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Embryological studies in *Melia azedarach* L.^{1,2}

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GALATI, B. G. (Cátedra de Botánica, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina), S. ROSENFELDT (DBBE, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina), AND G. M. TOURN (Coordinación Sede Polo Universitario Punilla Centro. Laboratorio Estación de Biología. FAUBA. Huerta Grande. Córdoba, Argentina). Embryological studies in *Melia azedarach* L. J. Torrey Bot. Soc. 138: 26–33. 2011.—Previous studies described the lateral flowers of *Melia azedarach* cymules as staminate, and the terminal one as perfect. This paper shows that all the flowers of the inflorescence present both gynoeceium and androeceium but only the terminal ones produce fruits. Both types of flowers are successful in developing megagametophytes and viable pollen. All flowers are fertilized but ovules of the lateral flowers of cymules degenerate after fertilization. The lateral flowers mature later than the apical ones. Therefore, it could be apical dominance by terminal flowers that prevents development of seeds from lateral ones as a possible energy saving. However, when the apical flower fails in its development after fertilization, lateral flowers can form seeds and fruits. This ensures good seed production in this species and that is what makes it a highly invasive species.

Key words: megagametophyte, *Melia azedarach*, perfect flowers, pollen.

Chinaberry (*Melia azedarach* L.) is a fleshy-fruited tree from Asia (Pennington 1981, Mabberley 1984, Miller 1990) that was introduced to Argentina to be used as a shadow-tree, timber, or locust barrier (Cozzo 1972, Ragonese and García 1980 cited in Mabberley 1984). In the Palmar National Park (Entre Ríos, Argentina) this species became an aggressive invader and is currently present in ca. 60% of the park with different degrees of invasion, from low (isolated individuals) to severe (dense thickets) (Movia and Menvielle 1990). Previous studies on this species focused on taxonomic features (see Mabberley 1984) but no description of those plant traits that may be relevant to understand its invasive capacity, were made. This species became an aggressive invader in Argentina that typically reproduces by seeds.

Papers about of the embryology in species of the family Meliaceae are scarce and there are no recent studies on this subject (Johri et

al. 1992). In *Melia azedarach* the development of the megagametophyte was described as Polygonum type (Nair 1959). The antipodals are ephemeral and at times they become multinucleate (Nair 1959).

The inflorescence is a many-flowered axillary panicle with cymose branchlets that develop from the basal nodes (preformed buds) of the current-year shoot unit (Tourn et al. 1999). The lateral flowers of cymules were described as staminate (Miller 1990), and the terminal one as perfect. Our preliminary observations showed that all flowers of the inflorescence contain both gynoeceium and androeceium. However, only the terminal ones produce fruits. The aim of this paper is to confirm the potential fo fruit and seed production by the lateral flowers of the inflorescence, which would ensure good seed production in the case of terminal flower abortion, and would help explain the invasive nature of this species. Additionally, we studied the ontogeny of the megagametophyte and the pollen grain, as well as, the fertilization and development of the endosperm comparatively between the lateral and terminal flowers of the cymules.

Materials and Methods. The study was conducted at El Palmar, National Park, Entre

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Ríos Province, Argentina (31° 55' S, 58° 10' W) in an area not subjected to prescribed fire and on the campus of the F.C.E.N, Buenos Aires University, Argentina.

Flowers from the cymules (apical and lateral) at different stages of development were collected, fixed in Formalin- ethanol- Acetic Acid (FAA), dehydrated in a graded ethanol series and embedded in Tissuemat (Fisher Scientific Co., New York, 56 °C melting point). A rotary microtome was used to obtain 10–12 µm thick sections, which were subsequently stained with safranin and fast-green (D'Ambrogio 1986).

The callosic walls were studied using aniline blue staining in low concentration (0.01%) which imparts a yellow fluorescence (Rost 1992). The photomicrographs were taken with Axioplan equipment, a Wild M20 microscope and a Canon PowerShot A650 IS digital camera.

