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Invasive tapetum and tricelled pollen in *Ambrosia trifida* (*Asteraceae*, tribe *Heliantheae*)

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Abstract: Staminate flowers of giant ragweed, *Ambrosia trifida* L. (*Asteraceae*, tribe *Heliantheae*, subtribe *Ambrosiinae*) were processed into resin and sectioned 1–2 μm thick. The invasive (amoeboid) anther tapetum remains parietal until microspores are released from tetrads, then it swells and invades the locule, merging gradually into a single protoplast that flows among the microspores. After the tapetal membrane ruptures at late microspore stage, tapetal debris fills the locule, then disappears as pollen matures. Pollen becomes tricelled before anthesis. The two sperm cell nuclei are slender and wormlike. The present report supports the two generalizations that invasive tapetum and tricelled pollen are attributes of the *Asteraceae*.

The tapetum is a layer of cells lining each of the four locules during pollen development. Two types of tapetum are recognized, one consisting of individual cells that remain peripheral in the locule (glandular or secretory type), and a strikingly different type in which tapetal cells enlarge, merge, and flow into the locule after meiosis, surrounding the microspores (amoeboid or plasmodial type). We prefer the neutral descriptive terms for these types coined by Heslop-Harrison (1969): parietal tapetum and invasive tapetum.

Pacini & al. (1985) recently reviewed the tapetal types and subtypes, including their physical characteristics, possible functions, and evolutionary aspects. An even more recent review (Albertini & al. 1987) indicates the high degree of current interest in the tapetum.

The distribution of tapetal types among angiosperm families was analyzed by Davis (1966) and supplemented later by Bhandari (1984). Their broadest generalizations are that the parietal tapetum is most common by far, and that the invasive type is more common in monocots but also occurs in some dicots. Sporne (1973) and Pacini & al. (1985) concluded that the parietal type evolved first, and that the invasive type is a derived form.

The *Asteraceae* is the largest family of dicots. It is of interest that virtually all tapetal descriptions among its diverse taxa are of the invasive type. Davis (1966) did not mention the number of reports contributing to her generalization that the

invasive (amoeboid) type pervades the family, but she indicated only one contrary report.

PULLAIAH (1984) reviewed all aspects of embryology of the *Asteraceae*. A column in his table 1 lists the type of tapetum reported in published studies, which are distributed widely among the tribes. Although not an exhaustive compilation, he included 106 species of 78 genera from 12 tribes, taken from about 80 different investigations. Of these, 98 species were reported to have an invasive (plasmodial) tapetum, and only eight were described as parietal (glandular or secretory). In his discussion of the tapetum, PULLAIAH mentions that re-examination of some species reported previously to have a parietal tapetum has revealed an invasive tapetum instead. He seems to doubt the validity of all reports of a parietal tapetum in this family.

One could carry this doubt further and question even the papers in which an invasive tapetum has been reported. Most published observations have been presented as drawings, usually sketchy and/or of very limited areas; some are merely statements lacking any documentation. The standard paraffin methods typically used often yield poor preparations of the fragile and sensitive tapetal cells, especially if they lack a cell wall. DAVIS (1966) warned against placing confidence in such methods, especially if fixation is poor and only a few sections are made. Two recent papers by VILLARI (1987 a, b), for example, report a "plasmodial" tapetum in *Helichrysum* (tribe *Inuleae*) but the accompanying photomicrographs of paraffin-sectioned anthers are not convincing.

DAVIS (1966) advocated greater use of "crushes and dissections" of whole anthers, preferably of fresh anthers, to study the tapetum accurately. Careful fixation methods, and the use of resin instead of paraffin as an embedding medium, will also yield excellent preparations, as demonstrated by the three most detailed, and probably most artefact-free, descriptions of the invasive tapetum in *Asteraceae:* HORNER (1977) and HORNER & PEARSON (1978) on *Helianthus annuus,* and SUN & GANDERS (1987) on several species of *Bidens*. These three studies were conducted on two genera of the tribe *Heliantheae*, each from a different subtribe (*Helianthus* in *Helianthinae*; *Bidens* in *Coreopsidinae*) according to the classification of STUESSY (1977).

We present here a description of the invasive tapetum in *Ambrosia trifida* L., giant ragweed, another species of *Heliantheae*, but from the *Ambrosiinae*, a distinctive subtribe specialized for wind-pollination. Since *Heliantheae* is the largest tribe of *Asteraceae*, with about 260 genera and 3 000 species (ROBINSON 1981), it is hardly redundant to add a third unambiguous example to the two existing ones. PULLAIAH (1984) noted in his table 1 that 32 species of 26 genera of *Heliantheae* had been reported on, with all claiming an invasive (plasmodial) tapetum. Only one brief mention, however, was from a member of *Ambrosiinae*, that of MAHESHWARI DEVI (1963) on *Xanthium strumarium*.

