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Histological Comparisons of Parasitism by *Schistonchus* spp. (Nemata: Aphelenchoididae) in Neotropical *Ficus* spp.[1]

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Abstract: Syconia (enclosed infructescences) infested with host-specific species of *Schistonchus* (Aphelenchoididae) were collected from six species of *Ficus* (Moraceae) native to Florida or Panama. They were sectioned and histologically examined to assess the effects of parasitism. Parasitism by *Schistonchus* spp. was associated with hypertrophied cells, tissue necrosis, and the presence of an exudate in all species. Occasional hypertrophy of the outer epidermal cells occurred on seed florets, wasp florets, and on the endothecial cells of male florets in *F. aurea* (subgenus *Urostigma*) from Florida. Aberrations of the inner mesocarp occurred under the hypertrophied cells on seed florets. In *F. laevigata* (subgenus *Urostigma*) from Florida, *Schistonchus* sp. infested immature male florets and was associated with hypertrophy of endothecial cells, epidermal cells of the anther filaments, and anthers. *Schistonchus* sp. also caused aberrations of the anther filament, anthers, and pollen. *Ficus poponoei* (subgenus *Urostigma*) and *F. glabrata* (subgenus *Pharmacosycea*), both from Panama, had hypertrophied outer epidermal cells on seed florets. *Ficus poponoei* also had *Schistonchus* sp. within the pedicel of an aborted floret, with hypertrophy of the cortical parenchyma. *Ficus trigonata* (subgenus *Urostigma*) from Panama had hypertrophy of the outer epidermis of seed florets. When the outer epidermis on these florets was missing, the inner mesocarp was hypertrophied. *Ficus maxima* (subgenus *Pharmacosycea*) from Panama had hypertrophy on the outer epidermis of seed and aborted florets. *Schistonchus* spp. were not found in wasp larvae or pupae in any of the *Ficus* spp. examined. Hypertrophy was never observed in the absence of *Schistonchus* spp.

Key words: Aphelenchoididae, *Ficus aurea, Ficus glabrata, Ficus laevigata, Ficus maxima, Ficus poponoei, Ficus trigonata,* fig, floret wall morphology, histopathology, life history, nematode, parasitism, *Schistonchus* spp.

There are more than 700 species of *Ficus* (Moraceae) (figs) worldwide. The New World *Ficus* comprise two subgenera and two sections: *Urostigma* section Americana with 120 species, and *Pharmacosycea* section Pharmacosycea with 20 species (Berg, 1989).

Ficus are mutualistically associated with host-specific fig wasps (Agaonidae) for pollination (Berg, 1989; Bronstein, 1992; Frank, 1984; Ramirez B., 1970; Wiebes, 1979). A high degree of coadaptation is apparent in each fig-fig wasp association. *Ficus* spp. produce enclosed infructescences (syconia) commonly called figs. About one-half of all *Ficus* spp. are gynodioecious, whereas the rest, including the Neotropical species, are monoecious, with both male and female florets occurring in an individual syconium. Monoecious syconia progress through five successive developmental phases that are synchronized with the life cycle of the pollinating fig wasp (Galil and Eisikowitch, 1968). Phase A occurs when the young syconium bears immature florets. This is prior to the loosening of the ostiolar scales that surround the opening of the syconium. In phase B the ostiolar scales relax and female florets within the syconium become receptive. Female wasps penetrate through the ostiole into the syconial cavity, pollinate, oviposit in accessible female florets, and then die. Monoecious *Ficus* are polymorphic with short- or long-styled female florets. The female wasp is able to oviposit into the ovaries of florets with short styles resulting in a female floret that contains a mutualistically associated wasp (wasp floret). Florets with a long style prevent the wasp from reaching the ovaries during oviposition, allowing for *Ficus* embryo development (seed floret).

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Phase C occurs when fig embryos and wasp larvae develop within their respective fig ovaries. In phase D the male florets mature. Male wasps emerge and mate with females while they are still in their galls. The female wasps then emerge from their galls, collect pollen, and exit the syconium via holes made by the male wasps. Finally, in phase E the syconium and seeds ripen. The mature syconium may then be consumed by birds, mammals, or fish, which disperse the seeds.

Potentially, most or all *Ficus* spp. could have an associated species of *Schistonchus* (Giblin-Davis et al., 1995). However, only two species of *Ficus* have been examined histologically to elucidate the parasitic association with *Schistonchus* spp.: *F. laevigata* Vahl (*F. citrifolia* Miller sensu DeWolf, 1960) (Giblin-Davis et al., 1995) and *F. carica sylvestris* L. (Vovlas et al., 1992; Vovlas and Larizza, 1996). *Schistonchus* spp. feed on syconial tissue and use the wasp pollinator for transport to new syconia. For example, in *F. laevigata,* mated entomophilic females are carried in the hemocoel of the female fig wasp *Pegoscapus* sp. to phase B syconia where the nematodes emerge and infest the immature anthers, causing epidermal and endothecial cell hypertrophy (Giblin-Davis et al., 1995). In contrast, *S. caprifici* (Gasperrini) causes hypertrophy of epidermal cells and necrosis in the cortical parenchyma of the pedicels of female florets and anther filaments of the gynodioecious fig, *F. carica* (Vovlas and Larizza, 1996). Eggs, juveniles, and adults may be carried in the hemocoel of the female fig wasp, *Blastophaga psenes* L. (Vovlas et al., 1992).

The floral structures and tissues affected by *Schistonchus* spp. feeding have varied in the two *Ficus* spp. that have been histologically examined. The purpose of this study was to determine the variation in pathology caused by Neotropical *Schistonchus* spp. in six species of *Ficus* from Florida and Panama: *F. aurea* Nuttall, *F. laevigata, F. poponoei* Standl., and *F. trigonata* L. of the *Urostigma* in the section Americana, and *F. glabrata* H.B.K. and *F. maxima* Mill. of the *Pharmacosycea* in the section Pharmacosycea.

Materials and Methods

Collections: Syconia of *F. aurea* were collected from May 1996 to January 1997 from several sites in Broward County, Florida. Syconia of *F. maxima, F. poponoei,* and *F. trigonata* were collected in June 1997 from islands in the Panama Canal near Barro Colorado Island, Panama. Syconia of *F. glabrata* were collected from the shores of the Chagres River near Gamboa, Panama, in June 1997.

Histopathology: Mid- to late-phase C syconia were cut in half. One half was placed into FAA (formalin, acetic acid, ethanol; 5:5:90), and the other half was chopped and placed in water for at least 20 minutes to determine whether it was infested with *Schistonchus* sp. Infested syconia halves were retained in FAA, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin (Johansen, 1940). Sections 15 µm thick were cut from embedded syconia halves, mounted on slides treated with Mayer's albumin (50 ml fresh egg albumin, 50 ml glycerin, 1 g sodium salicylate), stained at room temperature with 1% aqueous safranin for 90 minutes and 0.5% fast green in clove oil and 100% ethanol (1:1) for 15 seconds, examined, and photographed with a compound photomicroscope equipped with a camera lucida. Composite drawings from sections were done of floret walls from each species of *Ficus* for seed florets and wasp florets without *Schistonchus* infestations. Drawings were also done of *Schistonchus*-affected florets and other syconial structures. In addition, sections of *F. laevigata* from a previous study (Giblin-Davis et al., 1995) were re-examined for confirmation of pathological effects and tissue involvement. Due to microtoming artifacts and some breakage of the floret tissue, serial sections were used to construct the schematic illustrations presented (Figs. 1–3). We focused our largest sectioning efforts on the Florida species because of the availability of specimens.

