

Pistil Exudate Production and Pollen Tube Growth in *Lilium longiflorum* Thunb.

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Exudate production in the pistil of *Lilium longiflorum* was studied in relation to pollen tube growth, using scanning electron microscopy (SEM), transmission electron microscopy and light microscopy. In contrast with conventional fixation for SEM, during which the exudate of *L. longiflorum* largely washes away, the exudate remains present through freezing in case of cryo-SEM. Using the latter method we observed that exudate production on the stigma and in the style started before anthesis. Just underneath the stigma the exudate was first accumulated at the top of each secretory cell, followed by a merging of those accumulations as exudate production proceeded. Exudate is also produced by the placenta. It was however not possible to determine whether any of this fluid originated from the micropyle. Apart from the cell shape and the cuticle present in between the secretory cells, the ultrastructure of the secretory cells covering the placenta was comparable to those of the stylar canal. The transfer wall of the secretory cells of the placenta originated from fusing Golgi vesicles but the endoplasmic reticulum seemed to have an important role as well.

After pollination the pollen tubes grew across the stigma and entered the style through one of the slits in the three stigma lobes. The pollen tubes grew straight downward through the style and were covered by exudate. As the pollen tubes approached the ovary their growth was restricted to the areas with secretory cells. In the cavity the pollen tubes formed a bundle and they bent from this bundle in between the ovules towards the micropylar side. There they bent again to stay close to the secretory cells. After bud pollination the pollen tube growth was retarded. Later arriving pollen tubes had a tendency to grow close to the secretory cells of the style, which resulted in a growth between these cells and preceding pollen tubes. If there was still a little exudate produced, it resulted in a lifting up of the pollen tubes, out of the exudate. The relationship between exudate production and pollen tube growth is discussed. Both the speed and the guidance of the pollen tube seemed determined by the properties of the exudate.

Key words: Cryo-scanning electron microscopy, exudate, *Lilium longiflorum*, lily, ovary, pollination, pollen tube growth, secretory cell, stigma, style.

INTRODUCTION

The cells covering the stigma and the hollow style of *Lilium longiflorum* are secretory (Rosen, 1971) and also the placenta of *L. leucanthum* var. *centifolium* and *L. regale* is lined with secretory cells (Welk, Millington and Rosen, 1965). During conventional fixation and embedding of parts of the pistil of *L. longiflorum* the exudate produced largely washes away from the exudate producing cells. In the literature the development of the exudate producing cells at the stigma and in the style has been described with respect to their structure. At 3 d after anthesis the transfer wall in the secretory cells of the style has reached its maximal development (Rosen and Thomas, 1970). Such a wall was also found in *L. regale* where it was not present at 4 d before anthesis (Vasil'ev, 1970). Using cryo-scanning electron microscopy, in which the specimens are frozen before observation, the amount of exudate produced and the pollen tube growth in this exudate is studied.

In addition, we studied the ultrastructure of the secretory cells in the ovary (i.e. at the placenta) to compare them with those in the style (Rosen and Thomas, 1970; Vasil'ev, 1970; Rosen, 1971; Dashek, Thomas and Rosen, 1971; Dickinson, Moriarty and Lawson, 1982; Miki-Hirosige, Hoek and

Nakamura, 1987). The aim of this research was to relate exudate production to the pollen tube pathway and to gain insight in the extent to which exudate regulates and directs pollen tube growth from germination to ovule penetration.

MATERIAL AND METHODS

Plants of *Lilium longiflorum* Thunb. were grown throughout the year in pots in a greenhouse with additional illumination in the wintertime. Bulbs were stored at a low temperature (0 °C). The day temperature was 17–20 °C on average, with peaks of 30–35 °C in the summer, the night temperature had a minimum of 15 °C. Flower buds of cv. 'Gelria' were emasculated 1 or 2 d before anthesis and the stigma was covered with an aluminium foil cap. Unless otherwise stated, pollination with the compatible cv. 'White American' was carried out at 2 d after anthesis. After pollination the flowers were either left on the plant or put in a vase filled with water and transferred to a growth chamber with 16 h illumination at 24 °C.

Structure of secretory cells in the ovary

To study the ultrastructure of the placenta using the transmission electron microscope (TEM), parts of both

pollinated and unpollinated ovaries were dissected in 0.1 M phosphate buffer at pH 7.2 and subsequently fixed 4 h in 3% glutaraldehyde in the same buffer. After repeated rinsing in the buffer, the tissues were post-fixed 6 h in 1% (w/v) osmium tetroxide in the phosphate buffer containing 1.5% (w/v) $K_4Fe(CN)_6$ (Van Dort, Zeelen and De Bruijn, 1983). During dehydration in a graded ethanol series the material was stained 1 h in 0.5% (w/v) uranyl acetate in 70% ethanol. The tissues were embedded in low-viscosity resin (Spurr, 1969). Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Philips 301 or a JEOL JEM-1200EX II.

Pollen tube growth

The length of the pollen tube bundle in the style was measured after longitudinal dissection of the style followed by staining with water diluted cotton blue (Asano, 1980) or by isolating the bundle with a preparation needle. Sperm cell formation was observed using an aqueous solution of $50 \mu\text{g l}^{-1}$ DAPI (4'6-diamidino-2-phenylindole.2HCl) at pH 4.0.

To study the amount of exudate and the pollen tube growth in this substance stigmas, parts of the style and the ovary were frozen in solid nitrogen in an Emscope SP2000A. The tissues were cryo-fractured, followed by sublimation for 3 min at -80°C and sputtering with gold. The material was observed with a JEOL JSM-35C cryo scanning electron microscope (SEM).

