The Induction of Gall Formation in *Ageratina riparia* by *Procecidochares alani* (Diptera: Tephritidae). I. Gall Histology and Internal Gross Morphology of the Third Instar[1]

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ABSTRACT

Gall formation in the composite, *Ageratina riparia,* is induced by the presence and activity of the larval stages of the gall-forming tephritid, *Procecidochares alani* (Steyskal). The tephritid spends its entire larval stage in the subapical area of the stem and its activity causes the formation of a bulbous shaped gall. Histological examination of the gall reveals an abundance of secretory ducts external and sometimes internal to the vascular cylinder; doubled vascular bundles; "faisceaux d'irrigation"; an increase in phloem and pith parenchyma accompanied by a decrease in xylem; nutritive cells; and an increase in stem diameter due to hyperplasia of the pith area.

Morphologically, *P. alani* third instars possess structures generally characteristic of other cyclorrhaphous dipteran larvae. However, a yellowish membranous structure was found attached to the distal part of the most anterior pair of Malpighian tubules which appears to be unique to gall flies in the genus *Procecidochares.* This pair of structures, termed "yellow bodies," increases in size with each succeeding instar, decreases in size during pupariation, and is absent 2 to 3 days into the pupal stage. The proposed function of this structure is discussed.

Procecidochares alani Steyskal, a gall-forming tephritid commonly known in Hawaii as the Hamakua pamakani gall fly, was imported in 1974 for use on the island of Hawaii as a biological control agent against the noxious weed *Ageratina riparia,* which at that time covered more than 12,000 hectares of pastureland, rendering it unusable. *P. alani* typically produces galls on *A. riparia* which, if numerous on the weed, debilitate it. The galls are characterized by the presence of the cecidozoan (gall former) within the plant tissue from the onset of gall development.

This paper describes the comparative histology of normal and galled subapical stem sections of *A. riparia* and the internal gross morphology of third instar *P. alani.* This study formed a foundation on which further experiments were conducted in an effort to determine the mechanism of gall formation in *A. riparia.*

METHODS AND MATERIALS

A. riparia galls, both mid and fully mature in development and containing second and third instar *P. alani,* were collected from roadside vegetation in Ahualoa, Hawaii. Subapical areas of galled and normal stems were fixed in 95% ethyl alcohol-glacial acetic acid – 37% formaldehyde-H_2O (50:5:10:35, v/v), run through a standard dehydration and infiltration series, embedded in Paraplast, sectioned at 12 μ intervals with a rotary microtome, stained with safranin and fast green FCF, and mounted in Permount. Histological sections of plant and larval material made via the above procedure were examined under phase contrast microscopy.

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[1]Journal Series No. 2875 of the Hawaii Institute of Tropical Agriculture and Human Resources.

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Second and third instar *P. alani* were removed from gall material and placed in physiological saline. The larvae ranged 1–2 mm in length and were dissected under a stereomicroscope at 25–30=. The alimentary canal was removed by grasping the larval mouthhooks and gently pulling the viscera from the body trunk with fine dissecting forceps. Histological sections of larvae *in situ* within galls were also prepared for examination.

RESULTS AND DISCUSSION

Gall development occurs mainly in meristematic regions where histogenetic changes such as hypoplasy, redifferentiation and retrograde differentiation, form anomaly, hypertrophy, hyperplasy, tissue stretching and rupture, tissue and cell fusion, tissue regeneration and necrosis, and cytolysis have been observed (Mani 1964). Cell proliferation has been found to be most intense in parenchyma, vascular cambium, and medullary rays (Mani 1964, Callow and Ling 1973, Raman and Devadas 1977, Hapai 1981).

Cecidogenesis (gall formation) induced by insects has been attributed to: (1) tumor-causing chemicals released by the insect at the time of oviposition (2) mechanical irritation caused by larval burrowing through plant tissue causing activation of plant growth hormone synthesis and release (3) larval salivary secretions, and (4) larval excrement (Hutchins 1969).

The design of experiments in attempts to uncover the mechanism of gall formation in *A. riparia* is dependent on obtaining baseline information on the histological changes occurring in insect-induced galls with the objective of producing a profile which can be used in comparing natural with artificially-induced galls. Figures 1 and 2 show characteristic features of the gall induced by *P. alani* larvae. When compared to normal stem tissue sections, the following characteristics of the insect-induced gall were significant: (1) an abundance of secretory ducts external and sometimes internal to the vascular cylinder (2) the presence of doubled vascular bundles due to the infolding of tissues and "faisceaux d'irrigation" (new bundles directed towards the larval-made cavity, ending only as phloem elements) (3) an increase in phloem and pith parenchyma (4) more phloem present than xylem (5) the presence of nutritive cells with giant nuclei surrounding the larval-made cavity. The nutritive cells are found surrounding larvae first in the area of the adaxial and marginal leaf primordial meristems, followed by their presence in the subapical region of the stem, and eventually in the cambium of the vascular cylinder, and (6) an increase in stem diameter by hyperplasia of the pith area.