Results

Schistonchus presence in dissected synconia of Ficus: Re-examination of data from dissec-

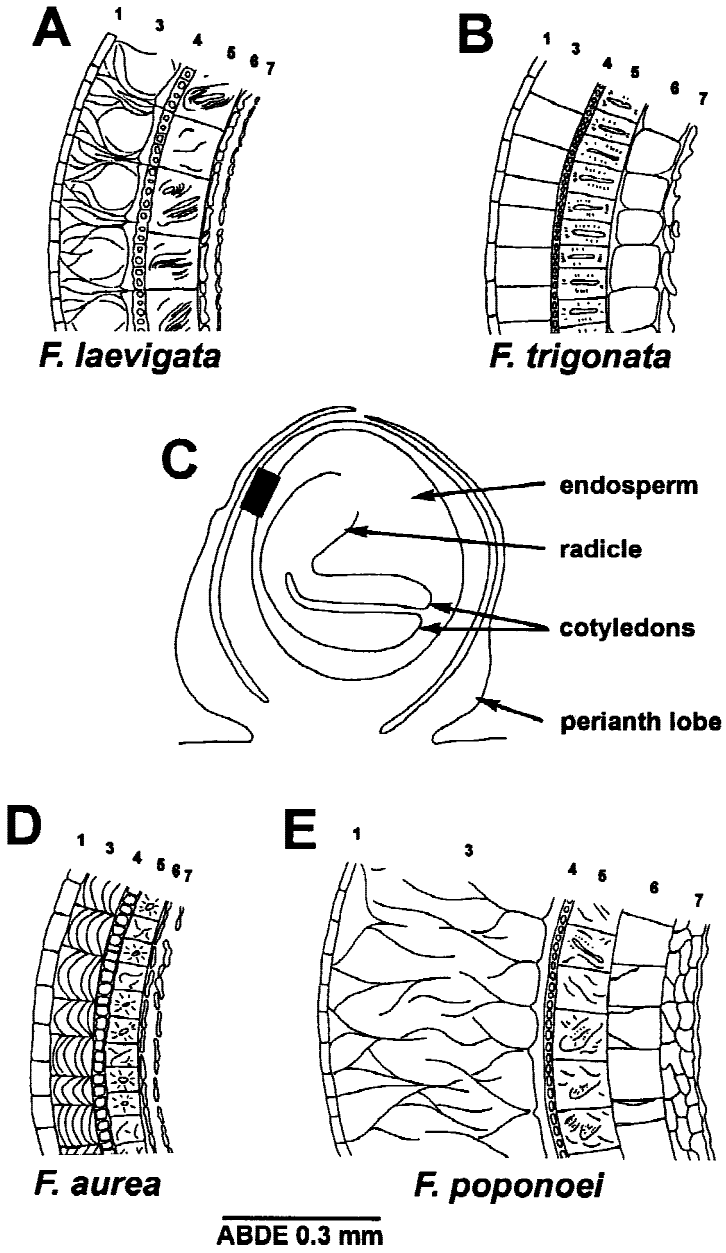

FIG. 1. Reconstructed camera lucida drawings of the drupe wall and seed coat of female florets with developing *Ficus* embryo (seed florets) from phase C syconia of *Ficus* subgenus *Urostigma* section Americana. A) *F. laevigata.* B) *F. trigonata.* C) Longitudinal section of a typical *Urostigma* seed floret (*F. laevigata*). Tissues were drawn from the black rectangular region in the illustration. D) *F. aurea.* E) *F. poponoei.* Tissue layers: 1 = outer epidermis, 3 = inner mesocarp. This layer determines whether the floret is a drupe (with an inner mesocarp) or an achene (without an inner mesocarp). At the end of floret development, this mucilaginous layer allows for seed anchorage in the hemi-epiphytic *Urostigma.* 4 = inner sub-epidermis, 5 = endocarp, 6 = outer embryo integument, 7 = inner embryo integument.

tions of *F. laevigata* confirmed that 20% of 414 syconia were infested with *Schistonchus* sp. Twenty-one percent of 100 *F. aurea* syconia were infested with *Schistonchus.* The four species of *Ficus* from Panama had a range of nematode infestation from a low of 27% in 11 syconia of *F. maxima* to a high of 50% in 12 syconia of *F. poponoei.*