Three different treatments (T1, T2, T3) were done in the inflorescences before anthesis. Three different trees were used and each treatment was performed in three different inflorescences from each one. Each treatment consisted of removing one or two flowers of inflorescence cymules (Fig. 1): T1= the apical flower of each cymule of the inflorescence was removed, T2= the apical flower and one lateral flower were removed, T3= one lateral flower was removed and C= control (cymules complete). At the end of fruiting season, the inflorescences were collected.

Results. The flowers are perfect. No morphological differences were observed between the terminal and lateral flowers of the cymules because both flowers have a gynoeceium and androeceium. The following descriptions are common to both types of flowers (lateral and terminal flowers). The distinctive features are specifically mentioned.

OVULE, MEGASPOROGENESIS AND MEGAGAMETOGENESIS. Ovule development, megasporogenesis and megagametogenesis were similar in all flowers analyzed.

The ovule is crassinucellate, bitegmic (Fig. 2A), with the micropyle formed by the inner integument. The nucellar epidermal cells divide to produce a nucellar cap (Fig. 2A). In the mature ovule, a hypostase is observed (Fig. 2B, C). The cell walls of this tissue stain red with safranin-fast green and they fluoresce

after staining with aniline blue indicating the presence of callose (Fig. 2D).

The megaspore mother cell divides meiotically and follows two successive divisions resulting in a linear tetrad (Fig. 2A). The three micropylar megaspores degenerate and the chalazal one develops into the megagametophyte. Three successive mitotic karyokineses give rise to the eight-nucleate female gametophyte. A central vacuole is formed and four nuclei are positioned in the micropylar end of the cytoplasm, and the other four nuclei in the chalazal end. After the 8-nucleate stage, the coenocytic megagametophyte becomes partly cellular. It consists of seven cells: egg cell, two synergids, central cell and three antipodal cells (Fig. 2B, C). These last ones are ephemeral. The egg cell and the synergids are small and slightly vacuolated.

MICROSPORANGIUM, MICROSPOROGENESIS AND MICROGAMETOGENESIS. The anther wall comprises the epidermis, endothecium, one or two middle layers, and tapetum of secretory type (Fig. 3 A, B). The epidermis is papillate (Fig. 3A–C). The endothecium becomes fibrous in the mature stage. The middle layers are ephemeral. The tapetum cells are multinucleate and their nuclei fuse. These cells persist until the young pollen grain stage (Fig. 3C).

The sporogenous tissue was distinguishable by the presence of isodiametric cells with prominent nuclei, dense cytoplasm and few intercellular spaces.

Microspore mother cell walls become thicker because of the deposition of callose between the plasmalemma and the primary wall. Subsequently, they come apart by the dissolution of the middle lamella and primary walls that keep the sporogenous tissue together (Fig. 3A). Each microspore mother cell undergoes simultaneous reductive divisions and gives rise to microspore tetrads with tetrahedral arrangement (Fig. 3B). Each individual microspore separates from the tetrad by the sudden dissolution of the callose wall. The deposition of sporopollenin begins immediately after the release of microspores into the anther locule. Consequently, a thick exine wall is formed (Fig. 3C). The first division of the microspore gives rise to a small generative cell and a large vegetative cell. After microspore mitosis the vegetative cell continues to grow, the vacuole gradually disappears and the cytoplasm fills with starch grains (Fig. 3D). At this stage pollen grains are shed.

