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ULTRASTRUCTURAL ASPECTS OF UNILATERAL INTERSPECIFIC INCOMPATIBILITY BETWEEN *LYCOPERSICUM PERUVIANUM* AND *L. ESCULENTUM* *

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INTRODUCTION

Whereas some information has been gained (VAN DER PLUIJM and LINSKENS 1966; DE NETTANCOURT *et al.* 1972) on the ultrastructural features of the self-rejection phenomenon in species with a gametophytic monofactorial pollen tube-style system of self-incompatibility, no description appears to have been made so far, at the electron microscope, of the unilateral interspecific incompatibility reaction which usually prevents the pollen tube of a self-compatible species to grow through the style of a self-incompatible one. Although it is generally implicitely assumed (LEWIS and CROWE 1958; MARTIN 1964), from observations with the light microscope, that pollen tube inhibitions are in the two cases identical, a number of genetic models have been presented (GRUN and RADLOW 1961; GRUN and AUBERTIN 1966; ABDALLA 1970; ABDALLA and HERMSEN 1972) which attribute unilateral incompatibility to the action of specific gones not belonging to the self-incompatibility locus. Such models are in opposition with those ascribing the occurrence of unilateral incompatibility to a direct involvement of S-alleles which may or may not include the partecipation of other genetic loci (for a review of the situation, see ABDALLA and HERMSEN 1972).

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** Biology Division of the European Communities.

Since the magnitude of the phenotypical differences between the two types of rejection can be expected to depend upon the magnitude of genetic differences between the two systems, we have thought that a comparison between the ultrastructural features of self and unilateral incompatibility could eventually bring to test the hypothesis that identical genetic units are governing the two reactions. As the ultrastructural aspects of the self-incompatibility reaction had been analysed recently (DE NETTANCOURT *et al.* 1972) in the species *L. peruvianum*, it was considered judicious, for comparison purposes, to examine the unilateral incompatibility barrier which prevents this species (MCGUIRE and RICK 1954) from accepting the pollen tubes of its self-compatible relative, *L. esculentum.*

MATERIAL AND METHODS

Test-plants: the plant material used in the experiment consisted of flowering individuals of *Lycopersicum esculentum* cv. Baldoni (self-compatible) and of its wild self-incompatible relative, *L. peruvianum* (Casaccia clone 10, S_1 S_4).

Pollinations: reciprocal crosses between the two species were made by means of hand-pollination on isolated emasculated flowers.