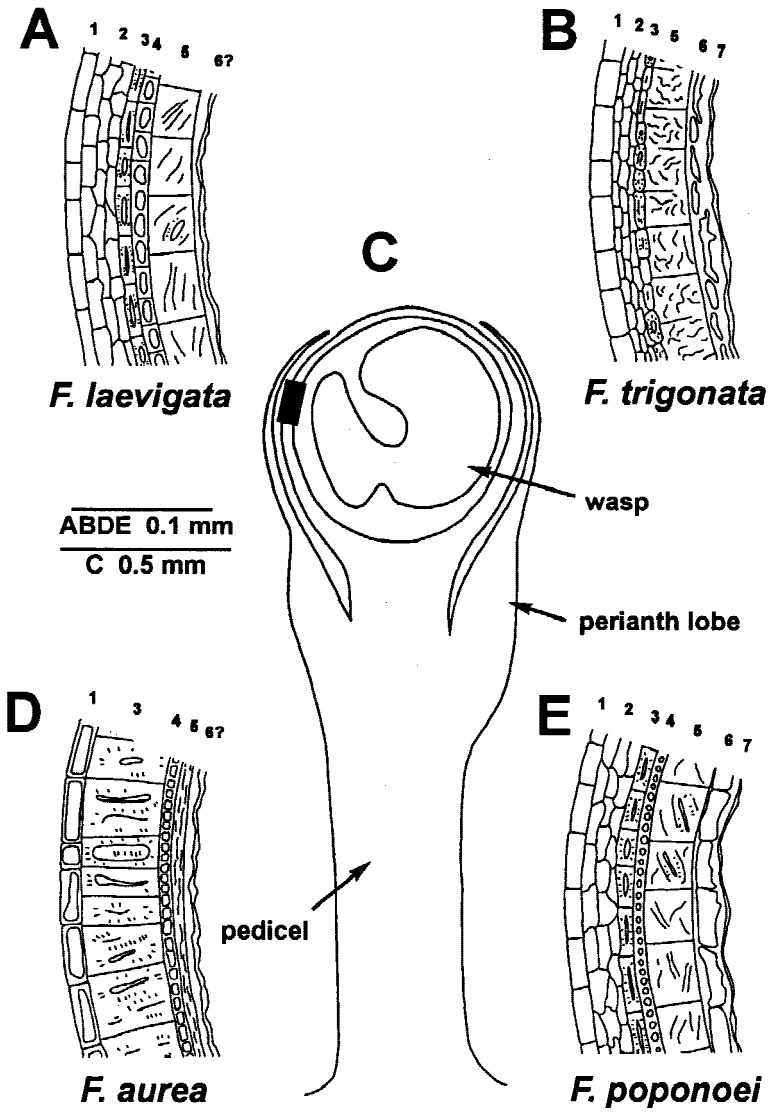

FIG. 2. Reconstructed camera lucida drawings of the drupe wall and seed coat from female florets with developing *Pegoscapus* spp. wasps (wasp florets) from phase C syconia from the *Ficus* subgenus *Urostigma* section Americana. A) *F. laevigata.* B) *F. trigonata.* C) Longitudinal section of a typical *Urostigma* wasp floret (*F. laevigata*). Tissues were drawn from the black rectangular region in the illustration. D) *F. aurea.* E) *F. poponoei.* Tissue layers: 1 = outer epidermis, 2 = outer mesocarp, 3 = inner mesocarp, 4 = inner sub-epidermis, 5 = endocarp, 6 = outer embryo integument, 7 = inner embryo integument.

Tissue layers in seed and wasp florets without nematodes: All the wasps within syconia were late instar larvae through newly eclosed adults, confirming that syconia were mid- to late-phase C. The late phase C floret wall had five discernible layers in *Urostigma* and four discernible layers in *Pharmacosycea,* with two additional layers comprising the seed coat. The outermost tissue layer of the floret was the outer epidermis. In all examined *Urostigma,* the outer epidermal cells were tanniferous (Figs. 1A,B,D,E; 2A,B,D,E). In some *F. aurea* wasp florets (Fig. 2D), these cells were only partially filled with tannins. In the subgenus *Pharmacosycea,* the tannin content was varible.

The second layer of the floret, the parenchymatous outer mesocarp, was observed in

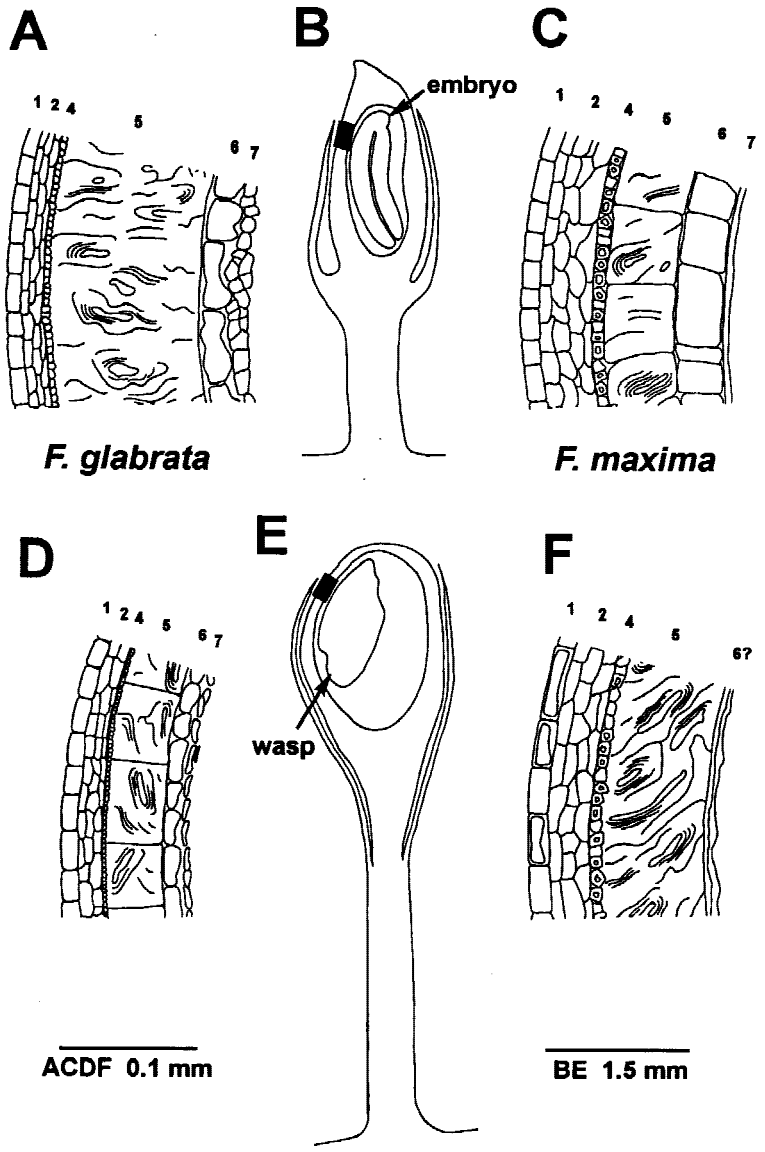

FIG. 3. Reconstructed camera lucida drawings of the achene wall and seed coat of female florets with developing *Ficus* embryo (seed florets) and female florets with developing *Tetrapus* spp. wasps (wasp florets) from phase C syconia from *Ficus* subgenus *Pharmacosycea* section Pharmacosycea. A) *F. glabrata* seed floret. B) Longitudinal section of a typical *Pharmacosycea* seed floret (*F. glabrata*). Tissues were drawn from the black rectangular region of the illustration. C) *F. maxima* seed floret. D) *F. glabrata* wasp floret. E) Longitudinal section of a typical *Pharmacosycea* wasp floret (*F. glabrata*). Tissues were drawn from the black rectangular region of the illustration. F) *F. maxima* wasp floret. Tissue layers: 1 = outer epidermis, 2 = outer mesocarp, 3 = inner mesocarp, 4 = inner sub-epidermis, 5 = endocarp, 6 = outer integument, 7 = inner integument.

both floret types in *Pharmacosycea* (Fig. 3A,C,D,F), and in the wasp florets of *F. laevigata, F. trigonata,* and *F. poponoei* (Fig. 2A,B,E).

The third layer was the inner mesocarp. It was absent in the *Pharmacosycea* (Fig. 3A,C,D,F). The appearance of this tissue varied with age in seed florets at phase C (Fig. 1A,B,D,E). In younger phase C syconia the cells were solid and quadrate. In older phase C syconia these cells developed a fine lamination that expanded and became mucilaginous. The expansion of the mucilage eventually ruptured the cell walls, sloughing the

outer epidermis. In contrast, these cells in wasp florets had thick walls, were finely laminated, and possessed a lumen. In the wasp florets of *F. aurea,* these cells were large and quadrate (Fig. 2D). In the other *Urostigma* spp., they were flat with a compressed lumen (Fig. 2A,B,E).

The fourth tissue layer was the inner subepidermis, which occurred in all florets examined except *F. trigonata* wasp florets (Fig. 2B). This layer consisted of small, sclerified cells with a small lumen. In *F. maxima,* a conspicuous diamond-shaped crystal was present in the lumen. Irregularly shaped crystals were observed in the lumina of *F. laevigata* and *F. poponoei.*

The fifth tissue layer was the endocarp and occurred in both seed and wasp florets in all species examined. This layer consisted of large, quadrate cells containing a lumen. The cells had thick secondary walls that were distinctly laminate and pitted. These cells became compressed and distorted, especially in wasp florets. In *F. aurea* wasp florets, the endocarp was completely flattened (Fig. 2D).

