Proc. VI Int. Symp. Biol. Contr. Weeds, 19–25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agric. Can., pp. 439–43 (1985)

Period of Maximum Host Stress During Development of Galls of *Urophora cardui* (Diptera: Tephritidae) on Canada Thistle (*Cirsium arvense*)

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Abstract

Morphological evidence suggests that stress exerted by the gall of *Urophora cardui* on *Cirsium arvense* is greatest during the growth phase of gall development. During the *c.* 20 day growth phase, undifferentiated host tissue proliferates. Following this, the maturation phase begins, wherein cell proliferation ceases and tissues differentiate into distinct nutritive and lignified zones. Using insecticide to kill larvae inside growth phase galls causes tissues near the larval chambers to revert to wound callus, whereas outer tissues enter into the maturation phase. However, there is little alteration of gall tissue morphology if larvae are killed in maturation phase galls. This indicates that larvae continuously maintain tissues is an active state throughout the gall growth phase, but cease provoking gall growth at the end of this phase, thus initiating gall maturation. The resulting stable tissues in maturation phase galls probably do not require as much of the host's resources as do the proliferating tissues of the growth phase gall.

Période de Perturbation Maximale des Plantes Hôtes Pendant le Développement des Galles de *Urophora cardui* (Diptère: Tephritidae) sur le Chardon des Champs *Cirsium arvense*

D'après certains preuves morphologiques, les effets perturbateurs de la galle de *Urophora cardui* sur *Cirsium arvense* s'exercent au maximum pendant le période de croissance de la galle. Pandant l'étape de croissance d'environ 20 jours, les tissus hôtes prolifèrent sans différenciation. Au bout de cette période, commence l'étape de la maturation, où la prolifération des cellules cesse et des zones distinctes de tissus nourriciers et lignifiés se forment. L'utilisation d'insecticides pour tuer les larves à l'intérieur des galles en période de croissance provoque la formation de callosités sur les tissus adjacents aux enveloppes larvaires, tandis que les autres tissue se rendent à maturation. Par contre, la modification de la morphologie des tissue des galles est négligeable lorsque les larves meurent dans les galles déjà à l'état de maturation. Ces observations indiquent que les larves maintiennent constamment les tissue vivants pendant la période de croissance des galles, mais cessent de favoriser la croissance de cells-ci à la fin de cette période, ce qui provoque la maturation des galles. Les tissus stables des galles à l'étape de maturation n'épuisent probablement pas autant les ressources des plantes hôtes que les tissus qui prolifèrent au cours de la croissance des galles.

Introduction

The gall-forming fly, *Urophora cardui* (L.) (Diptera: Tephritidae), was introduced into Canada in 1974 as part of an ongoing biocontrol program against Canada thistle, *Cirsium arvense* (L.) Scop. (Compositae) (Peschken *et al.* 1982). The narrow host range exhibited by gall-formers (Shorthouse and Harris 1985) and the successful use of a tephritid gall-former against a rangeland weed in Hawaii (Bess and Haramoto 1972),

influenced the decision to use this, and other gall-formers as weed control agents in Canada. Initial laboratory studies demonstrated that the gall induced by *U. cardui* causes a reduction in the growth of Canada thistle for about 4 wks after oviposition (Peschken and Harris 1975). However, subsequent field evaluations made in successful release areas indicated that the gall does not sufficiently stress its host to cause significant reduction in growth over the season and that other biological control agents against Canada thistle are required (Peschken *et al.* 1982).

Any thistle control program that employs several biocontrol agents such as *U. cardui* should be coordinated to maximize the deleterious effect of the agent on the weed and to minimize effects of interference between different agents. Therefore it is necessary to identify the period of time when *U. cardui* larvae are stressing their host and when the gall-formers are likely to be affected by other biocontrol agents. The purpose of this paper is to use results obtained from studies of the developmental biology of the *U. cardui* gall system (Lalonde and Shorthouse 1984, 1985; Shorthouse and Lalonde, unpubl. data), to identify these critical periods in gall development.

Methods and Materials

Galled and ungalled plants were obtained from a growth chamber culture maintained at Laurentian University. Ungalled host tissue and material from galled tissues at all stages of development were prepared for histological examination. Larvae were dissected from galls at all developmental stages, oven-dried, weighed and instar was determined. In addition, systemic insecticide was applied to plants bearing galls at different developmental stages. Gall tissue from insecticide-treated plants was then prepared for histological examination and compared with untreated gall tissue.

Results and Discussion

The gall induced by *U. cardui* larvae passes through four developmental phases from egg hatch to adult emergence from senescent galls in the spring. These phases are: initiation; the growth phase; the maturation phase; and dehiscence.