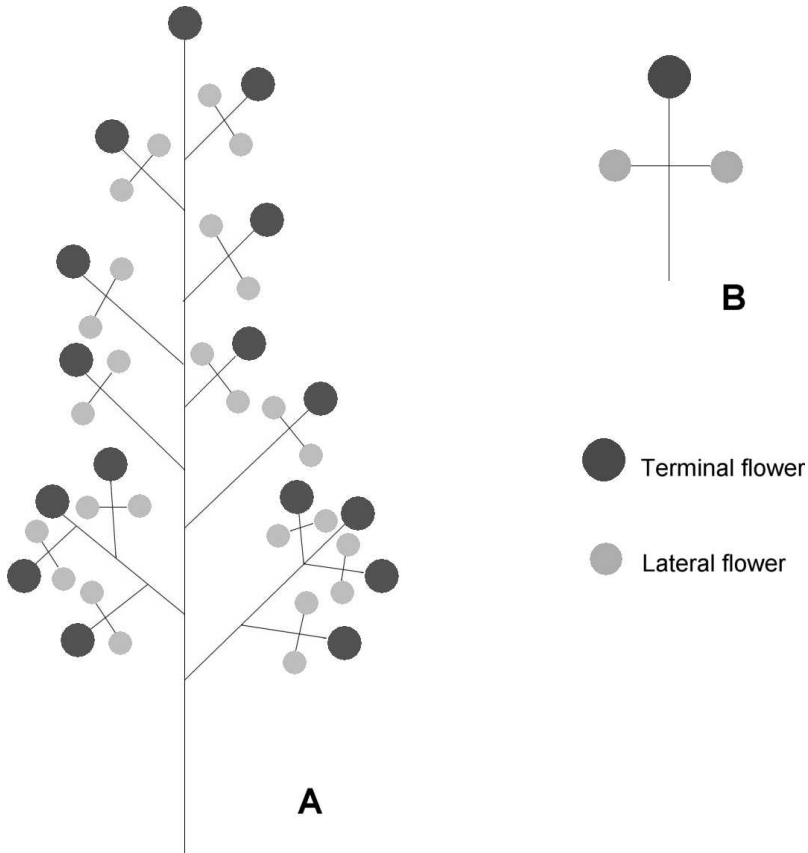


FIG. 1. *Melia azedarach*. A. The scheme represents the inflorescence. B. Detail of a cymule.

FERTILIZATION. The stigma is papillose. The papillae are unicellular (Fig. 4A, B). The style is hollow and has five and occasionally six canals coated by secretory epithelium (Fig. 4C). Several pollen tubes are observed growing through each styler canal (Fig. 4D).

The entrance of the pollen tube into the ovule micropyle is observed in the lateral and terminal flowers (Fig. 5A). Fertilization was completed in all observed flowers. After fusion, the primary endosperm nucleus and then the daughter nuclei undergo free nuclear divisions while the zygote remains without dividing (Fig. 5B, C). In this stage, the nuclear endosperm cytoplasm of the lateral flowers of the cymules shows abundant starch grains (Fig. 5B). These flowers aborted after fertilization. Conversely, the terminal flowers had little starch and continued their development to seed.

FLOWER REMOVAL TREATMENTS. The three different treatments (T1, T2, T3) and the

control (C) gave similar results because the formation of seeds and fruits was observed in all them. However, in T3 (with one lateral flower removed) and in C (control, cymules complete), only the terminal flower developed fruit. On the contrary, in T1 (the apical flower of the cymule removed) and T2 (the apical and one lateral flowers removed), the lateral flowers formed fruits and seeds. No unusual development was observed.

Discussion. The pollen and the megagametophyte development were coincident with the previous descriptions for the genus (Nair 1959, Johri et al. 1992).

A hypostase was observed in the ovule of *Melia azedarach*. This is the first report about the presence of a hypostase for the family Meliaceae. There are few reports about the presence of hypostases with callosic walls. *Ceiba insignis* Mill. presents a hypostase with these characteristics (Galati and Rosenfeldt 1997). The callose functions as a kind of