To follow pollen tube growth with the non-cryo SEM, material was fixed 4 h in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, with 0.25 M sucrose, rinsed in buffer with sucrose and postfixed for 6 h in an aqueous solution of 1% (w/v) osmium tetroxide. After rinsing and dehydration in ethanol the material was critical point dried over CO_2 and sputtered with palladium/gold. The material was observed with a JEOL JSM-5200 SEM.

To determine pollen tube penetration in the micropyle, ovules were cleared in a mixture of lactic acid, glycerol and water (1:2:1) at 80°C for 30 min, stained for 2 min in this solution supplemented with 1% (w/v) aniline blue at the same temperature and destained again in the clearing solution at 80°C (modification of Gerlach, 1977).

RESULTS

Exudate production at the stigma and in the style

The exudate production was studied in flower buds from 8 d before until 2 d after anthesis. At about 8 d before anthesis (i.e. bud length 80 mm, Fig. 1) the stigma was still almost dry, although exudate erupted locally at the surface of the stigmatic papillae as is shown in Fig. 2. From just underneath the stigma (Fig. 3) to the base of the style a thin layer of fluid covered the secretory cells, accumulating where two cells bordered each other.

At 6 d before anthesis, just underneath the stigma, the amount of exudate had increased at the top of the secretory cells (Fig. 4). Three to four days before anthesis some stigma papillae were covered together by one layer of exudate, while others appeared still solitary (Fig. 5). In the style the

local accumulation of exudate on the top of each secretory cell had spread out at a number of places, by flowing together with the exudate on neighbouring cells (Fig. 6). At other cells the accumulation had increased (Fig. 7). Halfway (Fig. 8) and at the base of the style (Fig. 9) this pattern of secretion was not observed, and the exudate gathered where the cells border each other.

Two days after anthesis (DAA) only the tops of the stigma papillae were visible above the thick layer of exudate (Fig. 10). In the top of the style an almost continuous layer was formed by fusion of the accumulations on top of the secretory cells. The stylar canal had three lobes and the exudate preferentially gathered in the most abaxial parts resulting in a smooth surface and a maximum thickness of $60 \mu\text{m}$ (Fig. 11). In between the lobes the surface of the exudate was irregular: secretory cells protruding into the stylar canal formed bumps in the thin exudate layer.

Structure of the secretory cells in the ovary

The shape of the secretory cells gradually shifted from long in the style towards shorter, more spherical cells in the ovary, which covered the placentas in each of the three cavities. Each placenta had two rows of ovules. Using TEM, the wall ingrowths of the placental cells appeared to border a more fibrillar cell wall (Fig. 12). The size of the wall ingrowths increased from 2–6 d after anthesis. During this period electron lucent (Fig. 13) and electron opaque vesicles (Fig. 14), both about 60 nm in diameter and possessing a membrane, fused with the plasmalemma. They probably originated from dictyosomes (Fig. 15). In between the wall ingrowths strands of plasma were present. The result was an irregular granular wall, with paramural bodies, osmiophilic islands and a fibrillar component.

Two days after anthesis the nucleus was surrounded by starch grains. The basal part of the cell, the side opposite the wall ingrowths, was more vacuolate compared to the upper part. The cytoplasm was rich in endoplasmic reticulum, both smooth and rough, mitochondria, mono- and poly-

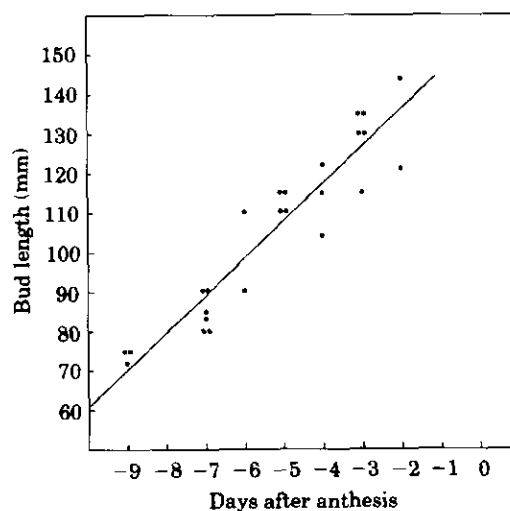
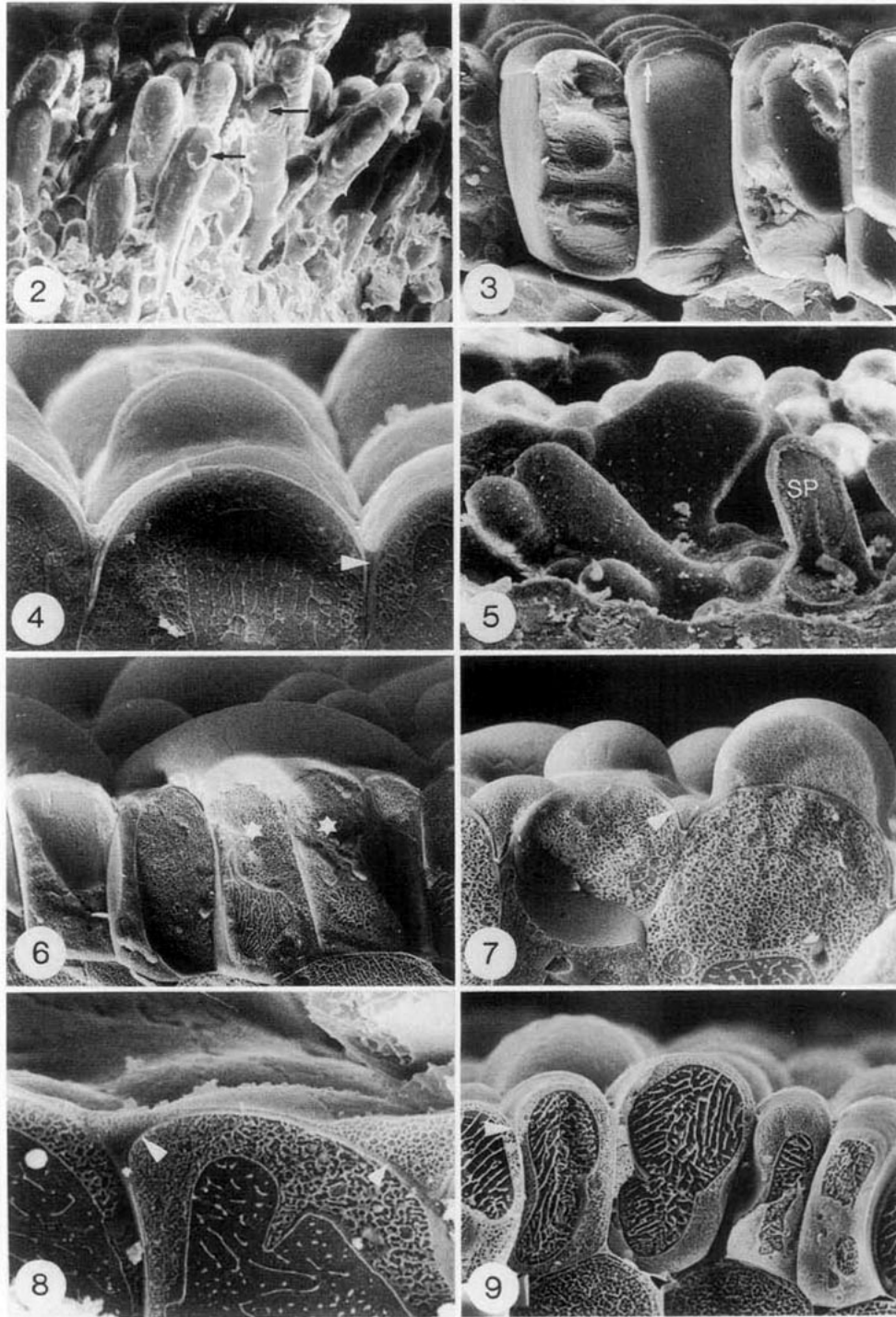


