Proceedings of the Sixth International Citrus Congress 431
Tel Aviv, Israel, March 6-11, 1988, R. Goren and K. Mendel (Eds)

Margraf Scientific Books, D-6992 Weikersheim
ISBN 3-8236-1136-4

An Ultrastructural Study on Development and Degeneration of Unfertilized Citrus Ovules

F.R TADEO AND E. PRIMO-MILLO

Department of Citriculture, Instituto Valenciano de Inverstigaciones Agrarias, Apartado Oficial, 46113 Moncada, (Valencia), Spain

Additional index words: Orange, integuments, nucellus, nucellus degeneration.

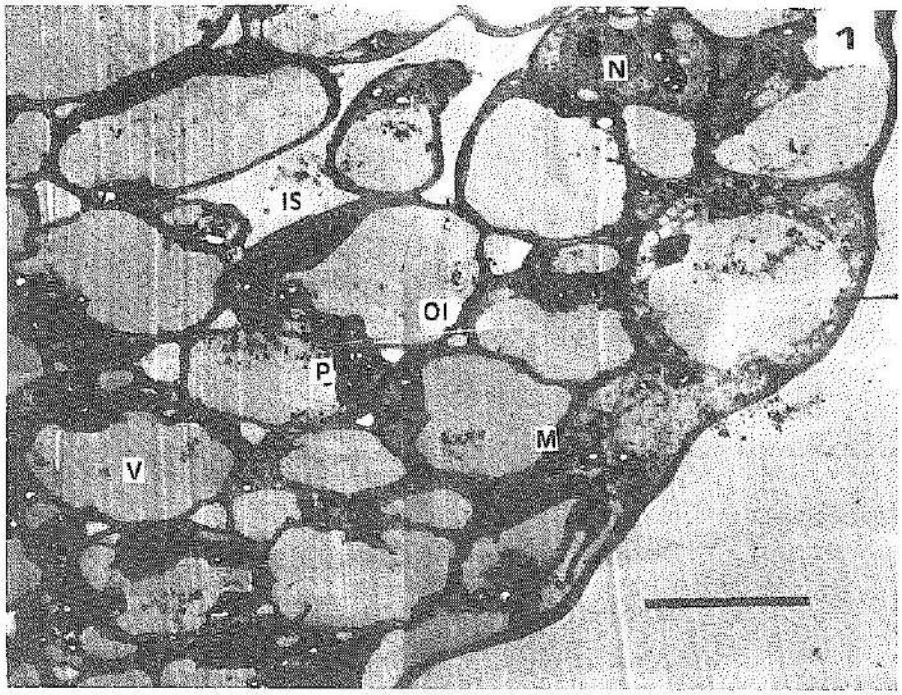
Abstract

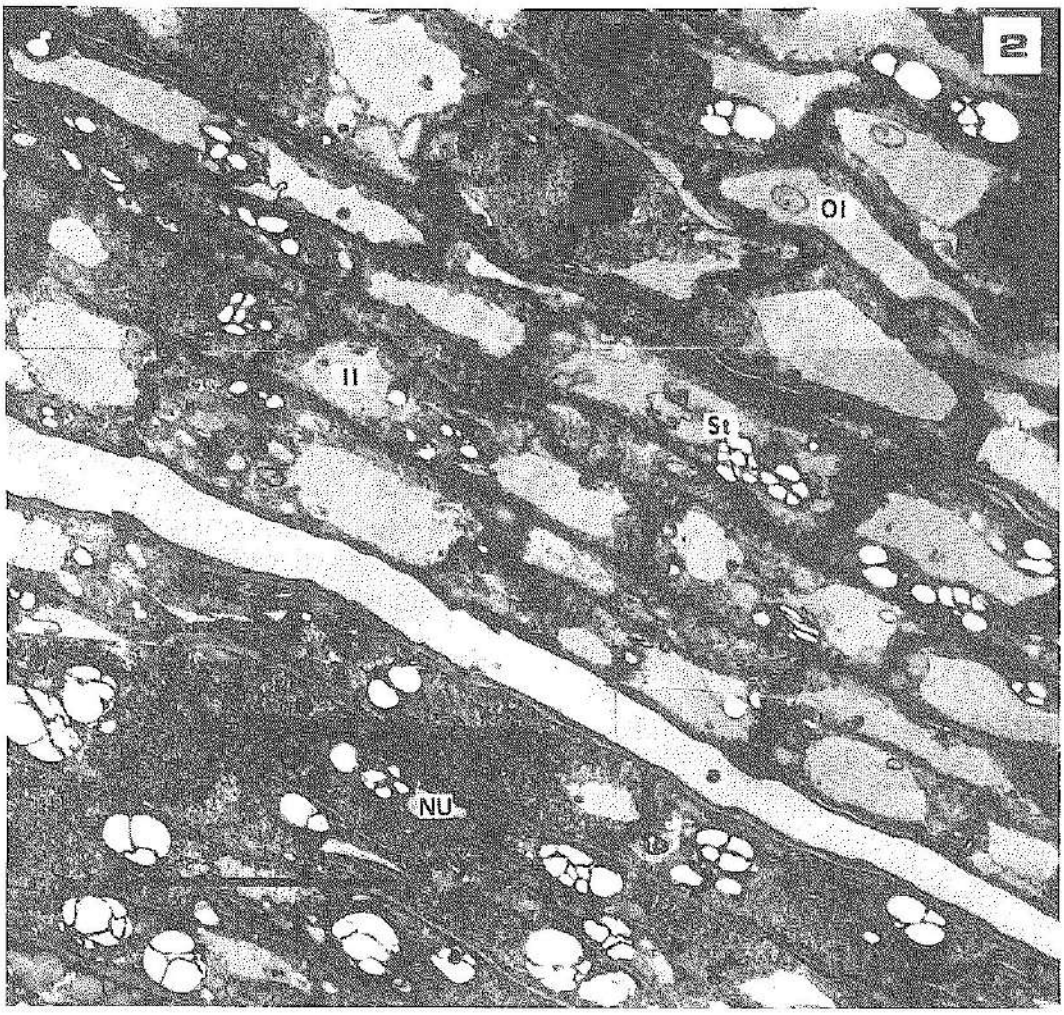
The ultrastructural changes of the different parts of citrus ovules (*Citrus sinensis* (L.) Osbeck, cv. 'Navelate'), outer and inner integuments and nucellus during their development, and the onset of following degeneration were studied. Remarkable changes in integument cells during development and degeneration were not observed. Both outer and inner integument cells have a large central vacuole and many amyloplasts containing large starch granules. Nucellus cells are rich in cytoplasm. At anthesis, their cytoplasm contains mitochondria, small profiles of endoplasmic reticulum and abundant free ribosomes and amyloplasts with large starch granules. Fifteen days after anthesis, the onset of nucellus degeneration takes place. Inside nucellus normal cells and cells with symptoms of degeneration coexist. Degenerating cells are distinguished by shrinkage of cytoplasm, disappearance of central vacuole and mixing of the different cytoplasmic constituents.