The sixth tissue layer, the outer integument of the embryo, occurred in seed and wasp florets in all species examined. The cells in this layer were tanniferous and became compressed except in the seed florets of *F. trigonata, F. poponoei,* and *F. maxima* (Figs. 1B,E; 3C), where they were large and quadrate.

The seventh tissue layer, the inner integument of the embryo, had cells that were greatly compressed, especially in wasp florets. This layer was not observed in wasp florets of *F. laevigata, F. aurea,* and *F. maxima* (Figs. 2A,D; 3F). It was unclear if the layer was missing or was compressed into layer six.

Histopathology of nematode-infested syconia of F. aurea: Florets (n = 878) were examined from serial sections of 23 *Schistonchus*-infested, late-phase C syconia of *F. aurea.* Hypertrophy of the cells associated with *Schistonchus* sp. was expressed by enlarged cell size, a granular cytoplasm, an enlarged single nucleus, and a prominent nucleolus. Unless otherwise noted, this constituted the typical appearance of hypertrophy in all *Ficus* spp. discussed. No syncytia were observed in any of the *Schistonchus*-affected *Ficus* spp. examined. No hypertrophy was observed in the absence of *Schistonchus* spp.

Thirty-eight percent (n = 330) of the florets examined were seed florets. Of these, two had hypertrophied cells of their outer epidermis. The affected areas were about 9 to 12 cells long and were covered by a perianth lobe. The cells of the inner mesocarp under the hypertrophied cells were enlarged and appeared empty. In one floret, the lateral cell walls of the inner mesocarp were wrinkled due to loss of turgor (Fig. 4C); in the other floret, these cells contained a layer at the bottom and appeared empty (Figs. 4E; 5A). The interior of the adjoining normal cells was laminate, typical for the inner mesocarp at this stage (Fig. 4A). The epidermis of the perianth lobe was hypertrophied on one floret, where it abutted the infested area of its floret. The hypertrophied cells of the perianth epidermis were slightly enlarged compared to unaffected cells.

Hypertrophy of the epidermis was also observed in two pedicels of seed florets (Fig. 4G). Normal epidermal cells of the pedicels that were usually small and rectangular became enlarged and rounded when hypertrophied. Epidermal cells of the pedicels were sometimes large and quadrate. When these cells hypertrophied, they were similar in size to normal cells. Hypertrophy often increased in descending order along the epidermal cells of the pedicel. Anteriormost cells on the pedicel were filled with tannins. Descending the pedicel, hypertrophication increased with cells that contained droplets of tannins progressing to cells with a completely granular cytoplasm. Four pedicels were observed with epidermal necrosis at their bases. This necrosis sometimes extended into the underlying cells, girdling the pedicel. Hypertrophy was observed in four other seed florets on the epidermis of the perianth lobes. These cells were similar to those in the perianth described above.

Eighteen percent (n = 162) of the florets were male. Two hypertrophied endothecial cells were found on one anther (Fig. 6A).

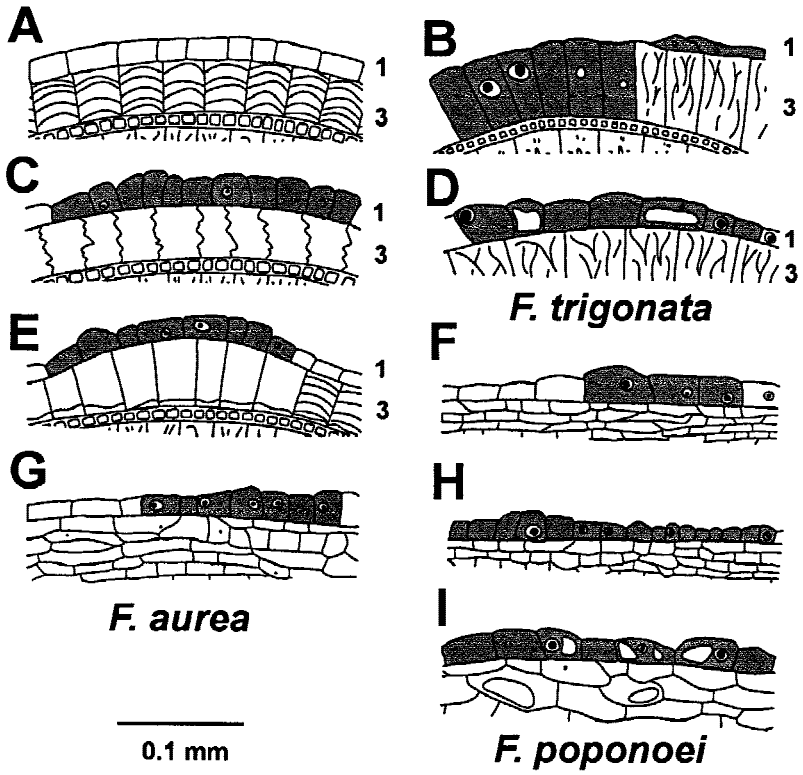

FIG. 4. Camera lucida drawings of *Schistonchus*-infested phase C syconia of *Ficus* subgenus *Urostigma*. Shaded cells indicate *Schistonchus*-induced hypertrophy. A,C,E, and G are *F. aurea*. A) Typical uninfested female floret with developing embryo (seed floret). C,E) Hypertrophy of the outer epidermis with aberration of the inner mesocarp of seed florets. G) Hypertrophy of the epidermis of a pedicel with a region of normal cells. B,D) *F. trigonata* seed florets. B) Hypertrophy of the inner mesocarp where the hypertrophied and collapsed outer epidermal cells were lost. D) Hypertrophy of the outer epidermis. F,H, and I are *F. poponoei*. F) Hypertrophy of the epidermis of a seed floret where it joins the perianth lobe with a region of normal cells. H) Hypertrophy of the epidermis of a perianth lobe. I) Hypertrophy of the epidermis of the syconial wall.

This floret was in a syconium that contained high numbers (ca. 500) of *Schistonchus* sp. These cells were not enlarged compared to neighboring normal cells. The cytoplasm was more lightly stained and the nucleus and nucleolus were slightly enlarged. The pedicel of this floret had hypertrophied epidermal cells that appeared similar to those in hypertrophied seed florets. The perianth lobe covering this floret was necrotic where it was in contact with a group of *Schistonchus* outside the floret, thus allowing access for the nematodes.

Twenty-nine percent (n = 256) of the florets were wasp florets. Of these, one short-pedicelled floret had hypertrophied cells on the outer epidermis. The affected area was under a perianth lobe and was about 10 cells long. Most of the cells were square and slightly taller than those of the normal outer epidermis with very dense, darkly stained cytoplasm. The nucleus and nucleolus were difficult to discern. A few cells were 2.5–3× the height of normal cells and irregularly globular in shape, exhibiting more typical hypertrophy. Cells underlying the hypertrophied cells appeared normal. Seven pedicels and one perianth lobe of other florets of this type had hypertrophied cells that were similar in appearance to those hypertrophied in seed florets.