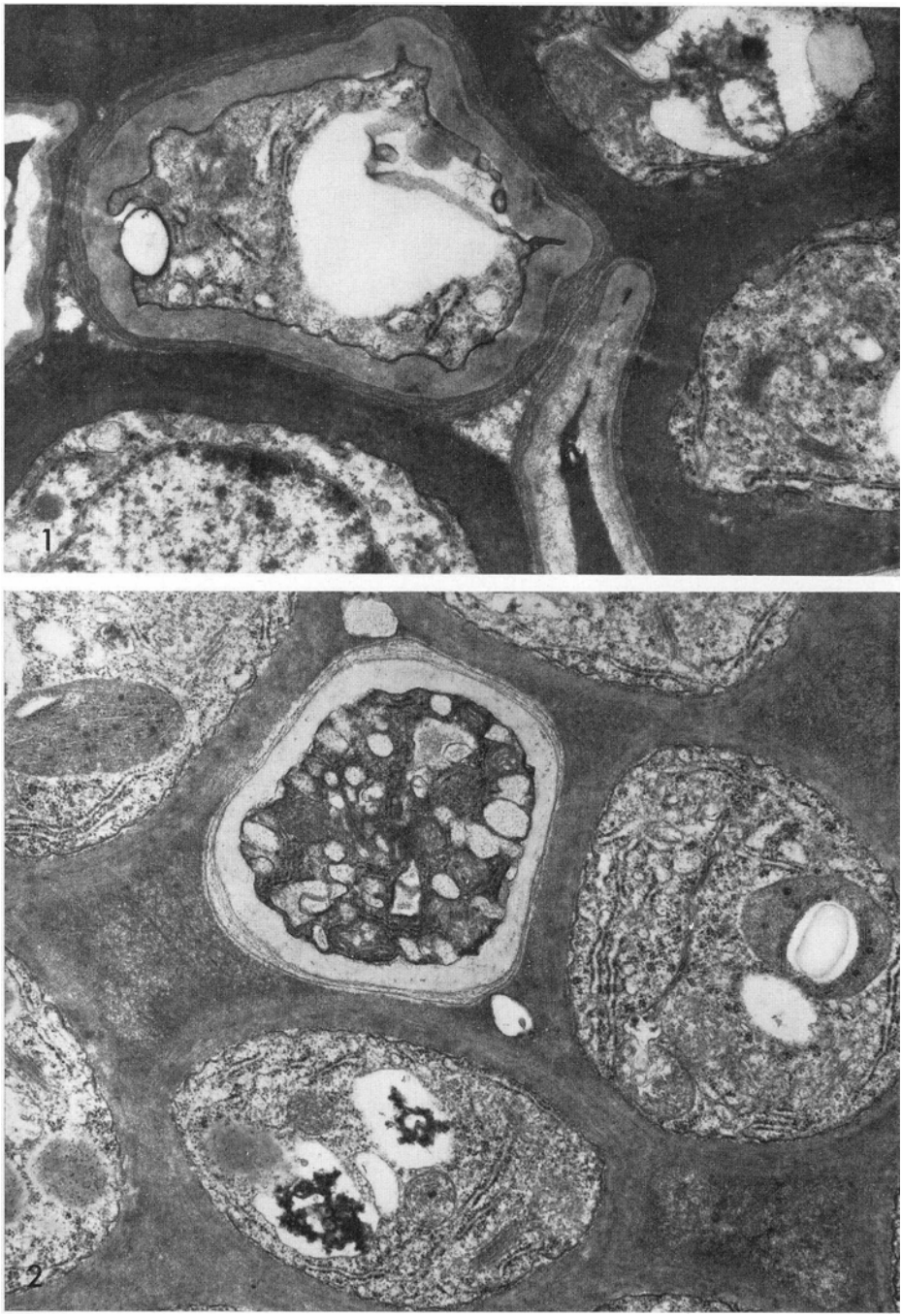
Self-pollinations on *L. esculentum* were carried out under isolated conditions but without any emasculation.

Harvest of pollinated flowers: a preliminary series of observations by fluorescence microscopy (MARTIN 1958) was made, at different harvesting times, to find out the time interval needed for the incompatibility reaction to occur and for localising, in the style, the region where most tube apices were inhibited in their growth. It was found that the unilateral incompatibility reaction usually had taken place 24 hours after pollination and that the pollen tubes were generally inhibited within the upper third of the style. The same material was used in an attempt to find out if the pollen tubes inhibited by unilateral incompatibility presented a specific pattern of callose deposition in their apical region.

Electron microscopy: style segments were collected 24 hours after pollination and fixed in 5% glutaraldehyde buffered by 0.065 M Sorensen's phosphate buffer, pH 6.9, for 90 minutes at room temperature. After post-fixation with buffered 1% osmium tetroxide for 3 hours, the tissue was dehydrated through a graded ethanol series and embedded in an Epon-Araldite mixture. Sections were cut with glass knives on an LKB ultramicrotome, stained with uranyl acetate (WATSON

Fig. 1. — Cross-section of the stylar conducting tissue of *Lycopersicum esculentum* 24 hours after self-pollination. Portions of two and all of one *esculentum* pollen tube are visible, and clearly display a bi-partite cell wall. (x 18,000).

Fig. 2. — Cross-section of the stylar conducting tissue of *Lycopersicum esculentum* 24 hours after cross-pollination with *L. peruvianum* pollen. One *peruvianum* pollen tube is visible which displays (as in Fig. 1) the normal appearance of compatible ones. (x 18,000).



1965) and lead citrate (REYNOLDS 1963), and observed with a Zeiss electron microscope EM 9 A.

