5.4 ± 4.7 ppb picloram in the soil at 10-20 cm.

Biocontrol value. Florets inhabited by larvae of *U. quadrifasciata* are destroyed and adjacent ones abort; however, there is no decrease in the number of capitula developed (Harris 1980*a*). Regression analysis indicated that each larva of *U. quadrifasciata* reduced production by 1.9 seeds in *C. diffusa* (Harris 1980*b*), so the caloric ratio of fly:displaced seed is 1:2.9.

Urophora quadrifasciata has spread rapidly (Story *et al*. 1987) and attacks isolated plants as well as stands. Roitberg (1988) showed that the greater dispersal of *U. quadrifasciata* than *U. affinis* was not related to their performance on a flight mill, so presumably the tendency of *U. affinis* to aggregate on dense stands of its host is behavioural. Average density in 74 samples of 100-200 capitula taken in September-October between 1978 and 1985 in British Columbia was 0.78 ± 0.08 (SE) larvae per capitulum on *C. maculosa* and 0.44 ± 0.08 (SE) on *C. diffusa.* This compares with 0.057 ± 0.13 (SE) larvae per capitulum for *C. maculosa* in Europe (H. Zwölfer, pers. com.).

Myers and Harris (1980) found that the presence of *U. affinis* in a capitulum tends to discourage attack by *U. quadrifasciata.* Nevertheless, the combination of both species enhances seed reduction. Indeed, the partial displacement by *U. affinis* may increase searching by *U. quadrifasciata* as indicated by our finding that in 1973-1975, a site in British Columbia had 73% of the capitula attacked by *U. quadrifasciata,* 54% by *U. affinis,* and 30.2% by both.

The biocontrol value of *U. quadrifasciata* is that it destroys some of the seed that escapes *U. affinis.* The fly also adds stability to the level of control between years because the relative success of the two flies changes annually with differences in synchronism of fly emergence and capitulum maturity by as little as a week (Berube 1980). The relative importance of *U. quadrifasciata* should increase as knapweed density declines, because it is less dependent on dense populations of knapweed. However, an equally abundant non-gall inducing insect with the ability to forage in the capitulum, of which there are several, would probably be more effective.

Nutrients supplied by callus formed after severance of vascular tissue

***Rhinocyllus conicus* (Froel.) (Coleoptera: Curculionidae).** *Rhinocyllus conicus is* a European weevil that develops in thistle capitula of *Carduus, Cirsium, Silybum,* and *Onopordum* (Zwölfer and Harris 1984). The weevil qualifies as a gall inducer rather than

a borer because it induces callus tissue on which the larva feeds preferentially. *Rhinocyllus conicus* was released in Canada in 1968 and subsequently in the United States, New Zealand, and other countries where it has controlled *Carduus nutans.* In Canada it is also established on *C. acanthoides* L. and *Cirsium* spp.

Carduus nutans and *C. acanthoides* are biennials or winter annuals dependent on seed production that, prior to the introduction of the weevil, formed persistent, almost pure stands up to 2 m tall in many places in North America. *Carduus nutans* produces capitula up to 7 cm in diameter in an early summer flush that coincides with the *R. conicus* oviposition period, but *C. acanthoides* starts flowering about 2 weeks later to produce a succession of capitula, up to 1.7 cm diameter, until frost. Only the early capitula of *C. acanthoides* are available to the weevil.

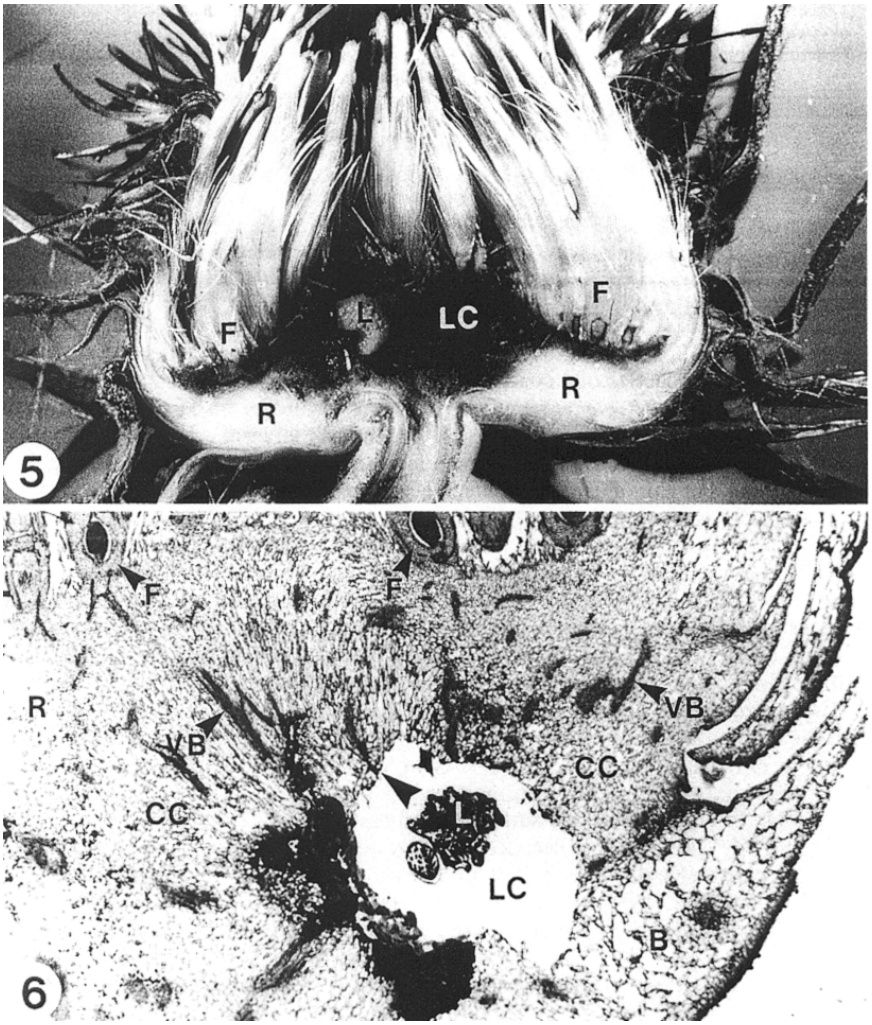
Biology. *Rhinocyllus conicus* emerges in the spring to feed on the host leaves and oviposits on the involucral bracts, mostly near the peduncle. In Europe, the number of eggs is closely correlated with capitulum size (Zwölfer and Preiss 1983), and although the dense North American populations of this weevil overload capitula with eggs, the number of weevils maturing is related to capitulum size (Zwölfer and Harris 1984). Each egg is covered with masticated thistle tissue which is used by the larva as an abutment for penetrating the capitulum (Zwölfer and Harris 1984). The larvae hatch in 6-9 days, tunnel down a bract and into the receptacle, and feed in the receptacle and peduncle (Shorthouse and Lalonde 1984). At the end of the fourth instar (Fig. 5), the larvae form pupal chambers of frass and faeces. The pupal stage lasts 8-14 days and the teneral adult remains in its chamber for several weeks before leaving to hibernate in the soil litter. About 10% of the attacked capitula in both Ontario and Saskatchewan have larvae in the peduncle. This has not been observed in Europe and appears to relate to the high density of *R. conicus.* In the peduncle, the larvae tunnel the pith without damaging the vascular bundles and no callus is formed.

In Europe, interspecific competition with other capitulum-infesting insects and parasitism are major causes of mortality (Zwölfer and Harris 1984). In Canada, larval parasitism, by *Bracon mellitor* Say (Hymenoptera: Bracondiae), is less than 1%. This parasite attacks a number of weevils and moths including *Homeosoma electellum* (Hulst) (Lepidoptera: Pyralidae) (Muesebeck *et al*. 1951), which is common in capitula of *C. nutans.* Parasitism was similar in the United States, but was 19% for larvae in the peduncle (Littlefield 1991). Parasitism was 5.3-15.7% in the capitula of *C. acanthoides,* compared with 1.8-2.5% in the larger capitula of *C. nutans* (Dowd and Kok 1982).

Development and structure of the gall. At the time of larval entry, the upper receptacle has small, closely packed, cytoplasmically dense cells that divide frequently whereas the lower receptacle has randomly arranged, occasionally dividing vacuolate pith-like parenchyma (Shorthouse and Lalonde 1984). Most larvae either tunnel into the upper receptacle, immediately below the ovules, or the region between the upper and lower receptacle. Both regions have extensive vascular systems, which the larvae sever as they feed. The severing produces a rapid proliferation of callus that surrounds the larva in 2-3 days (Fig. 6), but no new vascular tissue is formed. The larvae then feed on the callus and cease tunnelling. In the preferred upper receptacle region, the larvae sever more vascular tissue, callus is formed more rapidly, and there is less tunnelling than in the lower region. The fourth-instar larvae consume more callus than they induce and ultimately

consume it all. Depending on the size of the capitulum and the number of larvae, the receptacle may be reduced to involucral bracts, pappus hairs, and black pupal chambers composed of frass and faeces.

Gall physiology. All the requirements for callus production are present if the vascular tissue is severed in a plant nutrient sink (see Discussion). The greater production of callus in the upper than the lower receptacle and its absence in the peduncle presumably reflect nutrient distribution in these tissues. Weevils from *C. nutans* are smothered by callus if they attack *S. marianum,* which is exploited by *R. oblongatus* Cap. (Klein and Seitz 1994). In Saskatchewan, the number of larvae per capitulum and the attack of secondary capitula are lower when spring is dry (Zwölfer and Harris 1984), so moisture may be a limiting factor.



Figs. 5-6. Capitula of *Carduus nutans* modified by *Rhinocyllus conicus*: 5, mature capitulum with larvae, receptacle tissues have been consumed, severing the florets, ×3; 6, receptacle of *C. nutans* with larvae of *R. conicus* surrounded by proliferating callus, Safranin-fast green, ×28. Abbreviations: B, bract: CC, callus cells: F, floret; L, larva; LC, larval chamber; R, receptacle, VB, vascular bundles.

Impact of the weevil on seed production was measured on 2,135 capitula harvested in 1982 shortly before dehiscence from 60 plants of *C. nutans* growing on cultivated land at Regina, SK. *Carduus nutans* growing in competition with grass normally dies in early August, after the production of secondary capitula, but those in the test continued producing capitula, which had few *R. conicus,* until the plants were killed by frost in mid-October. The receptacular radius was measured and seed and weevil larvae in each capitulum were counted. Seed production varied between similarly sized capitula, possibly reflecting pollination success, but this did not obscure the impact of the weevil (Table 2). Multiple regression analysis showed that seed production increased with receptacular radius and each weevil reduced seed production by approximately 16 seeds in the attacked capitulum, and by one seed for each weevil in each of the five previously formed capitula. These findings suggest that at least 20% of the larval nourishment is obtained from assimilates diverted to the attacked capitula. This ability to import extra nutrients explains the small effect of larval crowding on weevil size, and the similar size of weevils developing in staminate and in pistillate capitula of *C. arvense* (Harris and Peschken, unpublished).