Abbreviations: CW = cell wall, G = Golgi area, II = inner integument, IS = intercellular space, M = mitochondrion, MV = multivesicular vaculoe, N = nucleus, Nl = nucleolus, NU = nucellus, OI = outer integument, P = plastid, Pd = plasmodesmata, R = ribosomes, RER = rough endoplasmic reticulum, St = starch grain, V = vacuole

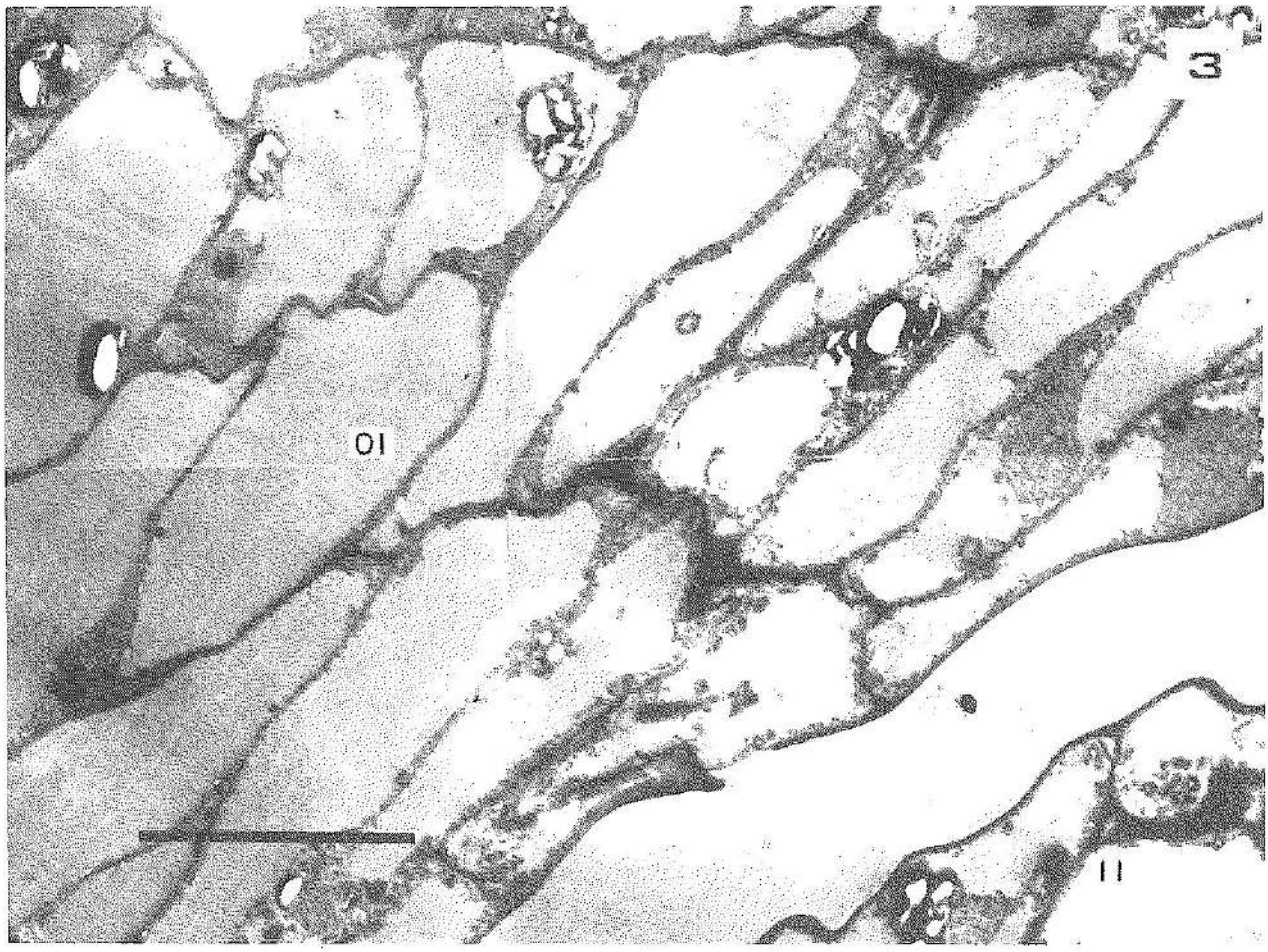
Introduction

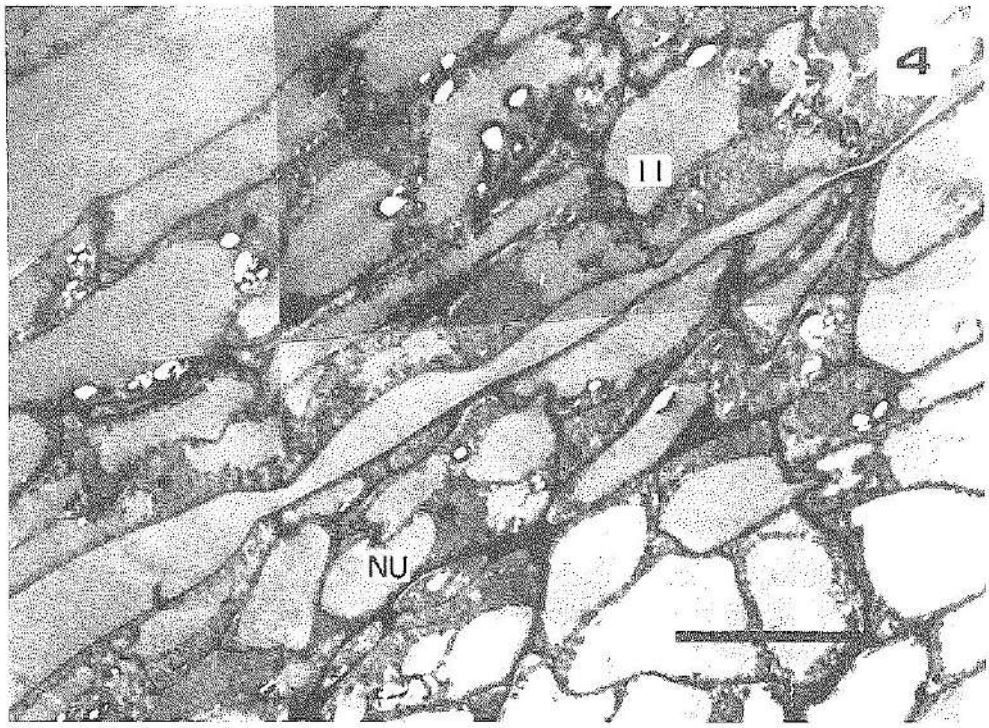
Parthenocarpic development of fruit is produced without seed development and therefore ovule abortion is a process that sooner or later will occur. However the precise time in which degeneration of citrus ovule tissues begins is unknown and whether ovules have some influence on setting and development of fruit before this phenomena takes place.



