Journal of Nematology 36(3):249–262. 2004.
© The Society of Nematologists 2004.

Histological Comparisons of *Fergusobia/Fergusonina*-Induced Galls on Different Myrtaceous Hosts[1]

R. M. Giblin-Davis,[2] B. J. Center,[2] K. A. Davies,[3] M. F. Purcell,[4] S. J. Scheffer,[5] G. S. Taylor,[3] J. Goolsby,[4] and T. D. Center[6]

Abstract: The putative mutualism between different host-specific *Fergusobia* nematodes and *Fergusonina* flies is manifested in a variety of gall types involving shoot or inflorescence buds, individual flower buds, stems, or young leaves in the plant family Myrtaceae. Different types of galls in the early-to-middle stages of development, with host-specific species of *Fergusobia/Fergusonina,* were collected from Australian members of the subfamily Leptospermoideae (six species of *Eucalyptus,* two species of *Corymbia,* and seven species of broad-leaved *Melaleuca*). Galls were sectioned and histologically examined to assess morphological changes induced by nematode/fly mutualism. The different gall forms were characterized into four broad categories: (i) individual flower bud, (ii) terminal and axial bud, (iii) 'basal rosette' stem, and (iv) flat leaf. Gall morphology in all four types appeared to result from species-specific selection of the oviposition site and timing and number of eggs deposited in a particular plant host. In all cases, early parasitism by *Fergusobia/Fergusonina* involved several layers of uninucleate, hypertrophied cells lining the lumen of each locule (gall chamber where each fly larva and accompanying nematodes develop). Hypertrophied cells in galls were larger than normal epidermal cells, and each had an enlarged nucleus, nucleolus, and granular cytoplasm that resembled shoot bud gall cells induced by nematodes in the Anguinidae.

Key words: Australia, Diptera, *Fergusobia, Fergusonina,* Fergusoninidae, fly, gall development, histology, life history, mutualism, Myrtaceae, Nematoda, nematode, Tylenchida.

Fergusobia (Currie) (Tylenchida: Neotylenchidae) nematodes and *Fergusonina* Malloch (Diptera: Fergusoninidae) flies are involved in the only known putative mutualism involving nematodes and insects (Giblin-Davis, 1993). This complex causes a variety of histioid (involving abnormal tissue production)-type galls in meristematic tissues of myrtaceous hosts in Australasia (Currie, 1937; Davies and Lloyd, 1996; Davies et al., 2001; Giblin-Davis et al., 2001b; Siddiqi, 1986; Taylor et al., 2004). Sequence comparisons within *Fergusonina* flies (mtDNA) and *Fergusobia* nematodes (rRNA) from different gall types, hosts, and geographical regions show strict host specificity within the Myrtaceae (Giblin-Davis et al., 2004; Scheffer et al., unpubl. data.).

The division of labor in the mutualism is still unclear, but the nematode appears to induce cecidogenesis before fly eggs hatch, and the fly, which may play a role in gall maintenance, is essential in sustaining and dispersing the nematode (Currie, 1937). All adult female flies contain nematodes but adult males do not (Currie, 1937). Female flies deposit eggs and juvenile nematodes in or near shoot or floral apices or leaf primordia. The nematodes feed and appear to initiate hypertrophied plant cells, and develop into parthenogenetic females (Currie, 1937; Giblin-Davis et al., 2001b). The fly eggs hatch after 1 to 2 months and the larva feeds on gall tissue and (or) secretions. The fly larva grows and molts twice while the nematodes lay eggs that produce male and female nematodes. Inseminated pre-parasitic female nematodes are infective and invade fully grown (3rd-instar) female fly larvae. Nematodes develop inside the fly into parasitic females without replacement of a new cuticle. The epidermis becomes hypertrophied with numerous epidermal microvilli that increase the absorptive surface area, putatively providing for more efficient nutrient acquisition by the parasitic nematode (Giblin-Davis et al., 2001a). The nematodes do not appear to over-exploit their fly host so some self-regulatory mechanism may be in effect. This parasitic stage develops large numbers of eggs that are deposited in the hemolymph of the fly. These nematode eggs hatch into female juveniles that invade the oviducts of the adult fly and are then deposited with fly eggs into a new plant host.

Currie (1937) and Tonnoir (1937) studied this interesting complex on *Eucalyptus* in Australia, and Currie (1937) designated several gall types as leaf, leaf–bud, axil-bud, leaf-stem, shoot-tip, stem-tip, and flower-bud. Morphology of gall types was recently re-examined (Taylor et al., 2004) and includes some additional, less common variants. Currie (1937) described the development of flower-bud galls formed on *E. macrorhyncha* (host of the fly *F. nicholsoni*) with brief references to flower-bud galls on *E. maculata* (host of the fly *F. eucalypti*), *E. blakelyi, E. camaldulensis,* and *E. tereticornis* (presumably all hosts of the fly *F. tillyardi*), and *E. hemiphloia, E. odorata, E. cebra,* and *E. melanophloia* (presumably all hosts of the fly *F. brimblecombei*). Flower-bud galls were

---

Received for publication 23 October 2003.

[1] Contribution of the Florida Agricultural Experiment Station, Journal Series R-09845.

[2] Fort Lauderdale Research and Education Center, Department of Entomology and Nematology, University of Florida/IFAS, 3205 College Ave., Davie, FL 33314.
[3] Discipline of Plant and Pest Science, and Centre for Evolutionary Biology and Biodiversity, Waite Campus, The University of Adelaide, PMB 1 Glen Osmond, South Australia, AUSTRALIA 5064.
[4] USDA Australian Biological Control Laboratory, PMB 3, 120 Meiers Road, Indooroopilly, Queensland, AUSTRALIA 4068.
[5] USDA-Agricultural Research Service, Systematic Entomology Lab, Bldg. 005, Rm. 137, BARC-W, 10300 Baltimore Ave., Beltsville, MD 20705.
[6] USDA-Agricultural Research Service, Invasive Plant Research Lab, 3205 College Ave., Davie, FL 33314.
E-mail: giblin@ufl.edu

The authors thank Jeff Makinson and Jim Plaskowitz for technical support and Bill Howard and Thomas Weissling for review of the manuscript. This project was funded, in part, by USDA-ARS Specific Cooperative Agreement No. 58-6629-9-004 from the USDA Invasive Plants Research laboratory and a USDA Special Grant in Tropical and Subtropical Agriculture CRSR-03- 34135-8478.
This paper was edited by S. Patricia Stock.

reportedly manifested in several ways: (i) multilocule gall, with individual membrane-bound locules derived from hypertrophied and fused-anther primordia and an unfused detachable operculum for release of adult flies (e.g., *E. macrorhyncha*); (ii) internally fused (nonmembrane bound) multilocule gall, with locules distributed throughout a hypertrophied and fused mass of staminal ring/floral disc/ovary origin with a fused operculum with fly release occurring through tissue disintegration (e.g., *E. maculata* and the *Eucalyptus* hosts of *F. tillyardi*); and (iii) partially internally fused multilocule with nonmembrane bound locules, with hypertrophied and fused mass of floral disc/ovary origin and nonfused operculum (e.g., the *Eucalyptus* hosts of *F. brimblecombei*). In addition, Currie (1937) briefly described "warty" multilocule galls on the leaf tips of *E. gomphocephala* and *E. maculosa,* multilocule shoot-tip or stem-tip galls on *E. rudis* (host of the fly *F. lockharti*) and *E. macrorhyncha* (host of Currie's *Fergusonina* sp. 3), and leaf galls formed by two young leaves fusing together on *E. stuartiana* (now *E. bridgesiana,* host of Currie's *Fergusonina* sp. 7). Recently, Giblin-Davis et al. (2001b) studied the development of shoot-bud galls induced by *Fergusobia/Fergusonina* from *Melaleuca quinquenervia,* and Taylor et al. (2004) described a wide variety of different *Fergusobia/Fergusonina* gall manifestations on members of the Myrtaceae.

The purpose of this study was to expand the histological observations of Currie (1937) and Giblin-Davis et al. (2001b) by comparing different gall types in the early-to-mid stage of development from host-specific species of *Fergusobia/Fergusonina* from species of *Eucalyptus, Corymbia,* and *Melaleuca* from Australia.

Materials and Methods

Uni- and (or) multilocule galls were collected during a 1999 survey of *Fergusobia/Fergusonina* isolates from different myrtaceous hosts from different geographical regions in Queensland and South Australia, Australia. Galls were halved to confirm *Fergusobia/Fergusonina* parasitism, and flies and nematodes from infested galls were collected as vouchers and for DNA sequencing. The other half of each gall was fixed in formalin, acetic acid, ethanol (5:5:90). Some specimens were retained for scanning electron microscopy (SEM), whereas others were dehydrated in a tertiary butyl alcohol series and embedded in paraffin as per Johansen (1940). In several cases, noninfested buds from the same tree were collected into fixative and processed for sectioning. Embedded buds/galls were sectioned 11 µm thick, mounted on slides treated with Mayer's albumin (50 ml fresh egg albumin, 50 ml glycerin, and 1 g sodium salicylate), stained with 1% aqueous safranin for 90 minutes and 0.5% fast green in clove oil and 100% ethanol (1:1) for 15 seconds, and then examined and photographed using a compound photomicroscope. Measurements were done using a calibrated ocular micrometer.

For SEM, buds were taken from the FAA fixative, rough-sectioned with a razor blade, rinsed twice in distilled water, post-fixed in 4% OsO_4, dehydrated through an ethanol series to 100%, and processed to dryness using liquid CO_2 with a critical point dryer. Specimens were mounted on stubs using double sticky tape, sputter-coated with gold-palladium, and viewed with a JEOL T300 scanning electron microscope at 15 kV.

Results and Discussion

Fergusobia/Fergusonina galls were recovered from 15 myrtaceous host plants from the subfamily Leptospermoidea: six species of *Eucalyptus,* two species of *Corymbia,* and seven species of *Melaleuca. Eucalyptus camaldulensis* and *Melaleuca nervosa* were each found to host two morphologically distinct gall types. Gall forms were broadly characterized into four categories based on external appearance and location of the gall: (i) individual flower bud, (ii) terminal and axial bud, (iii) 'basal rosette' stem, and (iv) flat leaf. The histology of each of these types of galls and their variants is described and discussed.

