Acta Bot. Neerl. 30(4), August 1981, p. 289–307.

THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 2. POLLEN-TUBE PENETRATION AND THE STIGMA RESPONSE IN SECALE

J. HESLOP-HARRISON and Y. HESLOP-HARRISON

Welsh Plant Breeding Station, Plas Gogerddan,
nr Aberystwyth SY23 3EB, United Kingdom

Key words: Rye, *Secale cereale*, pollination, pollen germination, pollen-tube growth, stigma reaction

SUMMARY

After an initial period of hydration on the stigma, viable pollen grains release an exudate which becomes continuous with the stigma surface secretions. This is accompanied by an enhancement of surface esterase activity, and eventually the stigma cuticle is eroded at the contact face. Simultaneously the pectin-rich outer layer of the wall of the stigma papilla gelatinises.

Germination of the grain begins with the gelatinisation of the pectins of the Zwischenkörper, which displaces the operculum. The emerging tip passes through the eroded stigma cuticle and enters the outer pectin-rich layer of the wall. Enzymic removal of the proteinaceous part of the stigma surface secretion before pollination interferes with the capacity of the tube tip to penetrate, but does not prevent germination. During further growth in the stigma, the tube passes through the intercellular material of the branch axis, which occupies some 10% of the volume of the branch before pollination. The advent of the tube brings about a rapid increase in the permeability of the affected cells, preceded in some species by the blockage of the plasmodesmata by callose. The membranes of the cells immediately adjacent to the pollen-tube pathway undergo dissolution, and the nuclei become pycnotic.

Calculations based upon the changes of volume of the grain during the first 500 μm of pollen tube growth show that the water requirement of the tube may be met entirely by transfer from the hydrated grain, and not by uptake from the stigma tissues. This implies that all of the initial events of germination, tube emergence and growth through the secondary stigma branches are driven by the hydrostatic pressure built up in the grain during the period of hydration following upon capture.

1. INTRODUCTION

In our first paper on pollen-stigma interaction in the grasses, referred to as Paper 1 (J. & Y. HESLOP-HARRISON 1980a) we have given an account of the principal fine-structural and cytochemical features of the virgin stigmas of *Hordeum* and *Secale*. The present paper is concerned with the events immediately following upon the receipt of compatible pollen in *Secale* and certain other genera, including the early interactions on the stigma surface, the penetration of the pollen tube and the growth of the tube through the secondary stigma branch. We deal with the responses of the stigma cells to the advent of the pollen and pollen tube, and also provide dimensional data for the stigma and tube upon which estimates of the rates of water movement during the period immediately following upon pollination may be based.

2. MATERIALS AND METHODS

The main observations were made on *Secale cereale* L. cv. Rheidol grown in field plots, with supplementary data from greenhouse-grown *Pennisetum glaucum* R. Br. and natural-source *Molinia caerulea* L. and *Gaudinia fragilis* (L.) Beauv. Inflorescences approaching flowering were brought into the laboratory and maintained in cool conditions until required, when anthesis was promoted by exposure to infra-red lamps. Where clean stigmas were required, the emerging anthers were removed to prevent selfing.

Controlled pollinations were carried out using entire excised pistils, or stigmas detached at the level of the top of the ovary. Time-series preparations were obtained by implanting the pistils or stigmas in 1% agar in petri dishes, which were then incubated at 24°C for the required periods after pollination. For the continuous observation of pollen germination, tube penetration and the subsequent growth of the pollen tubes through the stigma tissues, receptive stigmas were mounted in pollination chambers made from glass slips on microscope slides. The bases of the stigmas were moistened with filter paper wicks, but the stigmas themselves were left unenclosed to prevent excessive condensation. Pollinations were carried out with pollen from freshly dehisced anthers, and individual grains were applied to selected stigma papillae using a single human eyelash hair, either manually or with a Singer low-power micromanipulator. The preparations were viewed by epi-illumination, or combinations of epi- and transmitted illumination, with a Vickers M17 system. The temperature of the microscope stage was unregulated, but remained in the range 21–24°C during observation. Micrographs were taken at the required intervals with manual timing, or with an electronic timer controlling an automatic camera.

Permeability changes were followed in living stigmas in the manner described in Paper 1, and the cytochemical and optical- and electron-microscopic methods were also as in that paper.

Pollen-tube growth rates were estimated either by direct measurement on living material using a micrometer eyepiece with travelling graticule, or from time-lapse micrographs. The dimensions of the cells and tissues of the stigma were measured directly from whole mounts using a camera lucida. For the estimation of the areas of the cells and intercellular spaces in the pollen-tube transmitting tracts of the secondary stigma branches, measurements were made from electron micrographs.

Linear dimensions and complex areas were computed directly with a digitising data pad coupled to an Apple microcomputer.

Various pre-treatments were tested for their effect on pollen germination and tube penetration on the stigma of rye. Pistils were excised from florets at anthesis, and samples of stigmas were exposed to each of the following treatments: (a) the lectin concanavalin A (1 mg ml^{-1} in 0.05 M Tris/HCl buffer at pH 7.2 with 0.001 M $CaCl_2$); (b) pronase (1 mg ml^{-1} in 0.01 M phosphate buffer at pH 7.4 with 0.001 M $CaCl_2$); (c) trypsin (1 mg ml^{-1} in 0.05 M Tris/HCl buffer at pH 7.4 with 0.001 M $CaCl_2$), and (d) neuraminidase (1 mg ml^{-1} in 0.05 M acetate buffer at

pH 5.6). For each treatment controls were run using matched stigmas in the corresponding buffers without the lectin or enzymes. The stigmas were teased out in the media, placed under reduced pressure to remove residual air bubbles, and incubated for 30 min at c. 24°C. They were then rinsed three times in the appropriate buffer and finally in water, before being drained dry and mounted in pollination chambers. Single-grain manual pollinations were carried out with pollen from freshly dehiscing anthers, and the grains were placed on the stigmas and oriented so as to occupy a similar position on a papilla near the stigma tip in each instance. The preparations were scanned at intervals and the progress of germination and tube growth recorded during the succeeding four hours.

In the following account we use the terminology adopted in Paper 1 for the components of the grass stigma. In the species examined, the stigma is bifurcated, and the primary branches (stylodia) bear secondary branches composed of ranks of papillate cells. The pollen-receptive parts of the papillae bear a complex wall with thin surface secretions. The following strata are distinguishable by electron microscopy with the appropriate preparation methods: A, an outer proteinaceous secretion layer some 15–20 nm in thickness, overlying B, a second, mucilaginous, secretion of variable thickness; C, a discontinuous cuticle, overlying in turn D, a stratum staining for protein and pectic polysaccharides and containing a dispersed microfibrillar component, and E, an inner wall layer with staining properties suggestive of the presence of β-1,4-glucans, possessing a microfibrillar substructure as observed electron microscopically following standard fixation and post-staining procedures in *Gaudinia fragilis*, but appearing electron-transparent in *Secale cereale*. A further layer, F, of variable thickness and unknown nature can often be discerned between layer E and the plasmalemma. Layer D is continuous with material with similar cytochemical properties separating the cells and occupying the enlarged intercellular spaces in the core of the secondary stigma branches.