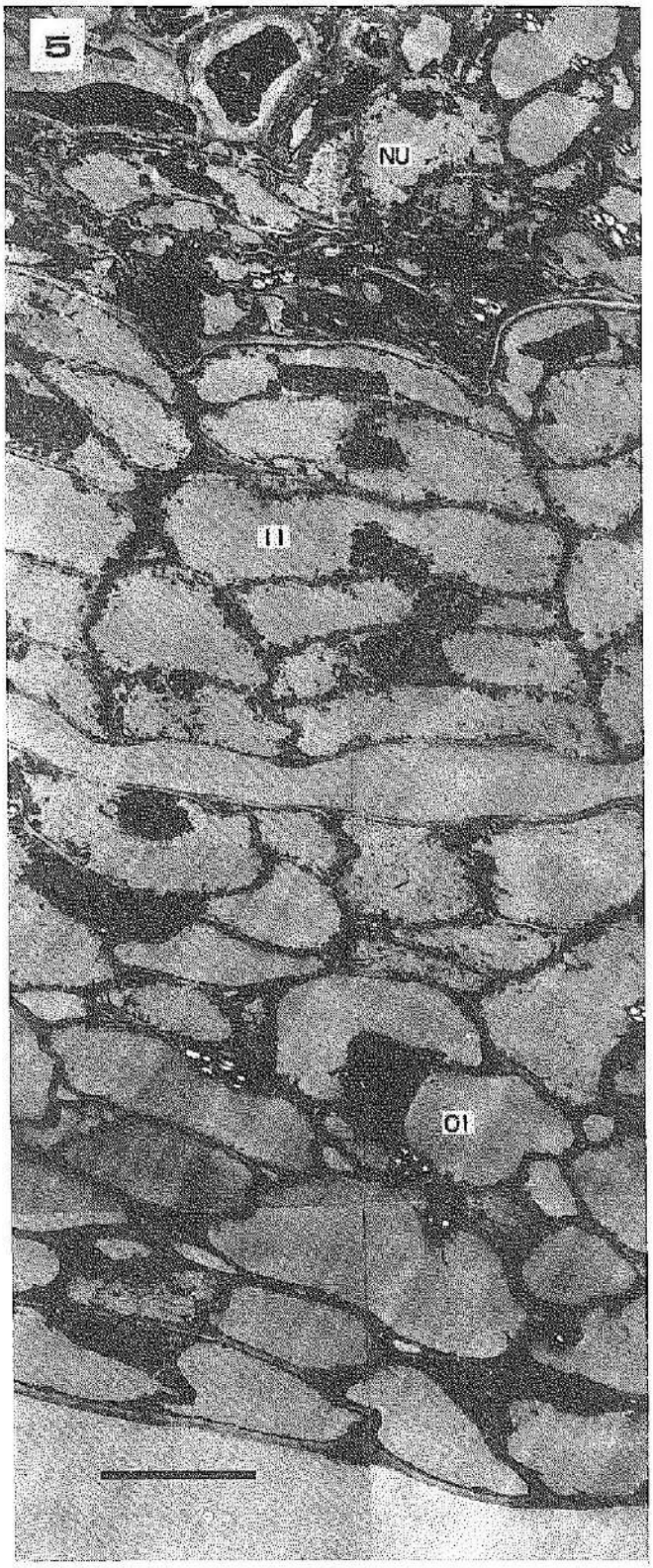


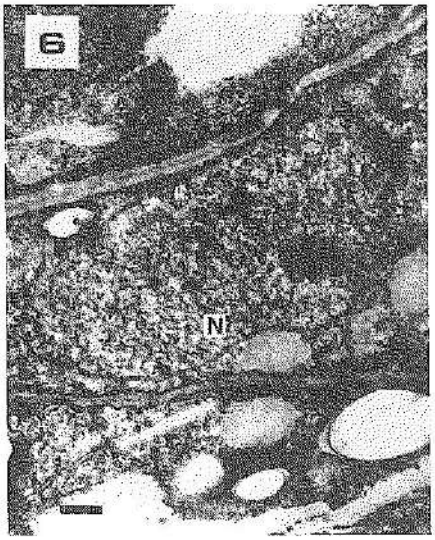
Figures 1 and 2: General partial views of outer integument (1) and inner integument and nucellus (2) from ovules in Stage I. Scale = 10 μm.





Figures 3 and 4: General partial views of outer integument (3) and inner integurment and nucellus (4) from ovules in Stage III. Scale bar = 10 μm.





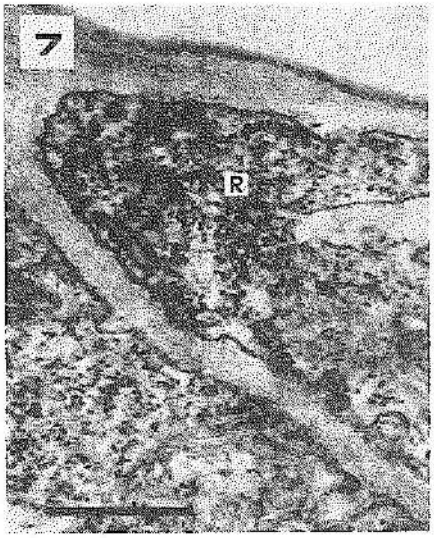
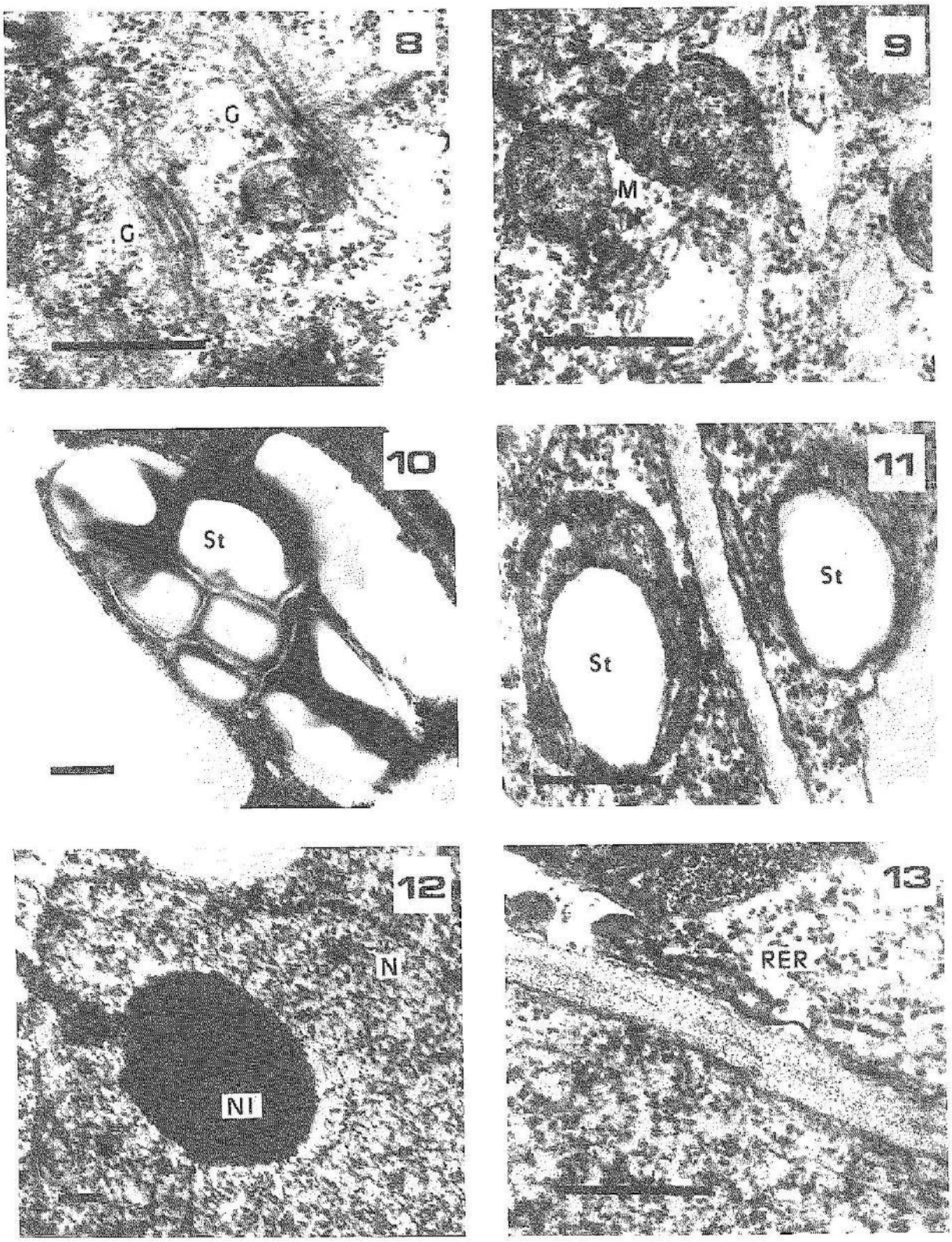


Figure 5: General partial view of outer and inner integument and nucellus from an ovule in Stage V. Scale bar = 10 µm.