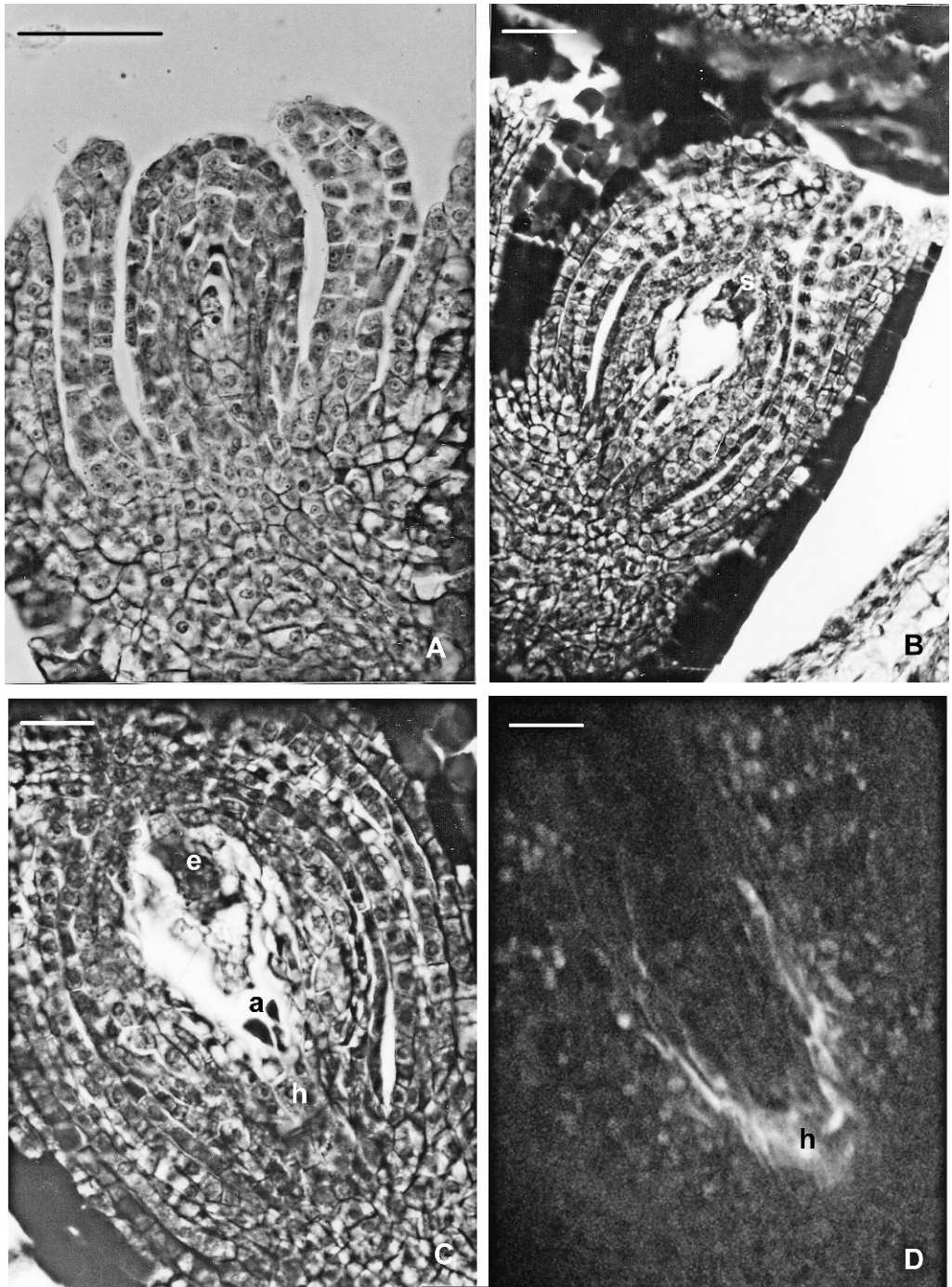


FIG. 2. *Melia azedarach*. Ovule, megalogonogenesis, megagametogenesis. A. Ovule primordium with tetrad of megaspores. Scale bar = 120 μ m. B-C. Mature ovule with megagametophyte. B. Detail of synergids. Scale bar = 77 μ m. C. Detail of egg cell (e) and antipodal cells (a); (h) hypostase. Scale bar = 30 μ m. D. Hypostase stained with aniline blue and observed with fluorescence microscope. Scale bar = 18 μ m.