3. OBSERVATIONS

3.1. The immediate post-pollination period

Detailed timings of the events following upon the attachment of freshly shed pollen to a receptive stigma of rye have been given in earlier papers (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978, J. HESLOP-HARRISON 1979a). The first observable event is the formation of a meniscus at the interface, usually within 20–30 sec after first contact in a laboratory temperature of 21–24°C. Thereafter, as the initial hydration of the grain proceeds, and usually within a further 20–30 secs, exudate appears on the surface of the exine, a phenomenon first reported by WATANABE (1955). The exudate is resorbed before the beginning of germination, which takes place in 70–150 sec following first contact with the stigma. In the pollen grain, germination begins with the gelatinisation of the pectins of the Zwischenkörper at the single aperture; this is followed by the lifting of the operculum, and the emergence of the tube tip (J. HESLOP-HARRISON 1979a, J. & Y. HESLOP-HARRISON 1980b). According to the orien-

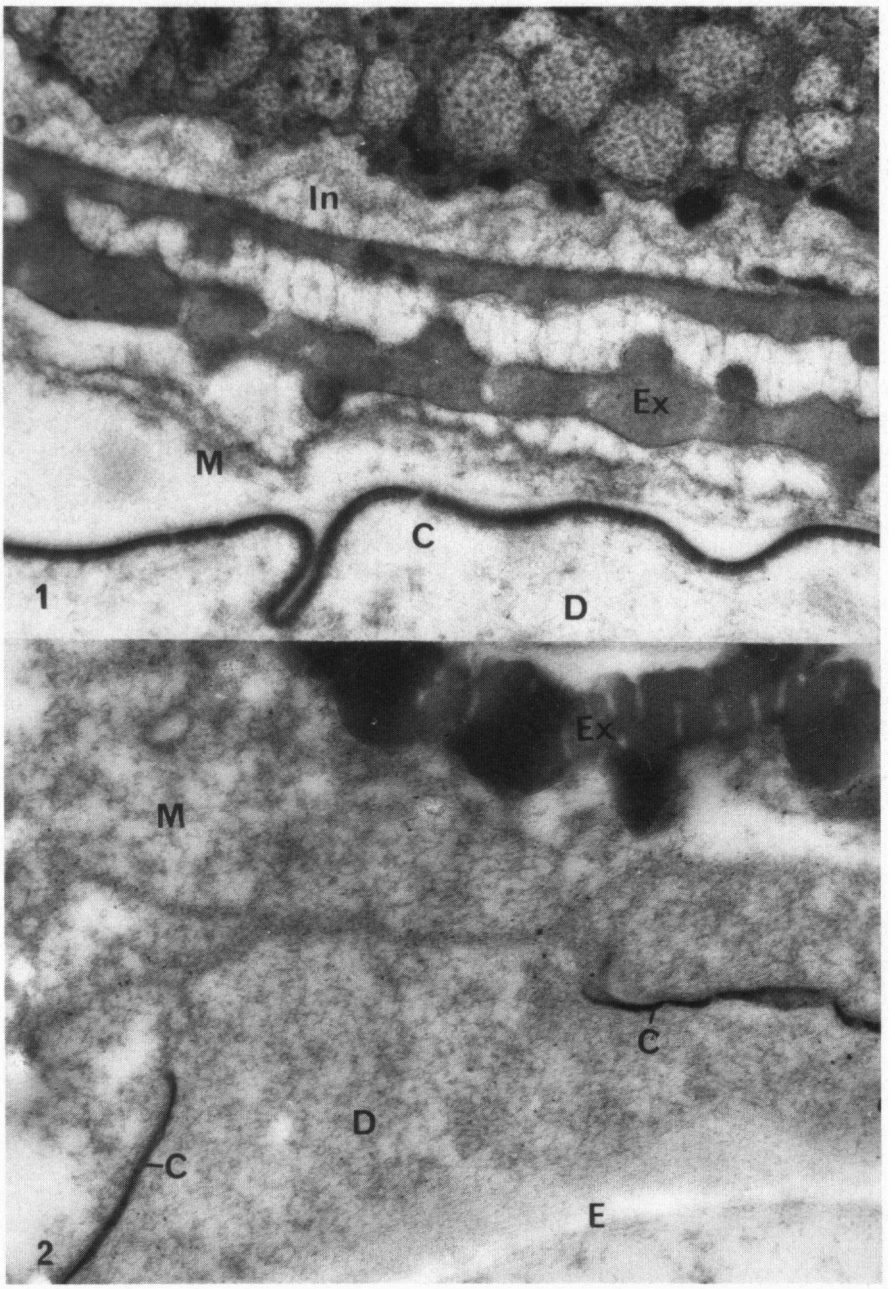


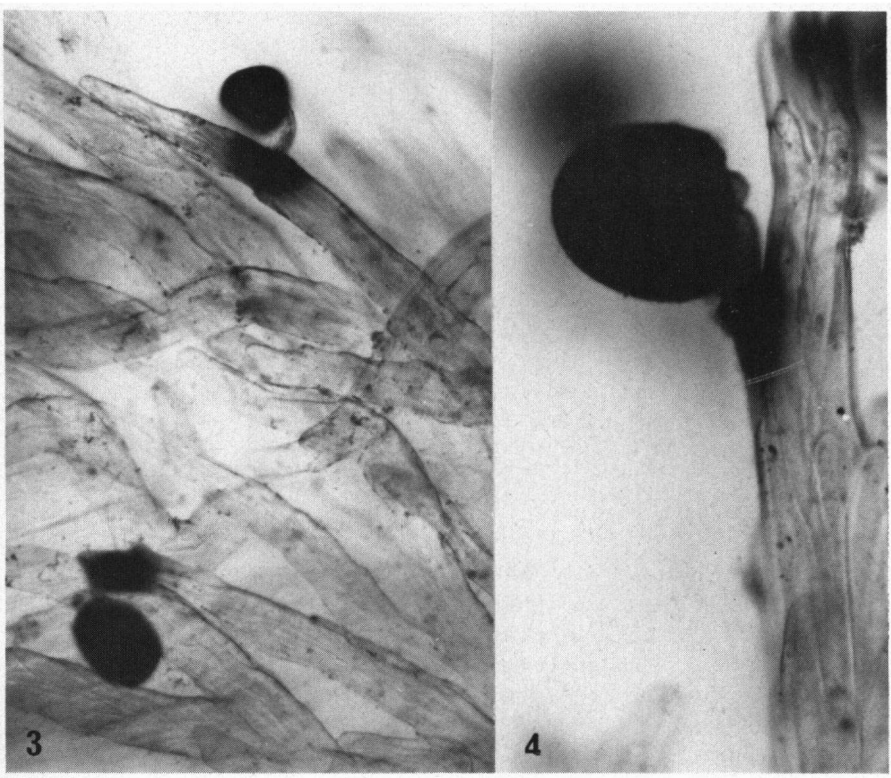
Fig. 1. Electron micrograph of the contact face between the pollen grain and stigma surface soon after pollination; *Gaudinia fragilis*. Ex, pollen exine; In, intine; C, cuticle of papilla; D, underlying wall layer, partly gelatinised. The material at the interface (M) includes both the surface secretions of the stigma and the pollen exudates. × c, 29,000.