The pith parenchyma found internal, and the cortical cells found external to the vascular cylinder in normal stems (see Fig. 3) are approximately equal in diameter. The number of pith cells increases in galled stems concomitant with a decrease in cell diameter, indicating increased cell proliferation accompanying the larval presence. The stem gradually increases in diameter when observed from the subapical area downwards. The subapical area then assumes a bulbous shape due to continued larval presence and hyperplasia of the pith area.

A longitudinal section of a typical third instar *P. alani* is shown in Fig. 4. This larval stage is found in mature galls located subapically on the shoot tip. The average length of the third instar is 2 mm. The mouth hooks, cephalopharyngeal skeleton, and cibarial pump were found in close proximity to one another in the anterior section of the larva. Imaginal discs were found inferior to the supraesophageal and ventral ganglia. Fat body cells lining the internal periphery of the hemocoel was a predomi-

nant feature. Being entirely phytophagous, the larva possesses an extensive alimentary tract that is seen in a variety of positions in longitudinal sections.

The salivary glands appear as strands of large, dark-red staining cells. Of interest was an unidentified structure located at the distal ends of the first pair of Malpighian tubules. In longitudinal section, they appeared to be heavily-tracheolated, membranous sacs enclosing dark, blue-staining material. In the unstained condition, a solid, yellowish-colored material was seen within the sac. These structures, which we also found in *Prececidochares utilis* Stone, a close relative of *P. alani*, were coined "yellow bodies." To our knowledge, no other structure of this type has been reported in the literature for tephritids.

Figure 5 shows the normal position of the viscera in third instar *P. alani*. A pair of salivary glands run posteriorly to about the midway point of the larval trunk, parallel to the esophagus. The salivary glands appear to open into the buccal cavity at the same proximity as the esophagus. The foregut continues posteriorly, eventually differentiating into the midgut. The midgut extends to the point where the Malpighian tubules bifurcate from the intestinal tract. The Malpighian tubules mark the beginning of the hindgut. The hindgut ends with the formation of a rectal sac, which further narrows into a rectum, terminating with the anal opening.

In general, then, *P. alani* third instars possess structures generally characteristic of other cyclorrhaphous dipteran larvae, with the exception of the "yellow bodies" mentioned above. In their normal position, the "yellow bodies" are attached to the distal ends of the most anterior Malpighian tubules by tracheae and tracheoles which make connection with the membranous sac enclosing each yellow body. The abundance of tracheae associated with the "yellow bodies" suggest a structure capable of high metabolic activity. The fact that they are in close proximity to the Malpighian tubules suggest that they also may be involved as a storage site for pigmented waste materials. That the yellow bodies are in dynamic transition is indicated by their increase in size with each succeeding instar, then decrease in size during puparation, and absence 2 to 3 days into the pupal stage.

Bioassays conducted to test for the presence of auxins, giberellins, and cytokinins have shown that IAA or IAA-like activity is present in the "yellow bodies" (Hapai 1981). Indeed, implantation of "yellow bodies" into the subapical area of *A. riparia* stems produced tumors characterized by cell elongations (Hapai 1981), an effect reminiscent of IAA activity in stems and coleoptiles. A hormonal tissue response in *A. riparia* was also suggested by a positive relationship between tumor size and the number of implanted "yellow bodies" (Hapai 1981).

From the preliminary information so far obtained, it is hypothesized that the "yellow bodies" in *P. alani* may be repositories or sites of synthesis for IAA (indole acetic acid) or IAA-like substances that may be partly responsible for inducing gall formation in *A. riparia*. It is interesting to note that *P. utilis* was also found to contain "yellow bodies," and it is tempting to speculate that the function of these structures may also be identical to that in *P. alani*. Indeed, all external and internal characteristics attributed to *A. riparia* galls can also be found in *Eupatorium adenophorum* galls produced by *P. utilis*. A more complete study on this aspect will be presented in a later report.