Flower bud galls: Fergusobia/Fergusonina-induced flower bud galls were several times larger than corresponding noninfested buds in an inflorescence. The smaller flower buds of *E. macrorhyncha, E. obliqua,* and *E. camaldulensis* (Figs. 1–3) allowed for multilocule production of 3 to 15 flies compared with the proportionally larger flower buds of *Corymbia ptychocarpa* (Fig. 4) where multilocule production of hundreds of flies was observed.

Histology of flower bud galls from *E. macrorhyncha* and *E. obliqua* appeared similar to the histological description of *E. macrorhyncha* by Currie (1937). The flies in sectioned material were second-instars, and the nematodes were males and parthenogenetic females. Infested flower buds were club-shaped as opposed to the diamond shape of noninfested flower buds (Figs. 1A;2A). In both of these hosts, locules appeared to originate from a proliferation of anther cells that were bounded by a layer of safranin-staining cells (Figs. 1,2). In *E. macrorhyncha,* there were eight or more locules per gall with outside measurements averaging 0.61 by 0.77 mm and lumen measurements averaging 0.27 by 0.32 mm. Two multilocule galls from *E. obliqua* were sectioned. In the gall with three locules, the outside measurements averaged 0.90 by 1.43 mm and their lumens averaged 0.52 by 0.85 mm. The gall with four locules was misshapen with concave locules averaging 0.61 × 0.97 mm, and their lumens averaged 0.33 × 0.37 mm. Each locule was attached to the floret wall by an anther filament. Filaments and their vascular elements in the infested florets were 2 to 3 times the diameter and

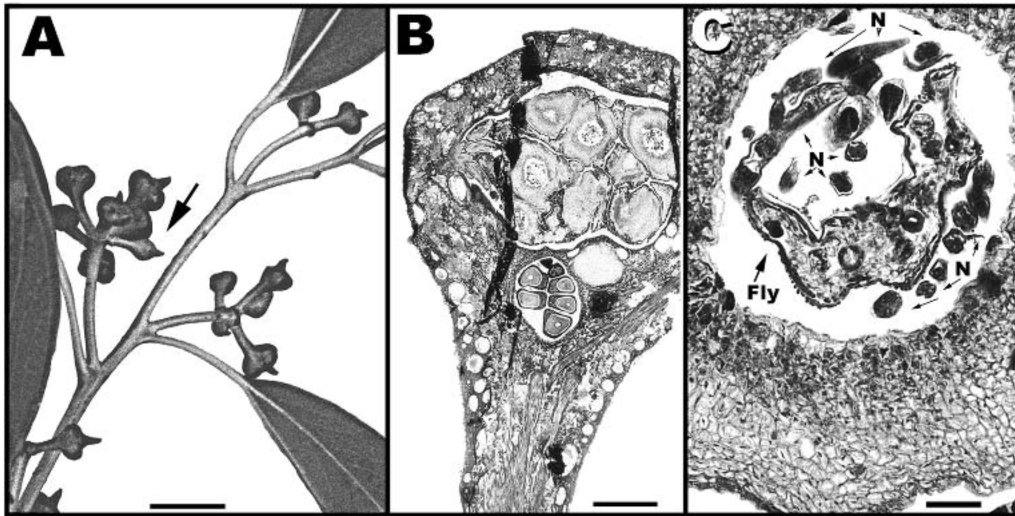

FIG. 1. *Fergusobia/Fergusonina*-induced flower bud galls on *Eucalyptus macrorhyncha.* A) Galled flower buds; arrow points to the only healthy flower bud. B) Histological section of a galled flower bud with several membrane-bound locules in anther chamber. C) Close-up of locule with typical hypertrophied cells lining the lumen and *Fergusobia* nematodes (N) and a larval *Fergusonina* (Fly) inside. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 50 µm.

stained more intensely than those in noninfested florets (Fig. 2B,C). In general, the cells on the outside of the locule were rounded and lightly stained but became larger and more granular and deeply stained with an increase in size of the nucleus and nucleolus near the lumen of each locule. These hypertrophied tissues were 4 to 7 cells thick in *E. macrorhyncha* (Fig. 1C) and 4 to 12 cells thick in *E. obliqua* (Fig. 2D). The cells at the surface of the lumen often appeared to have lost their integrity, producing purple-staining mucilage, which lined the lumen surface. In *E. macrorhyncha,* vascular tissues were scattered throughout the locule, but in *E. obliqua* they were seen only in the area where the filaments attached. The stigma in *E. obliqua* was developed, growing into the region where the locules were located. The stigma was not observed in the gall of *E. macrorhyncha.* The presence of an operculum in both hosts suggested that flies used it as a means of release from the gall.

Our observations of the histology of *Fergusobia/Fergusonina* induced flower bud galls from *E. camaldulensis* were similar to Currie's (1937) description for galls of *F. tillyardi.* The flies were first-instars, and the nematodes were juveniles and parthenogenetic females. The galled tissue appeared to be a proliferation of the disc and tissues from the base of the stigma (Fig. 3C). The ovaries appeared similar to those in noninfested florets (Fig. 3B). The anthers were not seen, but there were small pockets of red-staining and compressed-looking cells similar to filament cells in nonin-

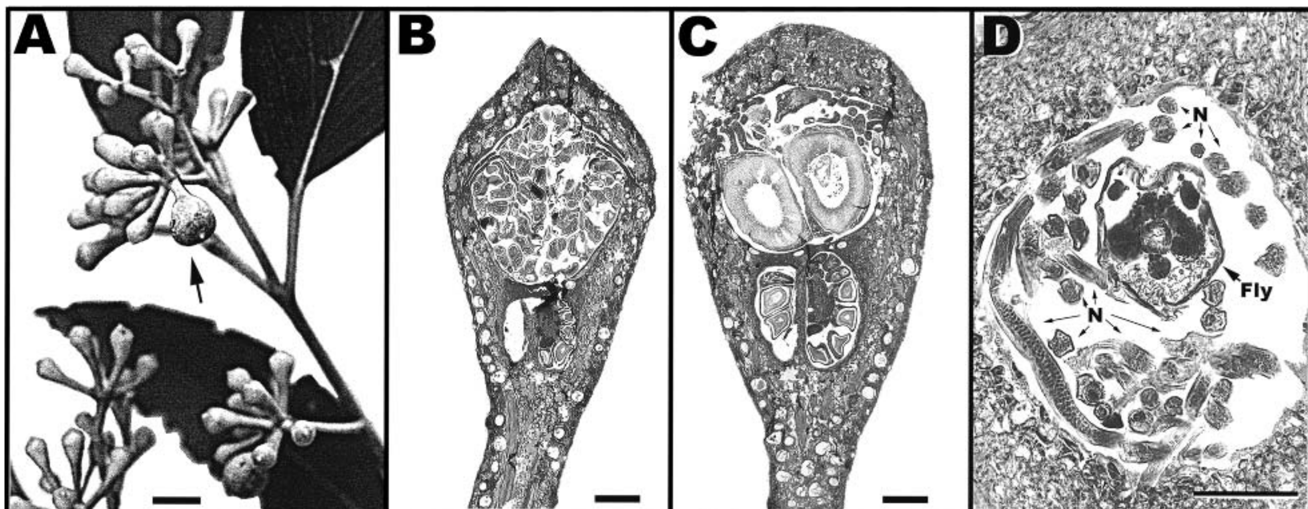

FIG. 2. *Fergusobia/Fergusonina*-induced flower bud galls on *Eucalyptus obliqua.* A) Galled and healthy flower buds; arrow points to a galled bud. B) Histological section of a healthy flower bud. C) Histological section of a galled flower bud with two membrane-bound locules visible in anther chamber. D) Close-up of locule with typical hypertrophied cells lining the lumen and *Fergusobia* nematodes (N) and a larval *Fergusonina* (Fly) inside. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 1 mm. D) Bar = 100 µm.

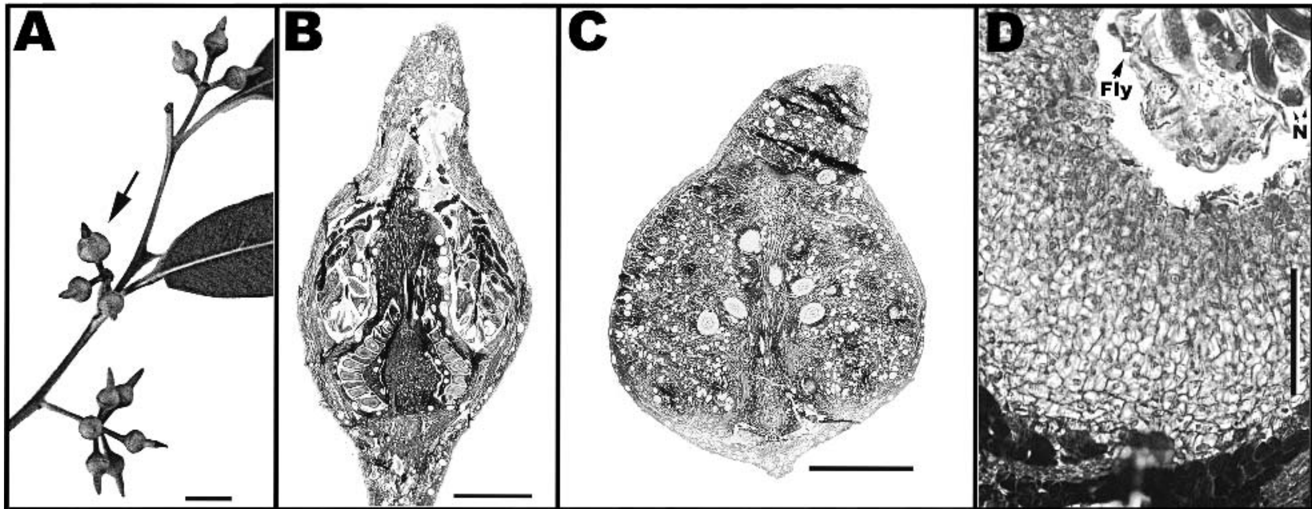

Fig. 3. *Fergusobia/Fergusonina*-induced flower bud galls (*F. tillyardi*) on *Eucalyptus camaldulensis.* A) Galled and healthy flower buds; arrow points to only galled bud. B) Histological section of a healthy flower bud. C) Histological section of a galled flower bud with several locules visible in proliferation of disc and stigma tissue. D) Close-up of locule with typical hypertrophied cells lining the lumen and *Fergusobia* nematodes (N) and a larval *Fergusonina* (Fly) inside. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 2 mm. D) Bar = 100 µm.

fested florets. The matrix of the gall was mostly safranin-stained cells with many scattered oil ducts and some vascular tissues. The locules scattered within this matrix were small, averaging 0.30 × 0.32 mm, with the lumen averaging 0.09 × 0.11 mm (Fig. 3C). Locules were bounded by a one- to two-cell-thick layer of purple-stained cells with an internal layer of granular, purple-staining cytoplasm. The cytoplasm of the cells of the locule appeared denser and more deeply stained near the lumen with the nucleus and nucleolus of each cell appearing enlarged (Fig. 3D). The lumens were not rounded like those in *E. obliqua* and *E. macrorhyncha* but had an irregular shape. The absence of an operculum suggested that flies employed a different method to escape from the gall.