Table 2. The effect on seed production by *Carduus nutans* of capitulum diameter, of the number of *Rhinocyllus conicus* in the capitulum, and of the number in the previous five capitula formed. Determined from a multiple regression of 2135 capitula collected in 1982 shortly before dehiscence from a stand of 60 plants at Regina, SK.

Parameter per mm	Seed production	Standard error	Prob > |T|
Radius	+33.1	1.2	0.0001
Per R. *conicus* in capitula	-15.9	1.0	0.0001
Per *R. conicus* in 5 previously formed capitula	-1.0	0.2	0.0001

Biocontrol value. According to Crawley (1989), the control of *C. nutans* with *R. conicus* is the fourth most successful weed biocontrol project in the world. The establishment of *R. conicus* in Canada reduced *C. nutans*'s seed production by about 50% and the thistle is now confined mostly to recently disturbed roadsides and to breaks in pasture (Zwölfer and Harris 1984). In Virginia, Kok and Surles (1975) reported that *R. conicus* reduced *C. nutans*'s density by 95%. The weevil has been less effective against *C. acanthoides* because, with the longer flower period, only early capitula are attacked. Consequently, seed reduction is about 10%. Rowe and Kok (1984) suggested that natural selection will increase the intensity of weevil attack of *C. acanthoides,* but population mixing may prevent this in regions with both thistles.

In conclusion, *R. conicus* is a successful biocontrol agent of *C. nutans* largely because it attacks a high proportion of the capitula. The weevil increases assimilate partitioning to the attacked capitula and consequently reduces seed production in the unattacked ones, but this impact is relatively small.

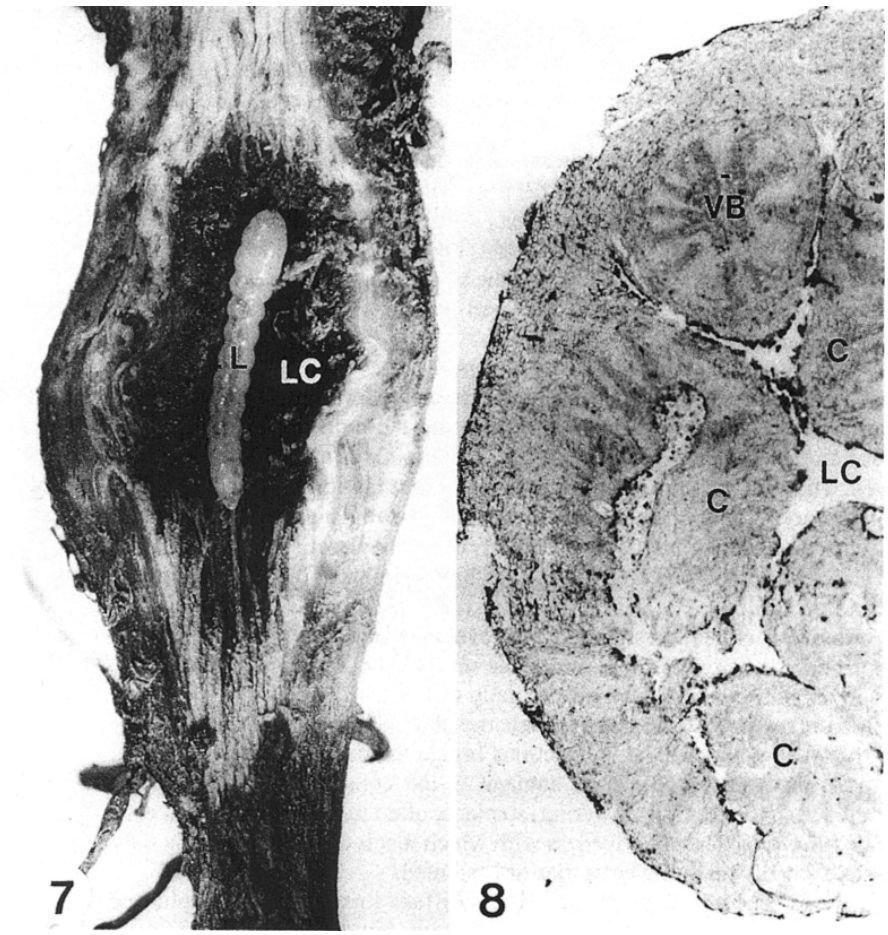
***Sphenoptera jugoslavica* Obenb. (Coleoptera: Buprestidae).** *Sphenoptera jugoslavica* is a beetle endemic to the steppes of east Romania, Bulgaria, northern Greece, and northeastern Turkey, where it forms callus galls in the roots of *C. diffusa* and closely related knapweeds (Zwölfer 1976). Beetles from northern Greece were released in British Columbia in 1976 and are now established throughout the dry belts of the province and the northwest United States. The host plant is described under *U. quadrifasciata.*

Biology. Adults of *S. jugoslavica* emerge in early July with the appearance of the first knapweed flowers (Powell and Myers 1988) and for about 2 weeks they can be sweep-netted on warm dry evenings. They feed on knapweed leaves, but usually eat only 0.25 cm^2 before departing (Powell and Myers 1988). Feeding is necessary for egg development and oviposition occurs from late July to mid-September. Eggs are wedged between tightly appressed petioles of rosettes with horizontal leaves (dormant plants). Rain during the 14-day incubation period causes eggs and newly hatched larvae to be dislodged by separation of the petioles or crushed by their expansion (Zwölfer 1976). In British Columbia, eggs are also placed in leaf axils of bolting stems up to 4 cm above the ground and the larvae bore down the stem into the root. Occasionally, well-developed larvae are found developing externally between the petioles of robust rosettes. The beetle is dependent on high temperatures: oviposition on 95% of knapweed rosettes took 8 days at 30º C and 24 days at 25º C (Powell and Myers 1988). Most plants receive several eggs, but usually only one larva survives and in rare cases where there are two larvae, the lower is stunted. Usually *C. maculosa* is not a host, but it is lightly attacked on alluvial sands at Castlegar, BC, where rosette growth is arrested by drought in July-August.

Freshly hatched larvae enter the petiole base and then bore toward the root. The second instar reaches the stem-root transition zone where some remain and others tunnel the root without causing noticeable swelling. However, most larvae penetrate the upper root to create a swelling (commonly about twice the root diameter) in which the larva feeds and overwinters (Fig. 7). Feeding resumes in the spring and pupation, which lasts 10-12 days, occurs in the root. The adult bores out in 2-3 weeks on a warm dry day in July-August.

In the fall, small rosette roots are consumed and so the plant and consequently the larva die. Best survival occurs in large roots, but in robust plants the larva is often found dead embedded in a mass of callus. Powell and Myers (1988) reported that in late August at White Lake, BC, all well-developed rosettes had eggs or larvae, but only 60% of the plants flowering the following year had been attacked. This can be explained by the eggs not having been laid on seedlings, some of which reached flowering size.

No parasitism or predation has been noted in British Columbia, but in Europe attack rate is low and parasitism high. Zwölfer (1976) dissected 100-500 rosettes from each of 30 sites on the Romanian Black Sea coast, but found the beetle at only three, which had 3-5% of the rosettes attacked. Over half the larvae were parasitized and there was predation by elaterid larvae and *Pterlonche inspersa* Staud. (Lepidoptera: Gelechidae) for a total mortality of around 70%.



Figs. 7-8. Upper root of *Centaurea diffusa* modified by *Sphenoptera jugoslavica:* 7, gall, with larva, which enlarges the root, ×5; 8, gall with callus protrusions, some of which contain vascular bundles, Safranin-fast green, ×20. Abbreviations: C, callus; L, larva; LC, larval chamber; VB, vascular bundles.

Development and structure of the gall. On consuming the pith in the stem-root transition zone, most second-instar larvae tunnel into the root and feed on parenchyma between the vascular rays. The pith cells lining the tunnel above the larva proliferate to produce clumps of densely packed callus. Supernumary cambia in this callus cause the root to swell. The main larval food is callus and its consumption creates the larval chamber. The larva commonly severs the secondary xylem, leaving pieces in the frass. The chamber surface becomes convoluted with irregularly expanding protuberances of callus (Fig. 8). The larva also feeds on the thick layers of light-brown callus at the chamber base.

Gall physiology. The gall is similar to that of *R. conicus* in that most of the feeding is on proliferating callus formed after the severance of vascular tissue. The knapweed root develops new vascular tissue to bridge the severed portions, which restores the root-shoot connection, but none is developed to supply the gall.

Biocontrol value. The larva tunnels the plant and severs vascular tissues in the upper root. However, new vascular connections bridge the damage and the tunnelling is of little structural importance. The main damage is the consumption of the assimilates being accumulated in the root for flowering, so plants often remain as rosettes that are attacked in the following year. The effectiveness with which this is done is apparently the reason that in a multiple attack, the lower larva dies or is stunted.

Measurement of the beetle impact on diffuse knapweed is complicated by the two seed-head flies *U. affinis* and *U. quadrifasciata;* but as these attack after the beetle has emerged, they have no direct effect on it. Powell and Myers (1988) found that, in 1983, *S. jugoslavica*-attacked plants produced 41% less seed than unattacked ones, and, in 1984, the reduction was 57%. Thus, one larva accounts for about half the assimilates destined for seed production. This is not its full effect because attack delays flowering in some rosettes; 2-5% of attacked rosettes die in the fall and 12-16% in the summer. In dry summers, feeding by adults increases seedling mortality.

The combined impact of the beetle and the two seed-head flies has reduced seed production at White Lake from around 35,000 per square metre in 1976 to 475 per square metre in 1989 (Harris 1991). This is reflected in a decline in knapweed cover from 52% in 1986 to 13.7% in 1992 (A. Sturko, pers. com.). Thus, the beetle has supplemented the impact of two seed-head flies to divert enough assimilates to reduce seed production to below the threshold needed to maintain the weed population. The need of the beetle for summer-dry sites is not a serious limitation because this is the habitat of its host.