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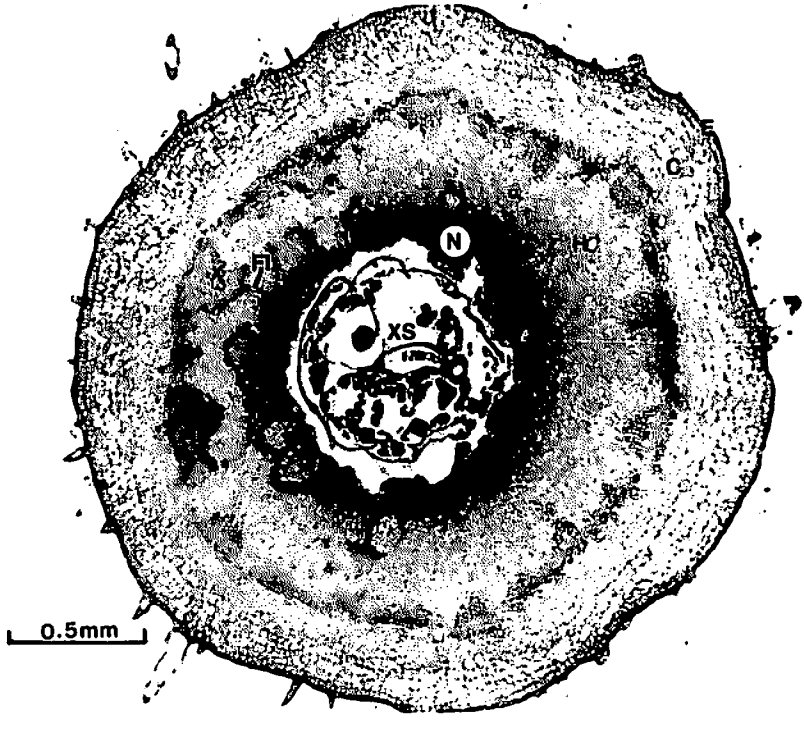

FIGURE 1. Cross-section of an early stage *A. riparia* gall containing a 2nd instar *P. alani.* Abbreviations: E, epidermis; C, cortex; H, hyperplasy of the pith parenchyma; FI, "fasceaux d'irrigation"; P, phloem; X, xylem; N, nutritive cells surrounding larval-made cavity; XS, larva within cavity.

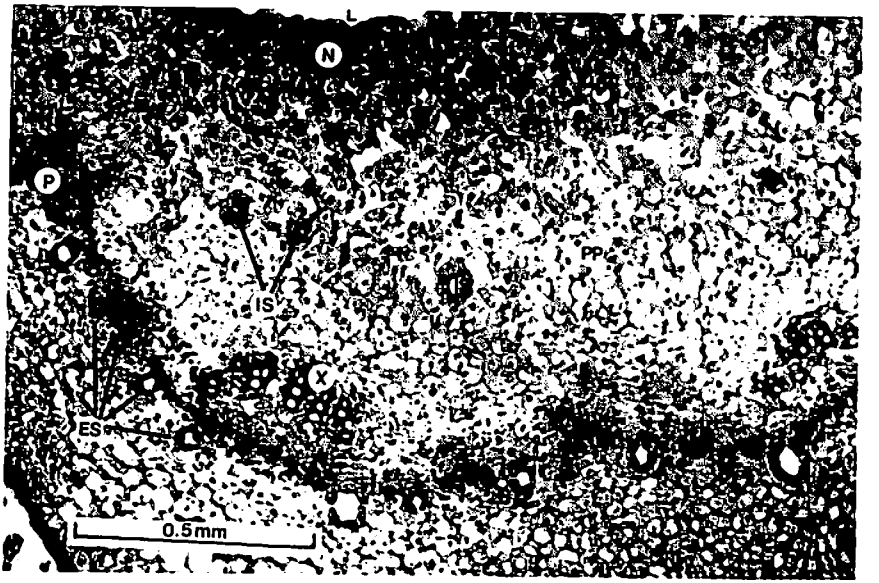

FIGURE 2. Stem cross-section of an insect-induced gall. The lower half of stem below larval cavity is shown. Abbreviations: X, xylem; IS, internal secretory duct; PP, pith parenchyma; N, nutritive cells; L, larval-made cavity; P, phloem; ES, external secretory duct.

