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# STRUCTURAL ASPECTS OF FEMALE STERILITY IN CITRUS LIMON

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SUMMARY

In *Citrus limon* structural aspects of female sterility have been investigated. The results obtained are compared with the development of the female fertile flowers. Pistils of female sterile flowers appear defective in all stages of development. In pistils of female fertile flowers only a few ovules reach a fertilizable stage, most ovules do not form a mature embryo sac, but the development of integuments and nucellus seems normal. In non-functional pistils a possible blocking of further stigma and style development is related to the presence or absence of receptive embryo sacs in the ovule.

## 1. INTRODUCTION

In *Citrus* two types of female sterility are observed. Firstly there are numerous flowers of which the pistils have not developed up to the functional stage. Secondly, the functional female flowers contain numerous ovules of which only a limited number develop into viable seeds. In the latter sterility can be due to gametic sterility or zygote and embryo abortion (FROST & SOOST 1968). With respect to gametic sterility, OSAWA (1912) found that in oranges degeneration can occur from the megaspore mother cell stage up to the embryo sac stage. Only a few embryo sacs reach full development and contain egg cells capable of fertilization.

According to UPHOF (1931) in lemon functional embryo sacs are not formed at all, although occasional viable seeds are produced (REECE & CHILDS 1962), apparently by apomixis. This paper describes the structural aspects of female sterility in *Citrus limon*, based on information from both light and scanning electron microscopy (SEM).

## 2. MATERIALS AND METHODS

Plants of *Citrus limon* (L.) Burm. were grown in the Botanical Garden of the University of Siena, Italy. Excised ovules were fixed in 2.5% glutaraldehyde in 0.066 M Na-cacodylate buffer, pH 7.2, for 2 hrs at room temperature, post-fixed

in 1% $OsO_4$ in 0.066 M cacodylate buffer, pH 7.2, for 2 hrs and dehydrated in a graded ethanol-propylene oxide series and embedded in Epon-Araldite. Pistils, fixed in 2.5% glutaraldehyde in 0.066 M Na-cacodylate, pH 7.2, for 2 hrs were dehydrated and embedded in glycolmetacrylate according to FEDER & O'BRIEN (1968). SEM was applied to both fresh and prepared material. The prepared material was fixed in glutaraldehyde, critical point dried and sputter-coated with gold.

## 3. RESULTS

The mature pistil is composed of the ovary at the base, a slender 8–16 mm long style, and the stigma (apical knob). The ovary usually consists of twelve carpels, each enclosing completely a locule.

In fertile flowers the stigma surface consists of long epidermal papillae, covered with sticky exudate *(fig. 1)*. The style contains stylar canals, as many as the carpels forming the fruit. The stylar canals start just beneath the stigma and reach the ovary locules. They have a lumen of 4 $\mu$m and a radial length of 200 $\mu$m, bordered by specialized canal cells. The canal cells are elongated with their long axis orthogonal to the canal. They are very rich in cytoplasm and their inner tangential wall is thickened *(fig. 2)*. The stylar canal is filled with material secreted by the canal cells. In the locules the epidermal cells of the placenta elongate and form papillae *(fig. 7)*. These papillate cells are rich in cytoplasm and have thickened cell walls.

In sterile flowers the stigma surface also consists of elongated epidermal papillae, which, however, are less well-developed. The papillae have smaller dimensions, they are more vacuolated, and exudate formation does not occur. Also the adjacent parenchym cells are reduced in size *(fig. 3)*. The development of the style stops before the canal cells become functional. The length of these cells is reduced to 2/3 of the normal size whereas they have many vacuoles, less cytoplasm and no wall thickenings *(fig. 4)*. Excretion products are not observed in these stylar canals. In the loculi the ovules are poorly developed *(fig. 8)*. Some small buds without any differentiation are formed. No epidermal papillae develop.