Nutrients supplied via a gall vascular system

***Urophora cardui* (L.) (Diptera: Tephritidae).** *Urophora cardui* is a European tephritid fly that induces a large spherical or irregular multichambered gall in the stems of *Cirsium arvense* (L.) Scop. The fly requires moist sites and is spreading along rivers in Germany at a rate of 100-800 m a year, but populations are genetically impoverished by only local dispersal by flight, high parasitism, and colonization of new areas by few individuals (Seitz and Komma 1984). The only hosts are *C. arvense* and *C. creticum* (Lam.) D'Urv. (an aquatic thistle) (White and Korneyev 1989). In Canada, *U. cardui* is established in six provinces, but on the prairies it is restricted to the edges of ponds and streams (Peschken 1990).

Cirsium arvense is a herbaceous perennial that reproduces by seed, vegetatively by horizontal roots, and, after cultivation, by root fragments, so it commonly grows in dense patches. The ability of the thistle to thrive in a variety of edaphic, climatic, and plant communities makes it a serious weed, which Peschken and Johnson (1979) estimated to cause losses of $23 million annually to wheat producers on the Canadian prairies.

Biology. Forsyth (1984) noted that in Quebec, *U. cardui* oviposited from mid- to late June, when the thistle had completed almost 80% of its growth in height. Eggs are laid into an apical leaf bud and hatch in about 10 days as second instars (the first-instar exuvium being left in the egg shell). The larvae tunnel down the stem until they reach the zone of vascular differentiation where they induce the gall. The tunnel, which expands as the gall grows, fills with callus (Lalonde and Shorthouse 1984). The newly hatched lar-

vae produce a secretion that presumably breaks polysaccharide chains as they sink in a pool if placed on agar gel. This secretion presumably plays a role in gall induction. The gall grows for about 30 days to enclose each larva in its own chamber where it feeds little until the gall is mature (Fig. 9). Larvae feed for 30-65 days, then overwinter in the gall, remaining dormant as long as the gall is intact, but pupating when exposed to air. In nature this occurs in the spring with the disintegration of the callus plug in the exit tunnel (Lalonde and Shorthouse 1982). Females mate within a day or two of emergence.

The size and number of larvae in the mature gall varies, but the clutch size is 14.0 ± 2.0 (SE) n = 47, regardless of stem diameter (Harris, unpublished data). Final gall size is a function of the number of larvae in it (Zwölfer *et al*. 1970; Lalonde and Shorthouse 1985). Forsyth (1984) noted that the dry weight of galls on main stems 4-7 cm tall was 3.3 ± 1.5 (SE) g compared with 1.0 ± 0.8 (SE) g for stems 7-13 cm tall and in the field, the length x width of galls on the main stems was 11.5 ± 5.0 (SE) cm^2 compared with 4.5 ± 2.4 (SE) cm^2 for those on laterals, which do not appear until main stem growth is complete. Thus, larval survival is best on young main shoots.

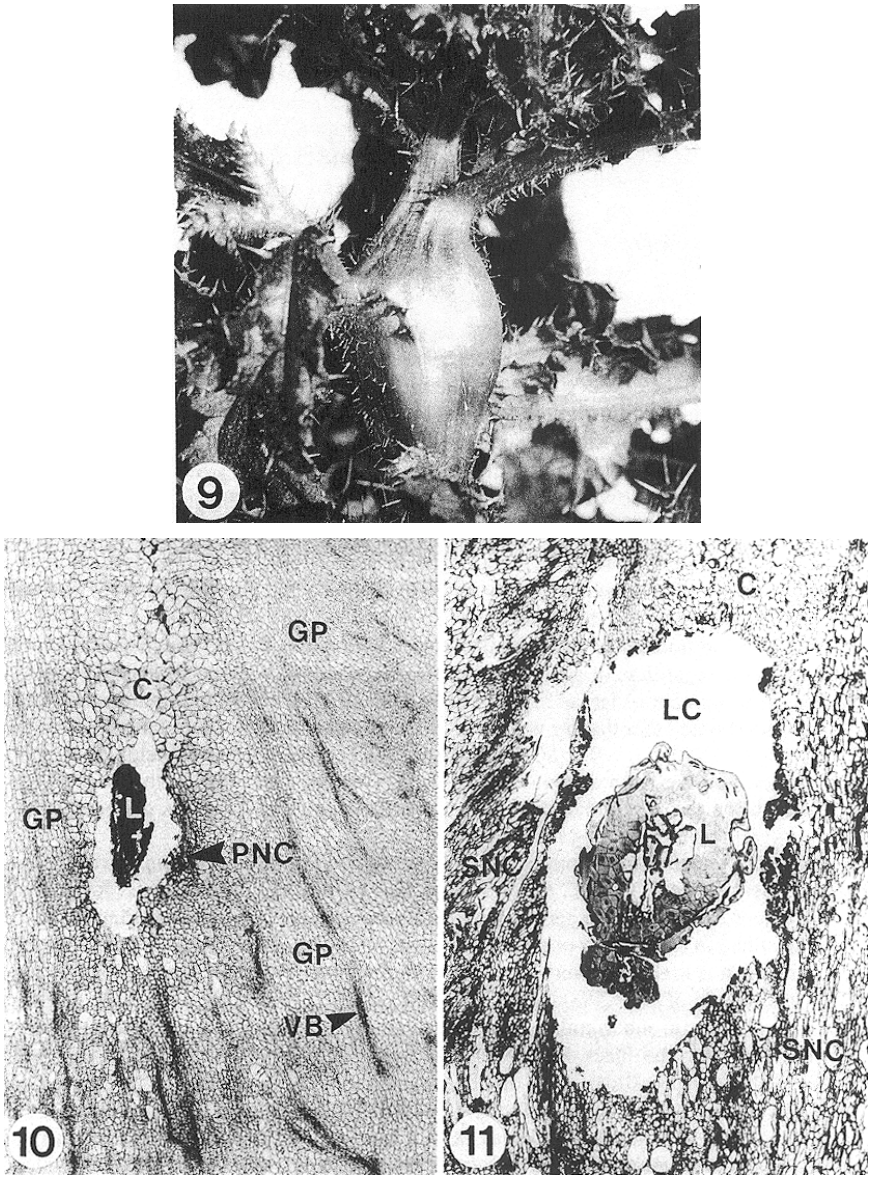
Galls of *U. cardui* in Europe are mainly parasitized by the following Hymenoptera: *Eurytoma serratulae* (F.) and *E. robusta* Mayr. (Euryatomidae), *Torymus* sp. (Torymidae), and to a minor extent by other species such as *Habrocytus elevatus* Walker (Pteromalidae). Total parasitism in established populations is over 60% (Zwölfer *et al*. 1970), but mortality from parasitism is higher as many larvae are killed without parasite development (Zwölfer 1979). In a sample of over 2000 larvae from 100 galls in Nova Scotia, 7.7% were killed by *H. elevatus* (Sampson and Ingraham 1990) which may have entered Canada in ships ballast with its main host, the black knapweed gall fly, *Urophora jaceana* L. (Graham 1969). Paradoxically, with parasitism, 43% of the larvae failed to emerge, whereas in other regions of Canada without parasites, mortality was 30-90% (Peschken, pers. com.). Thus, without parasitism, larval survival is similar to that in Europe with high parasitism. Parasitism reduces energy consumption in the unilocular gall of *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) by 4.3-8.7 kJ (Stinner and Abrahamson 1979). The effect is probably similar in *U. cardui,* which means that it should improve the nutrition of unattacked larvae and hence their winter survival.

Development and structure of the gall. The gall induced by *U. cardui*, which is described by Lalonde and Shorthouse (1985), is soft and fleshy while growing, but hard when mature. The gall is initiated by the larva severing vascular bundles and feeding on the interfascicular parenchyma, differentiating procambium and pith to form a chamber (Lalonde and Shorthouse 1985). A distinct zone of small, cytoplasmically dense cells forms around each larva (Fig. 10).

Gall growth is marked by the development of procambial strands that eventually become branched xylem, attached to the main vascular system, around the larval chambers. ‘Primary nutritive cells’ containing starch and lipids accumulate at the ends of the larval chamber. Some are eaten, but the larva grows little (Lalonde and Shorthouse 1985).

At gall maturation, division of the procambium produces ‘secondary nutritive tissue’ (Lalonde and Shorthouse 1984) with cells that have a large vacuole and accumulate lipids and proteins, but lack starch. Their formation coincides with rapid larval feeding on the remaining primary nutritive cells which leaves the larva surrounded by secondary nutri-

tive tissue (Fig. 11). Coincidentally, gall parenchyma beyond the nutritive layer becomes lignified. The gall vascular system increases and differentiates with the phloem on the inside of the xylem instead of in its normal external position. During the 36 days of gall growth, mean larval dry weight increases from 0.03 to 0.1 mg, but to 7.7 mg during the following 30 days. The larvae consume all secondary nutritive tissue by the end of the maturation stage, leaving each chamber lined with lignified cells.



Figs. 9-11. Stem gall of *Urophora cardui* on *Cirsium arvense*: 9, mature gall, ×1.0; 10, immature gall with larva surrounded by parenchyma with primary nutritive cells near the larva and callus above the larval chamber which forms the escape route for the adult, Schiffs reagent-fast green, ×25; 11, mature larva within its chamber surrounded by secondary nutritive cells, Safranin-fast green, ×15. Abbreviations: C, callus; GP, gall parenchyma; L, larva; LC, larval chamber; PNC, primary nutritive cells; SNC, secondary nutritive cells; VB, vascular bundle.

Gall dehiscence occurs when the callus tissues above the larval chambers degrade. This increases the air exchange in the larval chamber and induces pupation (Peschken and Harris 1975).

Gall physiology. The immature gall of *U. cardui* is a powerful metabolic sink. Alexander (1987) found it accumulated 22 times more picloram than the stem which resulted in cell wall thickening and production of small flies. Forsyth (1984) found that radioactive fructose was accumulated during the growth but not the maturation phase. Similarly, Thibodeau (1985) found that, during the growth phase, 12% of labelled assimilate from leaves accumulated in the galls at the expense of nutrients to vegetative and flowering apices. In contrast, less than 2% of the assimilates accumulated in the gall during rapid larval growth. Thus, the main assimilate loss to the plant occurs before the larvae do most of their feeding. The number of larvae in a gall affects its size and assimilate partitioning to the gall. One gall with a single larva accumulated 8.9% of labelled assimilates, whereas 25.4% accumulated in a gall with six larvae (Thibodeau 1985). Alexander (1987) found that during early growth, galls had 2.6-3.9 times more indoleacetic acid (IAA) than stem tissue, which, together with a high sugar concentration around the larva would account for the reversal of phloem and xylem positions in the gall (see Discussion). Sugar export from the gall has not been examined, but may occur, as phloem loading is stimulated by high IAA levels (Moorby 1977).