All *Asteraceae* have tricelled pollen before anthesis, according to DAVIS (1966) and BREWBAKER (1967). The latter concluded that "The uniformity of cytology and morphology of composite pollen is noteworthy" based collectively on his original observations and published (but uncited in his paper) reports from 46 genera, including an unnamed species of *Ambrosia*. We feel that it is useful to show photographically and in situ the tricelled pollen of giant ragweed.

Material and methods

Staminate inflorescences at various stages of development were removed from locally abundant plants of *Ambrosia trifida* and placed in a modified FAA killing and preserving fluid consisting of equal amounts of solutions A (250 ml 95% ethanol, 25 ml acetic acid) and B (175 ml distilled water, 40 ml formalin, 10 ml glutaraldehyde) combined just before use. Samples were placed, within a few minutes, under a 15 psi vacuum at room temperature for one hour, then stored in the same FAA at room temperature. Flowers of various lengths were removed from these inflorescences, dehydrated in ethanol, and embedded in L. R. White acrylic resin. Sections were cut 1 – 2 μm thick with glass knives, stained with toluidine blue O, and mounted permanently in piccolyte. Voucher specimens are deposited in ISC.

Results

Staminate flowers have five stamens, each with four locules per anther. There are four peripheral cell layers external to the sporogenous tissue: epidermis, endothecium, an ephemeral middle layer, and the tapetum (Fig. 1 a). The tapetum is a uniseriate layer of darkly-stained cells lining each locule by the time sporogenous cells have completed their last mitotic division and become microspore mother cells ready for meiosis (Fig. 1 a). The tapetal cells are uninucleate at this stage.

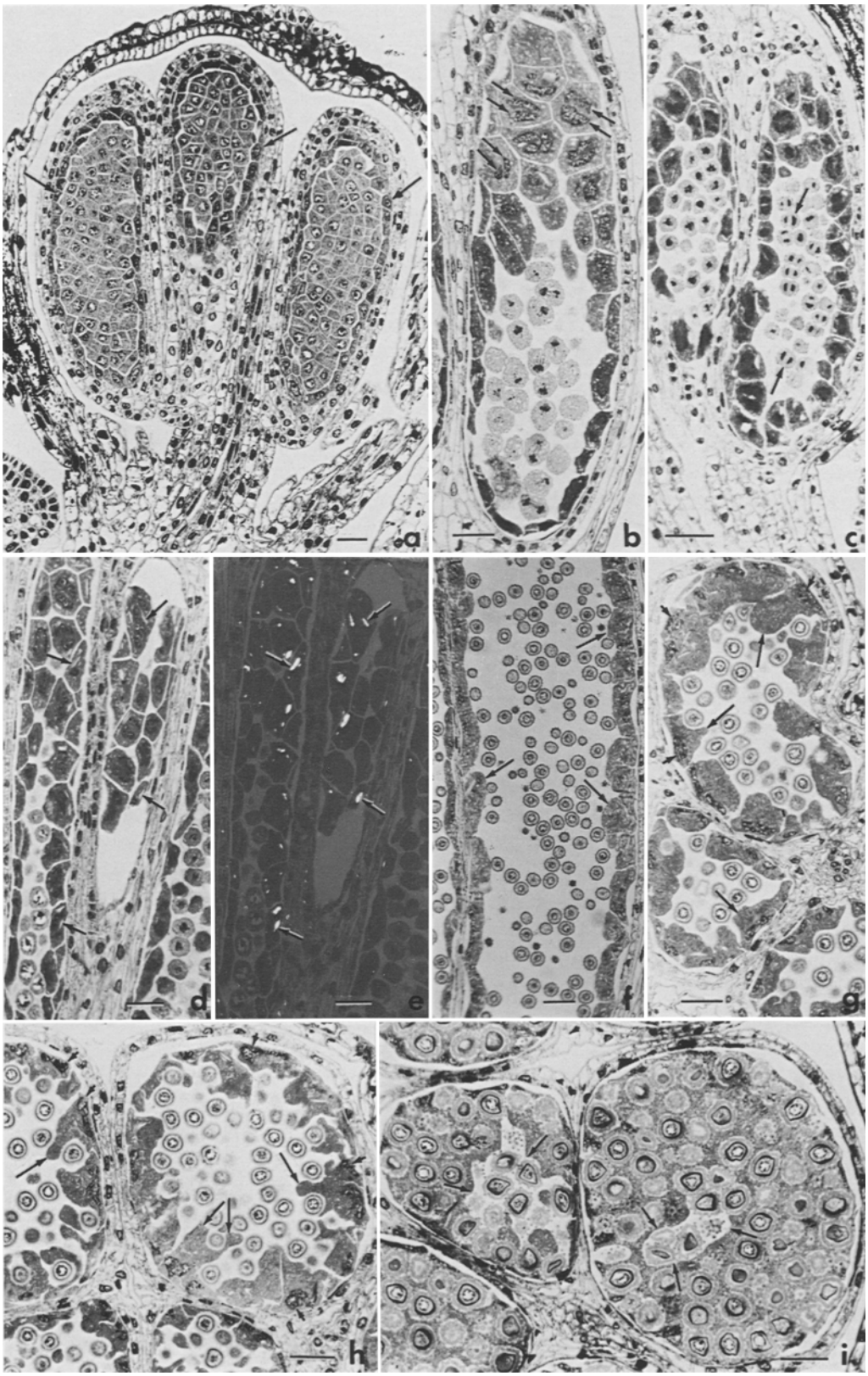
During first meiotic division of the microspore mother cells many, if not most, tapetal cells undergo mitosis and become binucleate (Fig. 1 b). The tapetal cell wall also seems to disappear during meiosis (Fig. 1 b, c), causing these cells to separate from each other.