FIG. 1. The growth of the tepals of buds of *Lilium longiflorum* 'Gelria'. The line represents $y = 9.3x + 154$, $r = 0.95$, $n = 33$, experiment carried out in September.



FIGS 2-9. Cryo-SEM observations.

FIG. 2. A stigma of a flower bud of length 80 mm. Arrows indicate what might be the first production of exudate. $\times 500$.

FIG. 3. In a bud of length 80 mm, just underneath the stigma, secretory cells are covered by a thin layer (arrow) of fluid. $\times 2400$.

FIG. 4. In the top of the style of a bud of length 100 mm, exudate gathers especially at the top of the secretory cell, but also, although to a lesser extent, in between the cells (arrowhead). $\times 5300$.

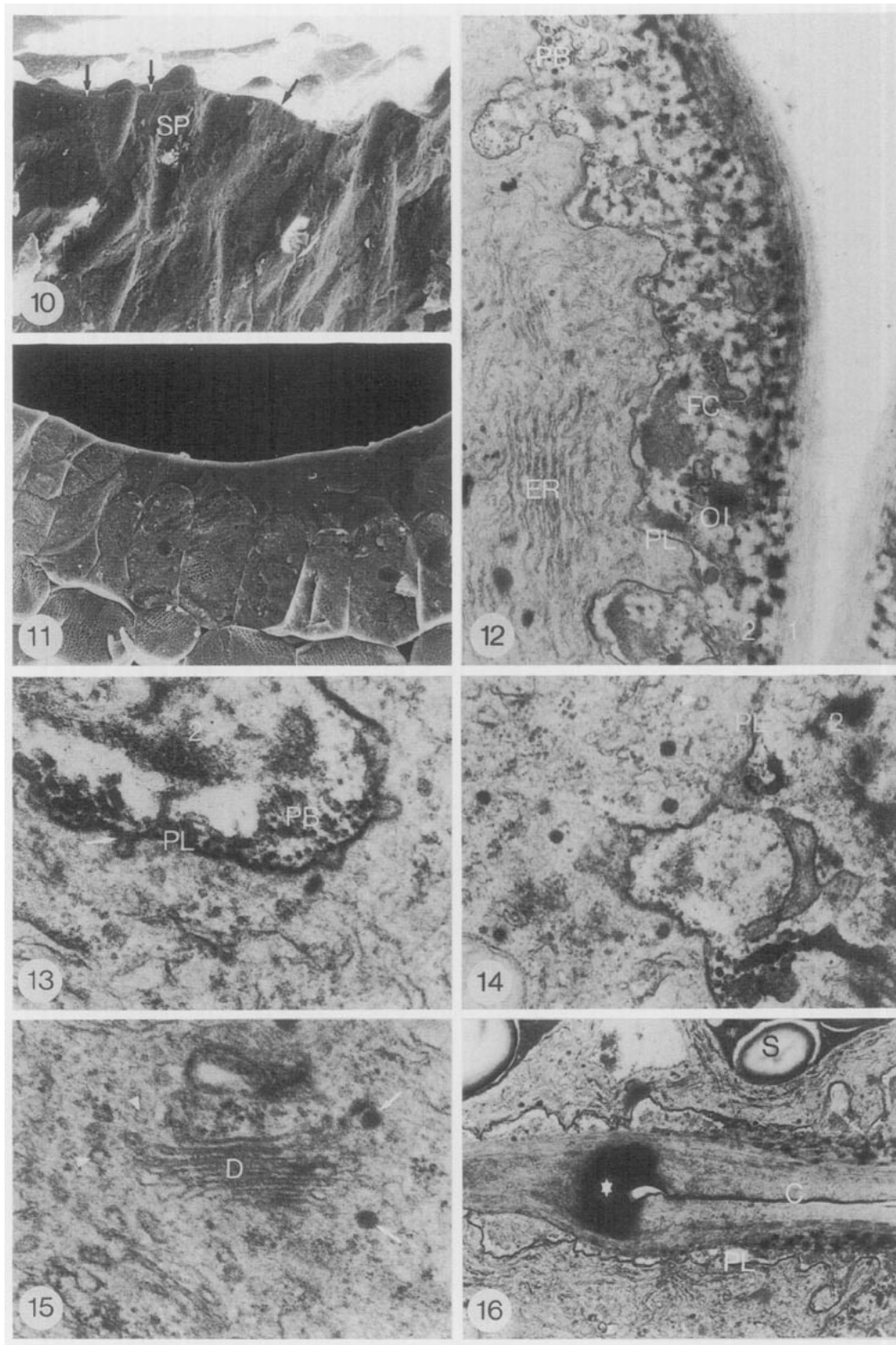
FIG. 5. Exudate covering the stigmatic papillae (SP) in a bud of length 120 mm. $\times 700$.