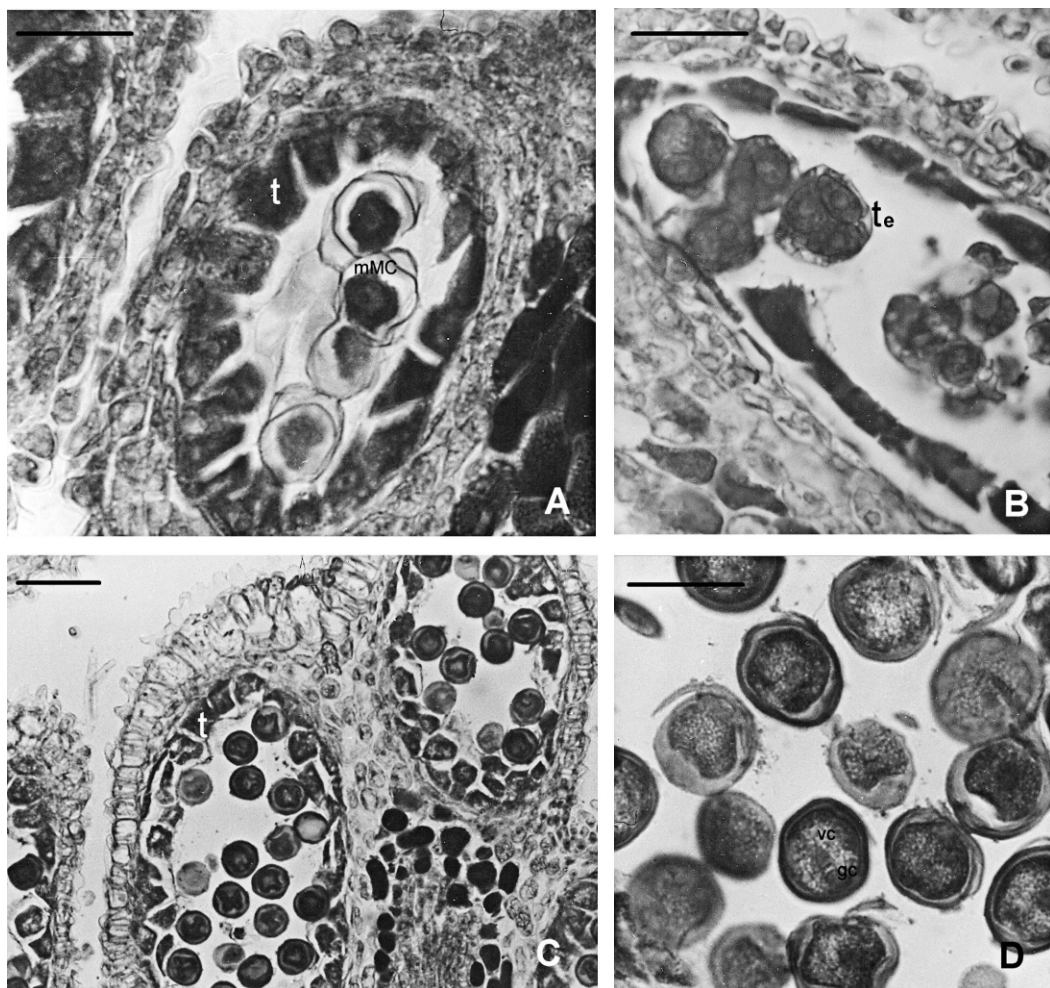


FIG. 3. *Melia azedarach*. Microsporogenesis, microgametogenesis. A. Microspore mother cells with callosic walls (mMC), tapetal cells (t) with numerous nuclei. Scale bar = 77 μ m. B. Microspore tetrads with tetrahedral arrangement (te). Scale bar = 77 μ m. C. Longitudinal section of an anther with young pollen grains. Scale bar = 150 μ m. D. Detail of bicellular pollen grain, vegetative cell (vc) and generative cell (gc). Scale bar = 77 μ m.

chemical isolation, established as a selective barrier or “molecular filter to the exchange of at least some macromolecules” (Bhandari 1984). Therefore, it is possible that the hypostase of *M. azedarach* acts as a barrier for the growing female gametophyte. It is known that callose is hygroscopic in nature, and has a pronounced ability to absorb water (Bhandari 1984). For this reason, this hypostase may help in the hydric regulation of the megagametophyte.