Fifteen percent (n = 130) of the florets examined were aborted. None of these florets had hypertrophy associated with the floret itself. All tissues in these florets and their perianth lobes retained safranin stain. Tis-

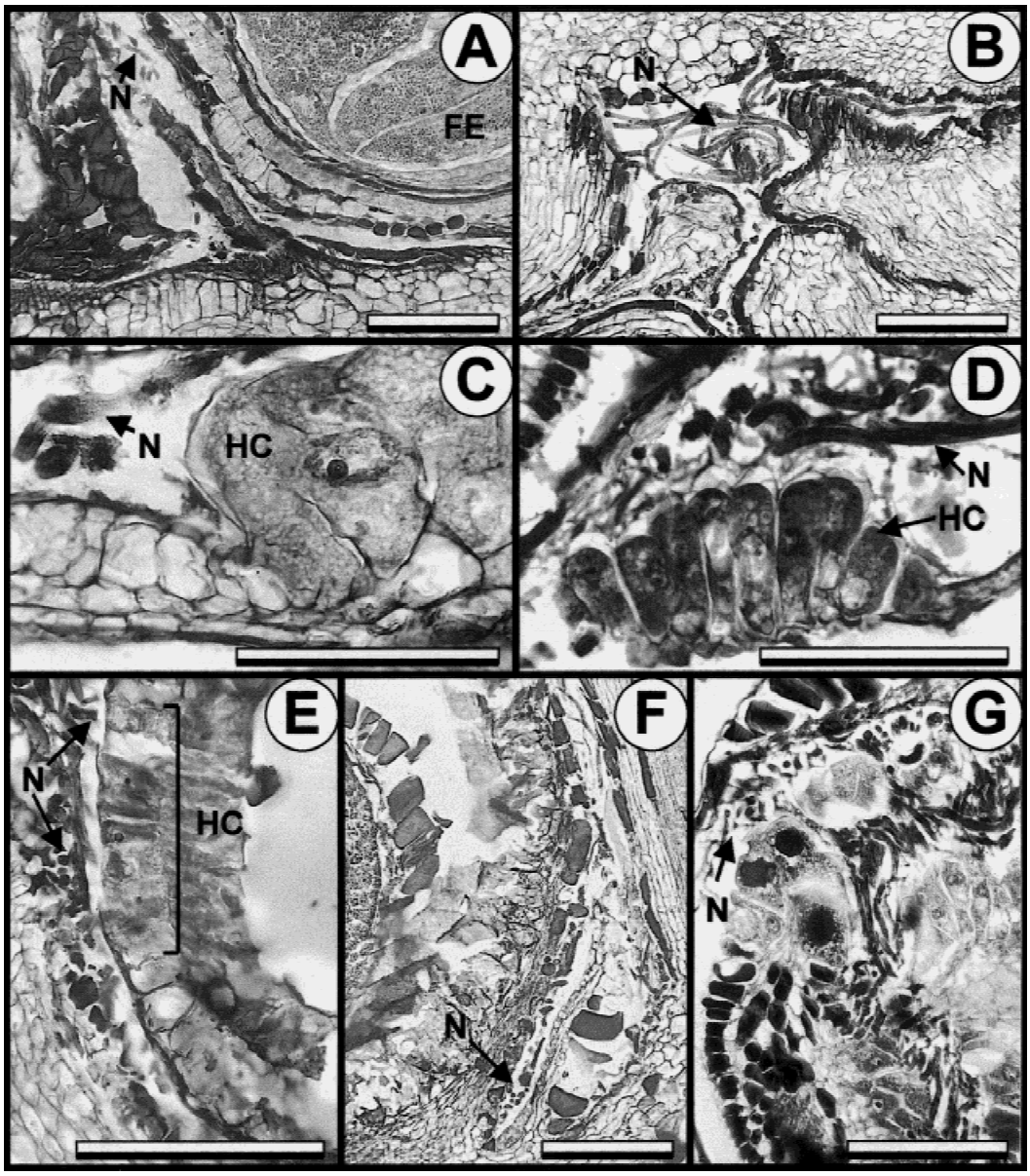

FIG. 5. Photomicrographs of *Schistonchus*-affected phase C syconia of *Ficus* spp. A) *F. aurea*. Hypertrophy of the outer epidermis of a seed floret. Cells of the inner mesocarp that underlie these cells were enlarged and appeared empty (FE = floret embryo). B) *F. glabrata* with *Schistonchus* sp. in a pocket of necrotic cortical parenchyma in the syconial wall. C,D) *F. maxima*. Hypertrophy of the outer epidermal cells of seed florets. E) *F. trigonata*. Hypertrophy of the inner mesocarp of a seed floret where the outer epidermis was lost. F) *F. poponoei*. Hypertrophy of the outer epidermis of a seed floret and of the epidermis on the inner side of its perianth lobe. G) *F. laevigata*. Hypertrophy of endothecial cells. Bar for A,B,D–G = 200 µm; C = 100 µm; N = nematodes; HC = hypertrophied cells.

sues to the interior of the outer epidermis of the embryos were undeveloped and collapsed while the outer epidermal cells retained their shape. Cells in the pedicels of some of these florets were deeply safranin-stained with compressed vascular tissues, and in other pedicels had a normal appearance with both safranin and fast green stain retained. If aborted florets had short pedicels, they became compressed by the growth of the surrounding, developing florets. *Schistonchus* were associated with 12 aborted flo-