FIG. 6. In the top of the style exudate accumulations are observed to cover more than one secretory cell (\star) (bud 120 mm long). $\times 1800$.

FIG. 7. Just underneath the stigma at the top of the secretory cell a large accumulation of exudate is present, but also in between the cells the amount of exudate is prominent (arrowhead) (bud 120 mm long). $\times 3400$.

FIG. 8. Halfway down the style the exudate gathers in between the secretory cells (arrowheads) (bud 120 mm long). $\times 5400$.

FIG. 9. At the base of the style the secretory cells developed into a more rounded shape and a small amount of exudate has gathered in between them (arrowhead) (bud 120 mm long). $\times 2800$.



FIGS 10 AND 11. Cryo-SEM observations in a flower 2 d after anthesis.

FIG. 10. The thick layer of stigmatic exudate (arrows) covers the papillae (SP) almost completely. $\times 500$.

FIG. 11. In the top of the style exudate is gathering in the lobes, thereby creating a smooth surface. $\times 1100$.

FIGS 12–16. The ultrastructure of placental cells 2 d after anthesis (DAA).

FIG. 12. The fibrillar cell wall of a placental cell with wall ingrowths of a different structure. $\times 13900$. ER, Endoplasmic reticulum; FC, fibrillar component; OI, osmiophilic islands; PL, plasma-lemma; PB, paramural body; 1: refers to the outer fibrillar layer and 2: inner granular fibrillar layer of the cell wall. Terminology according to Dashek *et al.* (1971).

FIG. 13. Fusion (arrow) of electron lucent vesicles with the plasmalemma (PL). Note the paramural bodies (PB). $\times 52100$.

FIG. 14. Electron lucent (arrowhead) and osmiophilic vesicles are of comparable size and present in the proximity of the wall of the secretory cell. $\times 32000$. PL, Plasma-lemma; 2: inner granular fibrillar layer of the cell wall.

FIG. 15. A dictyosome (D) with electron lucent (arrowheads) and electron opaque (arrows) vesicles in its proximity. $\times 41800$.

somes and dictyosomes which were actively producing vesicles. Some lipid bodies were present. The secretory cells were connected mutually and to the underlying parenchyma cells by plasmodesmata. The latter cells were also rich in starch. The secretory cells only partly bordered each other and at the place of disjunction an electron opaque site in their radial and transverse cell walls was observed (Fig. 16). The cuticle was usually present at this point but vanished towards the top of the cell.

Six days after anthesis the amount of starch in the placental secretory cells had decreased. No difference was observed between the placental cells at the micropylar side of the ovules and those situated at the central placenta or those present between two adjacent ovules.

Pollen tube growth at the stigma and in the style

After pollination at 2 d after anthesis, the pollen grains germinated and their tubes grew over the stigma followed by a passage through the slits in the stigma lobes towards the stylar canal (Figs 17 and 18). The growth over the stigma seemed to take place in all directions apart from places close to the slits. Arriving at the edge of the stigma, which was reached because of the undirected pollen tube growth, the pollen tubes remained growing in the exudate and because of the three lobed shape of the stigma, they finally grew towards one of the three slits. In a growth chamber the first sperm cell formation in this bicellate species occurred 13–16 h after pollination, when the pollen tubes had penetrated the style 15–25 mm. At 24 h after pollination only a few pollen tubes with an undivided generative cell could be found.

The speed of the pollen tube growth was determined by measuring the length of the pollen tubes reached at different time intervals after pollination and subsequent incubation in the growth chamber. After a slow start, the pollen tubes grew 2.4 mm h^{-1} from 6 h until about 48 h after pollination ($n = 72$ styles). Then the pollen tubes had reached the end of the style, after which growth speed was difficult to measure. The time of the day at which pollination was carried out did not influence the pollen tube length reached after 24 h, which was on average 47 mm ($n = 43$ styles). In the greenhouse this growth was slower.

Two days after pollination (i.e. 4 DAA) a huge number of pollen tubes could be observed in the exudate in the top of the style. Using cryo-SEM, contact between the pollen tubes and the secretory cells was only occasionally observed. The pollen tubes grew sometimes close to the exudate surface, especially at places where the exudate layer was thin. In the stylar lobes the secretory cells were first bordered by an exudate layer, followed by a layer with pollen tubes surrounded by fluid and then again a layer of exudate (Fig. 19). Two days after pollination some pollen tubes had grown halfway down the style. At places where much exudate was present, they seemed to grow freely in the fluid, being spherical in cross section. Where the layer was thinner, they more often had a flattened appearance (Fig.