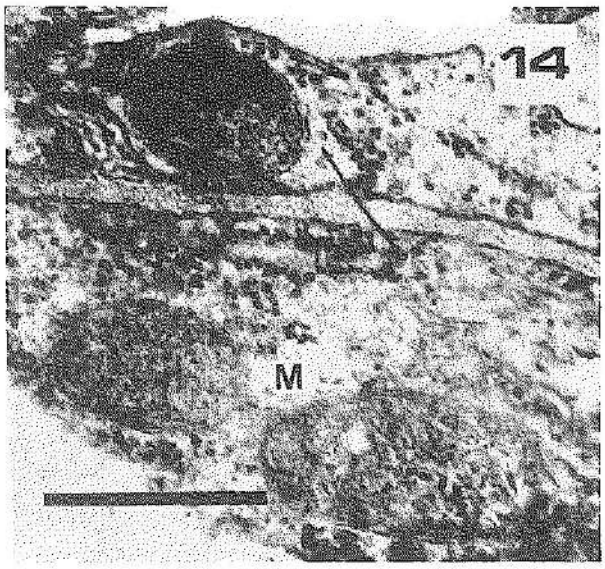
Figure 6: Nucleus of an outer integument cell from an ovule in stage I. Scale bar = 0.5 µm.

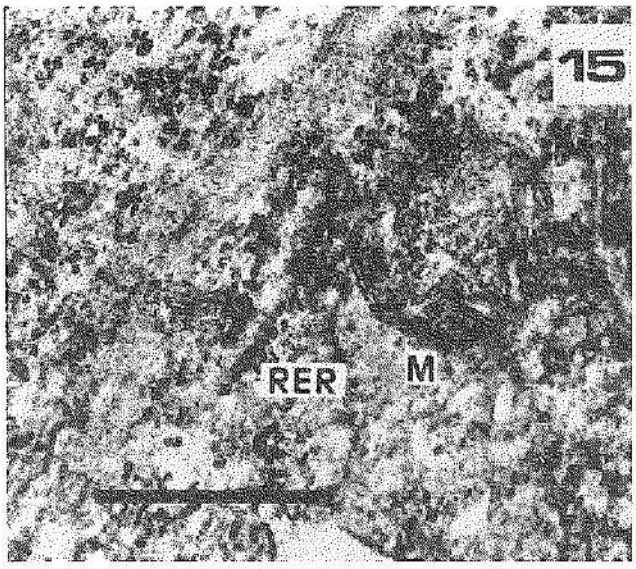
Figure 7: Portion of cytoplasm with abundant free ribosomes and RER of an inner integument cell from an ovule in Stage II. Scale bar = 0.5 µm.

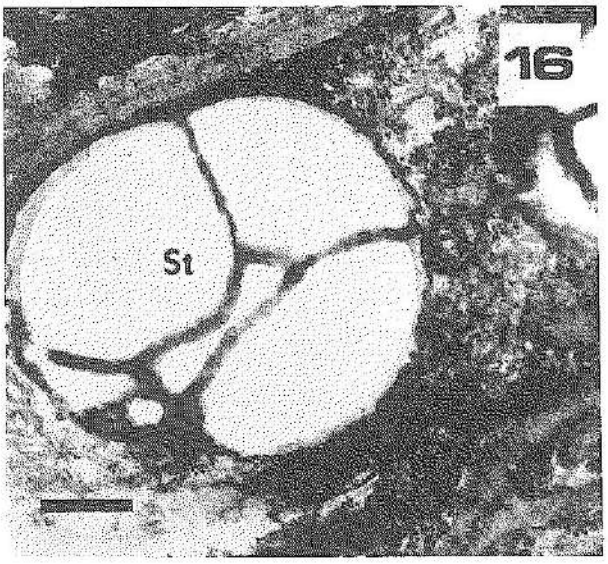


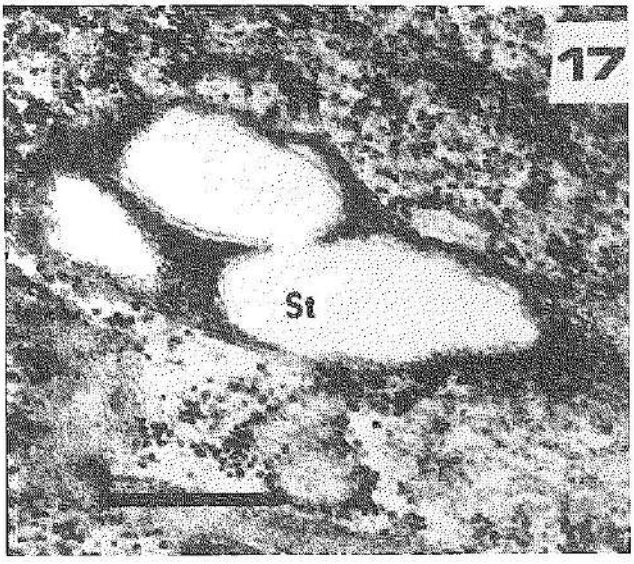
Figures 8 - 11: Portions of cytoplasm containing several cell organelles. (8) Golgi areas of an inner integument cell from an ovule in Stage I, (9) mitochondria of an outer integument cell from an ovule in Stage III, (10) amyloplast of an outer integument cell from an ovule in Stage II and (11) amyloplasts of an inner integument cell from an ovule in Stage I. Scale bar = 0.5 µm.