There is less potassium in the gall than the stem and the addition of 10 kg/100 m^2 in a strip through a thistle-infested field in New Brunswick had no significant effect on either numbers of galls per stem, larvae per gall, or larval size (Table 3). Similarly, Abrahamson and McCrea (1986) found that the gall inducer *E. solidaginis* did not benefit from the addition of potassium.

Stems of *C. arvense* have few stomata compared with the leaves [12.90 ± 0.73 (SE) per mm^2 vs. 57.09 ± 5.21 (SE) per mm^2] and there was no significant difference between the number of stomata on a gall and an equal length of ungalled stem. However, the aperture (both length and width) of the stomata on the gall increased with the size of the gall (Fig. 12) (Experiment 1, Appendix 1) and the stomata apparently do not close. The result was that gall transpiration loss was 47% greater than from an equivalent length of ungalled stem (Experiment 2, Appendix 1).

Table 3. The effect on *Urophora cardui* of fertilizing its host plant, *Cirsium arvense*, with potassium chloride.

	Treated ± SE	Untreated ± SE	$p > \|T\|$
No. of galls per stem	2.12 ± 0.25	1.44 ± 0.19	0.04
Dry gall weight (g)	0.66 ± 0.06	1.01 ± 0.12	0.005
No. of larvae per gall	3.98 ± 0.39	4.68 ± 0.58	0.3
Live larval weight (mg)	14.22 ± 0.43	15.16 ± 0.43	0.1
% potassium in galls	1.51 ± 0.05	1.13 ± 0.04	
% potassium in stem	2.11 ± —	2.04 ± —	

Blocking the gall stomata on plants with eight or more leaves enhanced the power of the gall as a metabolic sink, which is indicated by the larvae from these galls being significantly heavier than those from galls with open stomata. This suggests that the untreated galls on plants with many leaves suffer from the competing leaf transpiration (Fig. 13) (Experiment 3, Appendix 1). The finding of Lichter *et al*. (1990) that the galls of *E. solidaginis* on *Solidago altissima* L. were 10% smaller and had larvae that were 26% larger than those on *S. gigantea* Ait. may also be related to stomatal expansion and water loss from the gall. Patrick (1984) suggested there is an upper turgor limit regulated by an "off-switch", implying that if turgor was not limiting in the untreated galls of *U. cardui* on plants with few leaves, their growth would be similar to those with blocked stomata. Gall turgor is less likely to be limiting in the aquatic thistle *C. creticum* than in *C. arvense,* which grows on drier sites.

Gall turgor can also be increased by defoliation of plants with many leaves, as in Experiment 4 (Appendix 1). In this case both the number and total weight of larvae maturing per gall were significantly greater in the defoliated than in the undefoliated stems; however, the weights of the gall shells were not significantly different (Table 4). Larval nutrition depends on the development of nitrogen-rich nutritive cells (during gall maturation) which occurs after the stomata have reached maximum size. The shell of the gall is formed during gall growth and is lignified during the maturation stage. The mature gall of *U. cardui* has a shell with 9.0% acid-insoluble lignin and 4.1% acid-soluble lignin (Experiment 5, Appendix 1), so, although lignin has an important role (see Discussion), it comprises a relatively small part of the shell.

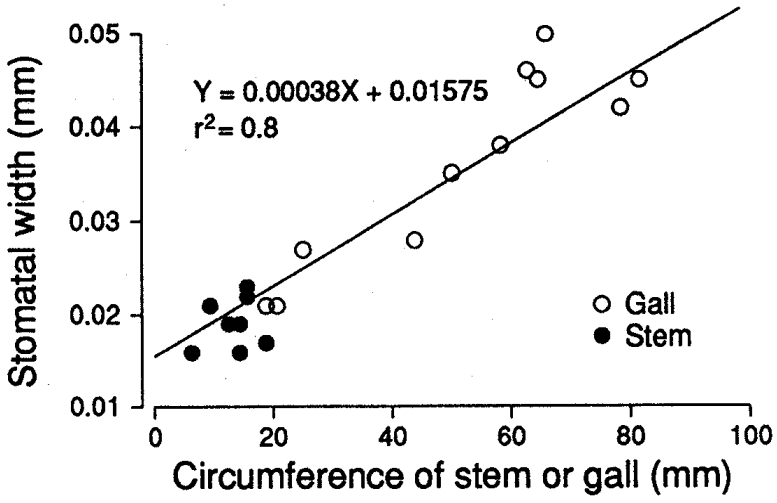


Fig. 12. Relation of *Cirsium arvense* stomatal width to stem and gall circumference.

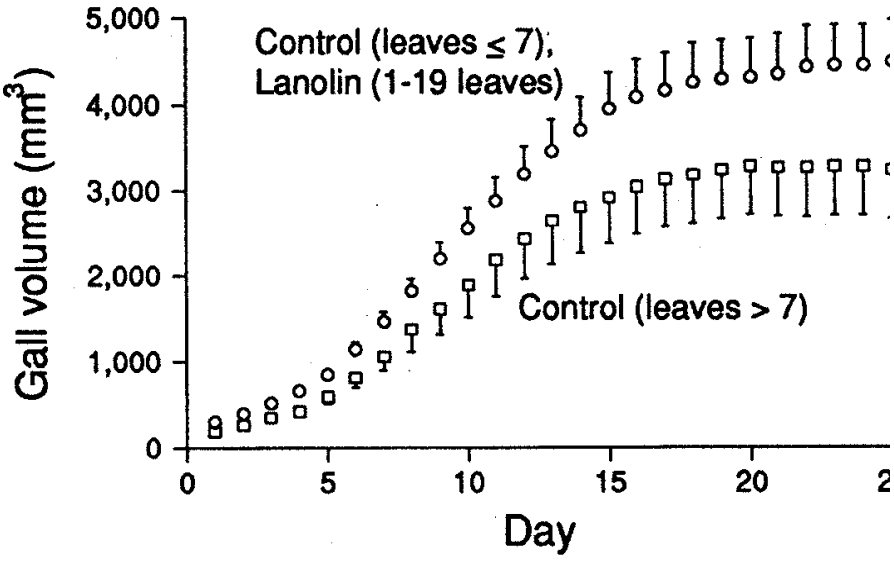


Fig. 13. Growth of *Urophora cardui* galls on *Cirsium arvense* stems. Untreated galls on stems with eight or more leaves vs. galls coated with lanolin on stems with 1-19 leaves plus untreated galls on stems with less than eight leaves.

Biocontrol value. The impact of *U. cardui* is affected by the age of the stem attacked. Thistle stems that were nearly mature when attacked were not significantly lighter than unattacked stems at the end of the summer (Peschken and Derby 1992). Similarly, Forsyth (1984) found no significant effect of galls on root weight or new ramet production in 27-cm-tall thistles, but when stems 4-7 cm high were galled, there was a 25% reduction in fresh root weight. After 11 weeks, galled plants had only 0.25 ramets each compared with 4.25 ramets in the controls. Similarly, Peschken and Harris (1975) reported a reduction of 66% in fresh root weight, and 50% in ramet production, 53 days after initiation of a single gall on stems 13 cm tall. Several galls per stem produced larger effects.

Table 4. The effect of defoliation on galls of *Urophora cardui*.

Treatment		Mean ± SE	$p > \lvert T \rvert$
No. of larvae developed per plant	Defoliated	8.71 ± 1.23	
	Non-defoliated	5.35 ± 0.7	0.01
Total pupal weight (g) per plant	Defoliated	0.097 ± 0.014	
	Non-defoliated	0.052 ± 0.007	0.003
Gall weight (g)	Defoliated	1.32 ± 0.219	
	Non-defoliated	1.06 ± 0.145	not sig.

Unfortunately for biocontrol, the power of the gall of *U. cardui* as a metabolic sink suffers because it is induced too late, when most of the thistle height growth is complete and transpiration from the many leaves reduces gall turgor. Consequently, the galls do not grow as large, receive less assimilates, and develop smaller larvae than they would on younger plants. Also, the lack of drought adaptation mechanisms for maintaining turgor, such as the accumulation of potassium or a hairy surface, restricts the gall to moist and shaded sites on the Canadian prairies. Consequently, *U. cardui* is of little biocontrol value in most agricultural situations.

***Subanguina pictidis* (Kirj.) (Nematoda:Tylenchidae).** *Subanguina picridis* is a nematode that induces bud, stem, and leaf galls on Russian knapweed, *Acroptilon repens* (L.) de Candolle. Galls range from small, pubescent protuberances on leaves to swollen and stunted stems that die in early summer. The nematode is endemic to Armenia-Tadzhikistan where its use for biocontrol was pioneered (Ivanova 1966; Kirjanova and Ivanova 1969). In Canada, *S. picridis* is established near Kamloops, BC, and there was a colony on the bank of the South Saskatchewan River near Leader, SK, until it was lost to erosion.

Acroptilon repens is a persistent herbaceous perennial introduced into Canada with Turkestan alfalfa seed in the early 1900's, but its spread is mostly by horizontal roots. Most infestations are on river benches in the dry regions of British Columbia, Alberta, and Saskatchewan where, on both irrigated and non-irrigated land, it forms stands of 11-64 stems per square metre that displace other herbaceous plants. The weed is toxic to horses and difficult to control with herbicides. More details are given by Watson (1980).

Biology. The biology and host range of *S. picridis* were studied by Watson (1986*a*, 1986*b*, 1986*c*) who suggested that the *Subanguina* spp. described from *Acroptilon, Cousinia, Rhaponticum, Centaurea,* and *Chartolepsis* should be synonymized with *S. picridis*. Thus, the host range includes several genera in the tribe Centaureinae. The infective second-stage larvae of the nematode concentrate in the upper 5 cm of the soil in early spring: Ivanova (1966) reported densities of up to 140 larvae per gram of soil. When the humidity is high, the larvae climb the hairs on emerging stems and penetrate the meristem to induce a gall in 3-4 days, but host recognition is poor and larvae penetrate most plants including wheat, sunflower, bean, and tomato in which they are absorbed (Watson 1986*a*). The shoot-bud galls (Fig. 14), induced before the plant has leaves, start as small, whitish, hairy swellings with 5-55 nematodes of both sexes (Ivanova 1966). Numerous eggs are laid and by the end of the second generation in late August, there may be over 11,500 nematodes in a gall on a stunted-leafless stem. Galls that form later, after the plant has many leaves, remain small and develop few nematodes. The rust disease, *Puccinia acroptili* P. and H. Syd., was particularly dense on galls at Leader, SK, where it sporulated both on the gall exterior and inside the chamber without apparent harm to the nematode.