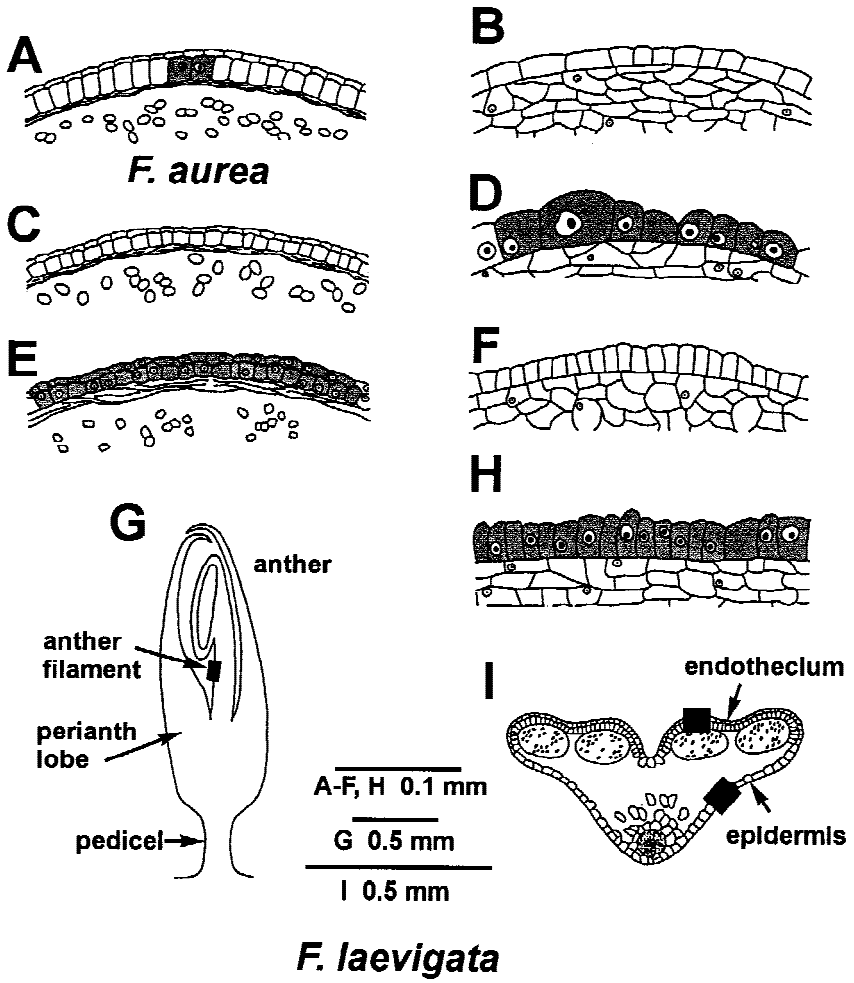

FIG. 6. Camera lucida drawings from *Schistonchus*-infested phase C syconia of male florets from *Ficus* in the subgenus *Urostigma*. Shaded cells indicate *Schistonchus*-induced hypertrophy. A) Hypertrophy of endothecial cells with normal cells of *F. aurea*. B–I) *F. laevigata*. B) Normal anther epidermis. C) Uninfested endothecial cells with normally developed pollen. D) Hypertrophied anther epidermis. E) Hypertrophied endothecial cells with pollen aberration. F) Normal filament epidermis. G) Longitudinal section through a typical *Urostigma* male floret. H) Hypertrophied filament epidermis. Tissues F and H were drawn from the black rectangular region in the illustration. I) Typical *Urostigma* anther cross section. Endothecial tissues A,C, and E and epidermis B and D were drawn from the black rectangular regions in the illustration.

rets and were frequently located between the floret and its perianth lobes. Six of these florets had short pedicels, five of which had no hypertrophied cells, necrosis, or exudate. One floret, which was filled with nematodes between the aborted embryo and its perianth lobes, had slight hypertrophy on the epidermis of the perianth lobes. The other six of these florets had long pedicels. In two instances, *Schistonchus* were associated with the stigmas but did not cause hypertrophy, necrosis, or exudate. One of these florets had hypertrophied cells on the epidermis of a perianth lobe that was next to the stigma. Two florets contained no hypertrophied cells or exudate. The other two florets possessed hypertrophied cells at the bases of their pedicels which were next to a group of nematodes at the syconial wall. *Ficus aurea* was the only species examined that had *Schistonchus* associated with what appeared to be dead tissue.

The syconial wall of *F. aurea* had 34 sites with *Schistonchus* infestations, of which 14

were hypertrophied. Hypertrophy was limited to the epidermal layer and was frequently contiguous with hypertrophied pedicel epidermis. Hypertrophied cells were square to rectangular, as long as or slightly longer than normal cells, were generally half again as high. Twenty sites with necrosis associated with *Schistonchus* also were observed on the syconial wall. Necrosis of the cells in the syconial wall was frequently observed alone or with hypertrophied cells. Necrotic pockets, filled with nematodes, sometimes extended several cells deep into the syconial wall, but hypertrophy was never observed beneath the epidermis. Hypertrophy of the epidermis was also noted on 10 pedicels and on three perianth lobes where the floret type could not be determined.

Schistonchus generally occurred in discrete groups within the syconia of *F. aurea*. Areas of numerous nematodes and extensive feeding damage occurred adjacent to large areas without nematodes, suggesting that the nematodes depended on hypertrophied cells for sustenance. *Schistonchus* were usually associated with an exudate, possibly leakage from tanniferous cells. Numerous sites with *Schistonchus* and exudate occurred in the syconial cavity and between florets of all types without observable cellular damage in the vicinity.

Histopathology of nematode-infested syconia of F. laevigata: As previously reported (Giblin-Davis et al., 1995), 262 florets were examined from serial sections of four *Schistonchus*-infested, late-phase C syconia of *F. laevigata*. We re-examined these sections in this study. Twenty-nine percent (n = 75) of these were seed florets, 43% (n = 114) were wasp florets, and 9% (n = 23) were aborted. Reexamination confirmed that no *Schistonchus,* necrosis, or hypertrophy was associated with these floret types.

Nineteen percent (n = 50) were male florets. Of these, 23 were observed to have *Schistonchus* and hypertrophy that affected the endothecial cells (Figs. 5G; 6E), the epidermis of the anthers (Fig. 6B,D), and anther filaments (Fig. 6F,H). *Schistonchus* infestation of only male florets was unique to *F. laevigata*. Hypertrophy was possible in all epidermal cells of the anthers and filaments, but the epidermal cells of the dorsal midrib of the filament usually were not affected. Hypertrophy in the epidermis was typical. Hypertrophied endothecial cells were the same size or slightly enlarged compared to normal cells (Fig. 6C,E). Compared with hypertrophied epidermal cells, the cytoplasm of the hypertrophied endothecial cells was not as deeply stained, and the nucleus and nucleolus were not as enlarged. When the numbers of *Schistonchus* were high, the hypertrophied cells appeared to lose their integrity.

Schistonchus damage also caused aberrations in the shape of the anthers, filaments, and pollen. Pollen production often appeared normal in one or more pollen sacs of lightly infested florets. However, in heavily infested florets, the four pollen sacs were completely deformed with little or no pollen. Hypertrophied cells were observed on the epidermis of one perianth lobe and at one site on the epidermis of the syconial wall.

Histopathology of nematode-infested syconia of F. poponoei: Florets (n = 117) were examined from serial sections from one *Schistonchus* sp.-infested, mid-phase C syconium of *F. poponoei*. Twenty percent (n = 24) of these florets were seed florets. Of these, four short-pedicelled florets had hypertrophy. *Schistonchus* and associated feeding damage occurred at the junction of the florets and perianth lobes, with the affected areas being about 10 cells long (Figs. 4F; 5F). This epidermal layer was contiguous with the outer epidermis of the floret but did not overlay the embryo. When the small, rectangular epidermal cells of these florets were hypertrophied, they were the same size to twice as high as normal cells with a granular cytoplasm. Hypertrophy in the tanniferous cells at the junction of the floret and the perianth lobes also resulted in similar-sized to slightly enlarged cells. These cells had a granular cytoplasm containing droplets, suggesting possible mobilization of the tannins. Three of the florets had hypertrophy on their perianth lobe epidermis opposite the affected area of the floret. These cells were the same size as normal cells or were very slightly enlarged (Fig. 4H). Two of the affected florets

had *Schistonchus* at the base of their pedicels with necrosis and hypertrophy of the epidermis.

