POLLEN-GERMINATION AND POLLEN TUBE GROWTH IN DIPLOTAXIS TENUIFOLIA AFTER CROSS-POLLINATION

MARIANNE KROH AND A. J. MUNTING

Botanisch Laboratorium, Universiteit, Nijmegen

SUMMARY

After cross-pollination of self-incompatible *Diplotaxis tenuifolia* the pollen tubes penetrate the wall cuticle of the epidermal papillae and grow inside the cellulose-pectin layer of the wall towards the base of the papillae. The tubes continue their growth in the middle lamellae of the stigmatic tissue and the intercellular spaces of the stylar transmitting tissue, both of which contain pectin.

1. INTRODUCTION

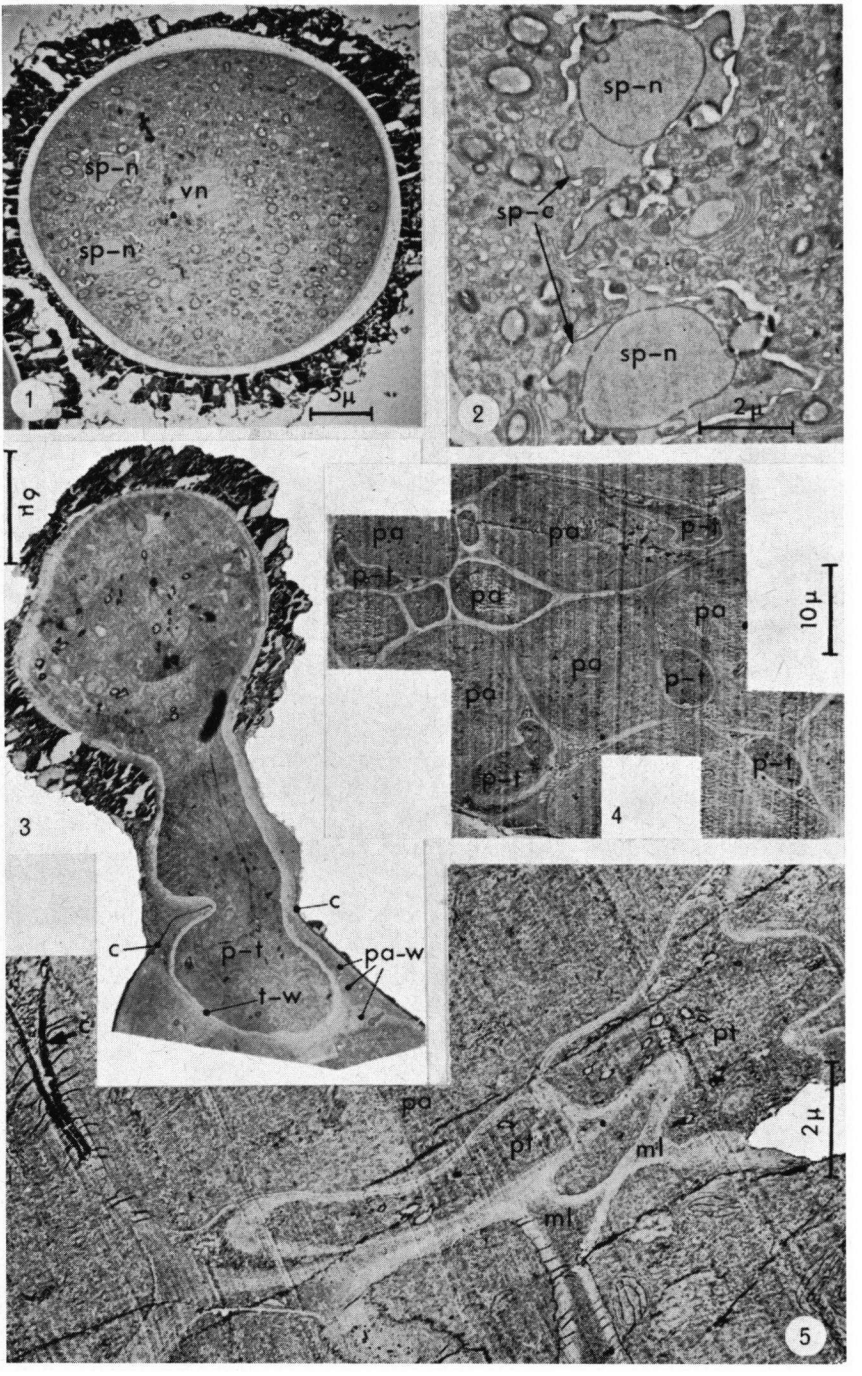
The first steps after cross-pollination were studied electron microscopically in *Brassica nigra* (KROH 1964). The pollen grains of *B. nigra* germinate on the epidermis of the stigma, the cells of which form papillae, a typical phenomenon for Cruciferae stigmas. After germination the pollen tubes penetrate enzymatically the wall of the papillae, which consists of a cuticle and a cellulose-pectin layer (CHRIST 1959), and grow inside the latter layer towards the base of the papilla.