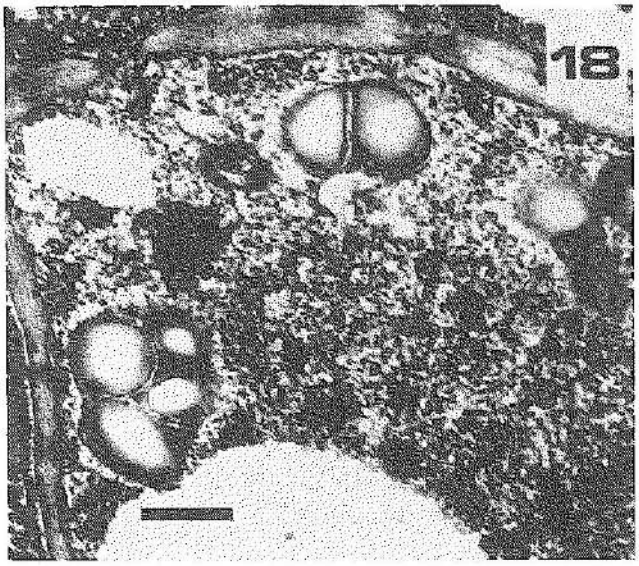
Figures 12 and 13: Portions of cytoplasm of nucellar cells from ovules in Stage I showing a section of a nucleus (12) and free ribosomes and RER (13). Scale bar = 0.5 µm.











Figures 14 - 18: Mitochondria of nucellus cells from ovules in stages I (14) and III (15), and amyloplasts from ovules in Stages I (16), II (17) and III (18). Scale bar = 0.5 μm.