Fig. 2. Electron micrograph of the contact face between the pollen grain and stigma surface, *Secale cereale*. Ex, pollen exine, M, meniscus material. The wall layers of the stigma are labelled according to the scheme given in Paper 1 (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1980a). The cuticle, C, has been eroded in the vicinity of the meniscus, and the pectinaceous layer D is continuous with the material of the meniscus. M. × c. 25,000.

tation of the aperture, the tip makes immediate contact with the stigma surface, or, in the absence of competition from other tubes, reaches the surface after growing over the exine for a varying distance.

In the course of the exudation from the grain, proteins held in the intine are transferred into and through the exine, both layers of which – sexine and nexine – are penetrated by micropores in the grass pollen grain (J. HESLOP-HARRISON 1975, 1978, 1979b). The outflow passes onto the stigma, and as the area of the meniscus enlarges, the secretion layers A and B of the surface of the papillate cells are dispersed into it (*fig. 1*).

As in species of other families with the "dry" type of stigma (Y. HESLOP-HARRISON 1977), the attachment of the pollen is accompanied by a rapid increase of the surface esterase activity in the vicinity of the contact face (*figs. 3 and 4*). Observations of the events following upon germination were made using secondary stigma branches pollinated manually with a single grain. In these circumstances, where there can be no competition between pollen tubes, the emerging pollen tube was found either to enter immediately at a site on the flank of the



Figs. 3 and 4. Optical micrographs of stigmas of *Pennisetum typhoideum*, 3–4 min following compatible pollination, showing enhanced surface esterase activity in the vicinity of the contact between the emerging tube tips and the stigma surface. Fig. 3, × c. 180; Fig. 4, × c. 380.

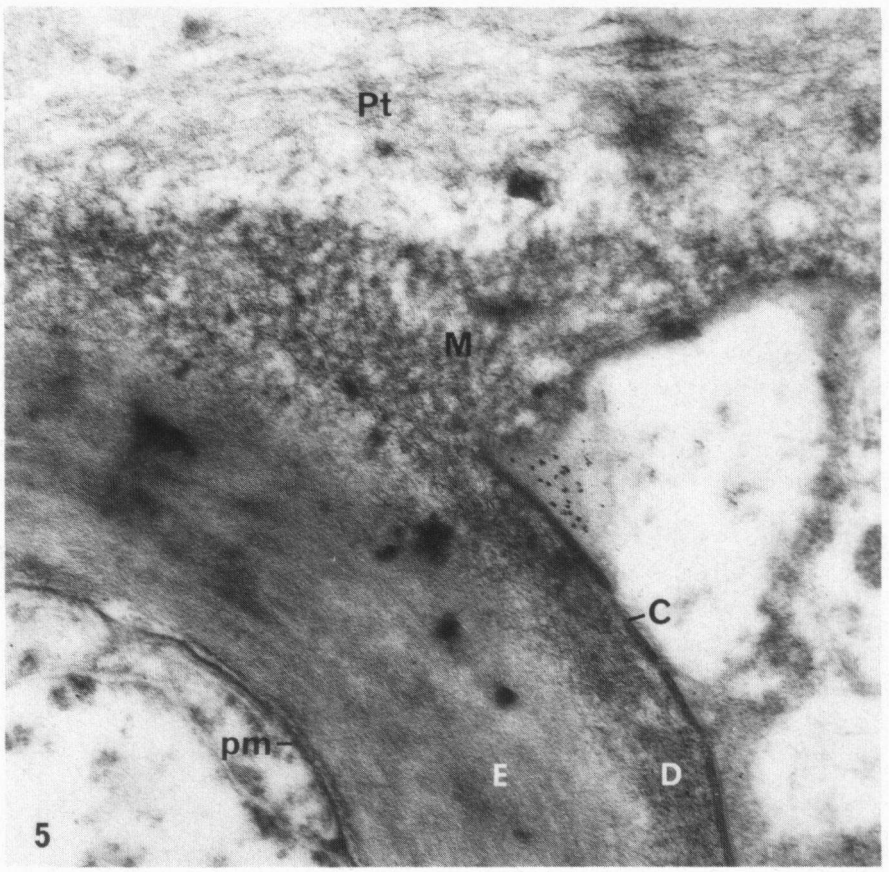


Fig. 5. Electron micrograph of the contact between the pollen tube tip (Pt) and the stigma surface during the early erosion of the cuticle; *Gaudinia fragilis*. The cuticle, C, is perforated at the contact face, and the material of wall layer D is now continuous with the meniscus material, M. In *Gaudinia fragilis* the inner wall layer, E, shows a microfibrillar structure following standard EM staining procedures; the corresponding layer in *Secale cereale* is electron-transparant (compare *fig. 2* of this paper, and figs. 3, 5, 21 and 22 of Paper 1). × c. 26,000.

contiguous papilla, or to grow along the surface to the junction with the next lowest papillate cell and enter there.

The entry is preceded by the detachment of the cuticle from the underlying layer E. This layer swells and gelatinises in register with the attached pollen and pollen tubes, suggesting a partial dissolution of the pectic component. It is known that pectolytic enzymes are released from hydrating grass pollens (J. HESLOP-HARRISON 1979a), so this is a comprehensible enough response. The cuticular layer C is eroded and perforated firstly in the vicinity of the tube tip, which then penetrates (*fig. 5*). Although the erosion is most rapid and extensive near the tip, the cuticle may also be perforated at the interface with the exine

Table 1. Effects of enzyme and lectin pretreatment of the stigma on pollen germination and tube penetration in *Secale cereale* (includes data from J. & Y. HESLOP-HARRISON 1978). Treatments and pollination method as in text.

Treatment	No. of pollinations	No. grains germinating	No. tubes penetrating
Pronase	5	5	0
Control (1)	7	7	6
Trypsin	8	8	1 (2)
Control	8	8	8
Neuraminidase (3)	8	8	8 (4)
Concanavalin A	8	8	0 (5)
Control	7	7	5

(1) In the controls, tube tips penetrated the stigma papillae in 5–7 min.
(2) No penetration after 25 min.
(3) Separate controls were not maintained for this experiment.
(4) One tube penetrated after 9 min; remainder grew extensively over the surface of the papillae.
(5) All tubes penetrated in under 10 min.
Three tube tips swelled in contact with the stigma surface; others looped repeatedly around the contiguous papillae.