Small prismatic or short styloid crystals first appear in tapetal cells at about the start of meiosis, but are absent from other parts of the stamen. The crystals can be seen as small but conspicuous white areas in tapetal cells in Fig. 1 b, c, but they are seen best in a face view of the tapetum in a locule sectioned obliquely longitudinally at meiosis I, in bright field (Fig. 1 d) and polarized light (Fig. 1 e), respectively. At later stages, crystals of the same size and shape also appear in other tissues of the stamen. These staminal crystals are in sharp contrast to the druse crystals that occur commonly in the bracts, pedicels, inflorescence axis, as well as in the stem cortex and pith of the same plants.

The tapetum remains parietal until after callose dissolves from around the tetrads and releases the microspores. Tapetal cells then begin to swell and intrude individually into the locule (Fig. 1 f). The microspores enlarge slowly, become vacuolate, and continue forming their complex pollen wall; tapetal cells enlarge faster and attain a much greater final size than the microspores, but they do not develop any vacuoles visible at the light microscope level. The swelling tapetal cells extend protoplasmic arms into the locule interior (Fig. 1 g, h), but the tapetal nuclei remain in a parietal position.

With deeper penetration of the locule, individual tapetal cells appear to merge into fewer, larger coenocytes and, at a somewhat later stage, it is evident that the tapetum now consists of either just a few large protoplasts or a single massive tapetal syncytium (Fig. 1 i). The spreading tapetum is still separated by a sharp boundary from the small area still unoccupied within the locule, which is indirect evidence that an enveloping tapetal membrane is still present.

At later microspore stages, after the developing pollen wall has formed the internal space (cavus) that gives it a conspicuously double-layered appearance, the tapetum inundates the entire locule with a uniformly dispersed granular mass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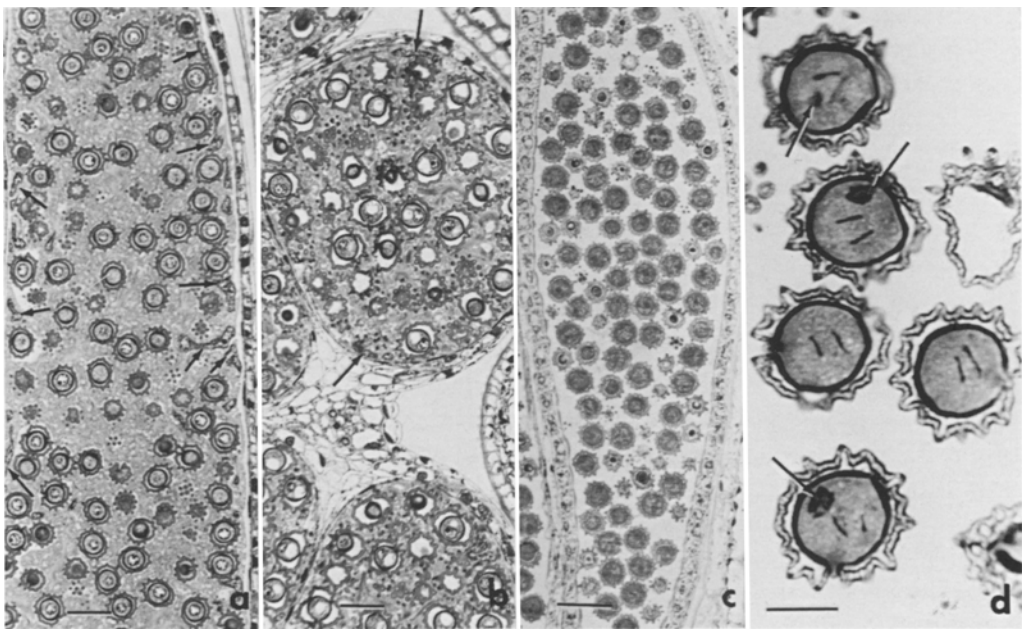