Second-stage larvae become dormant in mid-summer, the galls dry and eventually decay. The nematodes are revived by moisture and move into the soil. These pre-parasitic larvae have approximately half the proteolytic enzyme activity of the infective stage and they do not initiate galls until they have spent 6-7 weeks in moist soil, which in practice

means they overwinter. Larvae can be stored dry for at least 10 years and become active within minutes when placed in water. However, under field conditions they disappear after 2 years in soil (Watson and Harris 1984). No natural enemies have been reported.

Development and structure of the gall. The structure of the gall on *A. repens* is well defined 7 days after larval penetration. The central chamber with the nematodes is interspersed with debris of collapsed cells (Fig. 15). Next, there is a layer of non-vacuolated, cytoplasmically dense nutritive cells, each with a large nucleus and two or three nucleoli. The nutritive tissue consists of a broad zone of tightly packed parenchyma that radiates from the vascular bundles to the epidermis. Cells of the epidermis are slightly enlarged and have extensive hair development. The central chamber enlarges slightly and becomes increasingly irregular with age, but the tissue zones are maintained until the gall dries up.

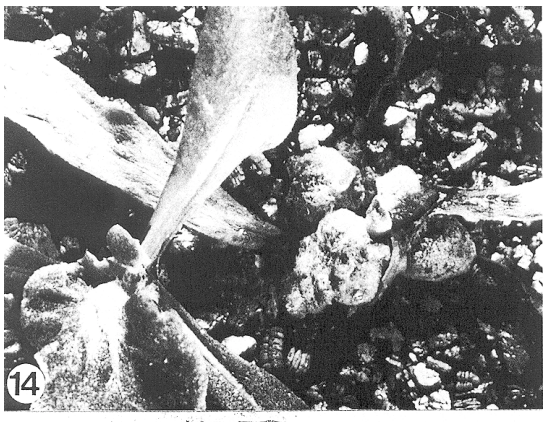
There are degrees of host suitability for *S. picridis* (Watson 1986*a*). On the best host, *A. picridis*, stems, buds, and leaves are galled and the galls have a broad, well-defined nutritive layer with little or no necrosis. On marginal hosts, the galls are confined to leaves, there is usually no nutritive tissue (but if present it is not arranged in a layer), and there is much necrosis. In tissue cultures of *A. repens* root tips, Ou and Watson (1992) found that the nematode did not induce galls or develop beyond the fourth larval stage. In stem callus on a high gibberellic acid culture medium, both males and females were produced, but they did not mate or produce eggs. However, they did so on shoot tips (Ou and Watson 1993) and the addition of gibberellic acid reduced developmental time from 6 to 4 weeks (Ou and Watson 1992).

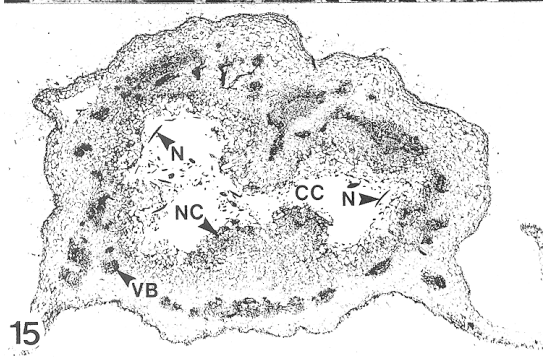
Gall physiology. The high proteolytic activity in the infective larvae suggests that galls develop in response to enzymatic rather than mechanical damage. The stem galls of *S. picridis* have enlarged stomata and transpiration is increased. The width of the stomata opening on ungalled stems was 0.9 ± 0.2 (SE) rm compared with 3.9 ± 0.2 (SE) rm for those in the enlarged bud galls (determined as in Experiment 1, Appendix 1). The galled stems collected in late August lost 0.083 ± 0.007 (SE) g at room temperature in 6 hours compared with 0.047 ± 0.007 (SE) g for those without galls (determined as in Experiment 2, Appendix 1). Thus, even at maturity, transpiration from the galls was 1.8 times that from an equivalent stem portion, so the early desiccation of attacked stems is not surprising.

The stem galls of both *U. cardui* and *S. picridis* increase transpiration, but the consequences are not the same. We suggest that a high potassium gradient (Tables 5 and 6) (Experiment 6, Appendix 1), which was not found in galls of the other species examined, allows the gall of *S. picridis* to be an assimilate sink under dry conditions (see Discussion). A high gradient of amino acids helps this process, and a high N level was found in the immature gall, although lost by July. Owens and Specht (1966) found that the root-knot galls of the nematode *Meloidogyne incognita* (Chitwood) (Nematoda:Tylenchida) had 20% more K, about half the free sugars, and over three times the free amino acids of ungalled tissue.

Biocontrol value. Assimilates are diverted to the gall of *S. picridis* for about half the growing season and this often kills its host. Ivanova (1966) reported that over 90% of stems of *A. repens* inoculated with crushed gall material (100 g/m^2) were galled, nearly 20% of them died, and there was severe damage to another 30%. At Regina, SK, in a series of dry years, less than 10% of the stems were galled, although continuous attack for 3 years killed the plants. On dry rangeland at Leader, SK, no galls were recovered, but at the edge of the South Saskatchewan River, where river fogs were common, shoots were often reduced to irregular galls 2 cm wide and 4 cm high. We conclude that infection by *S. picridis* requires high atmospheric humidity in the spring.

Subanguina picridis has been used little for biocontrol in North America, but it is promising for spring-moist and irrigated sites. The nematode can be used as a classical biocontrol agent or applied as a bioherbicide, and large numbers of nematodes can be obtained from field plots or increased 140- to 200-fold in 3 months on shoot tips of *A. repens* in tissue culture (Ou and Watson 1992).





Figs. 14- 15. Leaf galls of *Subanguina picridis* on *Acroptilon repens:* 13, typical leaf galls; 14, gall on leaf, Safranin-fast green, ×10. Abbreviations: CC, central chamber; N, nematodes; NC, nutritive cells; VB, vascular bundles.

Table 5. Nitrogen and potassium content of galls of *Subanguina picridis* and ungalled stems of *Acropti lonrepens.*

Tissue sample	Date	Mean % of dry tissue			
		N	SE	K	SE
Stem (ungalled plants)	Late June	1.07	-	2.33	-
Stem (galled plants)	Late June	0.83	-	2.00	-
Stem next to the gall	Late June	2.12	-	3.20	-
Gall	Late June	2.96	-	5.18	-
Stem (ungalled)	Early August	1.72a	0.12	1.72a	0.12
Stem (galled)	Early August	1.84a	0.03	1.78a	0.12
Gall	Early August	1.87b	0.79	2.12a	0.12

Means in a column with different letters are significantly different (Tukey's studentized range test, $p < 0.05$).

Table 6. Percentage potassium and nitrogen in stem and galls of *Subanguina picridis* in July.

	Tissue	Mean %	SE	N
Potassium	Gall	2.61a	0.18	9
	Stem	2.04b	0.12	14
Nitrogen	Gall	2.13a	0.24	9
	Stem	0.99b	0.07	14

Means with different letters are significantly different (Tukey's studentized range test, $p < 0.05$).

***Urophora affinis* (Frfld.) (Diptera: Tephritidae).** *Urophora affinis* is a tephritid fly that forms a unilocular receptacle gall (Fig. 16) in the *Centaurea* subgenus *Acrolophus*, which contains the weeds *C. diffusa* and *C. maculosa*, diffuse and spotted knapweed. The fly is native to Europe (White and Komeyev 1989). French stock from *C. valleseaca* (DC.) Jordon was released in Canada in 1970 and later supplemented with Ukrainian stock from *C. sterilis* Steven. The fly is established in British Columbia, Ontario, and Quebec where its impact has been summarized by Harris and Myers (1984). Biology has been reported by Zwölfer (1970), Story (1976), Harris (1980*a*, 1980*b*), Myers and Harris (1980), and Berube (1980). The two knapweeds are described under *U. quadrifasciata*.

Biology. Adults of *U. affinis* start emerging with formation of the first knapweed capitula (mid-June in British Columbia), and emergence peaks in the 1st week of July, when the largest capitula are about 3 mm long. Up to 120 eggs per female are laid in groups of one to five on top of the tubular disc florets 3.5-5.5 mm long (half-grown capitula) (Berube 1980) over a 3-week period (Zwölfer 1970). Multiple oviposition is restricted by the 2-3 days during which a capitulum is acceptable and, in contrast to the requirements of *U. quadrifasciata*, pollination is unnecessary for gall development.

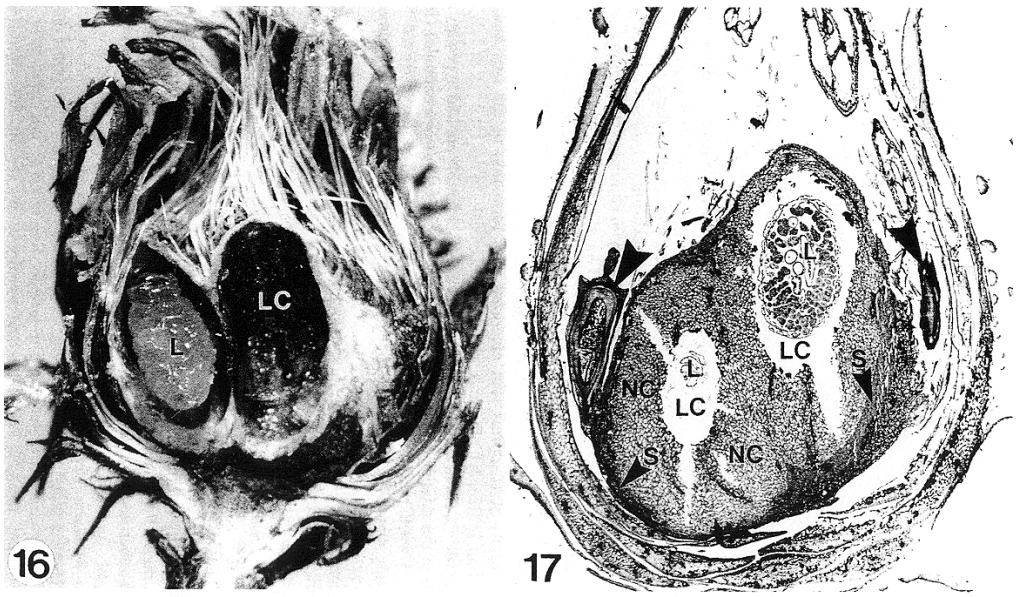
Eggs hatch in 3-4 days and the larvae tunnel down a floret to the receptacle surface where the gall is induced (Shorthouse 1989). Between 10 and 25% of the larvae pupate by 33 days and may emerge for a second generation in August; however, most overwinter as larvae in the gall. Larvae successfully overwintered at Regina, SK, so that the potential range of *U. affinis* is wider than the present distribution of knapweed. Densities can exceed 3000 galls per square metre and levels of over 1000 galls per square metre are common in British Columbia (Harris 1980*b*).