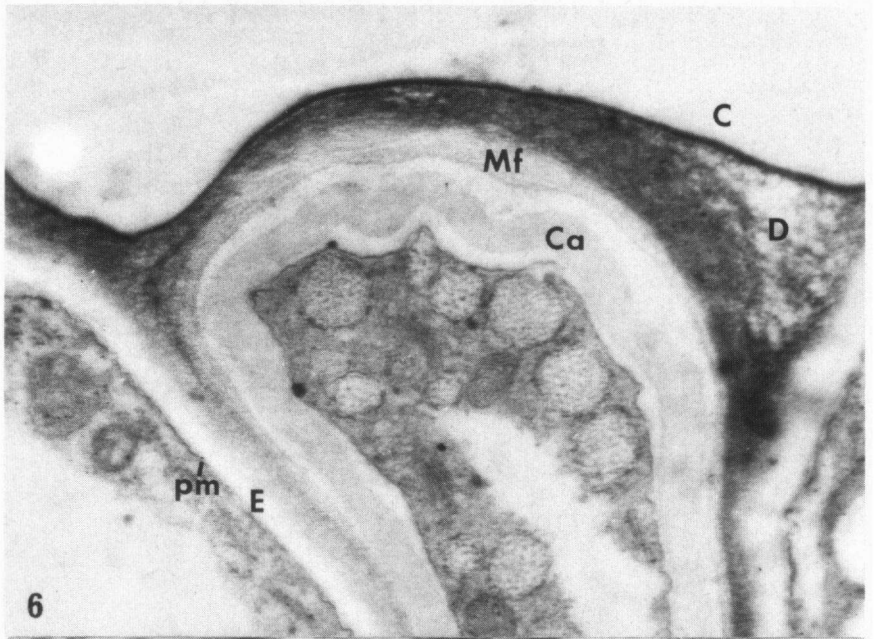
away from the tip (*fig. 2*). This indicates that the cutinase responsible for the dissolution of the cuticle, or its precursor, may be derived from other intine sites as well as from the tip itself. The penetration of the cuticle is always in the area of enhanced surface esterase activity (*figs. 3 and 4*), suggesting that the enzyme detected cytochemically is in fact a cutinase.

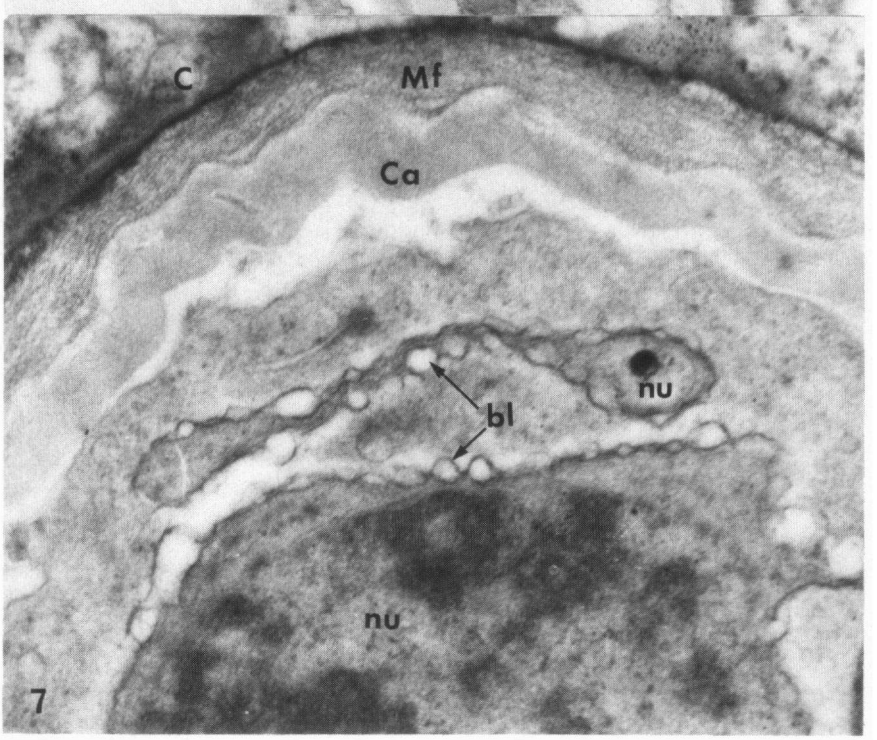
The effects of proteolytic enzymes on the penetration of pollen tubes in compatible pollinations of rye are shown in *table 1*. This treatment, which reduces the surface esterase activity of unpollinated stigmas presumably by partly dispersing the proteinaceous component of the secretion layer A, does not affect pollen germination. However, it was consistently found to interfere with the capacity of the tubes to penetrate the cuticles of the stigma papillae. As shown in *table 1*, a similar effect was observed with the lectin, concanavalin A, known to bind to the layer A of the grass stigma (Y. HESLOP-HARRISON 1976). The enzyme neuraminidase had no apparent effect on pollen germination or tube penetration.

3.2. Tube growth in the secondary stigma branches

In rye, the growth of the tube is suspended briefly during the period of penetration. On detached stigmas observed in pollination cells the process was found to take 30 to 120 sec. Thereafter the tube tip passes below the cuticle, and continues growth towards the primary stigma branch in the wall layer D.

The stratification at this time may be seen in the electron micrographs of *figs. 6* and *7*. The cuticle remains intact over the flanks of the tube, indicating that there is no further release of cutin-lysing enzymes from this site. As may be seen in *fig. 6*, the outer microfibrillar wall layer of the pollen tube is not sharply delimited


C
Mf
Ca
D
pm
E
6


C
Mf
Ca
nu
bl
nu
7

from the material of the stigma wall layer D. On the other hand, the callosic inner layer of the older parts of the pollen tube is quite clearly defined as a separate entity. The tube never penetrates the inner stigma wall layer E which, in rye, remains as an electron transparent sheath of varying thickness around the papillate cells. Accordingly the tube never comes into direct contact with the plasmalemmas.

The advancing tube tip usually – although not invariably – passes rapidly into the central part of the secondary stigma branch, growing onwards in the pectin-rich interstitial material of the axis, which forms the pollen-tube transmitting medium and is continuous with the outer wall layer D (Paper 1). The physical relationships may be judged from the optical micrographs of *figs. 8* and *10–12*. The callosic inner tube wall is specifically stained in *fig. 11*, which is to be compared with *fig. 12*, a micrograph of the same section from which the aniline blue has been removed before staining with calcofluor white. In suitably oriented sections the callosic layer is seen to have inclusions with pectin-like staining properties (*fig. 8*). These may correspond to the occasional microfibrillar inclusions in this wall layer visible in electron micrographs (*fig. 9*). The pollen tubes are readily detectable in the stigma branches through the presence of the typical pollen starch (*fig. 10*).