20). Halfway down the style the exudate layer had a smooth surface, in comparison with the top.

At the base of the style the pollen tubes grew from the stylar surface towards one of the three centrally situated exudate producing placentas. Here they generally followed the zone with secretory cells as well, which allowed them to spread out.

Pollen tube growth in the ovary

Two days after pollination (i.e. 4 DAA) exudate filled the space between the inner integuments of the ovules and the pericarp (Fig. 21). Unfortunately we could not observe whether any fluid originated from the micropyle. At 4 d after pollination the placenta was covered with an abundant amount of exudate (Fig. 22), in which pollen tubes grew.

The first few ovules in the top of the ovary were usually neglected by the pollen tubes. Pollen tubes grew in the exudate, present at the placenta, between the two rows of ovules and one by one bent 90° to grow in between two adjacent ovules towards the micropylar side (Fig. 23). After dissection of flowers at different intervals after pollination, it was concluded that later arriving pollen tubes either grew further down the ovary to penetrate still empty micropyles or bent towards the same or the first ovules. At 4 d after pollination the central pollen tube bundle still had not reached the base of the ovary.

Arriving at the micropylar side the pollen tubes bent again about 90° to reach the inner integument. They usually grew towards the base of the ovary, parallel with the pollen tubes in the central bundle, but an apically directed growth was also observed. In this way a small bundle of pollen tubes was formed at the micropylar side of the ovules. This narrow path was lined with secretory cells as well. To penetrate the micropyle again a slight change in growth direction was necessary (Fig. 24). Later arriving pollen tubes filled up the space between the inner integument and the pericarp. Pollen tubes also bent back towards the central bundle, and even a second return to the micropylar side of the ovules was no exception.

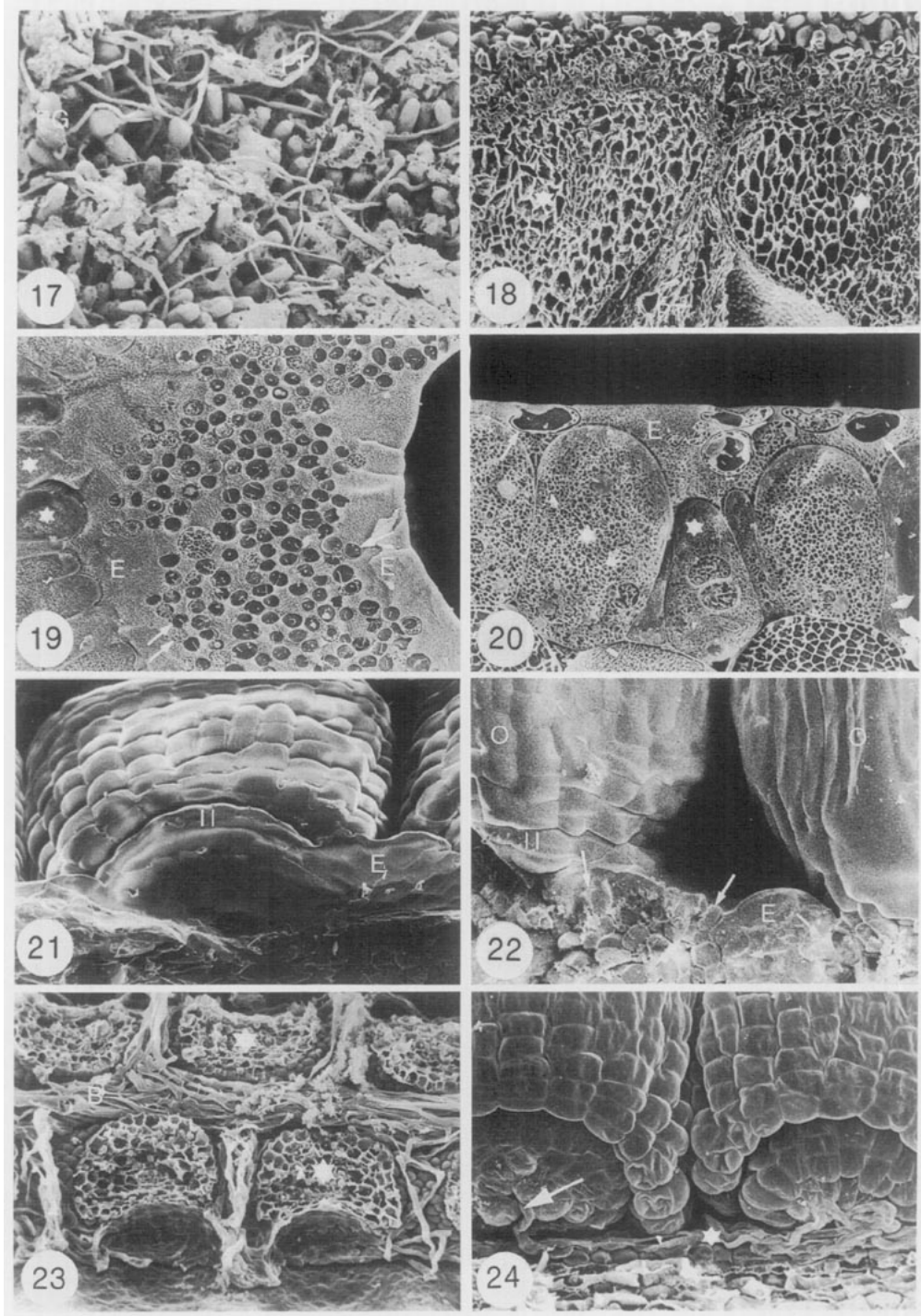
Penetration of the ovule

Pollen tube penetration of the ovules started 3 (growth chamber) to 4 (greenhouse) d after pollination. The percentage of penetrated ovules increased at least until 7 d of pollen tube growth (Table 1). This percentage varied with the time of the year and between different transverse parts of the ovary, with the second quarter from the top generally giving the highest score.