Fig. 2. Later stages of tapetum and pollen; bars: $a-c$ 20 µm; *d* and *e* 10 µm. *a* Longisection of locule at late microspore stage, with tapetal protoplasm uniformly distributed; tapetal nuclei remain at locule periphery (arrows). *b* Slightly later stage than *a*, in cross-sectional view; arrows indicate peripheral tapetal nuclei. *c* Longisection of locule with mature pollen; tapetal debris has disappeared. *d* Enlarged view of pollen grains, each with a large vegetative nucleus (arrows) and two darkly-stained elongate sperm nuclei

lacking any semblance of cellular structure except for the distorted tapetal nuclei, which remain peripheral (Fig. 2 a, b). HORNER & PEARSON (1978) observed by transmission electron microscopy that the tapetum in *Helianthus annuus* appeared disrupted from loss of the tapetal membrane and degeneration of tapetal organelles.

At some undetermined stage between microspore mitosis and pollen maturity, the tapetum disappears completely and the locule appears filled with spiny pollen

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Fig. 1. Invasive tapetum stages in *Ambrosia trifida*; bars: 20 µm. *a* Longisection of male flower including three locules, each with microspore mother cells and lined by a uniseriate tapetum (arrows). *b* Longisection of a locule in anaphase of meiosis I. Upper half shows face view of tapetum with binucleate cells (arrow pairs). *c* Longisection of two locules showing different meiotic stages (arrows to dyads) and tapetum still in parietal position. *d* Oblique longitudinal section of two locules in meiosis; arrows indicate a few of the many tapetal crystals that show as small white areas. *e* Same as *d*, seen under partial polarization, with arrows to same crystals. *f* Longisection of locule with young microspores recently released from tetrads; tapetum shows first signs of swelling (arrows). *g* Cross section of three locules showing tapetal cells more swollen and beginning to invade locule with microspores (arrows). Note tapetal nuclei against locule periphery (arrowheads), also in *h*. *h* Slightly later stage in cross section; tapetal protrusions (arrows) engulfing microspores. *i* Locules in cross section at later microspore stage; merged tapetal cells have invaded most, but not all (arrows at boundary) of each locule

grains (Fig. 2 c). The fate of the tapetal debris is unknown; HORNER & PEARSON (1978) suggested that, in *Helianthus annuus*, there is a correlation between tapetal disappearance and the engorgement of pollen with food reserves.

The two sperm cells of *A. trifida* pollen form before anthesis. We did not investigate their development, but their presence is indicated in mature pollen by their two wormlike nuclei. They are present in the pollen shown in Fig. 2 c, but a small area of one locule enlarged as Fig. 2 d shows them clearly.

Discussion

The invasive tapetum of *Ambrosia trifida* is remarkably similar to the tapetum of *Helianthus* and *Bidens*, even to the crystals, which were pointed out in the *Helianthus* tapetum by HORNER (1977) and which are evident, although not identified, in the bright-field photomicrographs of *Bidens* in SUN & GANDERS (1987). These are the only other studies of the tapetum of *Asteraceae* that have used superior resin-embedding and thin-sectioning methods. *Ambrosia, Bidens*, and *Helianthus* represent three widely separated subtribes within the *Heliantheae*, thereby strengthening the hypothesis that an invasive tapetum is characteristic at least of this tribe, if not of the family. The only other report from the *Ambrosiinae* (MAHESHWARI DEVI 1963) mentions a plasmodial tapetum and tricelled pollen in *Xanthium strumarium*, but these observations are only supported by two "generic" sketches that each represent nine species of eight genera.

The hypothesis that an invasive tapetum is a taxonomic character for this family can only be tested by a well-documented, adequate sample of tribes and critical subtribal groups of the *Asteraceae* supported by observations using unambiguous methods. An effort to obtain such a sample could yield significant results since present knowledge indicates that this type of tapetum is not very common in dicots as a whole. The usefulness of such information is evident on a smaller scale in the *Caprifoliaceae*, where a shift from parietal (secretory) to invasive (amoeboid) tapetum among certain groups of genera seems helpful to explain their evolutionary relationships (WEBERLING & HILDENBRAND 1982, 1986). For the *Asteraceae*, the most important task at present is to increase the sample size, and the quality of the observations, among the various tribes.

Note added in proof. Two recent papers show stages of invasive tapetum development in *Catananche caerulea* L. (tribe *Lactuceae*) clearly and convincingly using the novel approach of freeze-fracturing of anthers combined with scanning electron microscopy: BLACKMORE, S., BARNES, S. H., 1988, Ann. Bot. **62**: 605–614; and BARNES, S. H., BLACKMORE, S., 1988, Ann. Bot. **62**: 615–623.

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