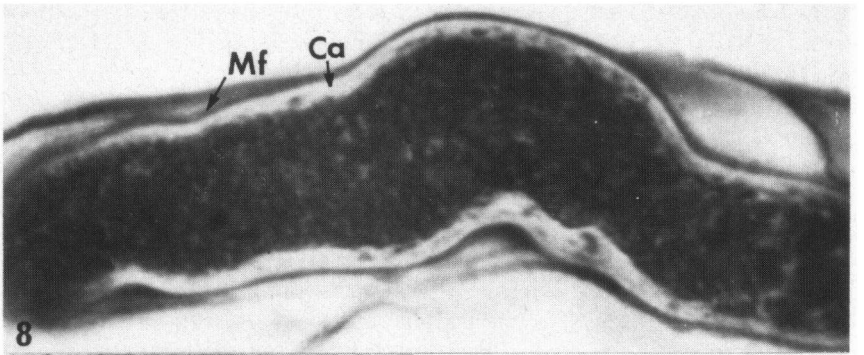
3.3 Responses of the stigmatic cells

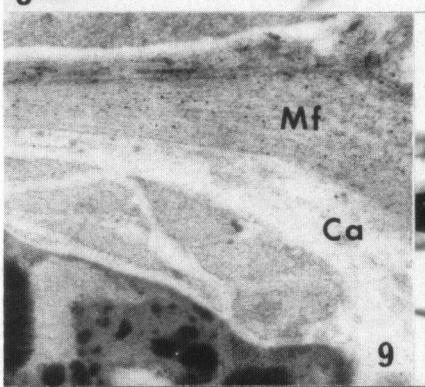
The receptive zone of the papillate cells of the stigma of rye bears a discontinuous cuticle, and is permeable to "vital" stains before pollination (Paper 1). Because of the considerable difficulty of manipulating individual grains after the first attachment to the stigma, the immediate consequences of the receipt of pollen have not been followed in any detail in rye. However, from observations on the stigma of *Molinia caerulea*, which has considerably larger receptive cells, it is evident that permeability is still further enhanced following rapidly upon the receipt of pollen. This greater permeability no doubt accounts for the increased propensity for acetocarmine staining recognised by KATO & WANATABE (1957) as being one of the earliest responses to pollination in the grasses examined by them.

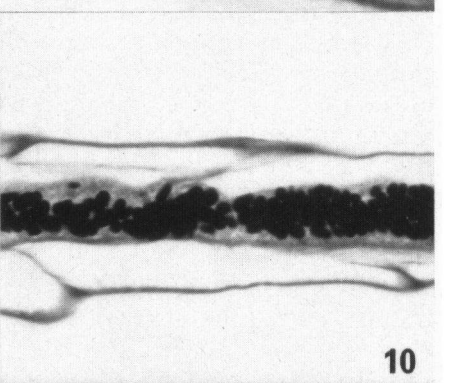
Figs. 6 &7. Electron micrographs of early pollen tube growth in the secondary stigma branch of *Secale cereale*.

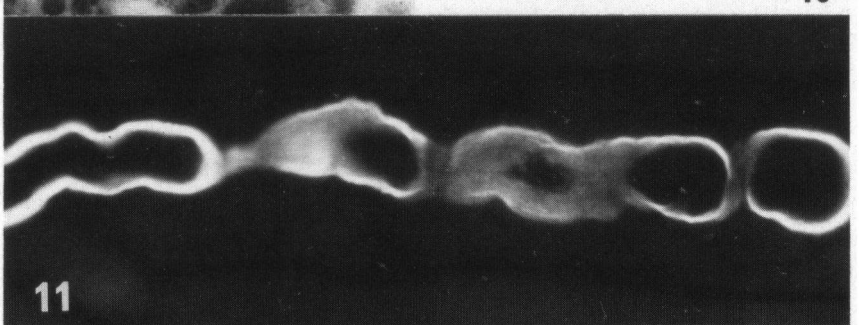
Fig. 6. Tube growing below the cuticle, C, in the wall layer D between two adjacent papillate cells. The wall layer E remains intact, and at this early stage stretches of the plasmalemma (pm) of the stigma cells are still apparent, although the tonoplasts are absent. The pollen tube wall shows the standard stratification at this level, with a well-defined inner callosic layer, Ca, and an outer microfibrillar zone, Mf, which is not sharply demarcated from the vaguely microfibrillar, and presumably pectic, material of layer D. × c. 29,000.

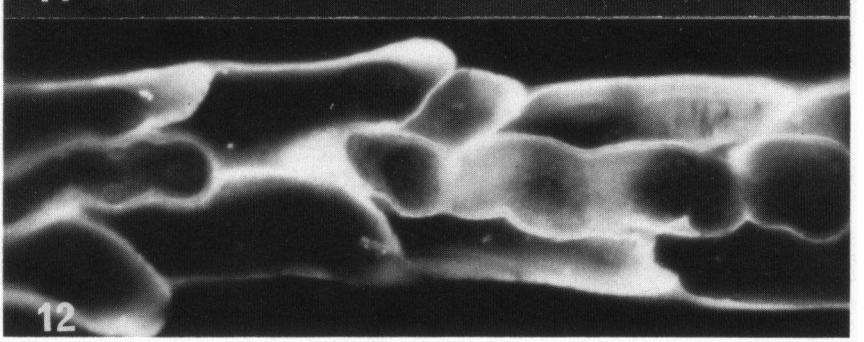
Fig. 7. As *fig. 6*, after the migration of the vegetative nucleus (nu) into the tube. This nucleus is deeply lobed and convoluted, and profiles appear twice in this section. The nuclear envelope shows conspicuous blebbing (bl). The callosic inner wall of the tube (Ca) is again well defined as a sheath separated from the outer microfibrillar layer, Mf. × c. 50,000-