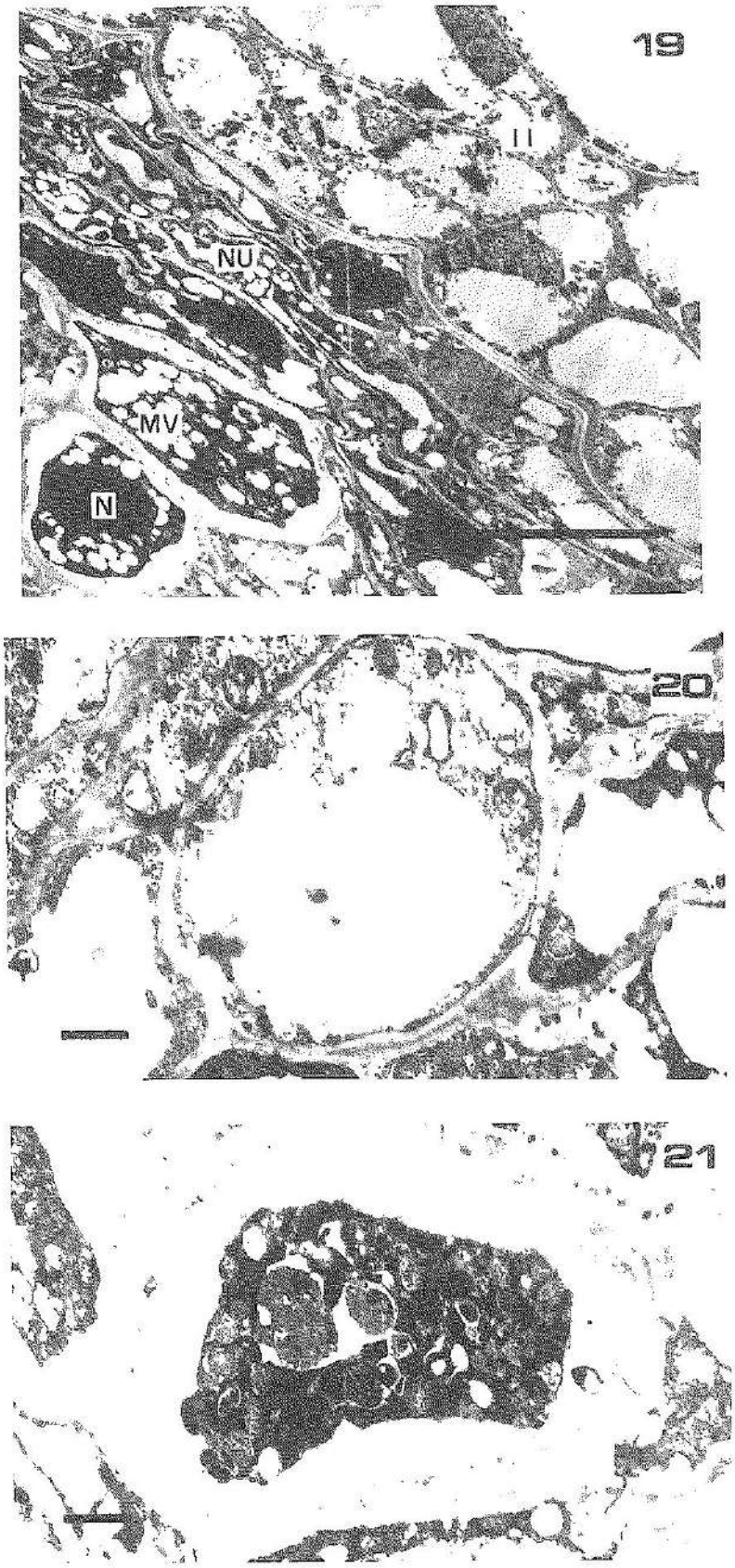
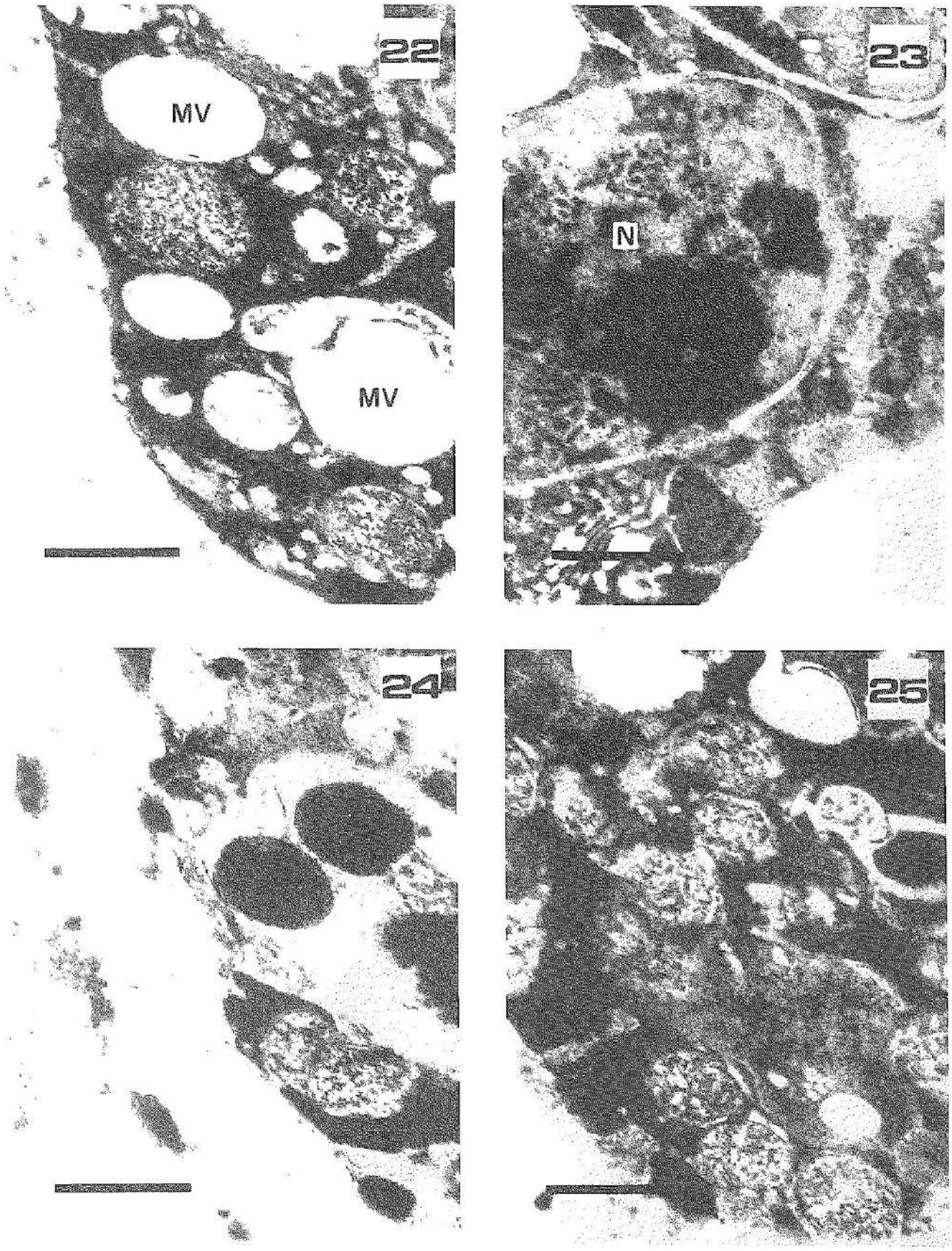


Figure 19: General partial view of nucellus from an ovule in Stage V. Note the presence of degenerating cells close to normal cells. Scale bar = 10 μm.

Figures 20 and 21: Nucellar cells from ovules in Stage V showing a normal appearance (20) and an advanced degree of cytoplasm disorganization (21). Scale bar = 1 μm.



Figures 22 - 25: Portions of cytoplasm of degenerating nucellar cells from ovules in Stage V. Note the presence of a pycnotic nucleus (23), multivesicular vacuoles and degenerating organelles. Scale bar = 0.5 μm.

Material and Methods

Ovules were obtained from ovaries and developing fruits of adult 'Navelate' orange (*Citrus sinensis* (L.) Osbeck) trees in five different stages of development: (I) closed flower ovaries 2.7 mm 0; (II) open flower ovaries 3.0 mm 0; (III) petal fall flower ovaries 4.0 mm 0; (IV) developing fruits 5.0 mm 0; (V) developing fruits 6.0 mm0. Sampling was made between April 15 and May 15, 1986.

Rows of ovules were dissected out from ovaries or developing fruits and fixed overnight in a solution containing 2% glutaraldehyde and 0.1M cacodylate buffer (pH 7.4). The tissue was postfixed in 1.25% Os04 in 0.1M Veronal-acetate buffer (pH 7.4) for 4 h and dehydrated in an ethanol series followed by propylene oxide. An intermediate step in dehydration was *the* en bloc staining with 2% uranyl acetate in 70% ethanol overnight. Dehydrated tissue was embedded in Durcupan. Gold-silver sections were cut with glass knives on a 8800 III LKB ultramicrotome and stained with lead citrate (8). A minimum of three ovules per sample were cut. Sections were viewed in a Jeol 100S electron microscope at 80 kV.