In fertile flowers the development of the ovules is highly varying. The ovules are anatropic, bitegmic and crassinucellate *(figs. 5, 9)*. A few ovules contain a complete and well developed embryo sac. *Figs. 10, 11, 12* show serial sections of one mature female gametophyte in which the seven cells can be seen: the three antipodals are already degenerating, the two synergids with wall thickenings at the micropylar side (Filiform Apparatus) and large vacuoles at the chalazal side, the egg cell with chalazal cytoplasm and nucleus and the central cell with the two polar nuclei near the chalazal part of the egg cell.

However, most ovules show an aberrant development. In some ovules already the megaspore mother cell degenerates *(fig. 13)*. In other ovules megasporogenesis is completed, but subsequently the functional megaspore does not develop

further *(fig. 14)*. It also occurs that a complete embryo sac is formed, which starts degenerating before maturity is reached *(fig. 15)*. The size of all these non-functional ovules becomes almost the same as that of the functional ovules, and integuments and nucellus are well developed. After the pistil has reached the receptive stage, degeneration of the nucellus of the non-functional ovules starts *(fig. 16)*, and the growth of the ovules increases.

Pollination results in the fertilization of the few functional ovules. The subsequent development of the fertilized ovules is very slow. Even 40 days after pollination the zygote is yet undivided, while the central cell has formed a thin layer of nucleate-endosperm located along the wall of the embryo sac *(figs. 17, 18)*. Around the embryo sac and in the chalazal area degeneration of nucellus cells occurs, the remaining cells, however, strongly expand and frequently divide.

## 4. DISCUSSION

The formation and development of functional pistils in *Citrus limon* is accompanied by a complex of cell differentiation and specialization, involved in the formation of a receptive stigma surface (CRESTI et al. 1982), pollen tube conducting stylar canal cells (CIAMPOLINI et al. 1981) and placenta epidermis, and the development of fertilizable ovules. Non-functional pistils of female-sterile flowers are defective in development and functions, although there seem to be some differences in developmental stage reached by the various parts. Ovule development is interrupted directly after the initial formation of a small nucleus; megaspore mother cell formation is absent and integuments are not formed. Style and stigma reach an advanced developmental stage, as papillate stigma epidermis cells and stylar canal cells are formed, which, however, do not become functional.

It is not known, whether these differences results from differences in the timing of aberrant development, or from differences in the stage at which development is interrupted.

In *Spinacia* the penetration of the pollen tube into the nucellus of the crassinucellate ovule is only possible after the embryo sac has reached the receptive stage (WILMS 1981). This means that the presence and developmental stage of the embryo sac can be of influence on the development and functioning of the other tissues. Possibly in the non-functional *Citrus* pistil a similar situation is present in which the absence of receptive embryo sacs leads to the blocking of style and stigma development and function.

In the functional pistils only few ovules reach a fertilizable stage. Most ovules do not form a mature embryo sac. The development can be blocked at the megaspore mother cell stage (mmc), megaspore stage (ms) and embryo sac stage. Functional and non-functional ovules can not be recognized by their external morphology. Integuments and nucellus seem to develop normally.

Apparently the formation and development of nucellus and integuments do not depend on the development of mmc, ms or gametophyte, at least up to the

mature ovule stage. On the other hand, the degeneration of mmc, ms or gametophyte is not a matter of generally poor development of the ovule, but must depend on more specific factors. A similar conclusion was stated for *Citrus sinensis* by OSAWA (1912) and FROST & SOOST (1968). Differences become only evident after the functional ovules have been fertilized.

In the fertilized ovules local degeneration of nucellus cells near the embryo sac and chalaza, and division and growth of the remaining ones are observed. In the non-functional ovules the entire nucellus starts degenerating. Obviously the futher development and regulation of the ovule is due to successful fertilization.

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## LEGENDS OF THE FIGURES 1–16

PLATE I.

Fig. 1. Longitudinal section through mature receptive stigma. Stigma papillae (SP) are covered with exudate (Ex). × 160.

Fig. 2. Stylar canal of a mature female-fertile flower. The canal cells (CaC) have a thick inner tangential wall and dense cytoplasm. The stylar canal is filled with dark material. × 160.