Pollen tube growth before anthesis

In buds from 7 d before anthesis pollen tube growth was possible. Their length reached in 24 h was however strongly reduced compared with pollination at or after anthesis. When pollinated from anthesis until about 7 d later the length of the pollen tubes, which was measured at 24 h after

FIG. 16. Electron opaque site in the cell wall (\star) where two placental cells separate. The cuticle (C) is present at this point but vanishes further towards the top of the cell. S, Starch; PL, plasmalemma. $\times 11200$.



FIGS 17–18. Compatible pollination of *L. longiflorum* 'Gelria' with 'White American' as observed by SEM.

FIG. 17. The pollen grains (PG) germinate and the pollen tubes (PT) grow over the stigma. $\times 240$.

FIG. 18. Pollen tubes (PT) grow in between the stigma lobes (\star) into the styler canal. $\times 100$.

FIGS 19–20. Secretory cells (\star), exudate (E) and pollen tubes (arrows) 2 d after compatible pollination as observed with cryo-SEM.

FIG. 19. In the top of the style, at both sides of the pollen tube bundle a layer of exudate is present. $\times 1000$.

FIG. 20. Halfway down the style a part of the pollen tubes has an irregular shape. $\times 2600$.

FIG. 21. An ovule after the pericarp was broken away, but leaving its print behind in the exudate (E). The exudate fills the space between the inner integument (II) and the pericarp. $\times 500$.

FIG. 22. The placenta (arrows) is covered with exudate (E) at 4 d after pollination (DAP). Inner integument (II), ovule (O). $\times 500$.

FIG. 23. Once arrived in the ovary the pollen tubes bend 90° from the central bundle (B) and grow over the placenta in between the ovules (here broken away, \star) towards the micropylar side. SEM, 6 DAP. $\times 190$.

FIG. 24. After growing from the central bundle and arriving at the pericarp, the pollen tubes bend again (\star) and form a bundle at the micropylar side of the ovules. To reach the micropyle (arrow) another change of direction from the micropylar bundle is necessary. SEM, 7 DAP. $\times 350$.

TABLE 1. Penetration percentages at different days after pollination (DAP), carried out at 2 d after anthesis, as determined after pollination and incubation in the growth chamber and in the greenhouse

DAP	Growth chamber	Greenhouse
2	0	0
3	38	0
4	76	29
5	70	63
6	82	80
7	90	92

Per flower three rows of ovules (on average 195) were checked after staining with Aniline Blue for pollen tube penetration. The results of two flowers were averaged apart from growth chamber 5–7 DAP, in which the percentages of four flowers were averaged. Experiment carried out in September.

pollination, was more or less constant at its highest level. In older flowers it dropped again. This tendency was also present when pollen tube growth after 40 h was observed, with a difference that pollen tube growth was found in even younger flower buds.

With cryo-SEM pollen tubes were observed in the top of the style at 1 and 3 d after pollination of a 120 mm flower bud (3–4 d before anthesis). One day after pollination the exudate layer had merged to a larger extent than the exudate in a flower bud of 120 mm. The accumulations on the top of the secretory cells were not observed. The pollen tubes grew both over the secretory cells and in between the cells, always covered by at least a thin layer of exudate. In cross section some pollen tubes protruded into the stylar canal at places where only a thin layer of exudate was present (Fig. 25). They were frequently stacked on top of each other. Three days after pollination, at about anthesis, far more pollen tubes had grown through the top of the style, compared with 1 d after pollination (Fig. 26). They were packed close together in the exudate and also here the pollen tubes directly bordering the stylar canal protruded into the latter. Sometimes there was even doubt whether a layer of exudate covered these pollen tubes (Fig. 27).

DISCUSSION

Exudate production in the pistil

Exudate production in the pistil was observed in buds of different lengths. The position and shape of the exudate accumulations in the top of the style in the younger bud stages pointed towards a restricted movement. The cuticle might have been a restricting force and bulged out during exudate production. According to Rosen and Thomas (1970) the exudate of *L. longiflorum* is captured by the cuticle until about the time of anthesis. Also in the study of Miki-Hirosige *et al.* (1987) it seems that the cuticle is lifted up or a large number of bladders were formed. Rosen (1971) proposed a dissolution or rupturing, Dickinson *et al.* (1982) a fragmentation and 'floating off' of the cuticle. A limitation of the cryo-SEM technique used was that the cuticle could not be distinguished from the exudate. The presence of the

remainder of the cuticle in between the secretory cells in the style, as was observed at the placenta using TEM, could indicate that it is torn, but reports on this were not found. If the cuticle was not torn, it might float away from the cells, which would then explain the pattern of fusion of the accumulations in the top of the style. The pattern of exudate deposition in the top of the style also showed that the exudate was produced there and did not flow down from the stigma, as was suggested by Rosen (1971). The production of exudate at the stigma and in the style started in the bud stage and was not triggered by pollination.

At the placenta cutinase produced by the pollen tubes was not involved in the disappearance of the cuticle, since the cuticle was also lost in unpollinated flowers. Because of the observation of thin fragments connected with the thicker ones covering the walls in between the secretory cells, the cuticle was presumably stretched and subsequently ruptured.