Mf
Ca
8


Mf
Ca
9


10


11


12

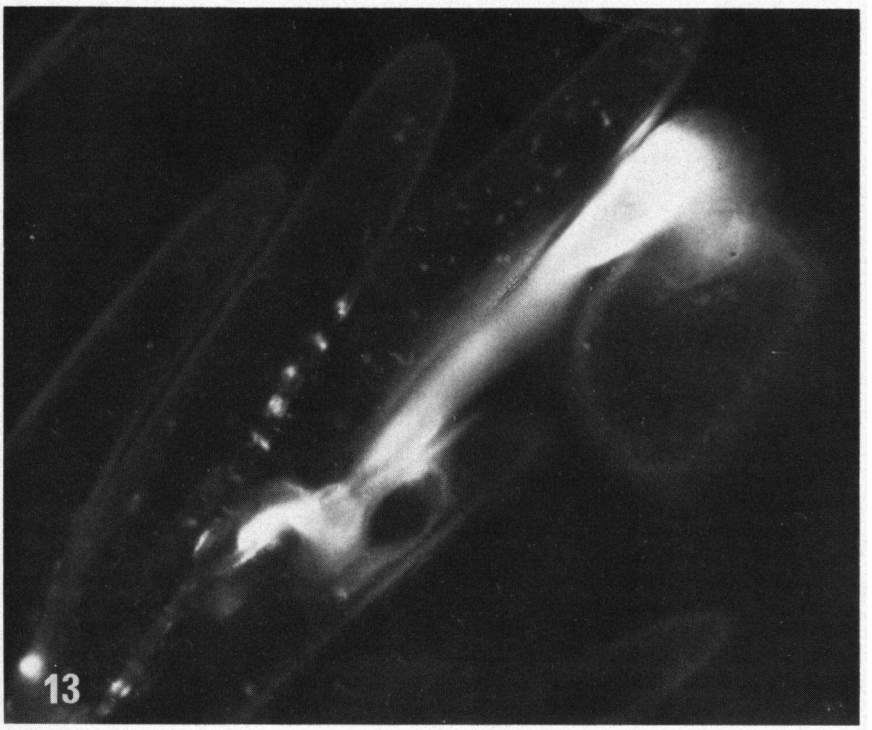


Fig. 13. Apparent callose blockage of pitfields in the vicinity of pollen tube entry, *Molinia caerulea*; fluorescence micrograph of a whole mount, decolourised aniline blue staining for callose. In this species, the effect is more conspicuous than in rye, and may occur also in contiguous cells. × c. 800.

As the tube tip passes through the cuticle and into the wall layer D, the plasmodesmata interconnecting the papillate cell with neighbouring cells become occluded with callose, and the effect may spread also to contiguous cells *fig. 13*, for *Molinia caerulea*). This reaction is rapidly followed by a change in the membranes in the cells in the vicinity of the tube. In the receptive papillate cells of

---

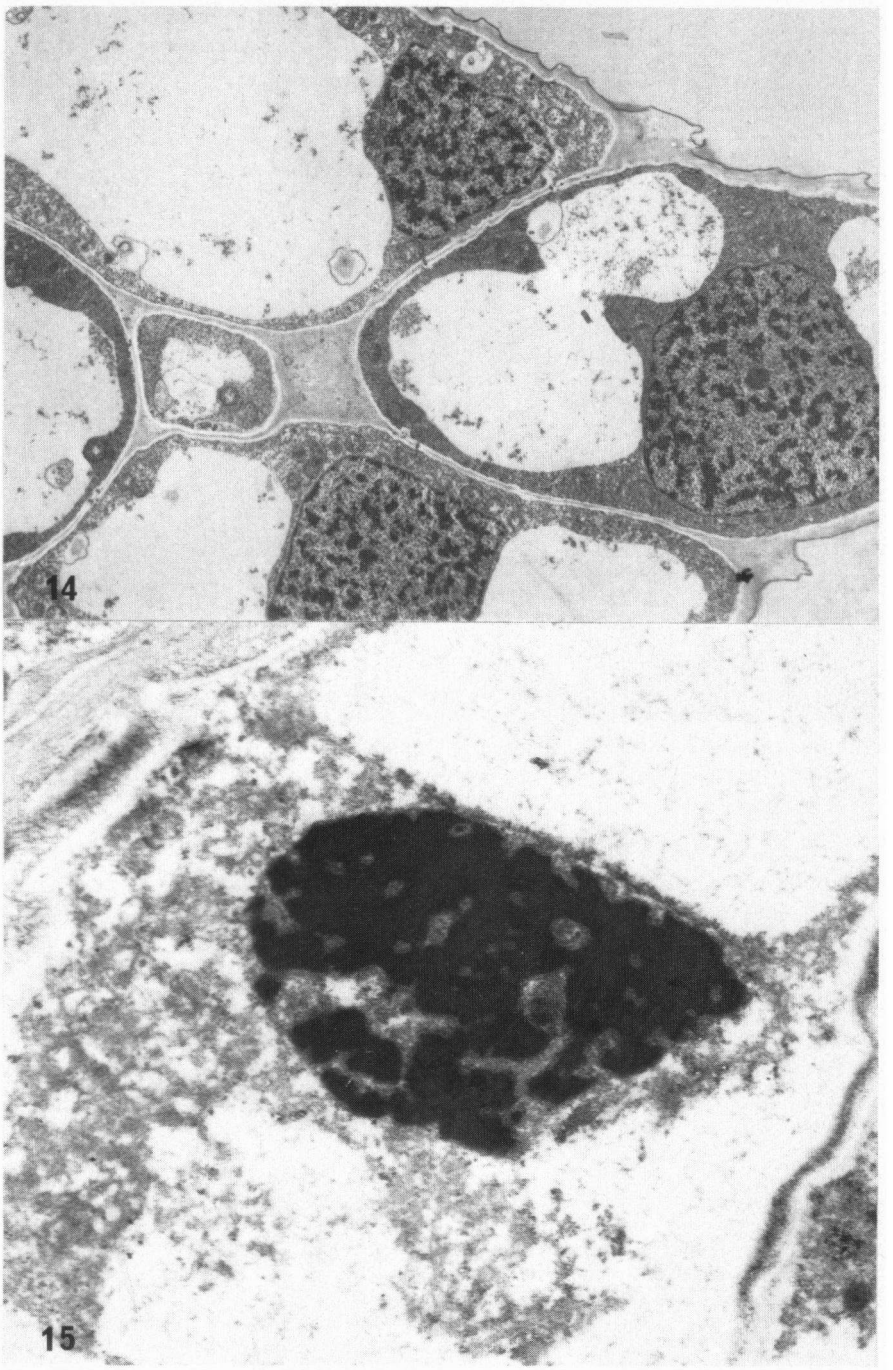
Fig. 8. Optical micrograph of a longitudinally sectioned secondary stigma branch of *Secale cereale*, with pollen tube (1–1.5 μm section, toluidine blue staining). The two wall layers of the tube are clearly differentiated, and the inner callosic layer (Ca) has inclusions with pectin-like staining properties. Mf, microfibrillar layer. × c. 2,000.

Fig. 9. Electron micrograph of a pollen tube wall corresponding to that of *fig. 8*, showing the microfibrillar inclusions in the callosic layer, Ca. Mf, microfibrillar layer. × c. 18,000.

Fig. 10. As *fig. 8*, PAS staining, showing the pollen tube starch. × c. 680.

Fig. 11. As *fig. 8*, fluorescence micrograph, decolourised aniline blue staining for callose. The inner wall layer of the pollen tube is sharply defined, and at this level in the stigma branch the branch cells are without callose. × c. 1000.