Fig. 3. Longitudinal section through stigma of mature female-sterile flower. Stigma papillae (SP) have not produced exudate. × 160.

Fig. 4. Stylar canal of a mature female-sterile flower. The canal cells (CaC) are less elongated and have centrally located cytoplasm and peripheral vacuoles. The stylar canal is empty. × 160.

PLATE II.

Fig. 5. SEM micrograph of a mature anatropous ovule.

Fig. 6. Cross section through young fruit. The loculi are filled with juice cells (JC) and ovules (OV).

Fig. 7. Part of the locule of a female-fertile flower. The placenta bears long epidermal papillae (EP) and well developed ovules (OV). × 60.

Fig. 8. Ovary of a female-sterile flower. The ovule primordia (OV) remain undifferentiated, and epidermal papillae on placenta do not develop.

PLATE III.

Fig. 9. Longitudinal section through ovule with mature female gametophyte. The outer (OI) and inner (II) integument enclose a multicellular nucellus (Nu). × 40.

Figs. 10–12. Serial sections of one mature female gametophyte. × 160.

Fig. 10. Central cell and degenerating antipodal cells (arrow).

Fig. 11. Synergids with vacuoles at the chalazal pole, Filiform Apparatus, cytoplasm and nucleus at the micropylar pole.

Fig. 12. Polar nuclei of the central cell and egg cell nucleus (arrow) together with a part of the synergids.

PLATE IV.

Fig. 13. Young ovule with a degenerating megaspore mother cell. × 110.

Fig. 14. Ovule with degenerating functional megaspore. × 60.

Fig. 15. Ovule with degenerating embryo sac. × 60.

Fig. 16. Ovule some time after embryo sac degeneration. The surrounding nucellus also degenerates. × 100.