Twenty-six percent ($n = 30$) of the florets examined were male florets. No *Schistonchus* were associated with this floret type.

Thirty-two percent of the florets ($n = 38$) were wasp florets. One wasp floret pedicel was observed with *Schistonchus* at the base with a small number of hypertrophied cells and necrosis on the epidermis of the syconial wall and pedicel.

Twenty-one percent ($n = 25$) of the florets examined were aborted. Five of these had normally developed floret wall layers. A gap was formed within the floret where the outer integument adhered to the collapsed embryo and was pulled away from the endocarp. One of these florets had hypertrophy on the outer epidermis and on the epidermis of its short pedicel. These were square cells about the size of normal cells. *Schistonchus* were also at the base of this floret causing necrosis of the syconial wall epidermis, and within the pedicel of this floret associated with hypertrophy of the cortical parenchyma. These cells were the same size as unaffected cells, had an enlarged nucleus and nucleolus, and had a grainy lightly-staining cytoplasm. When nematodes were present in high numbers, the cell walls appeared wrinkled, suggesting a loss in turgidity. This was the only instance of *Schistonchus* being found within plant tissues in the *Ficus* spp. examined. All other observed *Schistonchus* were in the syconial cavity, in necrotic pockets, or between florets and their perianth lobes.

In addition to the two described examples of syconial wall involvement, there were nine *Schistonchus*-infested areas on the syconial wall, including three with hypertrophy (Fig. 4I). Two of the hypertrophied sites were at the base of seed florets, and one was at the base of an aborted floret. These sites had few hypertrophied cells with extensive necrosis of the epidermis. The hypertrophied cells were the same size or slightly enlarged compared with normal cells. Necrosis of epidermal cells on the syconial wall was observed at the base of one other pedicel of an aborted floret, at the bases of four other pedicels whose floret type could not be determined, and at one site without a pedicel involved. No pockets of necrotic tissue in the syconial wall were observed in this species.

Histopathology of nematode-infested syconia of F. trigonata: Florets ($n = 238$) were examined from serial sections of one *Schistonchus*-infested, late-phase C syconium of *F. trigonata*. Thirteen percent ($n = 31$) of these were male florets, 39% ($n = 93$) were wasp florets, and 21% ($n = 51$) were aborted florets. None of these floret types had *Schistonchus* associated with the florets, pedicels, or perianth lobes.

Five of the 63 seed florets had *Schistonchus* sp. associated with them. All infested florets had short pedicels. Hypertrophied cells on the outer epidermis were either small and rectangular or enlarged with a rounded outer cell wall (Fig. 4D). The hypertrophied outer epidermis on some florets appeared to be falling apart and collapsing into a layer with enlarged nuclei visible within it. Cells of the outer mesocarp were hypertrophied when exposed to *Schistonchus* (Figs. 4B; 5E). Two florets had hypertrophied cells on their pedicels, and one had hypertrophied cells on a perianth lobe at the site of the floret infestation. The epidermis of the syconial wall had hypertrophied cells and necrosis at the base of infested floret pedicels. In one case a pocket was formed into the syconial wall that was filled with *Schistonchus*. At all sites with hypertrophy, the nematodes were lined up side by side.

Histopathology of nematode-infested syconia of F. glabrata: Florets ($n = 125$) were examined from serial sections of one *Schistonchus*-infested, mid-phase C syconium of *F. glabrata*. Thirty-five percent ($n = 44$) of these were seed florets. One short-pedicelled floret was observed to have hypertrophy on the outer epidermis (Fig. 7B), where it was covered by a perianth lobe. The hypertrophied cells were about twice as high as normal cells with a greatly enlarged nucleus. *Schistonchus* were present at the base of this floret at the syconia wall with hypertrophy and necrosis to the epidermis.

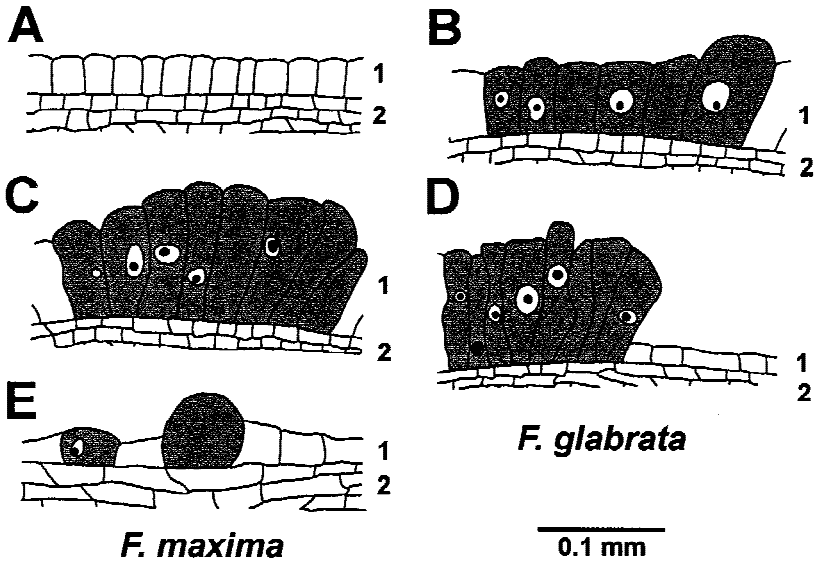

FIG. 7. Camera lucida drawings from *Schistonchus*-infested phase C syconia of *Ficus* subgenus *Pharmacosycea*. Shaded cells indicate *Schistonchus*-induced hypertrophy. A,C,E) *F. maxima*. A) Typical uninfested female floret with developing embryo (seed floret). C) Hypertrophy of the outer epidermis of a seed floret. E) Hypertrophy of the epidermis on a pedicel with a region of normal cells. B,D) *F. glabrata*. B) Hypertrophy on the outer epidermis of a seed floret. D) Hypertrophy of epidermal cells on a pedicel of a female floret with an aborted embryo (aborted floret) with a region of normal cells.

Twenty-four percent ($n = 30$) of the florets were aborted. One short-pedicelled floret with developed tissues was affected by *Schistonchus* feeding. The tissues of the outer integument were intact, but all tissues to the interior of it were collapsed, causing a gap where it was pulled away from the endocarp. Hypertrophy was present on the outer epidermis, the epidermis of the pedicel (Fig. 7D), and both sides of a stunted perianth lobe adjacent to this area. Hypertrophy and (or) necrosis (Fig. 5B) affected the syconial wall at the base of this floret. Hypertrophied cells on the outer epidermis of the floret and on the epidermis of the pedicel were four to five times the height of normal cells for these tissues (Fig. 7B,D), while the hypertrophied cells on the perianth were only slightly enlarged.

Twenty-seven percent of the florets ($n = 34$) were wasp florets. A few *Schistonchus* were observed between the style and the perianth lobes of one floret. No hypertrophy, necrosis, or exudate was present. Fourteen percent ($n = 17$) of the florets were male with no observed *Schistonchus* or damage.