Morphological and embryological studies of terminal and lateral flowers of the cymules showed that they are perfect or hermaphrodite and are successful in developing megagameto-

phytes and pollen. Both types of flowers were fertilized. However, the lateral flowers did not develop fruits and seeds when the apical ones were present in the inflorescence cymules. These observations are not coincident with the description of Miller (1990) who described the lateral flowers of the cymule as staminate.

The early formation of starch in the nuclear endosperm of the lateral flowers was observed. This fact could be related with the abortion of the seeds in the fertilized lateral flowers. Strittmatter et al. (2002) observed in *Consolea spinosissima* (Mill.) Lemaire megagametophytes, specifically in the central cell, numerous large starch grains while the egg apparatus

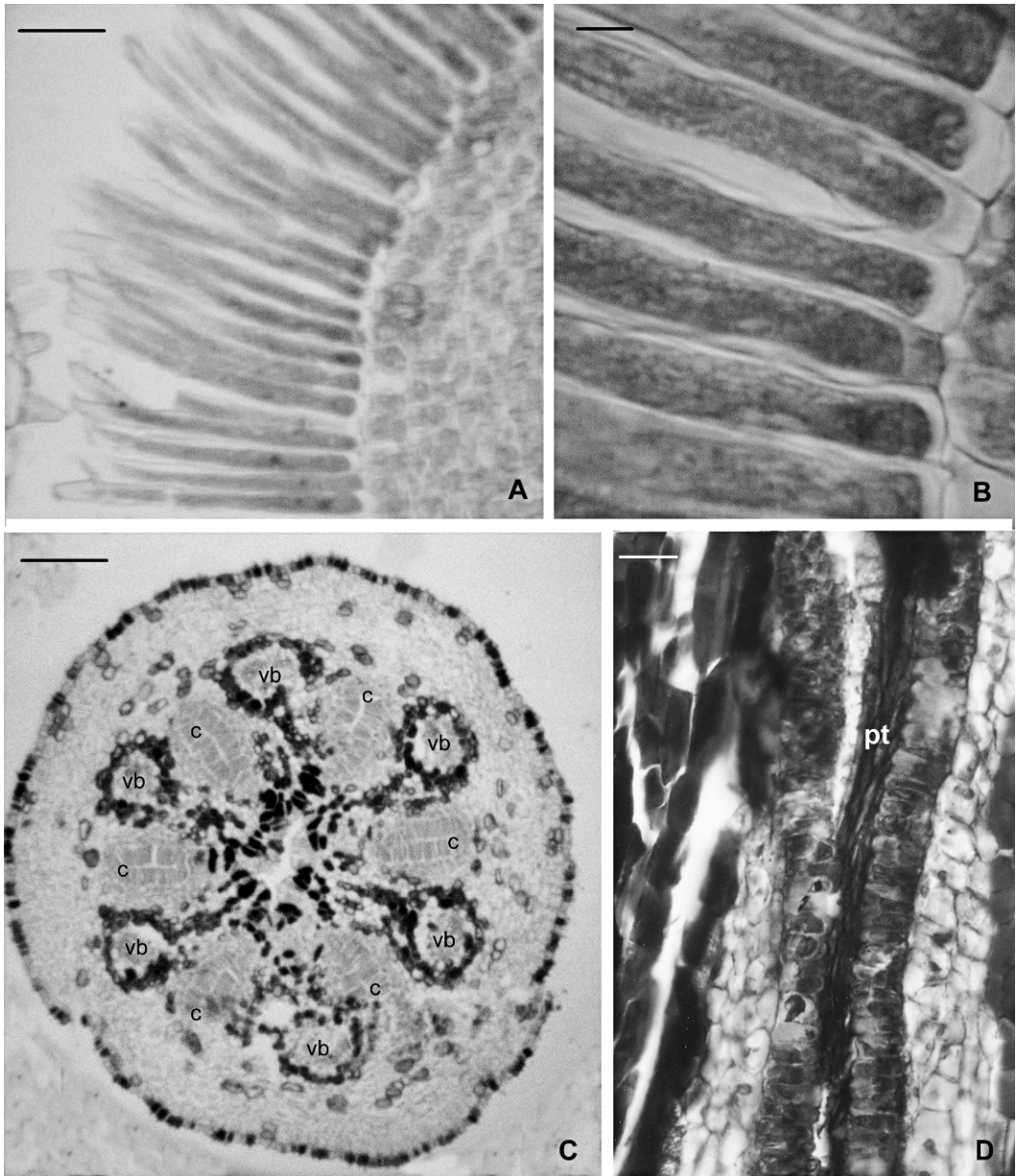


FIG. 4. *Melia azedarach*. A. Stigma with unicellular papillae. Scale bar = 150 μ m. B. Detail of the papilla base. Scale bar = 30 μ m. C. Transversal section of the style with six canals (c) and six vascular bundles (vb). Scale bar = 32 μ m. D. Longitudinal section of a styler canal showing secretory epithelium and pollen tubes (pt). Scale bar = 65 μ m.