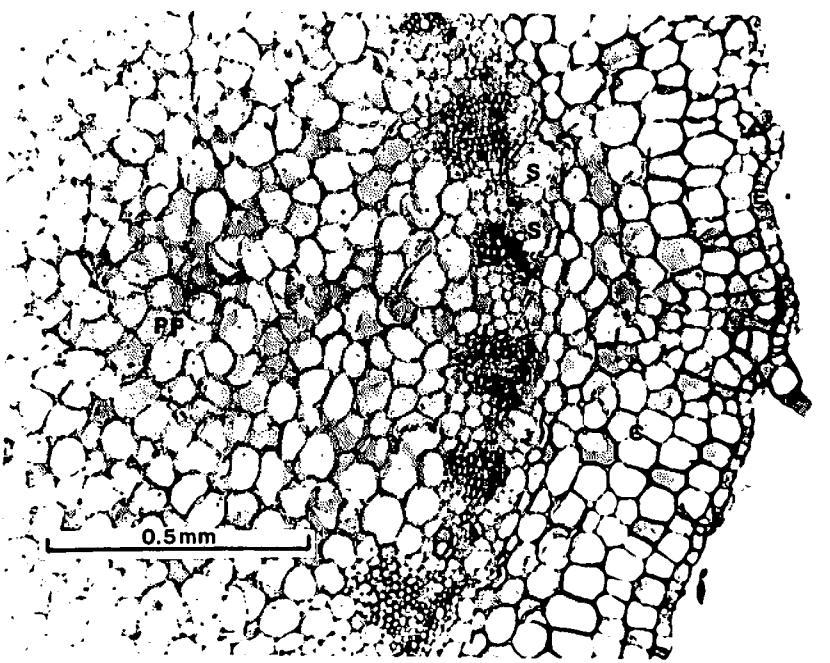

FIGURE 3. Cross-section of normal *A. riparia* stem showing normal size of cells within the central region of stem. Abbreviations: X, xylem; S, secretory duct; E, epidermis; C, cortex; PP, pith parenchyma; P, phloem.

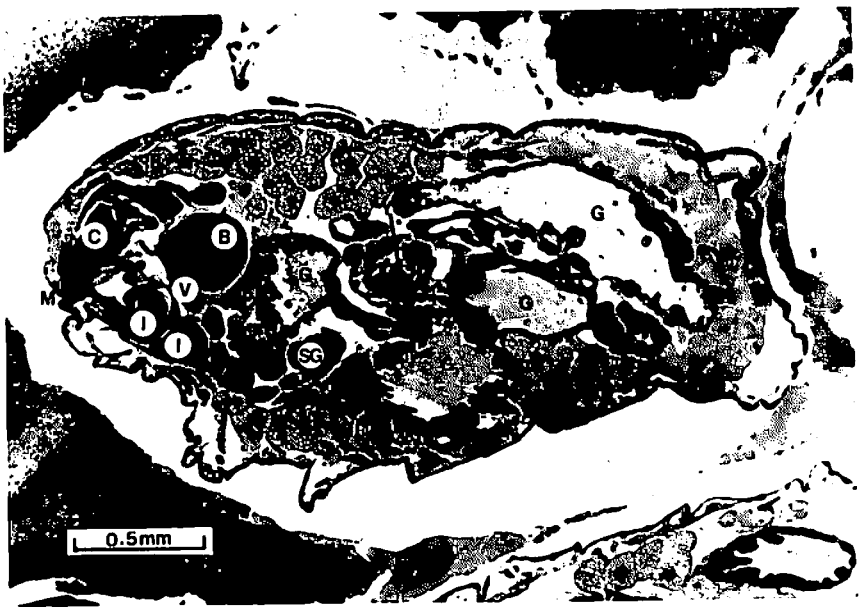

FIGURE 4. Longitudinal section of a 3rd instar *P. alani.* Abbreviations: G, gut; Y, yellow body; B, brain; F, fat body cells; C, cibarial pump; M, mouthhooks; I, imaginal disc; SG, salivary gland.

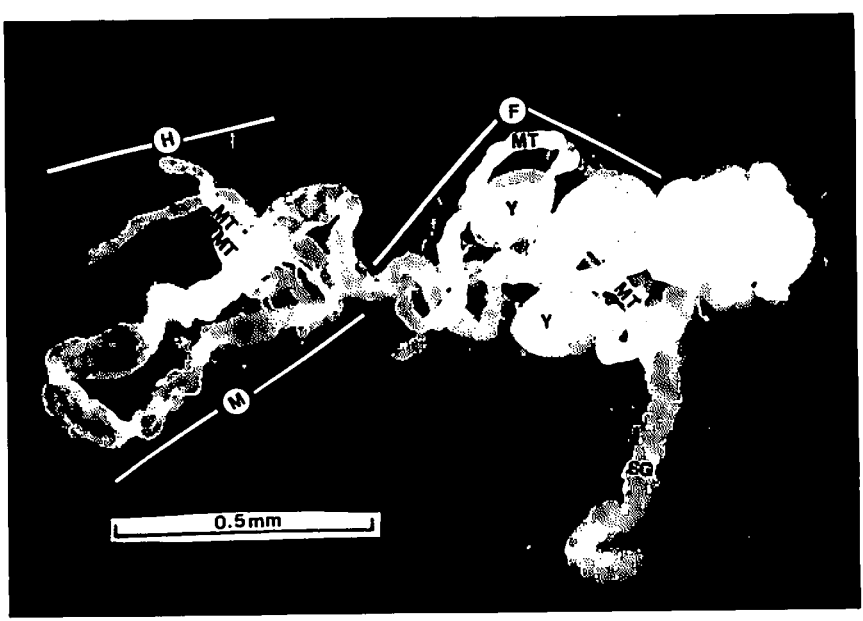

FIGURE 5. Internal organs of 3rd instar *P. alani* showing normal positioning of the viscera. Abbreviations: SG, salivary glands; Y, yellow body; MT, Malpighian tubule; F, foregut; M, midgut; H, hindgut.