Flower bud galls of *C. ptychocarpa* were not examined by Currie (1937). The flies were first- or second-instars or eggs, and the nematodes in the locules with fly larvae were males and parthenogenetic females. The locules appeared to originate from anther primordial cells as observed for *E. obliqua* and *E. macrorhyncha* (Currie, 1937). The disc tissue at the bottom of the floret with locules nearby was also proliferating (Fig. 4C). The locules were in a matrix of safranin-staining cells with scattered vascular tissues that were probably derived from anther filaments (Fig. 4C). Within this were small, flat-

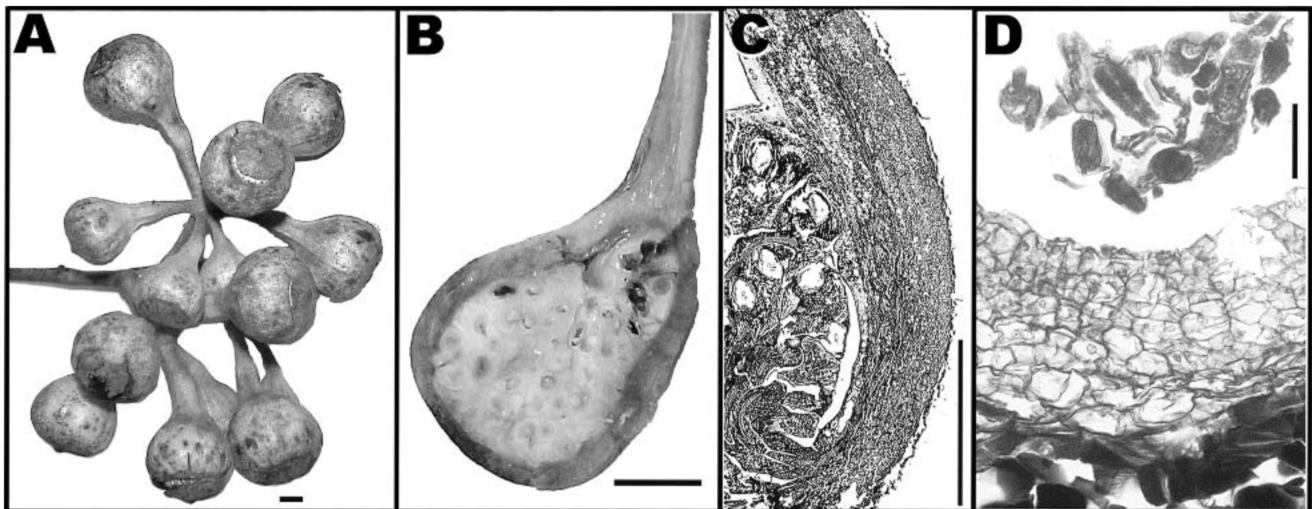

Fig. 4. *Fergusobia/Fergusonina*-induced flower bud galls on *Corymbia ptychocarpa.* A) Galled flower buds. B) Rough sectioned galled flower bud exposing many locules with *Fergusonina* larvae. C) Histological section of a galled flower bud with multiple locules in anthers and floral disc. D) Close-up of locule with typical hypertrophied cells lining the lumen and *Fergusobia* nematodes and a larval *Fergusonina* inside. A, B, C bar = 1 cm; D bar = 50 µm.

tened anthers with light blue-green staining cells, and the locules appeared to be in contact with these tissues. The locules with unhatched eggs were about half the size of the locules with first-instar fly larvae. These locules had small, dense, deeply stained cells with straight cell walls. The locules with first-instar fly larvae had cells that were less dense or clear with wrinkled walls, sometimes grading to cells that were flattened at the edge of the locule (Fig. 4D). The outside dimensions of these locules averaged 0.49 × 0.71 mm with their lumens averaging 0.19 × 0.29 mm. At the outer edge of the locules, the cells stained solidly purple, with the cells to their interior also staining purple but with granular cytoplasm and enlarged nucleus and nucleolus.

Terminal and axial bud galls: Terminal and axial bud galls were found on all three genera examined: *Eucalyptus, Melaleuca,* and *Corymbia.* In general, these galls involved the entire bud in a fused spheroidal mass of tissue with pupal windows for fly release. Galls were composed of a conglomeration of rigid but relatively soft plant tissue that was easily sectioned with a razor blade, except for the galls from *Corymbia* that were lignified and difficult to cut. In some forms, individual locules at the periphery of a multilocule gall could be observed as the gall matured, giving it the appearance of a bunch of grapes. Typically, these galls were 0.5 to 2 cm in diam. and ranged in color from different shades of green to mixtures of green and yellow, orange, pink, or red. Bud galls from *Eucalyptus* (Figs. 5,6) and *Corymbia* (Fig. 7) were glabrous, whereas galls on *Melaleuca* were trichomatous. Galls on *M. dealbata* (attributed to the fly *F. makinsoni,* Taylor, 2004) were usually hairy (Fig. 8), whereas galls on *M. quinquenervia* (*F. turneri*) and *M. viridiflora* (*F. burrowsi*) often had a large number of hairs although some did not. Galls on *M. fluviatilis* (Fig. 9) and *M. leucadendra* (*F. centeri*) (Fig. 10) usually had few hairs.

Terminal bud galls from Eucalyptus camaldulensis and E. diversifolia (Figs. 5,6): The *Fergusobia/Fergusonina*-induced terminal bud galls in both of these species varied in size (Figs. 5A;6A). However, the galls on *E. camaldulensis* were usually 2 to 10 times larger than bud galls in the broad-leaved *Melaleuca* species, *C. tessellaris,* and *E. diversifolia* (Fig. 5A). The flies in *E. camaldulensis* galls were third-instars or pupae, and many of them had left their locules and tunneled to the gall edge. The associated nematodes were heterosexual adults and juveniles. The flies in the *E. diversifolia* gall were third-instars, and the nematodes were heterosexual adults and juveniles.

The epidermis and matrix parenchymal cells of the *E. camaldulensis* gall appeared empty and stained light blue-green (Fig. 5B,C). The matrix had a few small vascular tissues scattered throughout (Fig. 5B,C). No oil glands were seen in these galls. There was no distinct demarcation between the locules and the matrix of the gall (Fig. 5C). The typical *Fergusobia/Fergusonina*-type hypertrophied tissue was two to nine cells thick and appeared wrinkled or collapsing (Fig. 5C). The cells around these locules had large nuclei and nucleoli. In the vacated *E. camaldulensis* locules, the cells either lacked hypertrophy or had hypertrophied cells that stained solidly red with nuclei that were not always readily visible. These locules also contained a few nematodes.

The *E. diversifolia* gall had a densely red-staining epidermis. Inside this cell layer was a layer of red-staining cells, five to seven cells thick, containing scattered oil

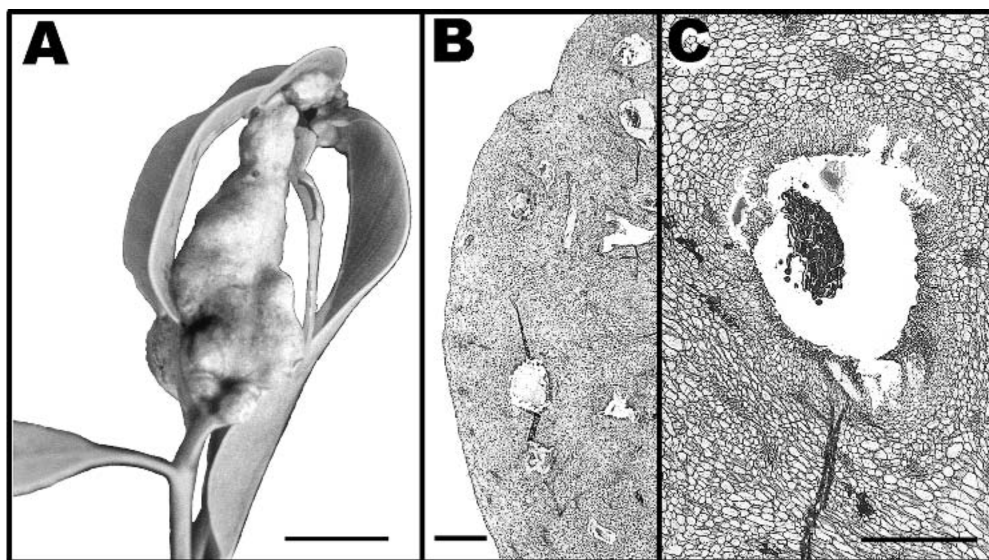

Fig. 5. *Fergusobia/Fergusonina*-induced shoot bud galls on *Eucalyptus camaldulensis.* A) Galled terminal shoot bud. B) Histological section of a galled shoot bud with many locules. C) Close-up of a locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and *Fergusonina* larva inside. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 500 μm.