RESULTS

1) *Compatible pollen tubes (L. esculentum selfed; L. esclulentum ♀ x L. peruvianum).*

As expected, the pollen tubes of *L. esculentum* and of *L. peruvianum* were found to grow without inhibition through *esculentum* styles and to display (Figs. 1-2) the same general appearance (infolding and flattening in all regions except the cylindrical apex) which had been described previously for the compatible tubes of *L. peruvianum* formed after cross-pollinations between plants of different S-genotypes (DE NETTANCOURT *et al.* 1972). The pollen tubes of the two species, when growing through *esculentum* styles, clearly displaid the bi-partite tube wall which had been observed after cross-compatible pollination within the species *L. peruvianum*. The fluorescence technique showed that the apical region of the tubes always contained callose.

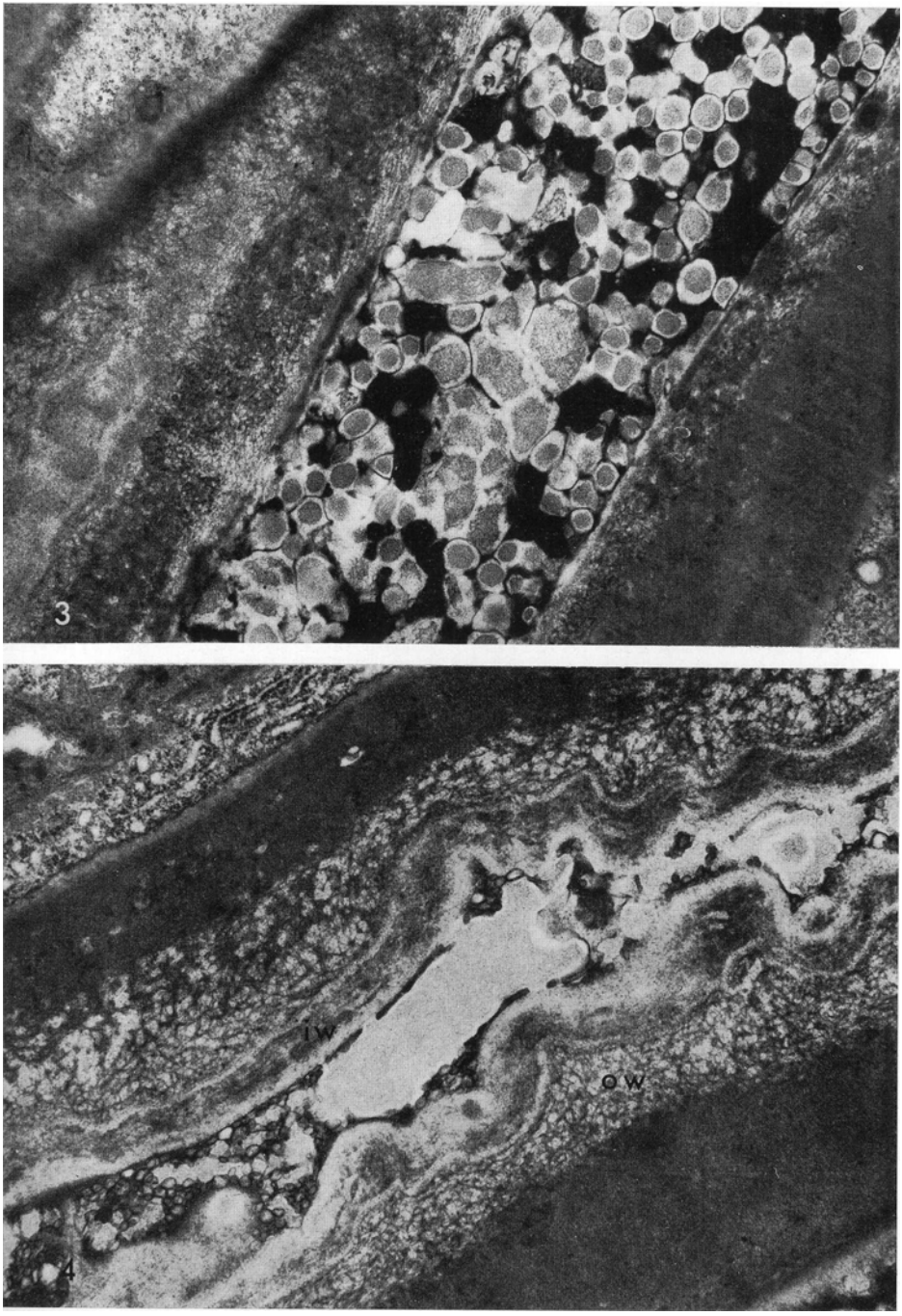
2) *Cross-incompatible pollen tubes (L. peruvianum ♀ x L. esculentum).*