Larval mortality in Europe from parasitoids in the genus *Eurytoma* and predation by *Pyemotes* sp. mites (Pyremotidae: Acarina) is usually over 50% (Zwölfer 1970). The mites tend to attack other insects in the capitula and then spread to *U. affinis* through the open apex of the gall. Turner *et al*. (1990) found that 1.3% of the galls of *U. affinis* on spotted knapweed in Montana were parasitized by *Microdontomerus anthonomi* (Crawford) (Torymidae: Hymenoptera), which is usually associated with weevils. The largest mortality in Canada is on spotted knapweed from the seed-feeding moth *M. paucipunctella*, which consumed 67% of the larvae in a capitulum and commonly attacked 50-80% of the capitula (Story *et al*. 1991). With lower parasitism and higher knapweed density in Canada, the population of *U. affinis* on spotted knapweed is at least 3.5 times greater than that in Europe and, on diffuse knapweed, almost 19 times greater (Table 7).

Table 7. Density of *Urophora affinis* in Canada and Europe.

	No. of samples	No. of capitula	No. of *U. affinis* per capitulum
Spotted knapweed			
Canada	74	100-200 each	1.44 ± 0.19 (SE)
Europe	45	4932	0.41 ± 0.19 (SE)
Diffuse knapweed			
Canada	26	100-200 each	0.62 ± 0.1 (SE)
Europe (excluding France and Germany)	55	8145	0.33 ± 0.13 (SE)
France and Germany	26	6258	0.005 ± 0.016 (SE)

Development and structure of the gall. Gall development was described by Shorthouse (1989). Freshly hatched larvae chew down a floret, consume the ovule, and then feed on the inner layer of the ovary wall. After about 12 days, they penetrate the base of the ovary to feed on the receptacle. Cells of the ovary wall and receptacle proliferate to enclose the larva in a thick layer of parenchyma. Vascular bundles extend from the receptacle into this layer which becomes nutritive tissue. The nutritive cells are thickest by about 25 days and, at about this time, a sclerenchyma layer forms around the gall except for the apex (Fig. 17). The larva feeds in an inverted position, but at the end of the fourth stadium it turns so the head is toward the sclerenchyma-free exit. Proliferation of the nutritive layer decreases with maturity and the larva, at about 28 days, is in a sclerenchyma-lined chamber.



Figs. 16-17. Galls of *Urophora affinis* within capitula of *Centaurea diffusa*: 15, capitulum with two larval chambers on the receptacle surface, ×12; 16, capitulum with two larvae surrounded by nutritive cells, sclerenchyma and aborted ovaries (arrows), Safranin-fast green, ×14. Abbreviations: L, larva; LC, larval chamber; NC, nutritive cells, S, sclerenchyma.

Gall physiology. The developmental time of *U. affinis is* about half that of *U. cardui,* because it avoids the month-long period of gall growth and nutrient accumulation. Presumably this is possible because the receptacle, in which the gall is initiated, is already a nutrient sink. The nutrient sink of the gall is powerful enough that larval crowding in a capitulum has little effect on their weight (Harris 1980*b*). Mature larvae in their gall were, on average, 117 kJ, and the value of the capitula increased directly with the number of larvae. The maximum number of galls that can develop is a function of the receptacle disc area, so more galls are produced in capitula of *C. maculosa* than in those of *C. diffusa.*

Much of the assimilate loss to the plant is represented by the gall shell. Each gall with a larva in spotted knapweed was, on average, 139 kJ and the mature larva was 66 kJ, so the lignified shell represents about 47% of the loss to the plant. Gall lignin content, as determined in Experiment 5 (Appendix 1), was 17.4% acid-insoluble and 1.1% acid-soluble, so it is mainly saturated (double bonded), which indicates that the gall should be a less effective accumulator of toxins than that of *U. cardui* . The power of the gall as a metabolic sink makes it vulnerable to many herbicides. Story *et al*. (1988) reported that 2,4-D, applied to knapweed during the flower-bud and flowering stages, reduced the emergence of *U. affinis*. Similarly, the herbicide, picloram, at levels sublethal to the plant, reduces the galls to small woody lumps that produced no flies, and in less affected galls, fly size is reduced. Thus, the effects are similar to those of *U. cardui*'s gall. Picloram did not affect the other two capitulum-inhabiting insects that do not form a gall shell, the gall-fly *U. quadrifasciata* (Table 1), or the non-gall moth *M. paucipunctella.*

The corolla is suppressed or absent in galled capitula and the stomata in the bracts are not enlarged, so transpiration losses from galled capitulum should be small. This is essential in a species of dry grasslands that lacks the elevated potassium levels of the gall of *S. picridis.* The gall K and N content was determined (Experiment 6, Appendix 1) by analysing capitula containing a known number of immature galls. The potassium content in galled capitula was 1.22 ± 0.04% (SE) and 1.24 ± 0.03% (SE) in the ungalled and did not increase with the number of galls. In contrast, the amount of nitrogen in the galled capitulum increased directly with the number of larvae (Table 8), so galling clearly increases the importation of nitrogen. According to Pate (1989), 97% of the nitrogen acquired by fruits is phloem-supplied so it is likely similar in the gall.

Biocontrol value. The florets tunnelled by larvae of *U. affinis* are destroyed and adjacent ones abort. However, Harris (1980*b*) showed that the main impact was the suppression of subsequent capitula and vegetative growth. In *C. diffusa,* each gall reduced production by about 13.7 seeds, and an average of 1.1 galls per capitulum reduced the above-ground dry weight of the plant by 71% as well as the average seed weight. The seed displaced by a single larva represents 372 kJ and mature larvae were, on average, 42 kJ. The damage ratio of 1:8.9 indicates an efficiency of less than one-third that of *U. quadrifasciata.* The more damage done by a weed biocontrol agent the better, so a low efficiency is desirable.

The combined effect of *U. affinis, U. quadrifasciata,* and *S. jugoslavica* at White Lake, BC, has been to reduce the number of capitula per square metre from 2100 in 1976 to 600 in 1988-1990 and seed production from around 33,000 to under 800 per square metre (Harris 1991). Roze (1981) determined that about 1500 seeds per square metre are

needed on rangeland to maintain knapweed density, so seed production is now considerably below this level.

The effect on the polycarpic *C. maculosa* is different. Near Chase, BC, there was no reduction in the number of capitula or above-ground biomass in the year of attack because most growth is complete before fly emergence. However, after several years, there has been a decline in the number of capitula and seed production has dropped from over 40,000 per square metre to an average of just over 3000 per square metre in 1987-1990 (Harris 1991). Berube and Myers (1982) suggested that control might now be achieved by establishing a strong competitor of knapweed, such as crested wheatgrass. Without competition, Schirman (1981) found that knapweed seed reduction would have to be over 99.9% to achieve control.

In conclusion, *U. affinis* is the most important seed-reducing agent established on diffuse and spotted knapweed. As a result, knapweed seed production is close to the threshold needed to achieve economic control, which can be achieved by establishing another agent and increasing interplant competition in pastures.

Discussion

We suggest that the impact of gall inducers on the host plant is affected by three interacting variables. First is the abundance of the gall which we discuss in terms of vulnerability to parasitism and to moisture stress. Second, we discuss the power of the gall as a metabolic sink, which in turn depends on the method of gall induction, the extent of vascularization, and lignification. Third, we discuss the ability of a gall to exploit a nutritional resource and suggest that the gall has logistic advantages over non-gall inducers for using resources that are diffuse in space and time.

Vulnerability of gall inducers to parasitism. Parasitism or predation that causes a major reduction in population density of a gall inducer obviously affects its efficacy as a biocontrol agent. A high proportion of parasitoids of gall inducers are idiobionts (Hawkins 1990) (those that develop on a dead or paralysed host), which tend to have a wide host range (Comell and Hawkins 1993). The implication is that native parasitoids are likely to attack introduced gall inducers used for weed biocontrol, but the contrary seems to be true.

Native, generalist, parasitoids attack 19% of larvae of *R. conicus* boring in petioles of *C. nutans* and almost 16% of those in the small capitula of *C. acanthoides.* However, losses of larvae inhabiting capitula of *C. nutans* are small, so apparently the mass of the surrounding tissue offers protection from generalists. The parasitoid of *U. cardui* in Canada is an adventive introduction on another *Urophora* sp. It does not cause high losses of *U. cardui* in Europe and it kills only 8% of the larvae in Nova Scotia. Predation by the introduced knapweed-seed moth, M. *paucipunctella,* destroys up to half the larvae of *U. affinis* and *U. quadrifas ciata,* but the loss occurs after the larvae have completed feeding, so it does not reduce the current impact of the gall inducer and it is not large enough to reduce the size of the subsequent generation. Parasitism by native species of the other gall inducers, except by Cecidomyiidae, is inconsequential and seems to be the result of accidental encounters.

Table 8. Effect of *Urophora afflnis* on the nitrogen content of capitula of *Centaurea maculosa.*

No. of larvae per capitulum	% nitrogen
0	1.24
1	1.32
2-3	1.45
4-5	1.48
6-7	1.74

On a world basis, the only example we can find of major attack by native parasitoids on a non-cecidomyiid gall inducer used for weed control is that of the tephritid stem-gall inducer *Procecidocharus utilis* Stone, in Australia. This species is ineffective as a biocontrol agent with parasitism of generally more than 30-40% and often 70% (Goeden and Louda 1976).

Cecidomyiidae is apparently the only family of weed biocontrol agents that routinely suffers high enough losses from native parasitoids to compromise its effectiveness. For example, in Alberta the leaf-gall midge *Cystiphora sonchi* L. on *S. arvense* is so heavily parasitized that it is difficult to find (Peschken, pers. com.). There is a high rate of parasitism of the spurge bract-gall midge *Spurgia esulae* Gagné, in North Dakota (Carlson and Mundal 1990). Up to 95% parasitism by *Mesopolobus* sp. of the late-season *Cystiphora schmidti* Rubsaamen reduces the midge's ability to control *Chondrilla juncea* L. in Washington State, but in Australia, parasitism by a native *Tetrastichus sp.* can reach 100% (Wehling and Piper 1988). The leaf-gall midge *Zeuxidiplosis giardi* (Kieffer) is established, but of little consequence, in California (Julien 1992), and at low elevations in Australia with parasitism of 60-80% (Goeden and Louda 1976). The low parasitism on the still localized populations of *Spurgia capitigena* (Bremi) on cypress spurge in Ontario is exceptional, but may not persist.