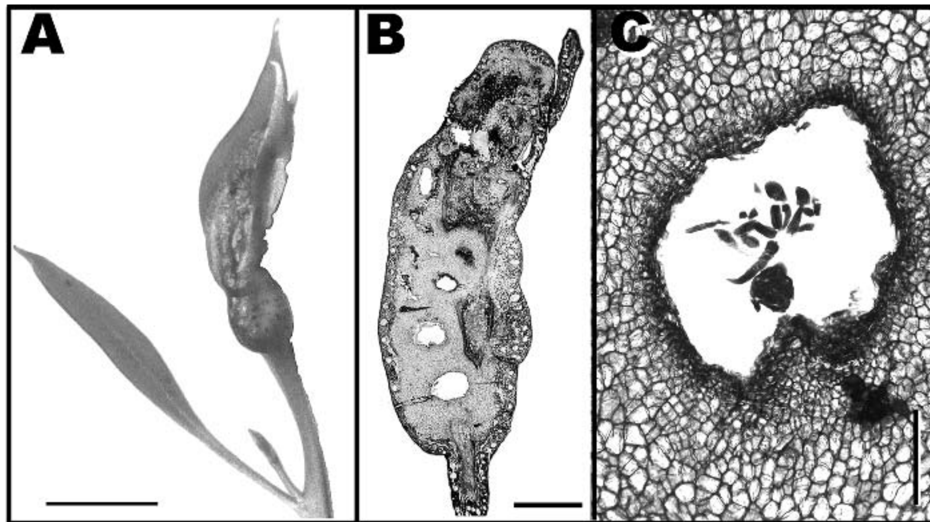

Fig. 6. *Fergusobia/Fergusonina*-induced shoot bud galls on *Eucalyptus diversifolia.* A) Galled terminal shoot bud. B) Histological section of a galled shoot bud with several locules. C) Close-up of a locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and *Fergusonina* larva inside. A) Bar = 1 cm. B) Bar = 2 mm. C) Bar = 200 μm.

glands. The matrix of the gall consisted mostly of red or blue-green staining, vacuolated-looking parenchymal cells with some large vascular tissues scattered within (Fig. 6B). There was an apparent flattening of red cells around the locules in this species (Fig. 6C). Typical granular-looking hypertrophied cells with enlarged nuclei and nucleoli lined the lumen of each locule two to six cell layers deep (Fig. 6C).

Terminal and axial bud galls from Corymbia tessellaris (Fig. 7): These galls were typically round to elliptical in shape and ranged in size from less than 0.6 to over 1.6 cm in diam. (Fig. 7A). As stated above, these galls were often very difficult to slice with a razor blade but yielded large numbers of nematodes and were often packed with much higher numbers of locules (>100) than similar-sized terminal and axial bud galls from *Melaleuca.* Preliminary morphological examinations of the nematodes from this gall type suggest that it is the rediscovered *Fergusobia magna* that was originally described by Siddiqi (1986) from shoot bud galls from an undetermined *Eucalyptus* species from Brisbane in Queensland, Australia.

Two *Fergusobia/Fergusonina*-induced bud galls were sectioned from this host species. The younger one was a terminal bud gall with first-instar flies and parthenogenetic female and juvenile nematodes. The older gall was an axial leaf bud gall with third-instar flies and nematodes that were mostly males with some parthenogenetic females. The young gall had a matrix of densely red-staining cells and lightly blue-green-staining parenchymal cells. Vascular tissues and oil ducts were seen only on the edge of this gall (Fig. 7B). The interior two

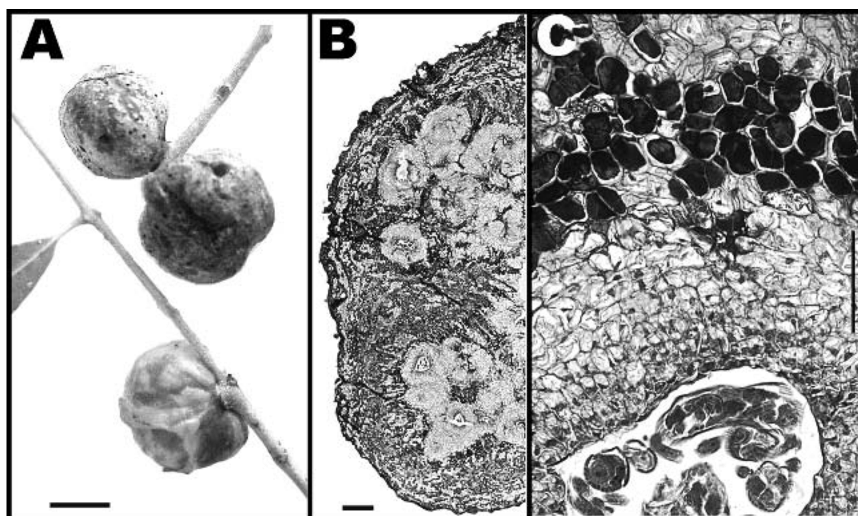

Fig. 7. *Fergusobia/Fergusonina*-induced shoot bud galls on *Corymbia tessellaris.* A) Top: two 'woody' axial galls after fly emergence. Bottom: galled axial shoot bud. B) Histological section of a galled shoot bud with multiple locules. C) Close-up of locule with hypertrophied cell lining the lumen and *Fergusobia* nematodes and a *Fergusonina* larva inside. A) Bar = 1 cm. B) Bar = 500 μm. C) Bar = 100 μm.

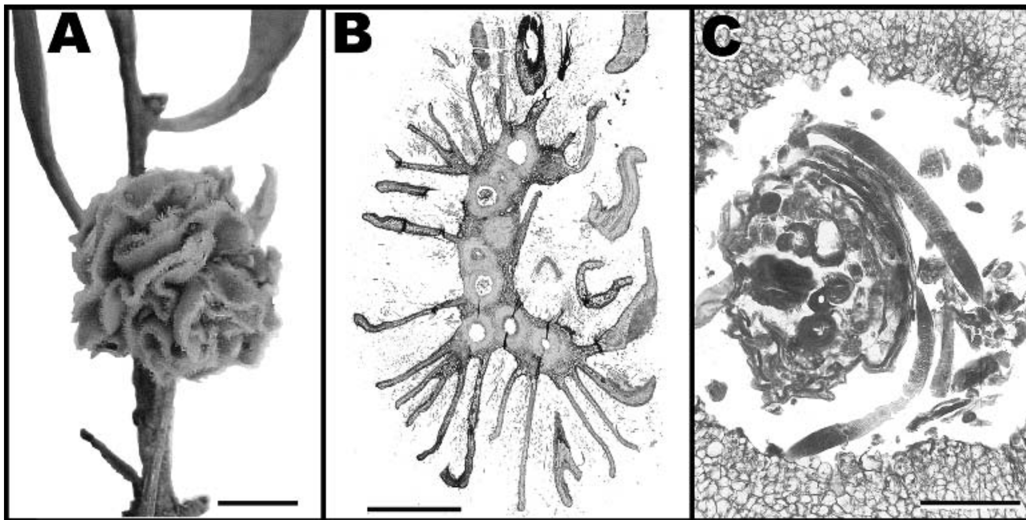

Fig. 8. *Fergusobia/Fergusonina*-induced shoot bud galls (*F. makinsoni*) on *Melaleuca dealbata*. A) Galled axial shoot bud. B) Histological section of a galled shoot bud with multiple locules. C) Close-up of locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and a larval *Fergusonina* inside. A) Bar = 1 cm. B) Bar = 2 mm. C) Bar = 100 μm.

thirds of this gall was composed mainly of the locules (0.21–0.40 mm lumens), many with coalesced edges (Fig. 7B). In some areas, the cells between the locules stained red and were compressed and broken down. The red-staining cells of the matrix were purple where they contacted the locules. Interior to this layer of cells,