Results and Discussion

Integuments were formed by cells of variable size and shape (Figs 1-5). Their cells were highly vacuolated containing a large central vacuole that filled an important portion of the cellular volume. Sometimes the additional presence of other vacuoles, smaller than the central one and in a variable number, included within the cytoplasm were observed. The cell walls delimiting protoplast were thin and passed through by a small number of plasmodesmata. Peripheral walls of the first integument layer were thicker than those of internal cells and were covered by a thin cuticle (Figs. 1-5). The presence of intercellular spaces between integument cells was only observed in the peripheral cells of the outer integument. Intercellular spaces were apparently non-existent in deeper zones of outer integument and in all inner integument.

The nuclei, that appeared rather spheric, were located preferably associated to internal walls (Figs. 1-5). They contained a single nucleolus and a small amount of condensed chromatin, with a larger part of chromatin being scattered inside the nuclei (Fig. 6).

With regard to the cytoplasm, the integument cells had presented a small content of it and it was limited to a narrow strip surrounding the central vacuole. No differences in organelle composition in integument cells were observed throughout the period of time studied. Ribosomes content was fairly high; these seemed to occur free, in clusters, or as constituents of rough endoplasmic reticulum (Fig. 7). A small number Golgi areas (Fig. 8) and mitochondria with normal appearance, although only a few profiles of mitochondrial crests (Fig. 9) were observed. Plastids (amyloplasts) had a starch content relatively high with a variable number of inclusions inside (Figs. 10,11). The outer integument was rich in starch, retaining it

throughout the period of time studied. Similar results on structure and composition of ovule integuments have been reported (6,9,10).

Nucellar cells were smaller than the integument cells although there was a considerable variation both in size and shape (Figs. 2,4,5). Nucellar walls were thin, whereas those walls that belong to peripheral cells were thicker and covered by a thin cuticle. Their degree of vacuolation was lower than that of integument cells and contained more abundant cytoplasm. Nucellus cytoplasm has a nucleus with a single nucleolus and a few condensed chromatin, abundant ribosomes, profiles of rough endoplasmic reticulum, mitochondria and plastids (amyloplasts) (Figs. 12-18). Similar structural features of nucellar cells have been described in cotton (2), *Quercus gambelii* (5), *Bathiochloa ischaemum* (6), avocado (1), and *Oenothera biennis* (7).

Throughout the late stages of flowering and the early stages of fruit setting, there were no visible signs of degeneration in nucellar cells, and all their cell components had stable features. However, nucellus from ovules of stage V (approximately 15 days after anthesis) had some cells with symptoms of degeneration that coexisted with other cells of normal appearance (Figs. 20,21). Degenerating cells were distinguished by a shrinkage of protoplast with the presence of an empty zone between the cell wall and the plasmalemma (Figs. 5.19). The cytoplasm became dark and disorganized, starch inclusions disappeared, the nucleus was pycnotic, and it was almost impossible to recognize most of the cell organelles. Central vacuole disappeared and multivesicular vacuoles free in the cytoplasm were observed (Figs. 22-25).

Pattern of nucellus degeneration of citrus unfertilized ovules resembled the processes that took place in nucellus of fertilized ovules of *Oenothera biennis* during the development of the embryo sac (7) and in cotton synergids after fertilization (3). Cytoplasm darkening was a typical symtom of cell degeneration and disappearance of the central vacuole and disorganization, and mixing of different cytoplasmic constituents could be regarded as the death of cells by autolysis (4). The results obtained showed that nucellus degeneration started approximately 15 days after flower antheses but the complete degeneration of the ovule will take place several weeks later.

Literature Cited

1. Endress, A.G. 1979. Plastid ultrastructure in the avocado nucellus. *Ann. Bot.* 44:511–512.

2. Jensen, W.A. 1965. The composition and ultrastructure of the nucellus of cotton. *J. Ultrastr. Res.* 13:112–128.

3. Jensen, W.A. and Fisher, D. 1968. Cotton embryogenesis: The entrance and discharge of the pollen tube in the embryo sac. *Planta* 78:158–183.

4. Matile, P. and Wiemken, A. 1976: Interactions between cytoplasm and vacuole. In C.R. Stoching and V. Heber (eds.) *Encyclopedia of Plant Physiology, New Series.* pp. 255–287. Springer Verlag, Berlin, Neidelberg, New York.

5. Mogensen, H.L. 1973. Some histochemical, ultrastructural and nutritional aspects of the ovule of *Quercus gambelii*. *Am. J. Bot.* 60:48–54.

6. Moskova, R.E. 1975. An electron microscopic study of the nucellus cells in *Bothriochloa ischaemum* L. *Caryologia* 28:295–300.

7. Noher de Halac, I. 1980. Fine structure of nucellar cells during development of the embryo sac in *Oenothera biennis*. L. *Ann. Bot.* 45:515–521.

8. Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17:208–212.

9. Rudramuniyappa, C.K. and Phnchaksharappa, M.G. 1976. Histochemical localization of polysaccharides and nucleic acids in the ovules of *Zea mays* L. *Karnatak University J. Sci.* 21:244–251.

10. Sedgley, M., Newbury, H.J. and Possingham, J.V. 1977. Early development in the watermelon: anatomical comparison of pollinated, auxin-induced parthenocarpic and unpollinated fruits. *Ann. Bot.* 41:1345–1355.