degenerated. Abnormal accumulation of starch grains was also noted in the degenerating megagametophyte of *Opuntia dillenii* (Ker Gawler) Haworth (Tiagi 1954), in the megagametophyte's central cell of the seedless *Ramosmania heterophylla* (Balf.f.) Tirv. and Verdc. (Owens et al. 1993), and in the central cell of the PS-1 mutant (female sterile) of

Glycine max (L.) Merrill (Pereira et al. 1997). Thus, the presence of large starch grains appears to correlate with megagametophyte abortion.

Ovules of the lateral flowers of cymules degenerated after fertilization on non-sectioned cymules. When the apical flower was sectioned, the lateral flowers had fruit and

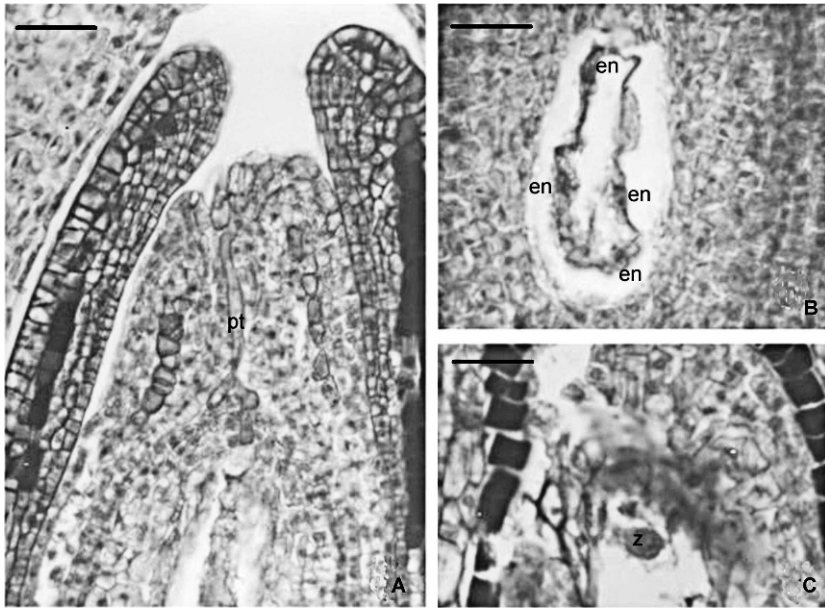


FIG. 5. *Melia azedarach*, lateral flowers. A. Detail of the mature ovule micropyle with a pollen tube (pt). B. Detail of nuclear endosperm (en, endosperm nucleus). C. Detail of the zygote (z). Scale bars = 125 μ m.

seed production. We consider the lateral flowers as functionally perfect. The lateral flowers mature later than the apical ones suggesting apical dominance by fertilized terminal flowers prevents development of fruits and seeds from lateral ones as possible energy saving. Therefore, when the apical flower fails in its development, the lateral ones can naturally form seeds and fruits. This ensures good seed production and may contribute to the success of this highly invasive species.

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