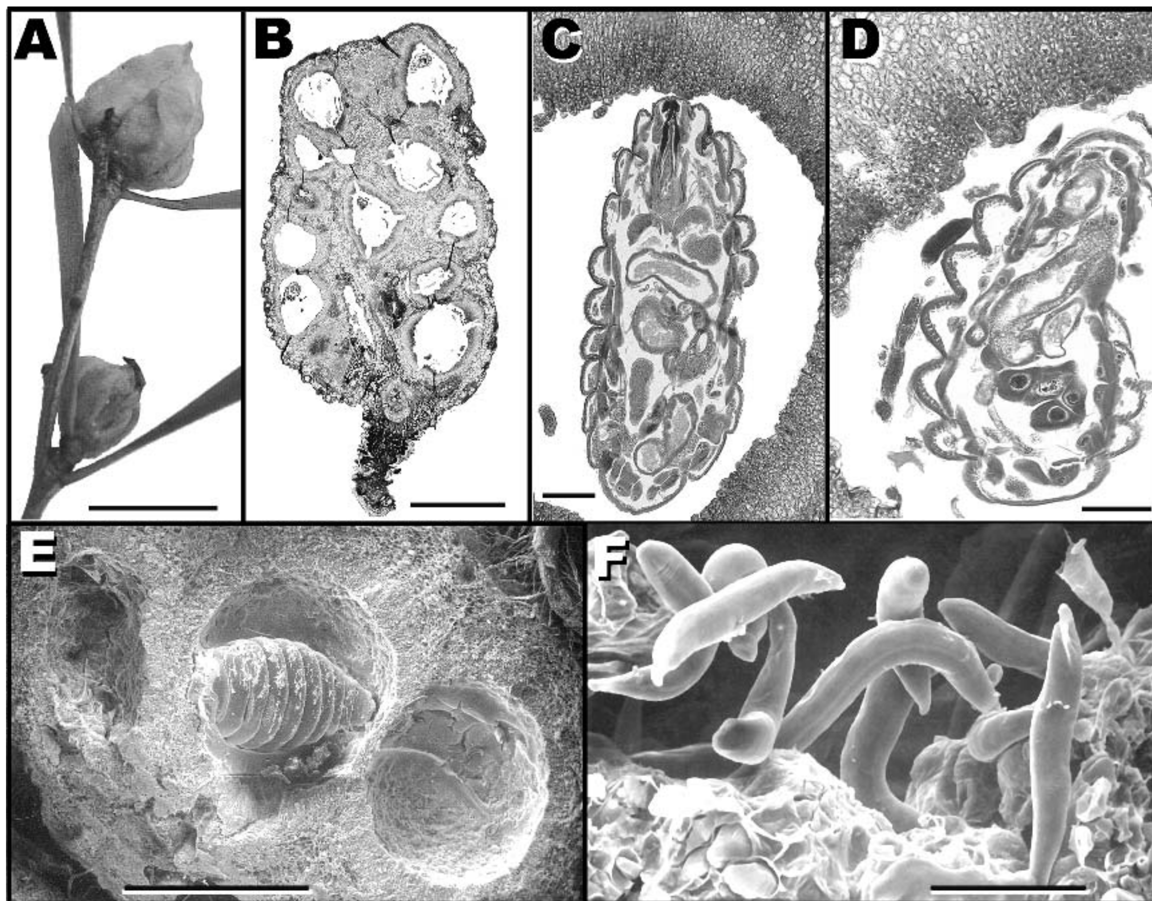

Fig. 9. *Fergusobia/Fergusonina*-induced shoot bud galls on *Melaleuca fluviatilis*. A) Galled terminal and axial shoot buds. B) Histological section of a galled shoot bud with multiple locules. C) Close-up of locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and a larval *Fergusonina* inside. D) Close-up of locule with hypertrophied cells, larva and nematodes. E) SEM micrograph of sectioned galled shoot bud. F) SEM close-up of nematodes on the lumen interior of a locule. A) Bar = 1 cm. B) Bar = 2 mm. C, D, F) Bar = 100 μm. E) Bar = 1 mm.

the hypertrophied cells stained purple with granular cytoplasm and enlarged nuclei and nucleoli (Fig. 7C). In some of these locules, the hypertrophied cells were irregular in shape and lost their integrity at the interior edge of the lumen. The surface of the lumen was often lined with a large amount of red or purple-staining exudate.

The older gall had a 15-to-20-cell-thick layer of solid, red-staining cells. Vascular bundles were regularly spaced around the edge of this layer. Interior to this, the matrix consisted of sclerofied cells with thick, pink-staining walls, which were either empty or filled solidly with a red-staining material. In addition, there were small numbers of light blue-green staining parenchymal cells within this matrix. The red contents of the sclerofied cells attained a purple color where they contacted the locules. Interior to these cells were purple-stained cells with thin walls. Within this layer, the cells stained blue with their nuclei and nucleoli becoming visible. The cells interior to these had granular cytoplasm that stained red or purple. The cell walls of the hypertrophied cells were crinkled, flattened, or torn. This gall consisted almost entirely of densely packed locules (0.80 to 1.12 mm lumens). Individual locules lacked the outer perimeter of red-staining cells seen in other types of galls.

Terminal and axial bud galls from Melaleuca dealbata, M. fluviatilis, M. leucadendra, M. quinquenervia, and M. viridiflora (Figs. 8–12): Two galls were sectioned from *M. dealbata*. The flies in the terminal bud gall were third-instars, and the nematodes were males and parthenogenetic females. The flies in the axial bud gall were third-instars and one second-instar that was molting to a third-instar. Male nematodes were observed in this gall. All *Fergusobia/Fergusonina*-induced bud galls observed from *M. dealbata* exhibited an extensive proliferation of leafy tissue from the central bud gall, giving the unique outward appearance of a head of bib lettuce (Fig. 8A,B). The sectioned terminal bud gall was long and slender with the locules arranged more or less in a row along the axis of the stem (Fig. 8B). The locules occupied most of the interior of the gall. Wrinkled fins of leafy tissue extended from the galled region (Fig. 8B). The matrix of this gall consisted mostly of densely staining red or purple cells with a few blue-green staining parenchymal cells. Oil glands and vascular tissues were scattered throughout these tissues. The outside dimensions of the locules averaged 0.85 × 0.87 mm with their lumens averaging 0.41 × 0.49 mm (Fig. 8B). The outside of the locules had blue-green staining cells. In the inner part of the locule for several cells deep, the cells exhibited typical hypertrophy, staining purplish-red with granular cytoplasm and enlarged nuclei and nucleoli (Fig. 8C). The cells on the surface of the lumen broke down leaving a blue-staining exudate. The axial bud gall was round with the locules evenly spaced inside. The matrix of this gall was similar to that of the terminal bud gall, but there were more and larger oil glands. The locules averaged 0.83 × 0.58 mm, and their lumens averaged 0.69 × 0.44 mm and were separated from one another and the matrix by a purple-staining ring of cells.

An axial bud gall of *M. fluviatilis* was sectioned and was found to contain third-instar flies and only male nematodes. This gall had a purple-staining epidermis and a layer of red, solidly staining cells two to seven cells thick. There were a few large oil ducts between these cells and the matrix of the gall (Fig. 9B). The matrix consisted of lightly staining blue-green parenchymal cells with scattered vascular tissues. The locules averaged 1.36 × 1.10 mm. There was no ring of purple cells where locules contacted the matrix. The lumen averaged 1.09 × 0.74 mm with deep furrows (Fig. 9B,D). The typical hypertrophied cells lining the locule lumen were pronounced and 4 to 14 cells thick (Fig. 9C,D). In outward appearance, these galls appeared typical for broad-leaved *Melaleuca* bud galls (Fig. 9A), except that as they dried they often exhibited a rugose surface. Observations with SEM demonstrated that hypertrophied cells near the lumen of each locule appeared denser than typical ground parenchymal cells (Fig. 9E). Also, nematodes were observed in each locule (Fig. 9E,F) but none were observed attached to the hypertrophied cells.

The gall sectioned from *M. leucadendra* was a terminal bud gall. The galls from this host were almost glabrous in appearance (Fig. 10A). The flies were third-instars. The nematodes were adults. The epidermis of the gall stained purple with a layer of solidly red or purple-staining cells one to six layers thick. Many oil ducts were located along the outside edge of the gall (Fig. 10B). The matrix of the gall was composed of empty, light purple-staining cells, red-staining cells, and solidly red- or purple-staining cells. Vascular tissue was observed in the matrix. The locules averaged 0.86 × 0.60 mm, and their lumens averaged 0.62 × 0.45 mm (Fig. 10B) and frequently contained groups of isolated plant cells. There was an apparent breakdown of hypertrophied cells adjacent to the lumen. Each of these cells had a large nucleus and nucleolus (Fig. 10C). The cells in the ring around the locules had thicker cell walls than cells of the matrix (Fig. 10C). The locule lumens were lined with a large amount of purple-staining exudate.

Two axial bud galls were examined for *M. quinquenervia*. The flies in both galls were first-instars and the nematodes were parthenogenetic females. These field-collected galls appeared similar to those observed with caged flies (Fig. 11A) (Giblin-Davis et al., 2001b). The epidermis was stained densely red. The matrix was composed of blue-green staining parenchymal cells and had vascular tissues scattered within it. The matrix cells near the locules also stained red. Oil glands were located along the edges of the gall. These locules averaged 0.41 × 0.35 mm with their lumens averaging 0.28 × 0.24 mm

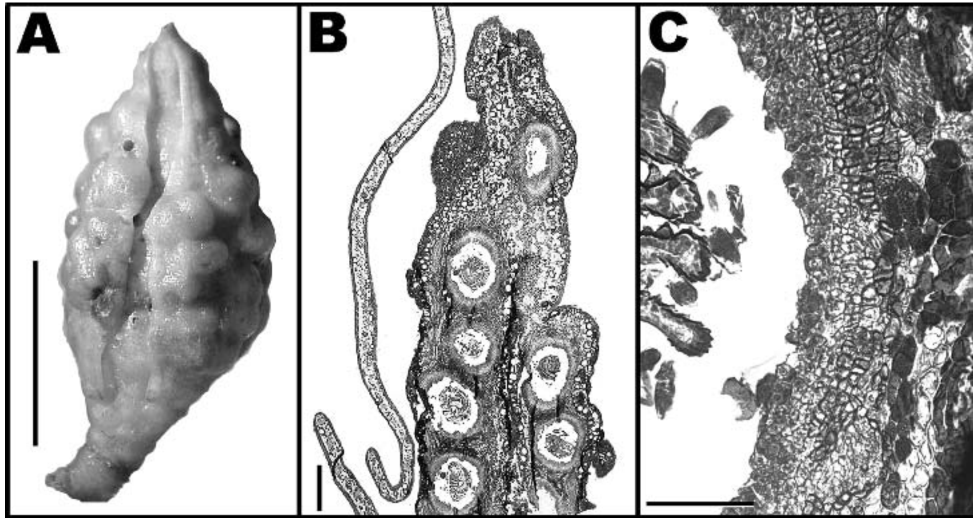

FIG. 10. *Fergusobia/Fergusonina*-induced shoot bud galls (*F. centeri*) on *Melaleuca leucadendra.* A) Galled terminal shoot bud. B) Histological section of a galled shoot bud with multiple locules. C) Close-up of locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and a larval *Fergusonina* inside. A) Bar = 1 cm. B) Bar = 2 mm. C) Bar = 100 μm.

(Fig. 11B). The typical hypertrophied cells that lined the locules stained red, were three to eight cells thick, and were irregular in shape (Fig. 11C). A purple- or red-staining exudate often occurred at the lumen surface.

One terminal bud gall was examined for *M. viridiflora*. The flies in the gall were third-instar larvae and pupae and the nematodes were heterosexual adults and parthenogenetic females, when present. The matrix consisted of lightly staining blue-green parenchymal cells with scattered vascular tissues (Fig. 12B). Because of the late developmental age of the gall, most of the locules had been stripped of hypertrophied cells by last-instar larvae (Fig. 12C) and small windows with only a single layer of purple-staining epidermis were observed that serve for adult fly escape. In general, this gall looked very similar to other broad-leaved *Melaleuca* galls (Fig. 12A).