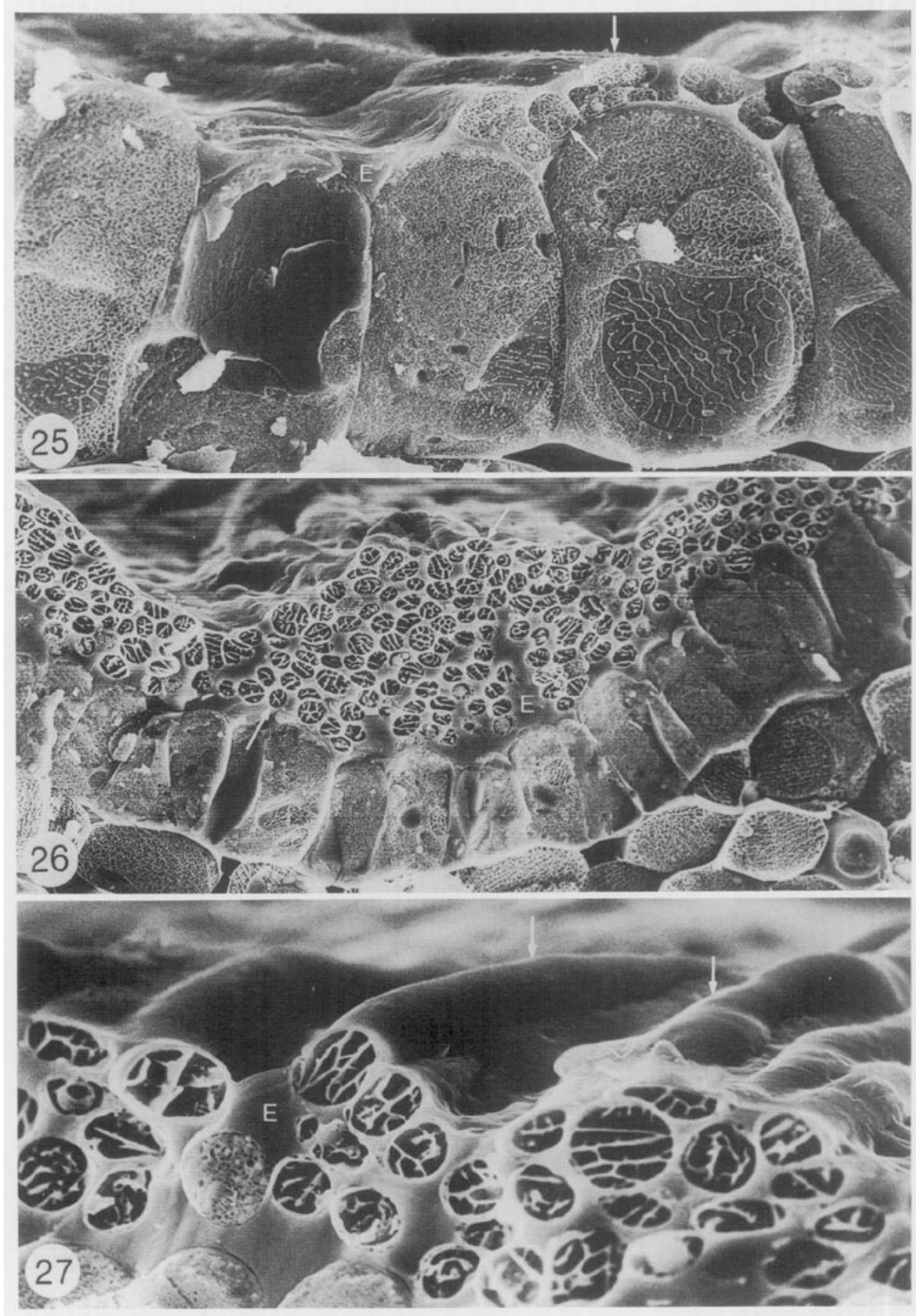
Apart from detecting stylar and placental exudate, the cryo-SEM method was also chosen to detect any fluid in the micropyle. In our specimens the plane of fracture never followed a cross section through the micropyle, so that we failed to trace such a substance. The observed substance at the locular end of the micropyle might have been of placental origin.

Structure of the secretory cells in the ovary

The secretory cells of the placenta were ultrastructurally similar to those of the style. In both locations these exudate-producing cells had a transfer wall and a comparable composition of the cytoplasm. The outer layer of the transfer wall might be of a cellulosic nature associated with the fibrillar component in the inner wall, which is more of a pectic nature (Dashek *et al.*, 1971). A sign of the fibrillar component in the inner wall is shown in Fig. 12. The inner wall might be built up from the contents of vesicles with a diameter of 70 nm, seen elsewhere in the cytoplasm close to the dictyosomes (Dickinson *et al.*, 1982). This size was comparable to the 60 nm vesicles found in our experiments. Apart from the predominant role of dictyosomes, endoplasmic reticulum (ER) is involved as well in the production of the cell wall of secretory cells in the style (Miki-Hirosige *et al.*, 1987). In the secretory cells of the ovary ER was abundant as well. If the exudate originates from the parenchyma cells underneath the secretory cells, as was discussed by Yamada (1965), the latter cells at least partly alter the exudate because they are metabolically very active (Rosen and Thomas, 1970). Miki-Hirosige *et al.* (1987) launched the idea that the exudate is first stored in the cell wall before secretion. Crystals as observed by Gawlik (1984) in *Lilium leucanthum* were not found in our specimens.

Pollen tube growth patterns

At the stigma, pollen tubes lacked a directed and preferential growth towards the slits which were present in the three lobes, except when they were in their proximity. They grew across the stigmatic surface, in between the stigmatic papillae and entered the style only when they randomly met a slit. Here they might face a mixture of



FIGS 25–27. Cryo-SEM observations in the top of the style after pollination of a bud of length 120 mm.

FIG. 25. One day after pollination only a few pollen tubes are detected and a straight growth over the complete surface of the secretory cells is observed. The pollen tubes (arrows) are covered by a thin layer of exudate (E) hereby protruding into the styler canal. $\times 3400$.

FIG. 26. Three days after pollination more pollen tubes (arrows) have invaded the style and a larger amount of exudate (E) has been formed. $\times 1300$.