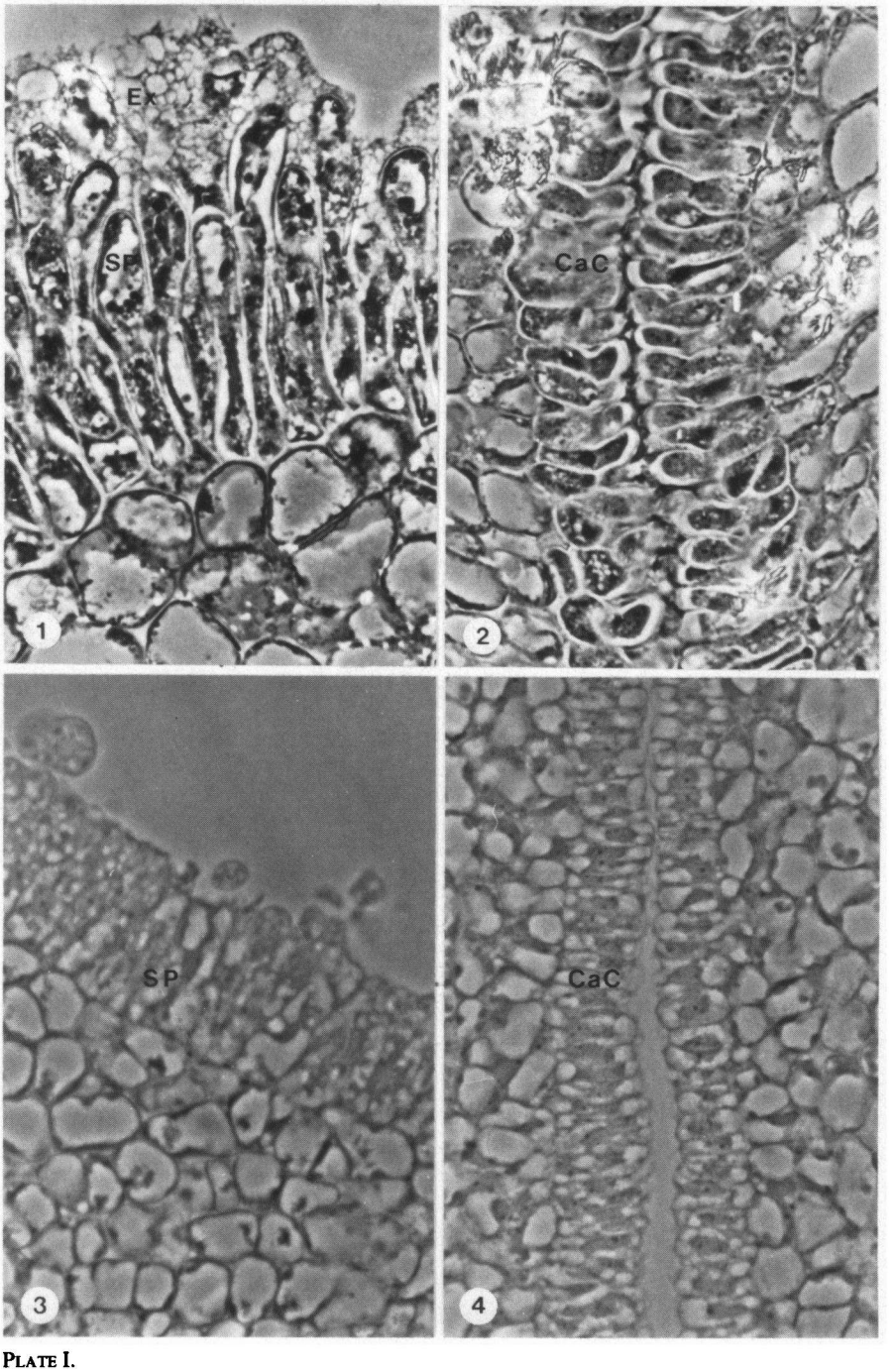