Terminal and axial bud gall, 'Leaf gall variant' from Melaleuca stenostachya (Fig. 13): Galls of this variant (attributed to the fly *Fergusonina* sp. 2, Taylor, 2004) exhibited extensive leaf folding (back and forth on itself) in the region of the infested bud (Fig. 13A). The whole gall measured 2.8 × 2.6 mm and contained one locule with a second-instar fly larva, and parthenogenetic female nematodes. The outer layer of the gall comprised rectangular, purple-staining leaf epidermal cells. Under the epidermal layer on one side of the gall there were about two layers of palisade parenchyma, which stained red with red-staining chloroplasts (Fig. 13B). Inside this layer and the epidermal layer on the other side of the gall were spongy parenchymal cells with blue-green-to-

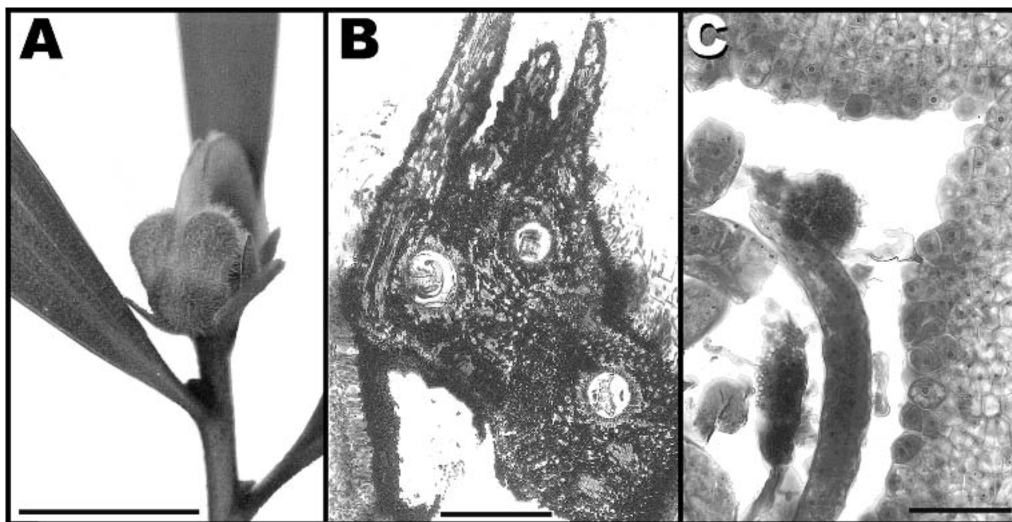

FIG. 11. *Fergusobia/Fergusonina*-induced shoot bud galls (*F. turneri*) on *Melaleuca quinquenervia.* A) Galled terminal shoot bud. B) Histological section of a galled shoot bud with three locules. C) Close-up of a locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and *Fergusonina* eggs inside. A) Bar = 1 cm. B) Bar = 500 μm. C) Bar = 50 μm.

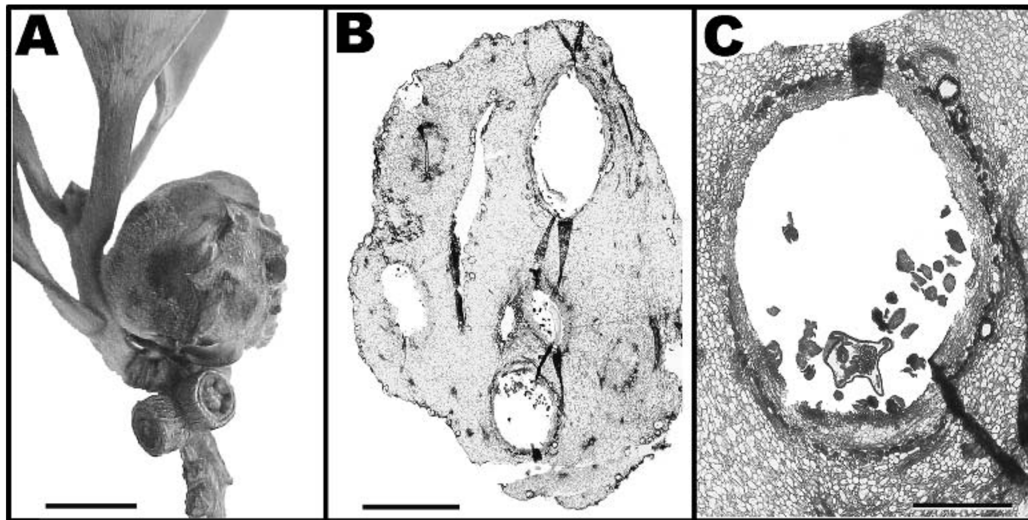

FIG. 12. *Fergusobia/Fergusonina*-induced shoot bud galls (*F. burrowsi*) on *Melaleuca viridiflora.* A) Galled and healthy shoot buds. B) Histological section of a galled shoot bud with multiple locules. C) Close-up of locule with hypertrophied cell lining of the lumen having been mostly removed by third-instar *Fergusonina* inside. A) Bar = 1 cm. B) Bar = 2 mm. C) Bar = 500 µm.

purple-staining cell walls and red-staining chloroplasts. Large oil ducts (0.13 to 0.18 mm) occurred between the palisade and spongy parenchymal layer. Vascular tissues were scattered in the spongy parenchyma along with areas of solidly purple-staining cells. The locule was in the spongy parenchyma with no clear-cut demarcation between it and the rest of the gall tissues. The locule was 0.61 × 0.78 mm, and the lumen was 0.43 × 0.38 mm (Fig. 13B). Typical hypertrophied cells were mostly 4 to 11 cells thick, extending 0.11 to 0.13 mm from the lumen. Most of the surface of the lumen had a thin layer of blue-staining exudate. A few cells on the edge of the lumen were about twice the size of other hypertrophied cells, each with an enlarged nucleus and nucleolus. The hypertrophied cells nearest the lumen had the most granular cytoplasm and the largest nuclei and nucleoli (Fig. 13C). The hypertrophied cells in the inner half of the locule were elongated vertically, whereas those in the outer half were more rounded with their nuclei flattened against a cell wall. Because of the presence of differentiated palisade parenchymal tissue in this gall type, gall manifestation may be partly due to the placement of fly eggs and nematodes between leaves of the developing bud. In the other broad-leaved *Melaleuca* bud galls, eggs and nematodes are deposited at the apical region of the bud (Giblin-Davis et al., 2001b).

'Basal rosette' axial stem galls from Melaleuca nervosa (Fig. 14): This gall type (attributed to the fly *F. goolsbyi,* Taylor, 2004) occurred at the base of the stem below the apical bud (Fig. 14A) and contains from one to five locules that encircled the stem and rendered it suscep-

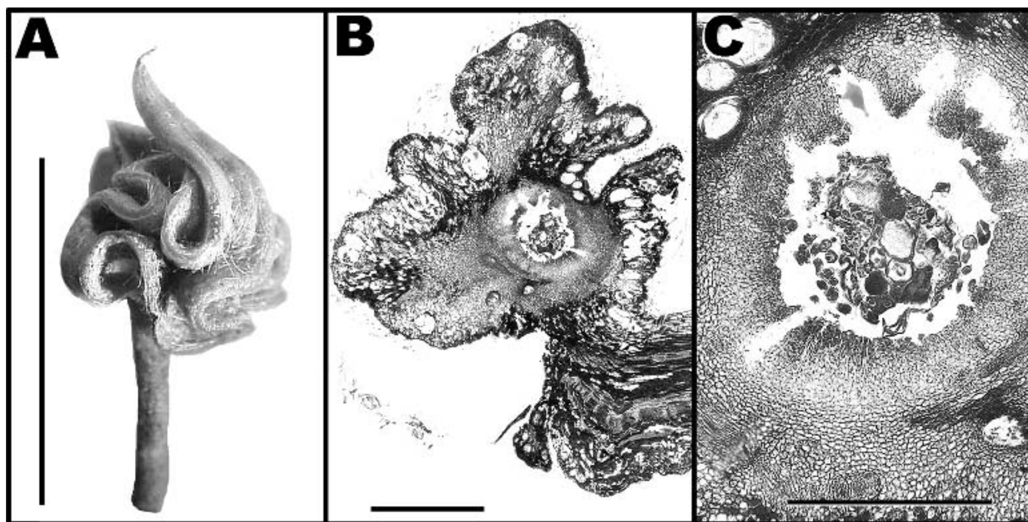

FIG. 13. *Fergusobia/Fergusonina*-induced 'leaf gall variant' of shoot bud gall (*Fergusonina* sp. 2, Taylor, 2004) on *Melaleuca stenostachya.* A) Galled terminal shoot bud. B) Histological section of a galled shoot bud with one locule. C) Close-up of a locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and a *Fergusonina* larva inside. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 500 µm.