The early evolution of incompatibility in the *esculentum* pollen tubes growing through *peruvianum* styles was found to progress in a manner very similar to that described for the self-incompatible tubes of *L. peruvianum* (DE NETTANCOURT *et al.* 1972) and to be characterized by a progressive disappearance of the callose rich inner wall and by an accumulation of particles in the tube cytoplasm (Fig. 3). As in the case of self-incompatibility, the majority of these particles was found to measure approximately 2,000 Å in diameter and to always display a clear bi-partite structure with a less electron dense outer-shell and a denser granular core.

At the same time or at later stages, however, some features could be observed which had not been detected previously in the case of self-incompatibility. These features may be briefly defined as follows:

— progressive breakdown of the outer wall which appears to become disaggregated in a great number of loose irregular particles (Fig. 4); such particles disperse themselves in the intercellular spaces of the stylar tissue;

Fig. 3. — Longitudinal section of an *esculentum* pollen tube in the style of *L. peruvianum*. The inner wall has disappeared and numerous perticles have accumulated in the cytoplasm. (x 15,000).
Fig. 4. — Longitudinal section of an *esculentum* pollen tube in the style of *L. peruvianum*. Note the accordeon-like folding of the inner wall (iw) and the disaggregation of the outer wall (ow). (x 19,200).



— accordeon-like folding of the tube inner wall higher up in the tube while the disaggregating outer wall remains nearly unfolded (Fig. 4);

— progressive disappearance of the thick callosic apical plug (Fig. 5), now in direct contact with the intercellular spaces of the style, which becomes gradually thinner at the very tip of the pollen tube (Fig. 6) and finally opens up (Fig. 7), allowing the tube contents to flow out between the stylar cells.

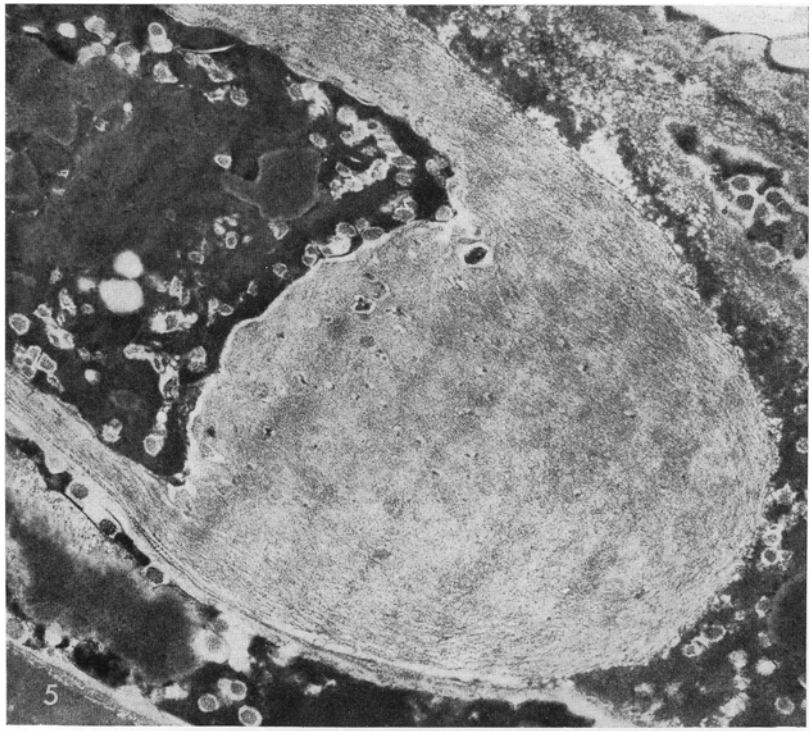
Observations by means of the fluorescence technique (Fig. 8) indicated that the elimination of the callose-rich inner wall did not involve, as in the case of self-incompatibility (DE NETTANCOURT *et al.* l.c.) the entire apical portion of the tube but only its extreme tip, a callose lining being clearly visible at the extremity of incompatible pollen tubes having accomplished their opening process. Such observations clearly confirm those which had been made with the electron microscope.