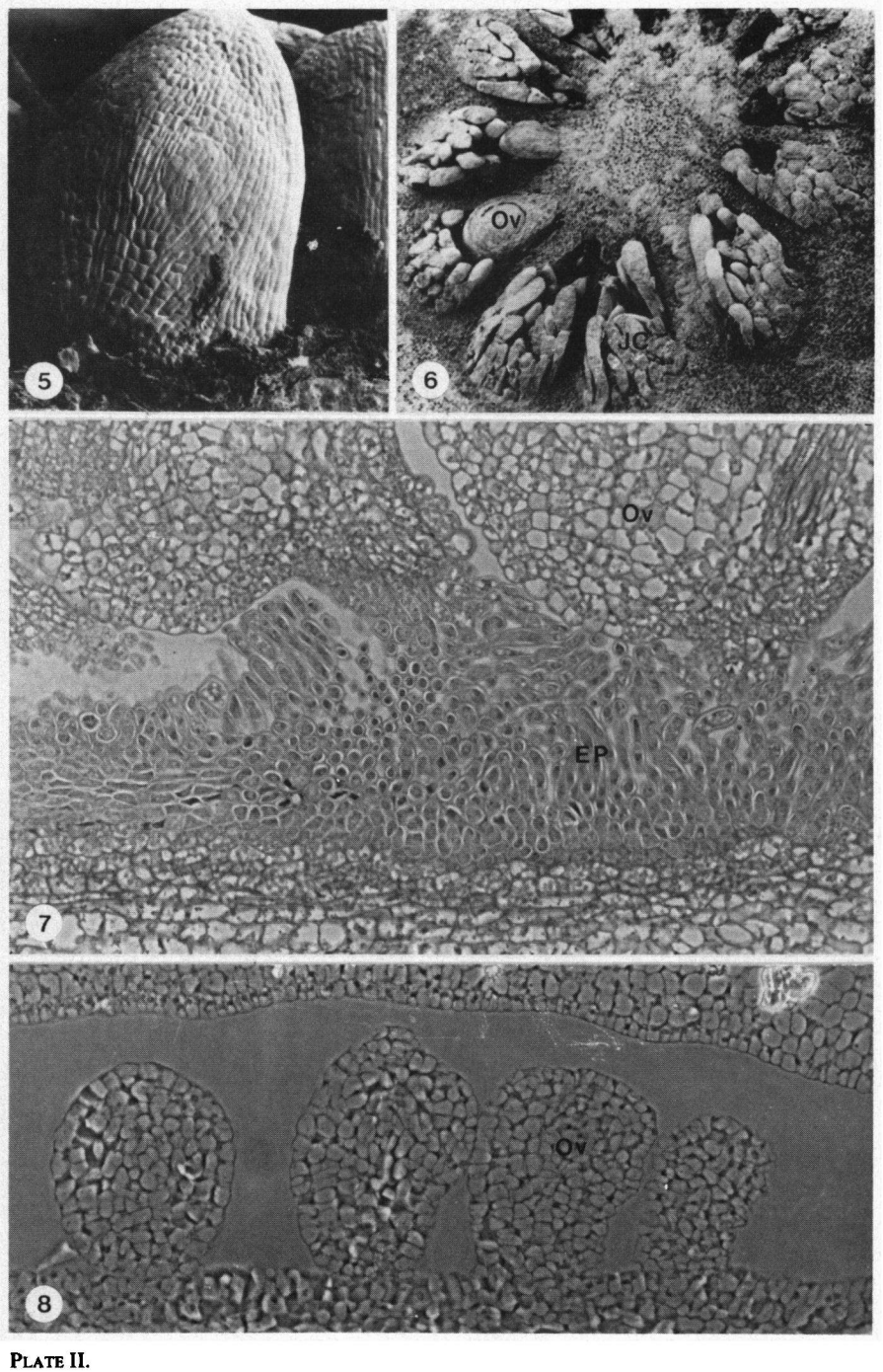