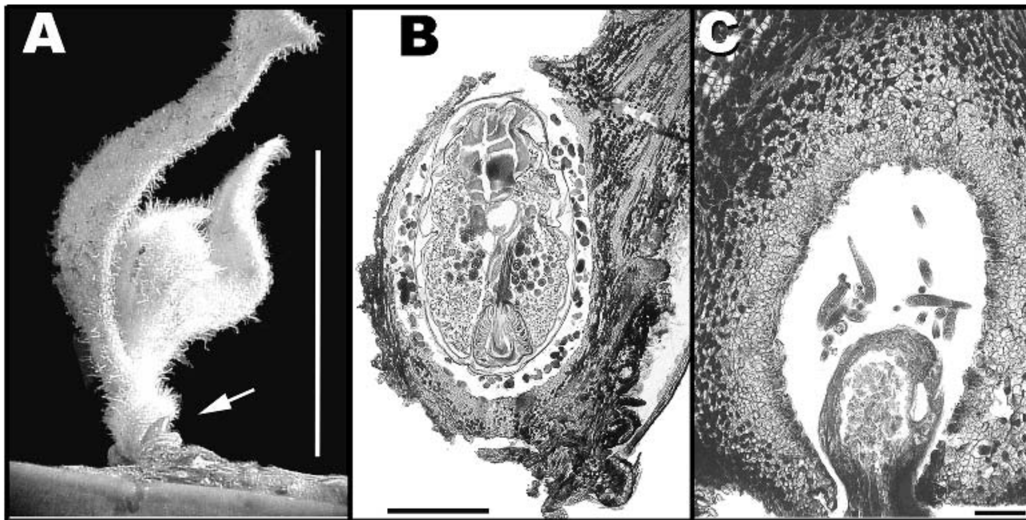

FIG. 14. *Fergusobia/Fergusonina*-induced 'basal rosette' axial stem galls (*F. goolsbyi*) on *Melaleuca nervosa*. A) Galled axial stem; arrow points at break in gall exposing *Fergusonina* pupa. B) Histological section of a galled stem with a locule containing a non-eclosed adult female fly (in puparium) infested with nematodes. Note: The inner lining of the lumen has been consumed by the fly larva leaving only frass between the locule lumen and the puparium. C) Close-up of a locule with hypertrophied cells lining the lumen and *Fergusobia* nematodes and *Fergusonina* larva inside. A) Bar = 1 cm. B) Bar = 500 µm. C) Bar = 100 µm.

tible to breakage. The sectioned gall contained an adult fly in its third-instar exuvium (Fig. 14B) and a third-instar larva with male and parthenogenetic female nematodes (Fig. 14C). The gall was formed in ground meristem or pith tissue below the apical bud. There was no distinct edge between the gall and the other plant tissues. The red, solidly staining cells of the ground meristem (or pith) became purple at the edge of the gall. Cells nearer the lumen stained purple with a granular cytoplasm and graded to a light blue-green at the lumen. The nuclei and nucleoli in these hypertrophied cells were not as distinct as observed in other *Fergusonina*-induced galls probably due to the maturity of the gall. Also, the dense, darkly staining hypertrophied cells were only 2 to 4 cells thick (Fig. 14C). Based upon molecular phylogenetic analysis, the 'basal rosette' axial stem galling *Fergusonina* from *M. nervosa* shared a distant ancestor with the well-defined *Fergusonina* from the broad-leaved *Melaleuca* terminal and axial bud clade (Scheffer et al., unpubl. data). The two distinctly different gall formers from *M. nervosa* (terminal and axial bud gall [attributed to the fly *F. schefferae*, Taylor, 2004, not-sectioned] vs. 'basal rosette' axial stem gall of *F. goolsbyi*) represent different sympatric species of *Fergusonina* (Taylor, 2004).

Flat leaf galls from Eucalyptus siderophloia (Fig. 15): This gall form involved a single layer of many locules in a mature leaf (Fig. 15A). One early flat leaf gall from *E.*

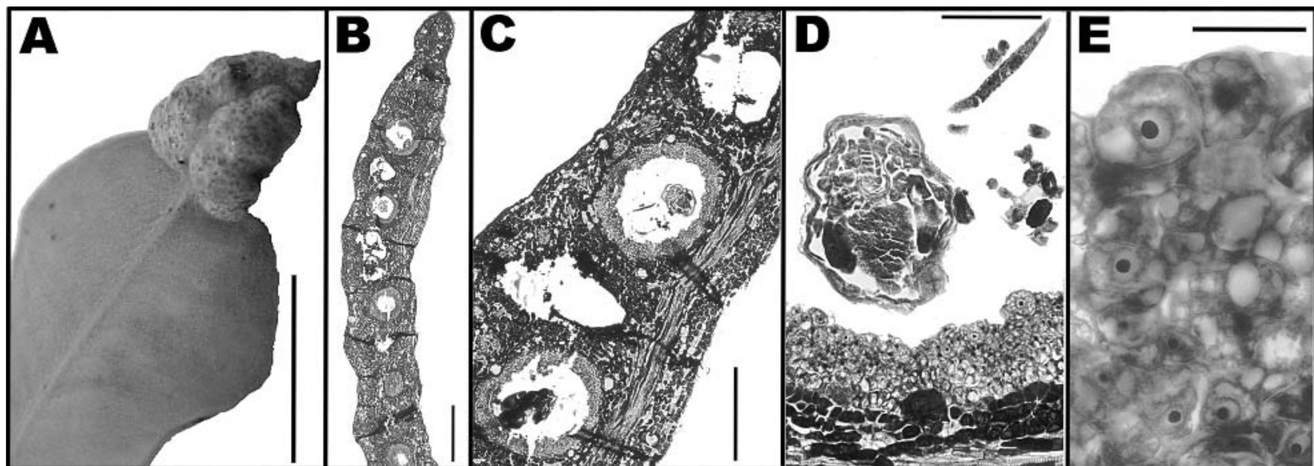

FIG. 15. *Fergusobia/Fergusonina*-induced flat leaf galls on *Eucalyptus siderophloia*. A) Galled leaf tip. B) Histological section of a galled young leaf with multiple locules (longitudinal to leaf axis). C) Close-up of four locules that alternate with and without hypertrophied cells lining their lumens (note that *Fergusobia* nematodes and a larval *Fergusonina* fly are present only in the locules with hypertrophied cells). D) Close-up of locule with hypertrophied cells, larva, and nematodes. E) Close-up of hypertrophied uninucleate cells lining the lumen of a locule. Each cell has an enlarged nucleus, nucleolus, and granular cytoplasm. A) Bar = 1 cm. B) Bar = 1 mm. C) Bar = 500 µm. D) Bar = 100 µm. E) Bar = 30 µm.

siderophloia was sectioned. The flies that were present in gall locules were second-instars and the nematodes were males and parthenogenetic females (Fig. 15B). The locules were formed in parenchymatous tissue, but there was no differentiation of cells into palisade and spongy parenchyma as was observed in the 'leaf gall variant' of bud galls of *Fergusonina* sp. 2 on *M. stenostachya* (see above). The epidermal layer stained solidly purple. Most of the other cells stained solidly red with scattered areas of lightly blue-green staining cells. Scattered in this matrix were vascular tissues and oil glands (Fig. 15C). This gall was composed of a series of locules along the leaf blade that generally alternated between those with typical hypertrophied cell layers and those with cells very similar to the matrix without hypertrophy (Fig. 15B,C). The lumens of locules with hypertrophied cells always contained a fly larva and nematodes, whereas the lumens without hypertrophied cells were empty or only contained a few nematodes. Surrounding the locules without hypertrophied cells there was a layer, 2 to 3 cells thick, similar to the red, solid-staining cells of the matrix, except that they were square and uniform in size (Fig. 15C). There was a small amount of blue-staining exudate at the surface of some of these. In some instances, there was some very slight purpling and granulation of the cytoplasm in the first layer of cells around the lumen. In contrast, the locules with flies and nematodes had a distinct ring of densely purple-staining cells where the locule touched the matrix cells (Fig. 15C,D). Inside this ring the cells were hypertrophied and had a granular cytoplasm, which became more deeply stained with larger nuclei and nucleoli (increasing hypertrophy) as the cells neared the lumen (Fig. 15D,E). Some of the cells adjacent to the lumen were twice the size of the other hypertrophied cells with much larger nuclei and nucleoli (Fig. 15E). There was often a large amount of exudate lining the lumen of these locules. There is strong phylogenetic support suggesting that flat leaf galling flies from *E. siderophloia* (sectioned) and *E. leucoxylon* (not sectioned) are part of a well-supported clade (Scheffer et al., unpubl. data).

Comparisons of the histology of Fergusobia/Fergusonina-induced galls: All of the four gall types identified in this study shared features relating to the type of plant tissue attacked and the histology of the gall. For example, flower bud galls involved individual flower buds only and were usually mutlilocule. As reported by Currie (1937), flower bud galls can be subdivided by the tissues used for locule production and whether or not the operculum of the flower bud detaches to release the next generation of flies. We confirmed that flower bud galls on *E. macrorhyncha* and *E. obliqua* could be grouped with galls with membrane-bound locules that were derived from anther primordia within the anther chamber with a detachable operculum for fly release. We also confirmed that the flower bud gall on *E. camaldulensis* could be grouped with galls with nonmembrane-bound locules derived from a fused mass of staminal ring/floral disc with a fused operculum requiring disintegration for release of the fly. Thirdly, for flower bud galls on *C. ptychocarpa,* we observed a mixture of the above groupings with galls possessing nonmembrane-bound locules derived from anthers and disc tissue with a detachable operculum requiring cracking and disintegration for fly release.

Bud galls typically involved fusion of tissue in the region of the bud apex and occurred in terminal and (or) axial shoot or inflorescence buds, leading to premature termination of the shoot in a fleshy spheroid gall. Bud galls ranged in hardness from relatively soft in *E. camadulensis* to very hard in *C. tessellaris.* Emergence of flies from this gall type appeared to be facilitated through the activities of the third-instar fly larva that created thin epidermal windows. The "leaf gall variant" of *Fergusonina* sp. 2 in *M. stenostachya* involved more differentiated leaves in the area of the bud leading to a back-and-forth folding of the leaves, whereas the leafy appearance of bud galls of *F. makinsoni* on *M. dealbata* was due to proliferation of leafy tissue from the central bud gall. Many of Currie's (1937) gall-type designations such as leaf–bud, axil-bud, and shoot-tip may fit into our broader category of bud galls.

The 'basal rosette' stem gall of *F. goolsbyi* on *M. nervosa* occurred at the base of an axial shoot and contained from one to five locules that encircled the stem. Fly release appeared to be caused by the gall windowing activities of the third-instar. The galls were formed in ground meristem or pith tissue below the apical bud and did not seem to fit Currie's (1937) designation of stem-tip galls. We have observed a variety of unilocule or small galls that might be characterized as 'basal rosette' stem galls or very small bud or leaf galls in the field on a variety of hosts (e.g., *M. armillaris*). Histological confirmation of gall type (stem vs. bud vs. leaf) may be necessary in these cases.