Fig. 12. Fluorescence micrograph of the same section as that in *fig. 11*, after removal of the decolourised aniline blue and restaining with calcofluor white. The outer wall layer of the pollen tube is now stained, and also the walls of the stigma cells. × c. 1000.


14
15

rye before pollination, the tonoplast is ill-defined and may not exist as a continuous membrane, but the plasmalemma, nuclear envelope and the membranes of the endoplasmic reticulum and organelles are readily resolved in electron micrographs of cells fixed and post-stained by the standard procedures (figs. 5 and 6 in Paper 1). The entry of the pollen tube into wall layer D leads to a dissolution of all of these remaining membranes. Because of the uncertainty of the timing of fixations for electron microscopy, it has not been possible to establish precisely the rate of the change; but estimates based upon the observed rate of tube growth indicate that it must begin within less than 60 sec of the approach of the tube tip. As observed by KATO & WATANABE (1957), the nuclei of the stigma cells become pycnotic soon after the passage of the tip. The electron microscopic appearance of the condensed and pycnotic nuclei may be seen in *fig. 15*, which is to be compared with *fig. 14*, a micrograph of a secondary branch of a receptive but as yet unpollinated stigma.

In the unpollinated stigma, the vacuoles of cells adjacent to the interstitial material in the branch axis which forms the pollen tube transmitting tract frequently contain numerous thick fibrils, probably proteinaceous (fig. 6 of Paper 1). These fibrils appear first in large cisternae of the endoplasmic reticulum (*fig. 16*), from which, presumably, they are released into the vacuoles (*fig. 17*). After the dissolution of the membranes, similar fibrils occur through the lumina of the cells of the stigma branch (*fig. 18*), but they were not observed in the intercellular spaces.

The micrograph of *fig. 19* shows the state of the cells towards the base of a secondary stigma branch after the passage of the tube; it may be compared with *fig. 14* of this paper, and figs. 5 and 18 of Paper 1. The cells lack membranes, but are still bounded by the continuous wall layer E. The callose of the inner wall of the part of the tube is homogeneous and of relatively low electron opacity with the standard staining procedure. As during the early passage of the tube (*fig. 6*), the outer fibrillar layer of the tube wall shows no sharp demarcation from the material of the wall layer D of the stigma.

3.4. Stigma volume and the water demand of the pollen tube

In the normal conditions of pollination, the hydration of the pollen grain must depend upon the entry of water from the contiguous papilla. Estimates of the maximum rate of water movement based upon the rate of enlargement during hydration have been given for rye pollen (J. HESLOP-HARRISON 1979a, 1979b), but the implications for the stigma have not previously been considered.

Figs. 14 & 15. Electron micrographs of secondary stigma branches of *Secale cerale*.

Fig. 14. Unpollinated but receptive stigma. The cells in this stage show cyclosis, although with normal preparation methods it is not possible to resolve a tonoplast (see Paper 1). × c. 7000.

Fig. 15. Base of a papillate stigma cell after the receipt of the pollen tube. All of the membranes of the cell have undergone dissolution, and the nucleus is condensed and heavily staining. × c. 8000.

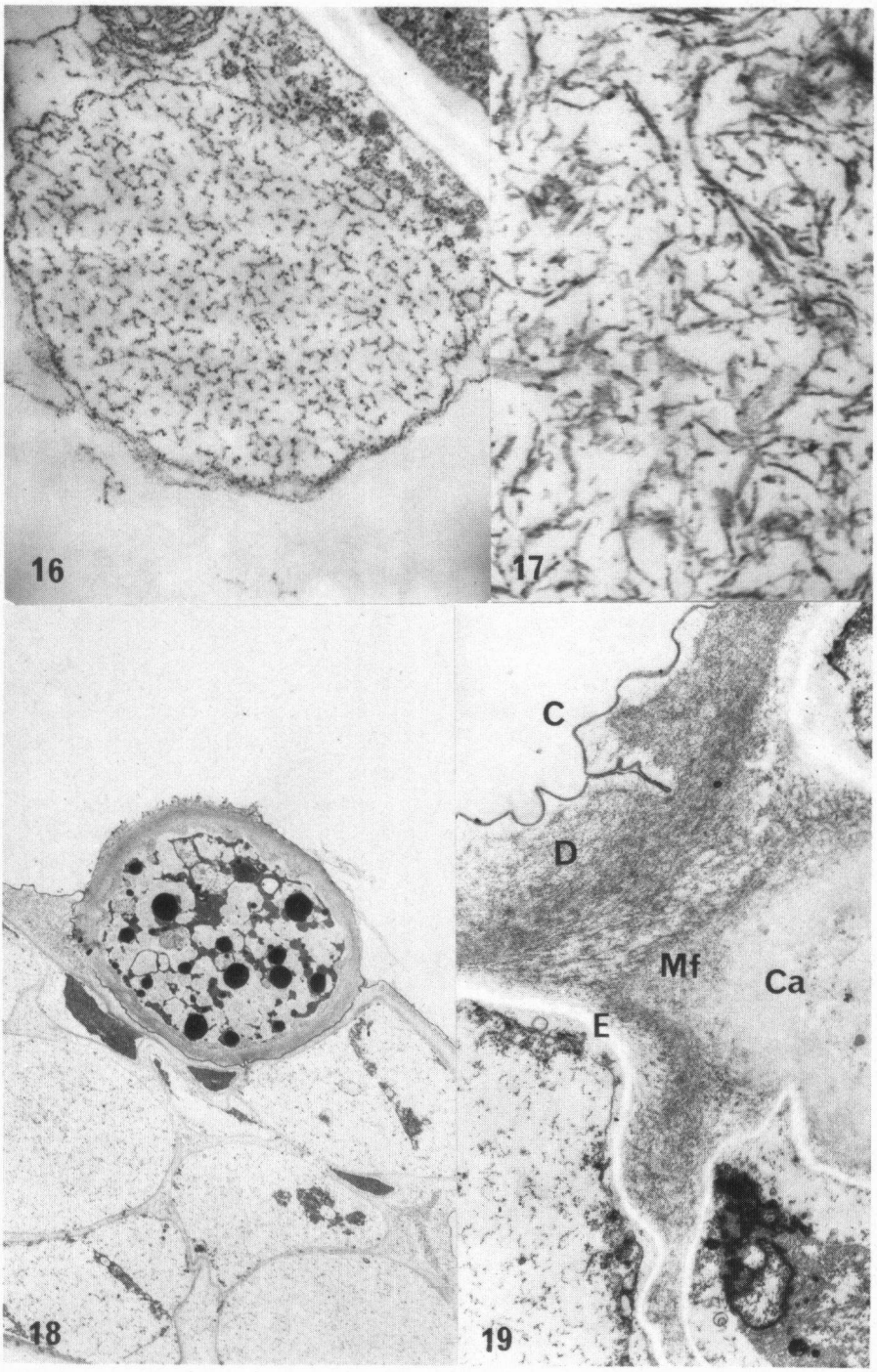

16
17
18
C
D
Mf
Ca
E
19

Table. 2. Dimensions computed from camera lucida measurements of whole mounts of secondary stigma branches of *Secale cereale* cv. Rheidol, in the receptive state but without pollen tubes.