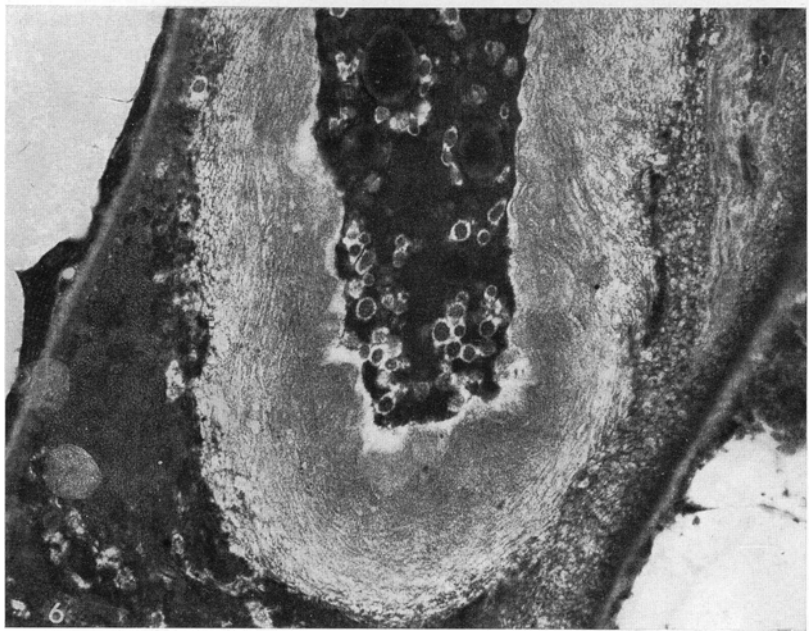
DISCUSSION

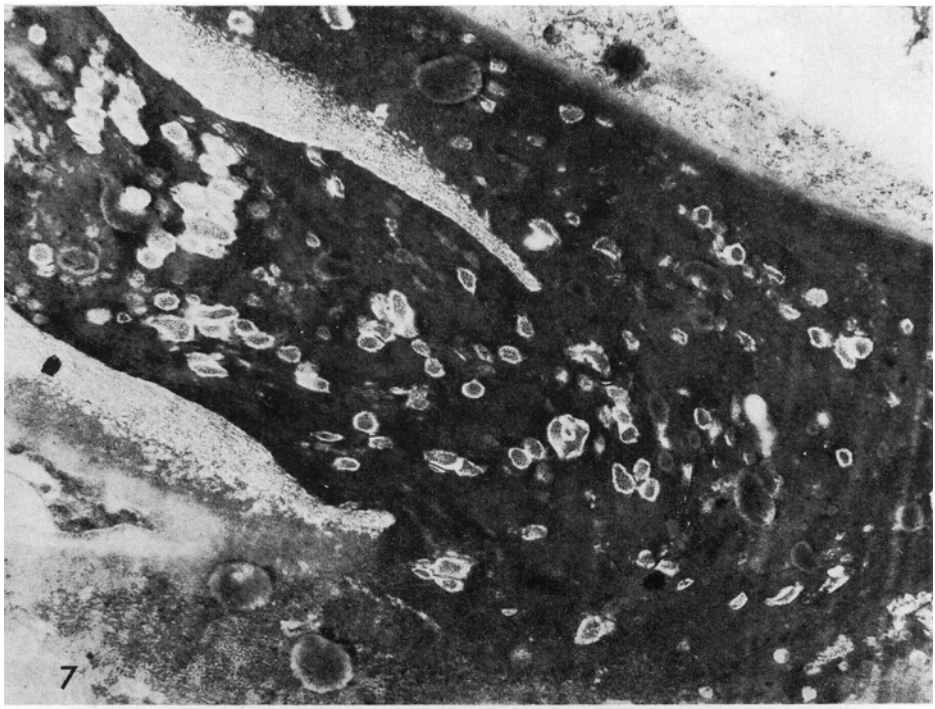
Whereas no apparent differences were found, at the ultrastructural level, between compatible pollen tubes growing through *esculentum* styles (pollinations with *esculentum* or *peruvianum*) or through *peruvianum* styles (cross-pollinations between *peruvianum* plants with different S-genotypes, as analysed by DE NETTANCOURT *et al.* 1972), a rather clear variation could be observed between unilateral interspecific incompatibility (this study) and self-incompatibility (DE NETTANCOURT *et al.* l.c.).