PLATE I.

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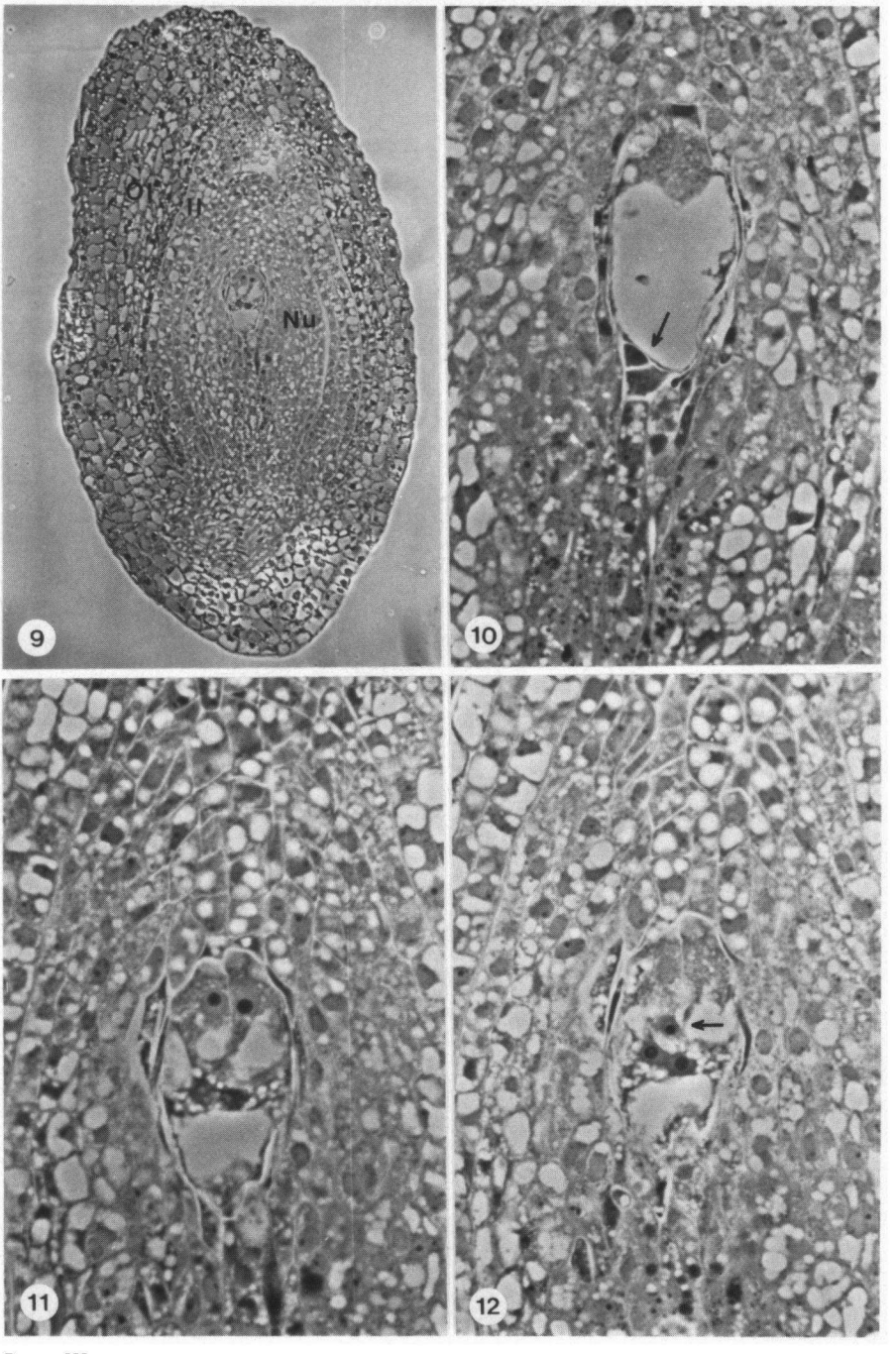