The flat leaf gall type on *E. siderophloia* involved a single layer of many locules in a mature leaf, suggesting that oviposition and nematode deposition may occur directly into leaves, confirmed by the presence of oviposition scars. The locules were formed in leaf parenchymatous tissue without differentiation into palisade and spongy layers. The presence of regions within or near locules with nematodes but without hypertrophied cells needs further examination. Currie (1937) also observed leaf galls that involved two leaves fusing together on *E. bridgesiana* (host of Currie's *Fergusonina* sp. 7). There are several variants of this gall type involving different part of leaves (Taylor and Davies, unpubl. data). In addition, there are other uncategorized gall types involving different host parts and manifestations, e.g., seedling stem galls (Taylor et al., 2004) that also should be investigated.

Fergusobia juveniles are deposited along with *Fergusonina* eggs during oviposition into specific locations of

a particular host plant at a critical time in its development. In *E. macrorhyncha,* Currie (1937) reported that a female fly deposits eggs and nematodes through the operculum of a young individual flower bud into the anther/stamen chamber and that many eggs and nematodes may be placed there by one or many flies. The nematodes feed on the primordia of the anther/stamens, causing rapid formation of patches of irregular cells. Newly eclosed fly larvae cut out small crypts between apposed masses of hypertrophied cells, and nematodes aggregate around the fly as the locule develops around them. In shoot buds of *M. quinquenervia* exposed to caged *Fergusonina* flies, nematode juveniles and fly eggs were placed internally near primordial buds. The nematodes apparently induced hypertrophied cells between young leaves, creating pockets of nematodes and fly eggs around which the leaves fused. When the fly eggs hatched between 44 and 66 days after exposure, the first-instar larva appeared to be a focal point for the development of individual locules (Giblin-Davis et al., 2001b). It is not clear where nematodes and fly eggs are deposited and how gall development proceeds in axial bud and flat leaf galls.

The morphology of the hypertrophied cells lining each locule appeared to be similar across all gall types examined, involving enlarged uninucleate and granular cells, each with an enlarged nucleus and nucleolus. Cell hypertrophy was most pronounced closest to the lumen of the locule. The morphology of the hypertrophied cells observed in this study was consistent with observations in a more detailed investigation of gall development on *M. quinquenervia* (Giblin-Davis et al., 2001b). Observed patterns are similar to those described for nematodes that induce seed galls in the Anguinidae (e.g., *Anguina agrostis*) (Stynes and Bird, 1982).

Although phylogenetic analysis is beyond the scope of this study, preliminary analysis of mitochondrial sequence data from species of *Fergusonina* indicates that gall type is only somewhat conserved and that some degree of convergence in gall morphology has occurred (Scheffer et al., unpubl. data). For example, although the flower-bud galling flies from *C. ptychocarpa* produce galls that are very similar morphologically/functionally to the flower bud galls of flies on *E. obliqua* and *E. macrorhyncha,* available evidence from both morphological and molecular analysis suggests that flies from *C. ptychocarpa* are phylogenetically quite distinct from the closely related flies on *E. obliqua* and *E. macrorhyncha,* respectively (Taylor et al., unpubl. data; Scheffer et al., unpubl. data). This pattern is also seen with the terminal and axial bud gallers; morphological and molecular data strongly support a *Melaleuca*-feeding clade of terminal and axial bud gallers as well as a distantly related clade containing terminal and axial bud gallers on *E. diversifolia* (sectioned) and *E. racemosa* (not sectioned). A more complete analysis of host-use evolution in *Fergusonina* and *Fergusobia* will be presented elsewhere (Giblin-Davis et al., unpubl. data; Scheffer et al., unpubl. data).

Phylogenetically, *Fergusonina* flies and *Fergusobia* nematodes are each monophyletic and together represent a unique mutualistic innovation that has led to a significant radiation of host-specific species (Giblin-Davis et al., unpubl. data). We are just beginning to understand the basic biology of the complex tritrophic interactions resulting in gall formation (Currie, 1937; Giblin-Davis et al., 2001b; Taylor et al., 2004), but the relative contribution of host plant, fly, and nematode genetics in determining gall morphology is unknown. Furthermore, cellular mechanisms resulting in gall formation may be very similar for the variety of myrtaceous gall manifestations observed such that the seemingly large outward differences in gall appearance may be largely due to the plant tissue selected and timing of placement of nematode and fly inoculum by the ovipositing fly. For example, we hypothesize that the 'leaf gall variant' on *M. stenostachya* may primarily be due to delayed timing of oviposition and nemaposition into a slightly more differentiated bud, placement of nematodes and fly eggs into unopened primordial leaves higher above the apical stem, a different host reaction to nematodes and (or) fly eggs, or a combination of these factors leading to locule development in differentiated young leaves. This could lead to a lack of apical bud fusion as seen in *M. quinquenervia* (Giblin-Davis et al., 2001b) and the bud galls sectioned herein from other species of *Melaleuca.*

Currie (1937) suggested that less specialized gall types, such as stem tip and leaf tip galls, were more likely to be parasitized by wasps than the highly specialized flower bud galls. This implies a relationship between gall morphogenesis and attack by natural enemies, including a variety of wasps (Currie, 1937; Goolsby et al., 2001; Taylor et al., 1996, 2003), and suggests that gall form may be under strong natural selection for survival of flies and nematodes. It would be interesting to map gall forms on to the phylogenies for the flies, nematodes, and myrtaceous hosts. Preliminary comparisons of gall forms with eucalypt phylogeny show that both less specialized forms such as axial leaf bud galls and more specialized flower bud galls have been collected from widely disparate clades within the myrtaceae (Taylor and Davies, unpubl. data), i.e. that there is no apparent correlation between host evolution and evolution of gall type.

Ultimately, gall formation in the *Fergusobia/Fergusonina*/Myrtaceae complex will be revealed as an interaction of genetic and phenotypic influences from all three of the organisms involved. Investigating the exact contributions made by each of these factors to gall development and morphology will provide an exciting challenge to current and future biologists.

Literature Cited

Currie, G. A. 1937. Galls on *Eucalyptus* trees. A new type of association between flies and nematodes. Proceedings of the Linnean Society of New South Wales, Sydney 62:147–174.

Davies, K. A., and J. Lloyd. 1996. Nematodes associated with Diptera in South Australia: A new species of *Fergusobia* Currie and a new record of *Syrphonema* Laumond and Lyon. Transactions of the Royal Society of South Australia 120:13–20.

Davies, K. A., J. Makinson, and M. F. Purcell. 2001. Observations on the development and parasitoids of *Fergusonina/Fergusobia* galls on *Melaleuca quinquenervia* (Myrtaceae) in Australia. Transactions of the Royal Society of South Australia 125:45–50.

Giblin-Davis, R. M. 1993. Interactions of nematodes with insects. Pp. 302–344 *in* W. Khan, ed. Nematode interactions. London: Chapman & Hall, Inc.

Giblin-Davis, R. M., K. A. Davies, G. S. Taylor, and W. K. Thomas. 2004. Entomophilic nematode models for studying biodiversity and cospeciation. Pp. 492–538 *in* Z. X. Chen, S. Y. Chen, and D. W. Dickson, eds. Nematology, advances and perspectives. New York: Tsinghua University Press/Springer-Verlag.

Giblin-Davis, R. M., K. A. Davies, D. S. Williams, and T. D. Center. 2001a. Cuticular changes in *Fergusobia* nematodes associated with *Fergusonina* flies. Comparative Parasitology 68:242–248.

Giblin-Davis, R. M., J. Makinson, B. J. Center, K. A. Davies, M. Purcell, G. S. Taylor, S. Scheffer, J. Goolsby, and T. D. Center. 2001b. *Fergusobia/Fergusonina*-induced shoot bud gall development on *Melaleuca quinquenervia*. Journal of Nematology 33:239–247.

Goolsby, J. A., C. J. Burwell, J. Makinson, and F. Driver. 2001. Investigation of the biology of Hymenoptera associated with *Fergusonina* sp. (Diptera: Fergusoninidae), a gall fly of *Melaleuca quinquenervia*, integrating molecular techniques. Journal of Hymenoptera Research 10:163–180.

Johansen, D. A. 1940. Plant microtechnique. New York: McGraw-Hill.

Siddiqi, M. R. 1986. A review of the nematode genus *Fergusobia* Currie (Hexatylina) with descriptions of *F. jambophila* n. sp. and *F. magna* n. sp. Pp. 264–278 *in* G. Swarup and D. R. Dasgupta, eds. Plant-parasitic nematodes of India, problem and progress. New Delhi: Indian Agricultural Research Institute.

Stynes, B. A., and A. F. Bird. 1982. Development of galls induced in *Lolium rigidum* by *Anguina agrostis*. Phytopathology 72:336–346.

Taylor, G. S. 2004. Revision of *Fergusonina* gall flies (Diptera: Fergusoninidae) from *Melaleuca* (Myrtaceae). Invertebrate Systematics 18:251–290.

Taylor, G. S., A. D. Austin, and K. A. Davies. 1996. Biology of the eucalypt gall-forming fly, *Fergusonina flavicornis* Malloch (Diptera: Fergusoninidae) and its associated hymenopterans in South Australia, with a description of a new species of *Bracon* (Hymenoptera: Braconidae). Transactions of the Royal Society of South Australia 120:131–146.

Taylor, G. S., K. A. Davies, and R. M. Giblin-Davis. 2003. Species-richness in gall-flies (Diptera: Fergusoninidae), nematodes (Nematoda: Neotylenchidae), and associated parasitoids and inquilines on Myrtaceae. Records of the South Australian Museum Monograph Series No. 7:249–256.

Taylor, G. S., E. R. Head, and K. A. Davies. 2004. Gall flies (Diptera: Fergusoninidae) on Myrtaceae: A mutualistic association between flies and nematodes. *In* A. Raman, C. W. Schaefer, and T. M. Withers, eds. Biology, ecology, and evolution of gall-inducing arthropods. New Hampshire: Science Publishers. In press.

Tonnoir, A. L. 1937. Revision of the genus *Fergusonina* Mall. (Diptera, Agromyzidae). Proceedings of the Linnaean Society of South Wales 62:126–146.