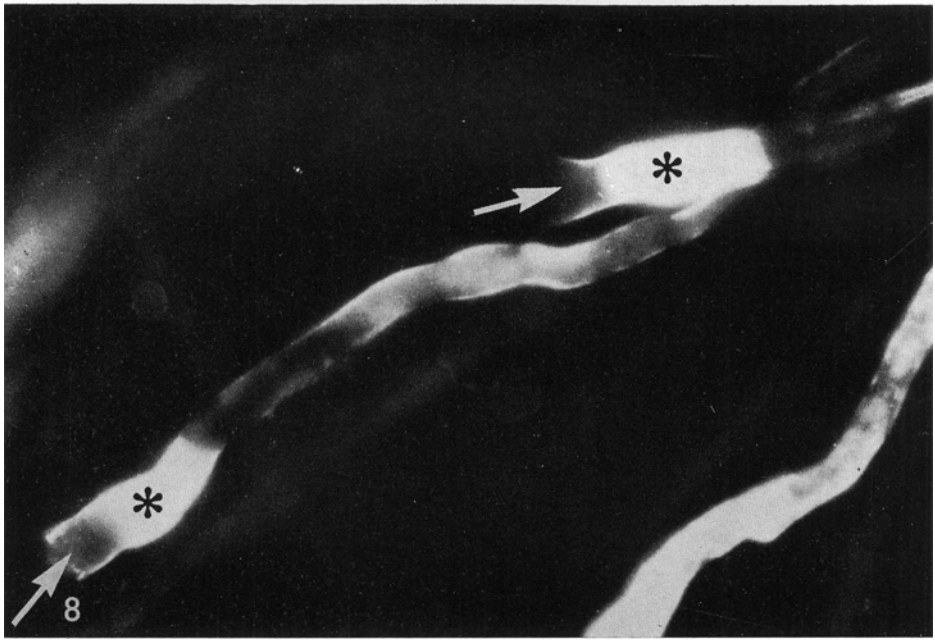
This variation does not seem to concern the accumulation of the typical bi-partite particles (DE NETTANCOURT *et al.* l.c.) in the tube cytoplasm which seems to characterize the rejection process of both types of incompatibility but essentially appears in the modalities leading to the opening of the incompatible pollen tube. In the case of self-incompatibility, the tube apex swells, forming a large apical bulge, and the inner callosic wall disappears while the outer wall expands and becomes, if anything, considerably thicker, the final opening of the tube giving the impression of a burst followed by the forceful expulsion of a cytoplasm which is extremely rich in the bi-partite particles described earlier. On the contrary, in the case of unilateral incompatibility, a smaller bulge is formed and the outer wall disappears completely while the

Figs. 5-7. — Longitudinal sections of the apical portion of *esculentum* pollen tubes in the style of *L. peruvianum*; the outer wall has almost completely disappeared. The inner callosic wall becomes progressively thinner at the very tip and finally disappears too.
Fig. 5. — Section of the apical plug before the dismantelling process. (x 15,600).
Fig. 6. — The apical plug at a later stage, already reduced in size (x 15,000).









apical callosic inner wall becomes thinner only at the very tip of the apex which opens without giving images similar to those of a real bursting phenomenum. As in the former case, however, the tube contents are finally poured into the intercellular spaces of the stylar tissue.