Diplotaxis tenuifolia belongs also to the self-incompatible species of Cruciferae. The intention of this paper was to determine if the processes of penetration and growth in the papilla wall are the same for both *Brassica* and *Diplotaxis* and how the further growth of the *Diplotaxis*-pollen tubes in stigma and style occurs.

2. MATERIAL AND METHODS

Two self-incompatible but cross-compatible clones of *Diplotaxis tenuifolia*, which were cultivated in a glasshouse, were used. *D. tenuifolia* was introduced into self-incompatibility research because this species has large stigmas, is a

Fig. 1. Section through a pollen grain of *Diplotaxis tenuifolia*. sp-n = sperm nucleus; vn = vegetative nucleus.
Fig. 2. Detail from *fig. 1* of the sperm cells (sp-c).
Fig. 3. The pollen tube (p-t) has penetrated the cuticle (c) and the cellulose-pectin layer of the papilla wall (pa-w). t-w = tube wall.
Fig. 4. Cross-section through the base of papillae (pa) with pollen tubes (p-t) growing inside the wall.
Fig. 5. Pollen tube (p-t) growing from the papilla (pa)-wall into the middle lamella (m-l) of the subepidermal stigmatic tissue. c = cuticle.


sp-n
vn
sp-n
1
5μ
sp-n
sp-c
sp-n
2
2μ
6μ
3
c
c
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t-w
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4
10μ
pa
pt
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5

perennial and can be easily propagated vegetatively. From one to three hours after cross-pollination the stigmas and styles were fixed in 5% glutaraldehyde in phosphate-buffer pH 7.2 for 1½ hours, postfixed in 2% $KMnO_4$ in A. dest. for 3 hours and embedded in Epon 812. To remove pectin and hemicellulose from the cell walls and to make visible the cellulose lamellae of the transmitting tissue, the styles were boiled in a 1:1 mixture of glacial acetic acid and 30% hydrogen peroxide for 20 minutes. After removing the methacrylate with amylacetate, the sections were shadowed with platinum. Thin sections were cut with a Porter-Blum ultratome and examined with a Philips EM-100 electron microscope (60 KV).

3. OBSERVATIONS

The pollen grains of *Diplotaxis tenuifolia* are trinucleate (*fig. 1*). The cytoplasm of the two sperm cells is surrounded by a plasma membrane which is separated from the plasma membrane of the vegetative cell in many places by an electron transparent space (*fig.2*). The sperm cells therefore appear to be surrounded by a wall-like structure. After cross-pollination the pollen grains swell and germinate. The pollen tube tips penetrate the cuticle of the papilla wall to grow in the cellulose-pectin layer towards the base of the papilla (*figs. 3, 4*). In *fig. 5* the pollen tube has left the papilla wall and has penetrated the middle lamella of the stigmatic tissue. The tube grows further intercellularly. Fig. 6 shows a cross section of the stigma in which the pollen tubes are lying intercellulary between the stigmatic cells. Due to the variations in rate of pollen tube growth, the tubes have been sectioned at different sites along their length. Sections through the proximal parts of the tubes (*fig. 6* I, II, III) give a high contrast caused by the presence of numerous plasma organelles. The distal parts of the pollen tubes (*fig.6* IV, V, VI, *fig. 7*) are less contrasted. Vacuoles appear in the cytoplasm and callose plugs are formed.

The stigmatic tissue is followed by the transmitting tissue of the style. This consists of cells separated by large intercellular spaces filled with pectin (*fig. 8*). If this substance is removed, only the cellulose lamellae of the walls remain (*fig. 9*). The pollen tubes grow to the ovary through the intercellular pectin substance of the transmitting tissue (*fig. 10*).