Vulnerability of the gall to moisture stress. There are mixed reports in the literature on the vulnerability of galls to drought, although most suggest it is detrimental. Waring (1986) reported that gall diversity and abundance were greatest on plants growing in warm dry regions with an adequate water supply. Waring and Cobb (1992) reported that in 73% of studies, gall inducers reacted negatively to drought.

Assimilates are partitioned within a plant to utilization centres, but water deficit redirects translocation to unstressed sinks. For example, Robinson *et al*. (1983) found that, in moist conditions, the gladiolus inflorescence received most of the plant's assimilates; but with a slight water deficit, the corm accumulated assimilates at four times the rate of that in well-watered plants. Similarly, Fay *et al*. (1993) found that turgor in shoots galled by *Antistrophus silphi* Gil. (Hymenoptera: Cynipidae) was greater than that of ungalled shoots in drought-stressed, but not in well-watered, plants. As a result, in dry conditions, photosynthesis continued in leaves near the gall after ceasing in the rest of the plant.

Without adaptations for resisting water stress and with enlarged stomata, *U. cardui* fares poorly in dry conditions and on plants with many leaves. Thus, in southern Saskatchewan, the galls are found only near water, whereas in the humid and cloudy regions

of western Europe, they are more widely distributed. The apparently poor adaptation of this insect to its host may be a consequence of evolutionary history: the genus *Urophora* evolved as capitular gall inducers (Zwölfer and Arnold-Rinehart 1993), in which gall induction does not enlarge the stomata and corolla reduction decreases water loss. Water is not limiting for the gall of *U. cardui* in the aquatic thistle *C. creticum* and high transpiration from the gall should increase its importation of nitrogen via the xylem. The fly does well on *C. arvense* in moist, forest-margin sites, but poorly in most places to which the thistle has been spread by agriculture. In contrast, the detrimental effects of enlarged stomata (inevitable in a gall induced in tissue with stomata) to the stem gall of the nematode *S. picridis* are moderated by two adaptations: a hairy surface, which is characteristic of many dry-region galls, and a high potassium content. The latter is similar to Australian mistletoes which commonly have enough potassium to increase turgor 3-8 bars over their host; one species has 39.0 mg/kg of potassium compared with 1.3 mg/kg for the host leaves (Lamont 1985). This allows the mistletoe to be an assimilate sink during drought (Ziegler 1986). Indeed, both mistletoes and galls with adaptations to maintain turgor may benefit from a moderate drought stress as resources are redirected to them from drought-stressed sinks.

Non-drought adapted galls without stomata apparently suffer with the plant during drought or periods of high transpiration from the leaves. For example, Dennill and Gordon (1990) reported that *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae), which has a gall with no stomata and a largely riparian host, thrives only in the shade in the dry interior of South Africa. Larson and Whitham (1991) found that removing pistilate poplar catkins, which are both transpiration and nutrient sinks, increased the success of the gall aphid, *Pemphigus betae* Doane, by 31%. Similarly, we found artificially reducing transpiration from galls improved the performance of *U. cardui* . Hence, the establishment of a defoliator on *C. arvense* should increase the impact of *U. cardui* by reducing leaf transpiration.

Metabolic sink strength and gall induction. Galls always contain modified tissues, and usually produce new tissue that increases the nutrient supply for the inducer. The inducer initiates these modifications by mechanical or secretory damage. In both methods of induction, IAA is likely important, as it causes cell wall loosening, allowing expansion in response to turgor pressure maintained by solute influx (especially K) (Ray 1987) and this may be the basis of bract enlargement in the gall of *T. dilacerata.* All plant tissues produce callus (if the usually autonomously produced cytokinin is not limiting) with water, nutrients, and IAA (Wilson and Wilson 1991). IAA is produced by the catalysis of tryptophan (Kutacek 1991) from the autolysis of dead and dying cells (Sheldrake and Northcote 1968) and accumulates when basipetal polar transport in vascular tissue is interrupted. Thus severing vascular tissue in a nutrient sink (thistle receptacle and knapweed rosette root) should provide both the necessary IAA and nutrients for callus production. Severed xylem can serve as a moisture source providing flow can be restored after cutting, which requires enough moisture to exert a positive pressure. This hypothesis is consistent with the greater number of larvae of *R. conicus* found in secondary capitula in moist summers. Furthermore, drought can be expected to affect *S. jugoslavica* less than *R. conicus,* because root turgor in knapweed rosettes tends to be maintained by leaf senescence. A potential problem for the gall inducer is that plant tissue subjected to severe or prolonged damage is likely to die; however, this could be countered by an increase in

cytokinin, which has a strong nutrient-sink and anti-senescence activity. This idea is consistent with Hewett's (1977) finding of high cytokinin levels in the callus of the stem gall formed by *Anetus virescens* (Doubleday), a moth that requires up to 5 years to develop. We speculate that cytokinin in the gall of *R. conicus* is responsible for both the increased nutrient supply, the delayed senescence in both the bracts galled by *S. arvensis* and the staminate capitula of *C. arvense,* and for maintenance of the nutrient sink in the galled roots of *C. diffusa.*

The production of IAA in non-severance galls appears to be a plant response to damage from enzymes produced by the inducer, although *U. cardui* initiates gall induction by severing vascular tissue. According to references cited by Hori (1992), these contain the following tissue-damaging substances: the tephritid *E. solidaginis* secretes protease; the cecidomyiid *Mayetiola destructor* Say, hemicellulase; and the siricid *Sirex noctilo* F., amylase, esterase, phenol oxidase, and protease. IAA is necessary for protein synthesis in plants (Stuart 1938; Moorby 1977), so it is assumed to be needed for the production of nutritive tissue in galls.

Vascular differentiation requires about 10 times the amount of IAA optimum for callus growth, with phloem developing as a result of high IAA – high sugar levels and xylem from high IAA - low sugar levels (Wilson and Wilson 1991), so the reversal of these tissues during the growth of *U. cardui*'s gall indicates a high IAA – high sugar concentration around the larvae. Sugar was not measured, but the IAA in *U. cardui*'s gall is about three times that in the stems (Alexander 1987).

In our study, the galls with vascular systems were all induced while the host vascular system was developing. Thus, the gall of *U. affinis,* which is initiated in a young capitulum, develops a gall vascular system, but it is rudimentary in galls of *U. quadrifasciata*, which are initiated after vascular differentiation in the capitulum.

The inducer-host synchronization for vascular development in galls is achieved in various ways. *Trichilogaster acaciaelongifolia* oviposits into dormant buds at the end of summer; the larvae emerge, but do not develop until the buds reach the critical development stage in the spring (Dennill 1987). In *U. affinis*, in which oviposition does not occur until the buds have started development, synchronism is achieved by restricting oviposition to capitula that will be at the correct stage when the eggs hatch. This allows more room for a mismatch, which may explain the annual changes in the relative abundance of *U. affinis* and *U. quadrifasciata*. The larvae of *U. cardui* achieve synchronism by boring down the stem to the vascular differentiation zone; however, the system is not perfect in that attack of younger stems would provide the gall with a larger resource.

Synchrony is usually less critical in species not inducing vascular galls. For example, *Phanacis taraxaci* (Ashmead) (Hymenoptera: Cynipidae) induces galls in 1-cm to fully expanded leaves, although it prefers the immature (Paquette *et al*. 1993). However, there are exceptions, such as *T. dilacerata*, for which a flower-bud is available only for about 2 days because attack of younger buds leads to their abortion and the involucral bracts are not susceptible to modification in older capitula (Berube 1978*a*). In vascular-severance galls the oviposition window depends on the persistence of the nutritive sink: it is narrower in the temporary receptacular sink of thistle capitula than in the long-term sink of diffuse knapweed roots, which may last for several months.

Lignification is essential for vascularization, so it should be found in any gall with vascular connections to its host. Lignin is formed from alcohols derived from phenylalanine (Bell 1981) and deposited during early secondary cell-wall thickening (Northcote 1972). It enables xylem to conduct water, provides rigidity by embedding cellulose fibre into a matrix, and binds metabolic wastes and many xenobiotic substances (Glasser and Kelley 1987) which include copper, zinc, iron, and nickel which were found in the shell by Bagatto *et al*. (1991) and Bagatto and Shorthouse (1991).

A lignified gall shell is similar to a seed coat because both have vascular tissue embedded in parenchyma of the surrounding shell and the nutrient supply is spatially and temporally distinct from the ovule or larval user. Indeed, nutrients are accumulated in the *U. cardui* gall before their use by the inducing larvae (Lalonde and Shorthouse 1985), much like the accumulation in receptacle tissue before ovule development. The main supply of these nutrients is phloem sap which has a carbon:nitrogen ratio of 15-200:1 (Pate 1980). Carbon is needed for cellulose formation during gall growth, but this need probably declines with gall maturation. A few cynipid galls secrete concentrated sugars (Bequaert 1924; Abe 1992) and surplus sugars may be exported via the phloem, as suggested earlier for the gall of *U. cardui*; but a more profitable use would be synthesis with ureides into amino acids. In seeds, phloem-imported ureides are synthesized into phenylalanine and tyrosine and then to amino acids, principally asparagine and glutamine, which are passed to the embryo (Thorne 1985). If the function of the gall shell is similar to that of a seed coat, it would provide both the high amino acid and IAA needed to form nutritive tissue, and to process any ureide wastes of the gall inducer into amino acids. The pathway for lignin synthesis is the same as that for aromatic amino acids except for the last steps (Neish 1960; Miflin and Lea 1977). In both seeds and galls there is rapid lignification at maturity, which we suggest results from switching production from aromatic amino acids to an inert substance, lignin, when the former are no longer needed. Extremely woody galls, such as that of *T. acaciaelongifolia*, have an extensive vascular system, so the degree of the gall lignification may be related to the strength of the gall as a metabolic sink.

In summary, we suggest the following largely new explanations. The induction of galls involves the local elevation of IAA which the inducer can elevate slightly by inflicting mechanical damage and particularly by severing vascular tissue. The severance of vascular tissue in a plant nutrient store normally produces substantial amounts of callus on which the inducer feeds. The impact of this type of gall increases with its persistence, which probably depends on a sustained level of cytokinin.