Initiation occurs after second instar larvae hatch (7–10 days after oviposition) and tunnel down into immature stem tissues. Larval tunnelling induces the formation of wound callus in stem tissues posterior to the larvae. Once larvae cease tunnelling, cells of pith and procambium nearest to their chambers lose their normal polarity and begin to divide. As soon as host tissue begins to proliferate under the direction of the gall insect, the gall is considered to have entered the growth phase.

Gall growth becomes apparent 12–20 days after oviposition as a lateral expansion of the stem beneath a vegetative shoot. During the gall growth phase, a network of procambial tissue develops within the proliferating gall parenchyma. This tissue anastomoses with the vascular system of the ungalled portion of the host stem. The callus tissue formed by the initial tunnelling of second instar larvae expands as gall growth proceeds to form a well-defined zone above each larval chamber. In addition, patches of cells adjacent to the larval chambers become cytoplasmicly dense and accumulate starch grains and lipid-like droplets. These cells are consumed in small quantities by the larvae and are termed primary nutritive cells. Throughout the gall growth phase, larvae remain in the second instar and grow very little.

When insecticide was applied to galls during the growth phase, cell proliferation ceased earlier than in untreated controls. Callus tissue already present remained unchanged and primary nutritive tissue rapidly changed to callus, expanded into the

larval chambers, and crushed the dead larvae. In the outer portion of the gall, gall parenchyma lignified and the procambial tissue matured into xylem and phloem (Fig. 1).

Approximately 20 days after the onset of the gall growth phase, cell proliferation ceases and the gall enters its maturation phase. During this phase, a thick zone of cytoplasmicly dense secondary nutritive cells develops from procambial initials in the tissue surrounding the larvae while outer gall tissues lignify. The region of callus above each larval chamber remains unchanged throughout this phase (Fig. 1). Shortly after the onset of gall maturation, larvae moult to third instar and begin feeding on secondary nutritive tissue. Larvae are fully grown within 24 days, or 60 days after oviposition.

When insecticide was applied to plants bearing maturation phase galls, larvae died, but most gall tissues remained unchanged (Fig. 1). However, this was not true for the secondary nutritive tissue. These cells did not lose their dense cytoplasm or revert to callus, but the internal cell structure gradually became increasingly amorphous, and bacteria often appeared within the cytoplasm.

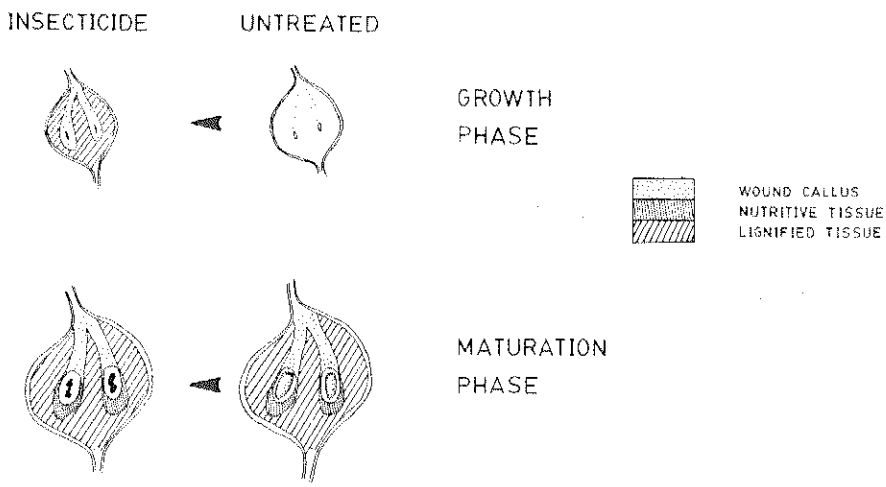

Fig. 1. Effect of application of systemic insecticide to growth phase and maturation phase galls of *Urophora cardui* (L.).

Dehiscence occurs in the spring when callus tissues in the gall degrade, opening channels through lignified tissue to the outside of the gall for emerging adults.

Of the four phases of gall development, the gall growth phase is probably the most critical, both in terms of the success of the gall-former and in terms of stress placed on the host plant. Once larval control is removed during this phase by insecticide treatment, gall tissues quickly become callus or lignify. This indicates that growth phase tissues are maintained in an active state by second instar larvae and that removal of larval influence allows the plant to convert gall tissue to inactive callus and halt further tissue proliferation. Also, the structural similarity between insecticide-treated growth phase galls and normal maturation phase galls suggests that normal gall maturation is triggered by a similar stimulus. Possibly the process of inducing formation of secondary nutritive tissue halts proliferation of gall parenchyma and initiates lignification.