Histopathology of nematode-infested syconia of F. maxima: Florets ($n = 244$) were examined from serial sections of one *Schistonchus*-infested, late-phase C syconium of *F. maxima* (Figs. 3C,F; 7A,C,E). Twenty-five percent ($n = 62$) of the florets were seed florets. Of these, six had hypertrophy on their outer epidermis. Four had short pedicels, and two had long pedicels. This was the only instance of hypertrophy in florets with long pedicels in the *Ficus* spp. we examined. Hypertrophy of the outer epidermis of the florets occurred over most of the floret surface (Figs. 5C,D; 7C). Hypertrophied cells occurred on the inner surface of the perianth lobe epidermis near the *Schistonchus* infestations. Where the perianth and pedicel joined, necrotic pockets were formed into the parenchymal tissues of these structures. Hypertrophied cells were on the epidermis of the pedicels (Fig. 7E), particularly at the top where the perianths joined and at the base at the syconial wall, where necrosis was extensive.

Thirty-one percent ($n = 75$) of the florets were aborted; two of these florets were affected by *Schistonchus*. One of these florets

was completely aborted and had hypertrophy on the outer epidermis where it was in contact with an infestation on a seed floret. This was the only instance where hypertrophy was observed on the outer epidermis of a completely aborted floret. The other floret had developed tissues similar in appearance to those described for other species. This floret had large hypertrophied cells on the outer epidermis, on the epidermis of its perianth lobes, and at the base of the pedicel. The syconial wall at the base of this floret was necrotic. *Schistonchus* were lined up, side by side, at all hypertrophied cells on the outer epidermis of florets with a large amount of exudate present.

Twenty percent (n = 48) of the florets were male florets, and 24% (n = 59) were wasp florets. No *Schistonchus* or hypertrophy was associated with these floret types.

Discussion

Schistonchus spp. were found in 20% to 50% of the syconia of the *Ficus* spp. examined. All *Schistonchus*-infested syconia exhibited hypertrophy in close proximity to nematodes. The location of the parasitized sites within the syconia varied among species but appeared to be more constant among geographical locations than among the *Ficus* taxonomic groupings.

The appearance of floral tissues changes with syconial maturity (Verkerke, 1986). We attempted to collect syconia of the same maturity, however, the length of phase C can range from 15 to 100 days in different *Ficus* spp. (Ramirez B., 1974). Thus, some maturity differences probably existed between the syconia of the *Ficus* spp. that we examined.

The *Schistonchus* parasitism levels for the Panamanian *Ficus* spp. may not be typical, since few synconia were examined. The morphology of the host response could be density-dependent and, if so, more samples would be needed for meaningful comparisons. For the Florida species, numerous syconia were available at various parasitism levels. The tissues targeted by *Schistonchus* spp. in *F. aurea* and *F. laevigata* were specific and consistent at different infestation rates, but parasitic effects were more pronounced with higher numbers of *Schistonchus*.

The effects of *Schistonchus* parasitism on *Ficus* reproductive biology were not quantified. However, several qualitative trends were apparent. The present study confirmed that in the *F. laevigata-Schistonchus* association, immature male florets are infested exclusively. *Schistonchus* was associated with extensive hypertrophy of anther epidermal cells and subsequent damage to pollen formation. The extent of the damage suggests that nematode feeding on the immature anthers causes changes that are developmentally magnified. Of the *Ficus-Schistonchus* associations observed, this was the most potentially damaging to the reproductive success of a *Ficus* host. If pollen availability is limited, pollination efficiency could be affected. Conversely, *Schistonchus* caused the least pathology to *F. aurea,* with less than 1% of florets showing hypertrophy. In this species, most of the pathology involved necrosis and hypertrophy of the syconial wall.

In the two Panamanian *Ficus* subgenera examined, the sites of *Schistonchus* parasitism were similar to *F. aurea* from Florida. In general, hypertrophy in the florets was limited to the outer epidermis of the seed florets and to aborted florets with developed tissue layers. It is not clear whether *Schistonchus* caused the embryos to abort or infested aborting florets. Secondary involvement seems most likely because many aborted florets with developed tissue layers were observed without *Schistonchus.* Developmental studies are needed to clarify this point.

In all *Ficus* spp. examined except *F. laevigata, Schistonchus*-associated pathology appeared to be superficial, without real damage to the reproductive success of its host plant or to its pollinator wasp. *Ficus* syconia are protogynous and female florets are more mature than the male florets at the time of *Schistonchus* infestation (phase B). This may explain the differences in *Schistonchus* pathology.

Occasionally in seed florets the cells of the inner mesocarp had aberrations when they were underneath hypertrophied outer epidermal cells. Where the outer epidermis was

missing, this tissue layer was sometimes hypertrophied (Fig. 4B). In all observations, except for the endothecial cells, hypertrophy was only one cell deep. The endothecial cells of *F. laevigata* were sometimes hypertrophied under the epidermis (Fig. 6E), and in the one instance of endothelial hypertrophy in *F. aurea* these cells were under normal epidermis (Fig. 6A). All of the embryos in parasitized seed florets appeared healthy when compared with neighboring uninfested seed florets. In the *Ficus* spp. examined, only one of the 594 wasp florets was found to have hypertrophy on its outer epidermis (in *F. aurea*). In contrast to *F. carica,* where *S. caprifici* develops in the immature or adult pollinator wasp, *B. psenes* (Volvas and Larizza, 1996), none of the Neotropical *Schistonchus* were found inside developing wasps. Hypertrophy of the cortical parenchyma in a pedicel of *F. poponoei* was the only instance of *Schistonchus* occurring within plant tissue. More sectioned material would need to be examined to determine if this is typical for this nematode-plant relationship.

Plant-parasitic aphelenchoidids that have been studied so far cause cell death and necrotic lesions at their feeding sites (Dropkin, 1969; Jones and De Waele, 1990). The genus *Schistonchus* may be unique in this family by inducing hypertrophy. The appearance of *Schistonchus*-associated hypertrophy was similar in all the *Ficus* spp. that we examined. Hypertrophy of the epidermal cells of the pedicels and perianth lobes, and of the outer epidermis of female florets and female florets with aborted embryos, resulted in enlarged cells (typically 2–5× larger) that had a granular cytoplasm with an enlarged nucleus and a prominent nucleolus. Hypertrophy of the epidermis of the syconial wall, the endothecial cells in the anthers of *F. laevigata* and *F. aurea,* and in the cortical parenchyma of *F. poponoei* was similar, but with less-pronounced symptoms. The enlarged cell size, granular cytoplasm, and enlarged nucleus and nucleolus in hypertrophied cells suggest increased metabolic activity and a specialized function. These cells may be analogous to uninucleate feeding cells, which are stimulated by secretions of some phytoparasitic nematodes. Necrotic areas often were associated with *Schistonchus,* possibly resulting from the breakdown of hypertrophied cells.

This study brings the total number of *Ficus-Schistonchus* associations examined histologically to seven species in three sections of three subgenera from three geographical locations. Considering there are about 700 species of *Ficus* worldwide (Berg, 1989) in four subgenera and 17 sections, generalizations about coevolutionary trends must be limited until further work is done.

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