Gall inducers can cause a greater elevation of IAA, which is necessary for the development of new vascular tissue, by inflicting enzymatic damage. Vascularly supplied galls, which are powerful metabolic sinks, result from enzymatic damage done during vascular differentiation in the host tissue. We suggest that the pre-lignified shell of these galls synthesizes amino acids and hence helps nourish the inducer. It is likely that gall shell lignification at inducer maturity is the result of switching from amino acid synthesis, when it is no longer required, to lignin, an inert substance. For biocontrol, the more lignin produced the better, because it diverts assimilates from plant growth and reproduction.

Effectiveness of resource exploitation by gall inducers. The proof of a gall inducer's worth in weed biocontrol is the reduction in the domination of the plant commu-

nity by the weed. The ability of the gall inducer to achieve such a change depends on a combination of the power of the gall as a sink, the distribution of the resource on the plant, and the abundance of the inducer.

It seems to us that the powerful-sink gall has a particular advantage for exploiting a resource that is produced in many small units over a long period, such as the capitula of *C. diffusa.* Logistically these are more difficult for a non-gall inducer to exploit than a resource concentrated into a few large capitula. Harris (1991) calculated that the receptacle gall fly *Urophora solstitialis* L. would reduce seed production by 50% more than would *R. conicus* in *C. acanthoides,* a thistle that produces many small capitula over a long season, but only 10-25% more in *C. nutans,* which produces fewer but larger capitula. In Italy, the main exploiters of the thistle *Cynara scolymus* L., which produces still larger and fewer capitula, are the non-gall weevils *Larinus cynarae* F. and *L. scolymi* Oliv. The gall fly *U. terebrans* Loew is comparatively uncommon (H. Zwölfer, pers. com. 1993). The justification for selecting gall inducers for biocontrol on this basis has still to be demonstrated, but Australian studies, cited by Woodburn (1993), suggest that the gall fly *U. solstitialis* would be superior to *R. conicus* for the biocontrol of *C. nutans.*

The vascular severance gall of *R. conicus* in the temporary nutrient sink of receptacle tissue obtains about 20% of its assimilates at the expense of later-developing capitula. In pistilate capitula of *C. arvense,* the importation of nutrients is likely greater than in *C. nutans,* but the sink is ephemeral, so the plant is affected only during a short part of the growing season. However, because *R. conicus* is abundant, well-synchronized with *C. nutans* flowering, and has little parasitism, it halves the seed production by *C. nutans,* but has less impact on *C. acanthoides,* which produces smaller capitula over a longer season. The vascular-severance gall induced by *S. jugoslavica* in the long-term nutrient store of diffuse knapweed rosette roots reduces seed production by about 50%, but it does this at a lower agent density than required by *R. conicus* for a similar impact.

The galls induced by *T. dilacerata* and *U. quadrifasciata* develop little or no new vascular tissue so they are both dependent on the normal assimilate supply to the attacked capitulum. Mortality from natural enemies is not important in either species, but *U. quadrifasciata* is of biocontrol value as it has become abundant and supplements damage done by *U. affinis.* Even if *T. dilacerata* had become established in Canada, it is unlikely to have been of much biocontrol value because it is not abundant in Europe in spite of little parasitism.

The impact of galls with vascular connections to the host can be large. It was shown by Staden and Bennet (199 1) that the vascularly supplied stem gall of *P. utilis* changes assimilate partitioning and even increases the amount of nutrients delivered to the shoot above the gall. A total of 59% of the assimilates from a leaf below the gall went to the gall and plant apex, 30% to the lower shoot, and 11% to the root. Partitioning in stems without galls, and in those with galls from which flies had emerged, was 21% to plant parts above the leaf, 53% to vegetative tissue below the leaf, and 26% to the root. This ability of galls with a vascular system to change partitioning has been well documented by Fourcroy and Braun (1967), Paclt and Hassler (1967), Jankiewicz *et al*. (1979), Skuhravy *et al*. (1980), McCrea *et al*. (1985), Brewer *et al*. (1987), Andersen and Mizell (1987), Larson and Whitham (1991), Hartley and Lawton (1992), and Paquette *et al*. (1993).

Unfortunately for the ease of selecting effective biocontrol agents, the nutrient diversion by galls with a vascular system varies widely. The unilocular tephritid stem gall of *E. solidaginis* on *Solidago canadensis* L. utilises about 7% of ramet net production (Stinner and Abrahamson 1979). The multilocular receptacle gall of *U. stylata* reduces seed production of *Cirsium vulgare* (Savi.) Ten. by about 60% (Harris and Wilkinson 1984) and accounts for 85% of the caloric loss from the capitula if the lignified gall tissues are included (Zwölfer 1985). Multiple attacks by the unilocular gall of *U. affinis* reduce knapweed seed production by at least 75%, whereas the bud-gall wasp *T. acaciaelongifoliae,* on less than 50% of the branches of its host, reduces seed production by 89-95% (Dennill 1988). Galls of the latter species contain up to 23 times the energy normally spent on seed production, and vegetative growth ceases during gall development, with the result that the gall accounts for 21% of annual dry biomass production by the tree (Dennill 1988).

Conclusions

1. To have a major impact on the target weed, galls should be powerful metabolic sinks. The most effective galls induced by severing vascular tissue persist over most of the growing season. The most effective galls with a vascular system develop a heavily lignified shell.
2. Gall inducers are particularly advantageous for exploiting a resource, like diffuse knapweed capitula, which is divided between many small centres over a long season.
3. Stomata on a gall are likely to be detrimental in dry sites, although they may be beneficial if moisture is not limiting. Some galls with hairy surfaces and high potassium levels are adapted to dry conditions and may benefit from moderate water stress that reduces translocation to competing sinks.
4. Except for gall midges, parasitism is generally not a problem for gall inducers introduced as weed biocontrol agents.

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Appendix I

Experiment 1. Size of stomatal aperture on *Urophora cardui* galls and ungalled *Cirsium arvense* stems

Methods. Mature galls of *U. cardui* were collected from three locations in the Qu'Appelle Valley, SK. Comparisons of stomata on the gall and stems were made by painting the surface with nail varnish which was peeled when dry and placed on a microscope slide. The width and length of the aperture (inside the guard cells) of three stomata was averaged from each peel for four galls and three stems from each site.

Results. The results (Fig. 12) show that width of stomatal apertures increased with the circumference of the gall at the measurement site. Indeed, the gall circumference accounted for 80% of the variation in stomatal width. The length of the stomatal opening also increased with the gall circumference (Y=0.09x + 0.34, R^2 = 0.76) but the slope was not as steep as for the width. Thus both the width and the length of the stomatal apertures increase with the diameter of the gall at the stomatal] location.

Experiment 2. Transpiration losses from *Urophora cardui* galls and an equal length of stem from below the gall

Methods. A galled stem, an equal length of ungalled stem from below the gall (approximately 10 cm), and an ungalled stem of similar diameter were cut under water and all the leaves removed. The lower end was then sealed in a vial of water with plasticine. After 30 hours in a growth cabinet at about 23º C, the vials were reweighed to determine transpiration losses. The test was replicated seven times. The dry weight of each gall tested was determined as a measure of its size.

Results. The transpiration losses from the ungalled stems and the stems from below the gall were similar, so they were combined for comparison with the losses from the galls. The average transpiration losses from the vials with galls was 2.72 ± 0.29 (SE) g compared with 1.86 ± 0.21 (SE) g for the stems, $p > 0.03$ for a 47% larger loss from the galls. The gall losses were not correlated with gall weight.

Experiment 3. The effect of blocking stomata on *Urophora cardui* galls

Methods. Galls of *U. cardui* on plants of *C. arvense* grown in pots in a greenhouse were coated with lanolin shortly after initiation (n = *58*) and their growth compared with that of untreated galls (n = 57) by measuring daily the diameter and length of each gall. Daily gall volume was calculated from the daily measurements on the assumption that the galls were cylinders. The mature galls were harvested after 2 months. The number and weight of larvae in each gall were recorded and whether the plants had less than eight leaves. This resulted in four approximately equal groups.

Results. The growth of the lanolin-treated galls, whether the plant had few or many leaves, was not significantly different from the untreated galls with few leaves, so they are combined for Figure 13. Growth was slow in the first 5 days, rapid to about 14 days, and then slow to about 20 days when the galls attained maximum size. The untreated

galls with many leaves followed the same schedule, but growth was significantly less (p <0.001), following Mead and Curnow (1983).

Larvae from the treated galls were significantly heavier 11.37 ± 0.33 (SE) mg vs. 9.9 ± 0.37 (SE) mg (p < 0.005), but the numbers of larvae per gall (5.00 vs. 4.16) were not significantly different.

Experiment 4. Effect of defoliation at gall initiation on *Urophora cardui*

Methods. Adults of *U. cardui* were released on a caged thistle stand. When gall development was first observed, 26 thistles were paired for height and stem diameter. One plant of each pair was then defoliated. Approximately 2 months later, the galls containing mature larvae were harvested, dissected, the larvae counted and weighed after they had pupated, and the gall material dried and weighed.

Results. The number of larvae maturing per gall and the total pupal weight from the defoliated stems were significantly greater than from the undefoliated stems (Table 6). The average weight of the gall shell was not significantly different, so defoliation had increased larval survival and nutrition, apparently by reducing transpiration loss from the plant.

Experiment 5. The lignin content of mature of galls of *Urophora cardui* galls

Methods. The lignin content of mature galls was determined by extraction with neutral detergent (Van Soest *et al*. 1991) to isolate cell walls. These were subsequently subjected to a two-stage acid hydrolysis (Kaar *et al*. 1991) to determine acid-insoluble (klason) lignin. Acid-soluble lignin in the filtrate was quantified by spectroscopy (TAPPI 1989).

Results. The cell wall content of acid-insoluble lignin was 9.0% and acid-soluble lignin, 4.1%. The 31% content of acid-soluble lignin indicates that double bonding was incomplete.

Experiment 6. Potassium and nitrogen content of *Subanguina picridis* galls.

Methods. Bud galls with their nematodes, gall stem cores, stem sections from above and below galls, and ungalled stems were collected in 1987 from Leader, SK, in late June, and from Regina, SK, in early and late August. Galls were also sampled from Regina in early July 1992. The galls were dried, and ground for N and K determination by autoanalysis.

Results. The levels of K and N were higher in the June galls than the stems (Table 5), but by July levels of K and N were not statistically different in galled and ungalled stems, but were significantly lower than those in galls (Table 6). By early August, N levels were similar and the difference in K was reduced (Table 5). By late August, K and N levels (not shown) in galls and stems were similar.