FIG. 27. The pollen tubes (arrows) being closest to the styler canal seem not to be covered by exudate (E). Three days after pollination. $\times 4300$.

exudates from the stigma and the style, directing them into the style.

Using cryo-SEM the ultrastructure of the pollen tubes appeared to be badly preserved. Despite this, the analysis of cross fractures in the top of the style at different time

intervals after pollination enabled us to distinguish between older and younger pollen tubes. The thus deduced stacked growth of younger pollen tubes below older ones, especially after pollination before anthesis, indicated that pollen tubes grew preferentially close to the secretory cells. Due to the

continuous production of stylar exudate they were pushed to the centre of the stylar canal, after which the most recently produced exudate (i.e. close to the secretory cells) was occupied by subsequently arriving tubes. In *L. philippinense* Crang (1969) also observed that younger pollen tubes grew closer to the secretory cells. The reason for this preference probably found its basis in the consumption of substances from the exudate by the pollen tubes, which was demonstrated using radioactive labels (Kroh *et al.*, 1970*a, b*; Labarca and Loewus, 1972, 1973), forcing later arriving pollen tubes to choose the most nutritive (fresh) exudate. The consumption might cause a gradient which can serve as a guidance for later arriving pollen tubes.

The general morphology of the pistil can direct the pollen tubes as well. Because of the trichome structure of the stigma of *Pennisetum typhoides* most of the pollen tubes grow towards the ovary and there is no reason to suggest a chemotropic guidance in this species (Heslop-Harrison and Reger, 1988). In spinach the pathway of the pollen tubes through the style is determined by the structure of the cell walls and the morphology and distribution of the central core of the transmitting tissue (Wilms, 1980). The production of exudate leading to a shift of the pollen tube towards the centre of the style might have led to the rare occurrence of direct contact between the pollen tubes and the secretory cells in *L. longiflorum*. Guidance of pollen tubes by the shape of the secretory cells, as a phenomenon to explain the downward directed growth in *L. auratum* (Iwanami, 1953), might also have taken place in our lily specimens.

Mascarenhas (1975) launched the idea that pollen tubes grow straight as long as no external stimuli are changing this direction. In *L. longiflorum* this implies that once arrived in the style, the pollen tubes grow straight downward. But in the ovary the pollen tubes bent aside toward the micropylar sides of the ovules while a straight growth was still possible. At the micropylar side they bent again, forced by their tendency to stay close to the secretory cells of which the zone ended here due to the beginning of the pericarp. Here external stimuli must then change their direction.

Chemotropy has frequently been suggested to explain directional changes of pollen tubes on the placenta and especially near the micropyle. Chao (1971) found a water-soluble periodic acid–Schiff–positive substance in *Paspalum orbiculare*. The pollen tubes of *Beta vulgaris* might be directed towards the embryo sac by a presumed secretory activity of the micropylar nucellus (Bruun and Olesen, 1989). A secretory function of the nucellar cells has also been suggested for *Gasteria verrucosa* (Franssen-Verheijen and Willemse, 1990). In *Ornithogalum caudatum* a micropylar exudate is secreted by the nucellar cap and the inner integument (Tilton, 1980). Unfortunately we failed to demonstrate a micropylar exudate using cryo-SEM. The ultrastructure of the secretory cells of the placenta did not change depending on their position relative to the pollen tube pathway. Together with the observed pollen tube growth from the micropylar side back to the central bundle this reduces the chances of the involvement of a gradient along the placenta. Instead it might be that the pollen tubes first use their reserves from the style and subsequently start curving. A difference between the two exudates was also

indicated by the difference in speed of pollen tube growth in the style and in the ovary as discussed below.

The speed of the pollen tubes

Hiratsuka and Tezuka (1979) studied germination and the first 6 h of pollen tube growth at the stigma of *L. longiflorum*. After 6 h the longest observed pollen tube was 1 mm. This initial growth is much slower than the 2.4 mm h⁻¹ that we measured in the style from 6 h after pollination. During sperm cell formation the speed was not altered.

In *Petunia hybrida* Mulcahy and Mulcahy (1982) found two phases of pollen tube growth, differing in speed of growth and in the appearance of callose plugs, and related them to a shift from autotrophic to heterotrophic metabolism. Rosen and Gawlik (1966) observed a change in the ultrastructure of the pollen tubes in compatible pistils of *L. longiflorum*. Since this change was absent in incompatible ones, they related it to a relatively autotrophic first phase (i.e. growth on its own reserves) and a more heterotrophic second phase (i.e. uptake of substances from the pistil). The self-incompatibility reaction occurs between 12 and 24 h after pollination (Ascher and Peloquin, 1970). In our experiments the change in growth speed occurred within 6 h. It is therefore unlikely that this change is related to the shift from autotrophic to heterotrophic as described above. The difference between the stigmatic and the stylar exudate (Dickinson *et al.*, 1982; Miki-Hirosige *et al.*, 1987) is more likely the cause of the slow start. The complex nature of the stigmatic exudate might be a good medium for acceptance and germination of pollen grains, but not of the right composition and/or concentration for a rapid pollen tube growth.

Two days after pollination the pollen tubes reached the end of the style. After another 2 d they had not yet reached the base of the ovary. The style was on average 9 cm long and the ovary between 4 and 5 cm. Although the pollen tubes bent to reach the micropyles this pointed towards a slower growth of the pollen tubes in the ovary. This might be caused by a difference in exudate composition. In flower buds the speed of pollen tube growth in the style was reduced as well. Exudate as a growth medium was already present, although there was not as much as after anthesis. Concerning the preference of pollen tubes to grow close to the exudate producing cells, the exudate composition in combination with its abundance is influencing pollen tube behaviour.

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