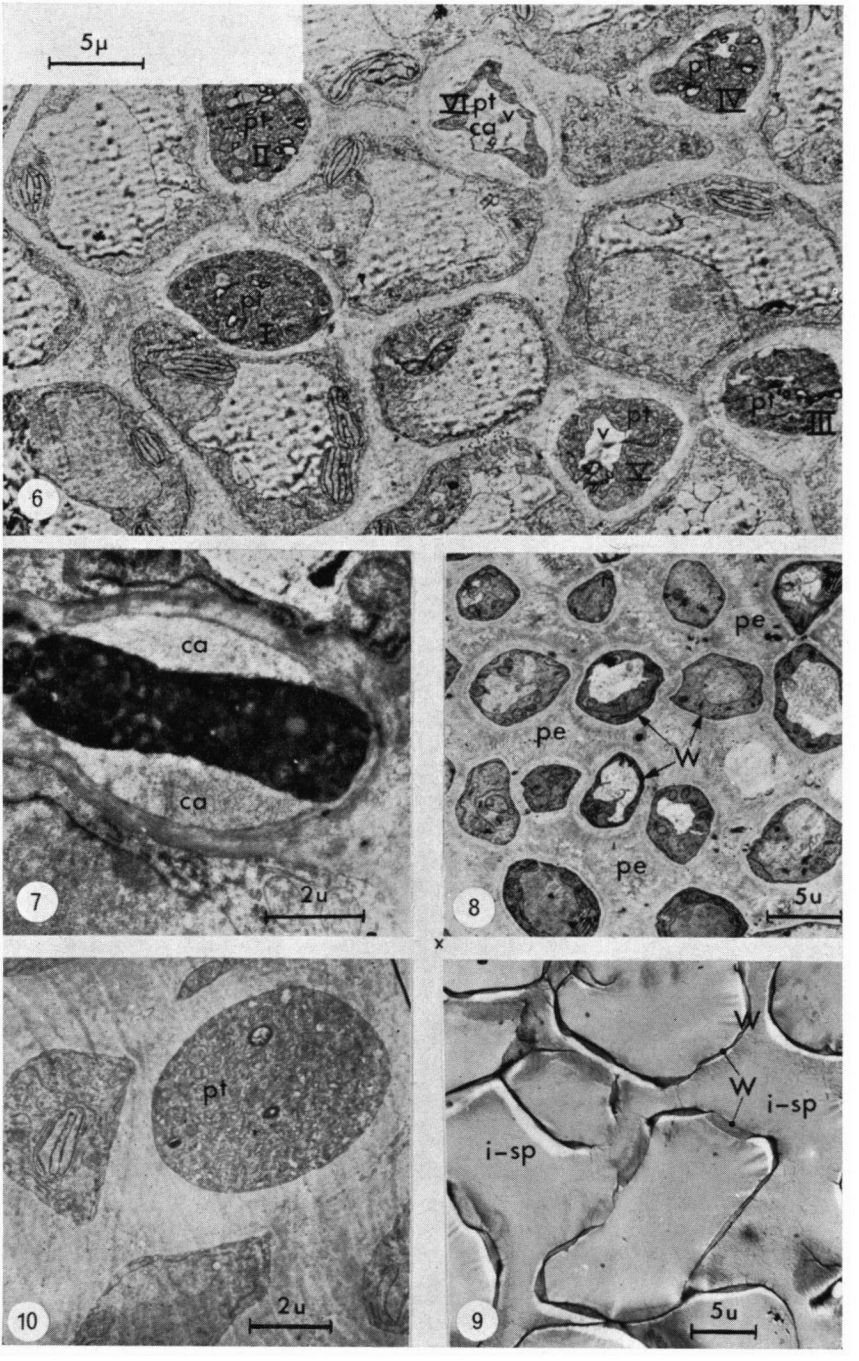
Fig. 6. Cross-section through the stigmatic tissue containing intercellularly growing pollen tubes. I, II, III proximal, IV, V, VI distal parts of pollen tubes. v = vacuole; ca = callose.

Fig. 7. Section through the distal part of a pollen tube. The cytoplasm of the tube is compressed by a callose (ca) plug.

Fig. 8. Cross-section through the transmitting tissue of the style. pe = pectin; w = cell wall.

Fig. 9. Transmitting tissue of the style after removal of the non-cellulosic components. i-sp = intercellular space; w = cell wall.

Fig. 10. Cross-section through the transmitting tissue of a style containing a pollen tube (p-t).


5μ
pt
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8
5u
pt
10
2u
W
W
i-sp
i-sp
9
5u

4. CONCLUSIONS

It is known from light microscopic investigations that pollen grains of Cruciferae are trinucleate (BREWBAKER 1957). This observation is now repeated electron microscopically. As in *Oenothera* (DIERS 1963) and in *Petunia* (SASSEN 1964) the sperm nuclei of *Diplotaxis* are surrounded by cytoplasm which is separated from the vegetative cytoplasm by a wall-like structure. GÓRSKA-BRYLASS (1967) described several plant species with the generative cell temporarily surrounded by a callose wall. Possibly the electron-transparent space between the plasma membrane of the vegetative and generative cytoplasm is the place where callose was present.

No differences could be observed between *Brassica nigra* and *Diplotaxis tenuifolia* concerning the means of penetration of the pollen tube into the papilla wall and its further growth towards the base of the papilla. Therefore, we expect a similar behavior of pollen tubes after cross-pollination in other Cruciferae species. For penetration and the growth inside the papilla wall, the pollen tubes probably need a cellulose-splitting enzyme in addition to cutinase and pectinase, which were discovered in pollen by PATON (1912) and LINSKENS & HEINEN (1962). The cellulase is necessary at two places: at the point of entrance into the cellulose-pectin layer of the papilla and at the point of leaving this layer before reaching the middle lamella of the stigmatic tissue. In the papilla wall itself the tubes are believed to force their way between the cellulose lamellae by dissolving the non-cellulose constituents of the wall (KROH 1964). While the growth of the pollen tubes in the epidermal papilla occurs inside the wall, it continues intercellularly. The pollen tubes grow through the middle lamellae of the stigmatic tissue and the intercellular spaces of the conductive tissue of the style by dissolving the pectic substances. Probably the pollen tube tips resorb the material set free under the action of pectin dissolving enzymes and use it for the construction of their wall (SCHOCH-BODMER & HUBER 1947; LINSKENS & ESSER 1960; LINSKENS & VAN DER PLUIJM 1966).

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