Proliferation of host tissue during the growth phase draws on plant resources which could otherwise be committed to flower production or to augmentation of root reserves. Stored carbohydrates in roots of *C. arvense* are at their lowest level during the early

phases of inflorescence development (Hodgson 1968), when early stage galls are likeliest to be present (Zwölfer *et al.* 1970). Consequently, it appears that galls of *U. cardui* develop on their host when it is most susceptible to stress.

There is apparently little or no tissue proliferation in the *U. cardui* gall following formation of secondary nutritive tissue. The minimal reaction observed when insecticide was applied to maturation phase galls also indicates that gall tissues are no longer maintained at a very high level of activity. Because minimal stress is exerted on the host once the maturation phase begins, thistles are probably able to begin making up reserves lost while the gall was growing. This may explain the aforementioned discrepancy between results obtained by Peschken and Harris (1975) and Peschken *et al.* (1982).

The 20 day period of the gall growth phase encompasses the time when *U. cardui* larvae may be sensitive to interference from other weed control agents. Gall tissue accumulated during this phase is converted to food for growing third instar larvae in the form of secondary nutritive tissue. If thistles are seriously stressed by other insects or pathogens while *U. cardui* galls are still growing, larvae may be unable to induce the growth of sufficient gall tissue. I believe that this would result in a reduced production of secondary nutritive tissue in the maturation phase, ultimately producing weaker, less fecund adults.

Galls accumulate all larval food in the form of secondary nutritive tissue at the onset of the maturation phase. Secondary nutritive tissue is stable, even in the absence of living larvae. Thus third instar larvae feeding in maturation phase galls are unlikely to be affected by interference from other thistle biocontrol agents. Larvae will only be seriously impaired during the gall maturation phase if their host is stressed to the point of death and nutritive tissues desiccate before they finish feeding.

The inclusion of *U. cardui* in a thistle control program thus may require selection of other biocontrol agents which attack the weed either prior to oviposition by adults or after galls have ceased to grow. Such a strategy would maintain a constant level of stress on thistles without adversely affecting *U. cardui* larvae. Further research should be undertaken to quantify the stress levels imposed by *U. cardui* galls on Canada thistle and to determine if interference from other biocontrol agents seriously affects the success of *U. cardui*.

Acknowledgments

This research was done while the author was under the supervision of J.D. Shorthouse of Laurentian University. I am grateful for his many helpful suggestions given throughout this study. I also thank B.D. Roitberg, S.A. Allan, and A. Furnell for their comments on the manuscript. Financial support came from Natural Sciences and Engineering Council of Canada Operating Grant No. AO230 and NSERC Equipment Grants No. E3956 and No. E660 awarded to J.D.S.

References

Bess, H.A., and Haramoto, F.H. 1972. Biological control of pamakani, *Eupatorium adenophorum*, in Hawaii by a tephritid gall fly, *Procedidochares utilis*. 3. Status of the weed, fly and parasites of the fly in 1966–71 versus 1950–57. *Proc. HI Ent. Soc.* **21**: 165–78.

Hodgson, J.M. 1968. The nature, ecology and control of Canada thistle. USDA Tech. Bull. 1386, 32 p.

Lalonde, R.G., and Shorthouse, J.D. 1984. Developmental morphology of the gall of *Urophora cardui* (Diptera, Tephritidae) in the stems of Canada thistle (*Cirsium arvense*). *Can. J. Bot.* **62**: 1372–84.

_____. 1985. Growth and development of larvae and galls of *Urophora cardui* (Diptera: Tephritidae) on *Cirsium arvense* (Compositae). *Oecologia (Berl.)* **65**: 161–5.

Peschken, D.P., and Harris, P. 1975. Host specificity and biology of *Urophora cardui* (Diptera: Tephritidae) a biocontrol agent for Canada thistle (*Cirsium arvense*) in Canada. *Can. Ent.* **107**: 1101–10.

Peschken, D.P., Finnamore, D.B., and Watson, A.K. 1982. Biocontrol of the weed Canada thistle (*Cirsium arvense*) releases and development of the gall fly *Urophora cardui* (Diptera: Tephritidae) in Canada. *Can. Ent.* **114**: 349–57.

Shorthouse, J.D., and Harris, P. 1985. Use of gall inducers in the biological control of weeds. *In*: Biology of Insect Galls. Shorthouse, J.D., and Rohfritsch, O. (eds.). Praeger Press, NY (*in press*).

Zwölfer, H., Englert, W., and Patullo, W. 1970. Investigations on the biology, population ecology and distribution of *Urophora cardui* L. Weed Proj. for Canada, Prog. Rep. No. 27, Commonw. Inst. Biol. Contr., Delemont, Switzerland, 17 p.