PLATE III.

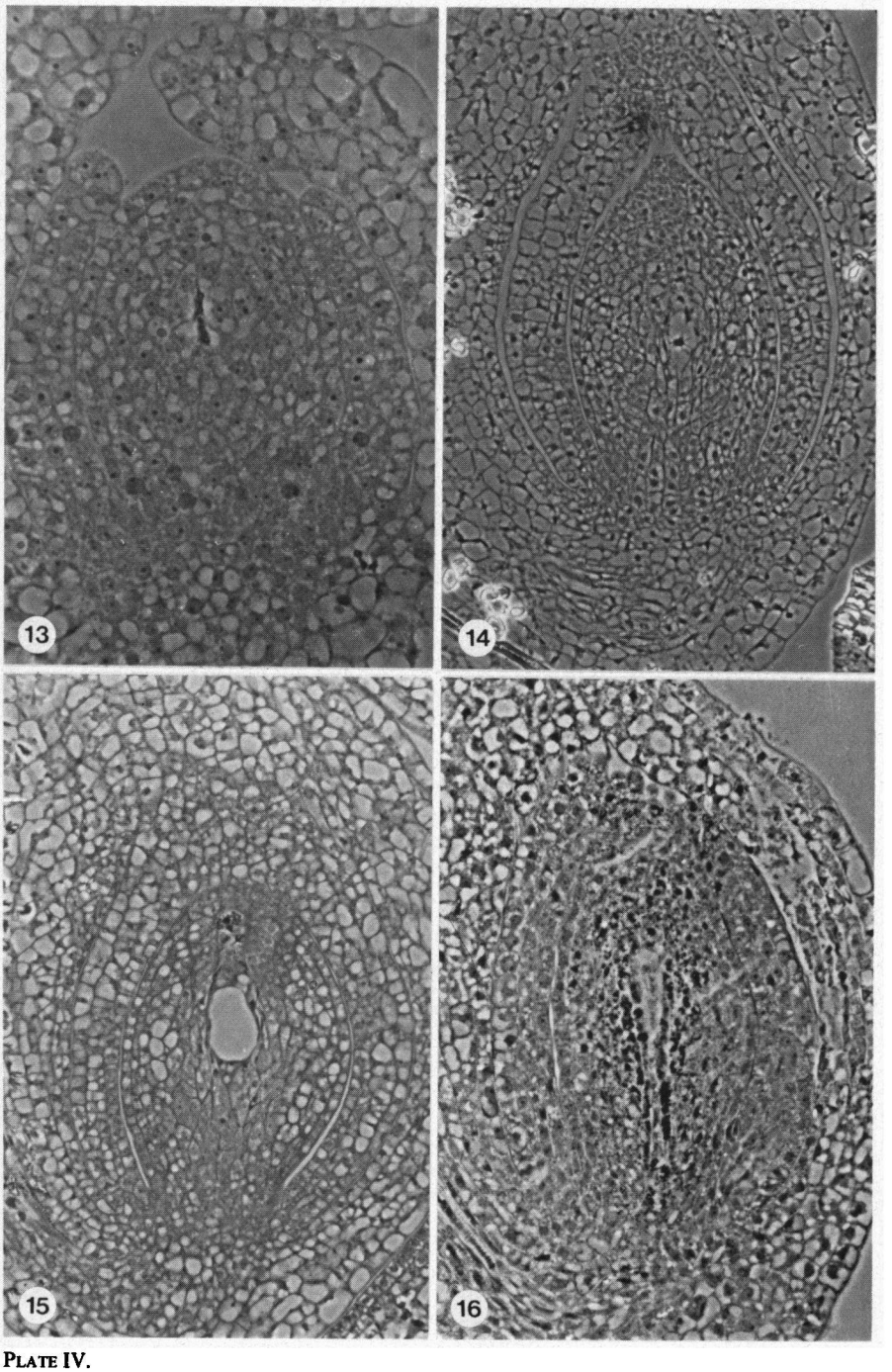


PLATE IV.

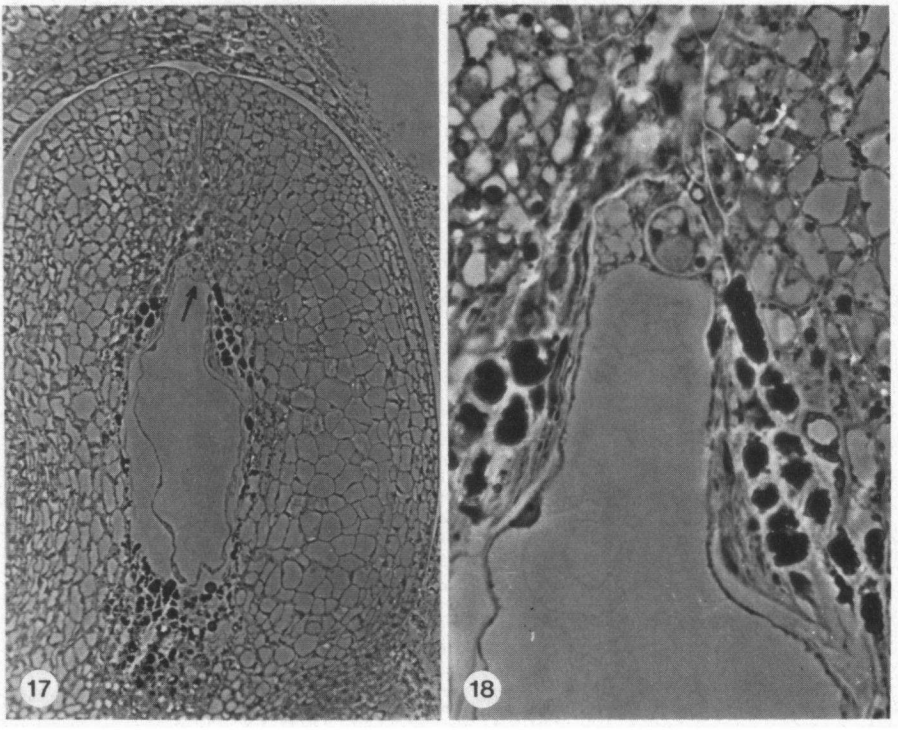


PLATE V.

Fig. 17. Survey of fertilized ovule, 40 days after pollination; arrow points to zygote. × 60.

Fig. 18. Detail of micropylar part of the fertilized embryo sac of *fig. 17*, showing the zygote and nucleate endosperm. Adjacent nucellus partly degenerates. × 160.