There is little doubt, from these observations, that both basic similarities and basic differences do exist between unilateral and self-incompatibility which strongly suggest that the genetic systems governing the two types of reactions are neither completely unrelated nor exactly identical. Hence, it appears improbable that unilateral incompatibility should only be governed by specific genes which do not belong to the self-incompatibility locus (for an extensive discussion of the genetic problem see MARTIN 1964; PANDEY 1969; ABDALLA and HERMSEN 1972). For the same reason, it seems rather unlikely that only the S-alleles should be involved in the reaction. Yet, it is interesting, in this case, to recall the model by LEWIS and CROWE (1958) in which unilateral incompatibility involves the binding by a stylar S-antigen of a « free » pollen enzyme which is not, as in the pollen of self-incompatible species, bound to a S-specific pollen protein. On the basis of such a model and of LINSKENS' report (1965) that the tube wall is the site of action for the incompatibility proteins, our results would imply that the « free » pollen enzyme which is implicated in unilateral incompatibility can bind to the stylar protein within both the inner and the outer wall of the tube whereas the protein-enzyme association present in self-incompatible tubes complexes itself with the stylar protein only inside the inner wall of the tube.

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Fig. 7. — The apical plug has disappeared and the open tube releases its contents in the stylar tissue. (x 15,000).

Fig. 8. — Pollen tubes of *L. esculentum* in a *peruvianum* style stained with aniline blue and observed by means of fluorescence microscopy. The callose lining is still visible along some portions of the pollen tube, even if rather thin. The apical regions (asterisks), instead, are very rich in callose except at the very tip (arrows) where the callosic plug has become much thinner and has finally opened (compare with Fig. 7). (x 1,500).

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SUMMARY

Observations have been made, at the electron microscope, of the pollen tubes present in the styles of *Lycopersicum esculentum* and *L. peruvianum* after reciprocal crosses between the two species.

The unilateral incompatibility barrier which isolates the two species when *L. peruvianum* is used as pistillate parent was then compared to the processes of pollen tube rejection which have been recently analysed (J. Cell Sci., 1972) after self-pollination in this self-incompatible species. Such a comparison, which was also carried out by means of fluorescence techniques, has permitted to find out that for both types of incompatibility the rejection process was characterised by a progressive disappearance of the callose-rich inner wall of the pollen tube and by an accumulation of bi-partite particles in the tube cytoplasm.

In the case of unilateral incompatibility, however, the tube outer wall is gradually disaggregated while the callosic inner wall remains quite thick at the tube apex, becoming thinner and finally opening only at the very tip of the apical zone. As a result of this complete degradation of the apical wall the cross-incompatible pollen tube merely opens in the stylar tissue and does not accomplish the bursting process which had been found so typical of the self-incompatibility reaction.

These observations support the hypothesis that unilateral incompatibility is governed by a mechanism which is related but no identical to the one controlling self-incompatibility.

RIASSUNTO

Vengono presentate le osservazioni effettuate al microscopio elettronico sui tubi pollinici presenti negli stili di *Lycopersicum esculentum* e *L. peruvianum* in seguito a incroci reciproci tra le due specie.

La barriera di incompatibilità unilaterale, che isola le due specie quando *L. peruvianum* funge da madre, è stata confrontata ai processi di rigetto dei tubi pollinici in seguito ad auto-

impollinazione di questa specie auto-incompatibile (vedi J. Cell Sci., 1972). Tale confronto, effettuato anche per mezzo di tecniche impieganti la fluorescenza, ha permesso di scoprire che per entrambi i tipi di incompatibilità il processo di rigetto è caratterizzato da una progressiva scomparsa della parete interna, ricca di callosio, del tubo pollinico e da un accumulo, nel citoplasma del tubo, di particelle a struttura doppia.

Tuttavia, nel caso dell'incompatibilità unilaterale, si ha una graduale distruzione della parete esterna, mentre la parete interna callosica rimane molto ispessita nell'apice del tubetto assottigliandosi, fino ad aprirsi, solo nella parte più distale della zona apicale.

Come risultato di questa degenerazione completa della parete apicale, il tubo pollinico di *Lycopersicum esculentum* si apre nel tessuto stilare senza dar luogo al processo di scoppio che è tipico della reazione di auto-incompatibilità.

Queste osservazioni corroborano l'ipotesi che l'incompatibilità unilaterale è regolata da un meccanismo correlato ma non identico a quello che controlla l'auto-incompatibilità.