	Mean length (overall) μm	Mean diam. (axial) μm	Mean vol. μm^3
Secondary stigma branch (1)	329.3 + 9.8	18.7 + 0.55	10.09×10^4 (2)
Papillate cell (3)	50.8 + 1.7	9.1 + 0.28	2.85×10^3 (4)

(1) Central zone of the stylodia.
(2) Includes the volume of the reflexed tips of the papillate cells.
(3) Central zone of the secondary branches.
(4) Volume includes reflexed tip treated as a cone.

Dimensional data for the fully expanded, receptive but not pollinated, secondary stigma branches and papillate cells are given in *table 2*. These data, obtained directly from whole mounts using a camera lucida digitiser, can be regarded as reasonably accurate; but it may be noted that the areas computed by this means were found to be some 10–15% greater than those obtained by measurement from electron micrographs. However, the measurements from the micrographs are likely to be inaccurate due to shrinkage during the preparation of the material for microscopy, and possibly also because of errors in the calibration of the microscope itself. Nevertheless, electron micrographs of stigma secondary branches sectioned transversely provide a satisfactory basis for estimating the relative cross-sectional areas of the cells and the intercellular material in the axis. In the three micrographs from which measurements were made, the intercellular spaces accounted for 7.9–11.5% of the cross sectional area, suggesting that before pollination some 10% of the internal volume of the branch is occupied by the interstitial material. Diameter measurements of single pollen tubes taken at intervals of 8–10 μm in the axes of secondary stigma branches gave a mean value of 8.89 + 0.25 μm. A single tube traversing the axis therefore occupies, at each level, approximately 23% of the volume of the branch before pollination. Linear growth rates measured in tubes growing through the axes of secondary branches lay in the range 1.2–2.25 μm sec^{-1}, so that the volume of the tube under the conditions of the present observations increases by 7.5–14 $\times 10^{-11}$ cm^3 sec^{-1}.

Figs. 16–19. Electron micrographs of stigma secondary branches of *Secale cereale*.

Fig. 16. Base of a papillate cell of a receptive but unpollinated stigma, with a large embayment of the endoplasmic reticulum containing proteinaceous fibrils. × c. 11,000.

Fig. 17. Protein fibrils in the vacuole of a papillate cell. × c. 33,000.

Fig. 18. Secondary stigma branch after the passage of a pollen tube (pt). The cells are necrotic, and the lumina are filled with fibrils like those of *fig. 17*. × c. 3500.

Fig. 19. As *fig. 18*, detail of walls of stigma cells and pollen tube. Labelling as in *fig* 6. × c. 14,000.

4. DISCUSSION

There appear to have been no previous attempts to compute the rates of water movement associated with pollen hydration and tube growth in the grasses, or for that matter in any angiosperm family, so the data presented in this paper and in an earlier one dealing with rye pollen (J. HESLOP-HARRISON 1979a) stand alone. It has been evident throughout the work that the interacting elements are subject to a certain amount of variation – for example, we have found that the behaviour of both pollen and stigma can be affected by the previous history of the parent plants – so that even in controlled laboratory conditions the rates of hydration, germination and subsequent tube growth may vary between experiments. Notwithstanding this and other sources of error, which we certainly do not wish to minimise, it seems permissible to draw one or two conclusions.

The highest rate of water uptake during the initial period of hydration of the rye pollen grain on the stigma has been estimated at 5.3×10^{-10} cm^3 sec^{-1} (J. HESLOP-HARRISON 1979a). After the penetration of the cuticle of the papilla and the onset of tube growth through the secondary stigma branch, the uptake of water into the tube is in the range $7.5–14 \times 10^{-11}$ cm^3 sec^{-1}, according to the calculations in the present paper. However, tube growth in the secondary stigma branch is accompanied by a shrinkage of the hydrated grain, indicating that water is abstracted from the grain during the early extension. In fact, the shrinkage measured (J. HESLOP-HARRISON 1979a) is adequate to account for rather more than 500 μm of tube growth. This implies that the water required for the passage through the full length of a secondary branch could be supplied entirely from the hydrated grain. Even allowing for a considerable margin of error in the estimates of grain volume, it seems certain, then, that the water needed for tube growth is mainly acquired during the initial hydration period, and not from the stigma tissues after the entry of the tube.

This conclusion is perhaps surprising at first sight; but in fact it provides an explanation for a commonly observed event on the grass stigma, namely that where there is competition between tubes, some may fail to enter and will then continue growth on the surface of the branch, or even in the air. In these circumstances there can be no question of a direct transfer of water into the tube from the stigma tissues, indicating that the supply must come exclusively from the hydrated grain.

The conclusion carries another significant implication. The time taken for an individual grain to hydrate on the stigma depends on its state at the moment of capture. A freshly shed pollen grain of rye with water content of 30–35% will germinate in 100 sec, while a grain from the same source allowed to dry out for 2 h at 23 °C in 50% RH, after which its water content will have fallen to 20–25%, may take up to 7 min to rehydrate to an ellipsoidal form and a further 2 min to germinate (J. HESLOP-HARRISON 1979b). Yet the tubes from each will achieve the same growth rate through the stigma tissues. Evidently the important factor in controlling the rate of germination is simply the capacity of the stigma to supply the need of the grain during the period of hydration. In this connection, it may be

noted that the figure already mentioned for the water flux during hydration up to the point of germination in rye may give a guide to the maximum flow rate achievable through a secondary stigma branch, which is of course without vasculation.

In the grass pollen grain, germination depends on achieving the appropriate level of turgidity, and also on various preparatory events at the aperture. The most significant early episode is the gelatinisation of the Zwischenkörper, a zone of compacted pectic materials lying between the operculum and the intine at the aperture site (J. & Y. HESLOP-HARRISON 1980b). The gelatinisation is due to the hydration of the pectins, which expand and push off the operculum, and then diffuse away. The process is easily observed in pollen germinating in optimal medium in culture, but if the pectins are stabilised by a higher than normal concentration of calcium in the medium, the operculum is not lifted off, and the grain continues to inflate without germinating, although continuing to show cyclosis and other signs of metabolic activity (J. HESLOP-HARRISON 1979a).

The Zwischenkörper gelatinises normally in dead pollen in an appropriate medium, but the next step, the emergence of the apertural intine and the adoption of tubular growth, is only seen in viable pollen. The formation of the tube tip requires a re-orientation of the cellulose microfibrils of the intine in the apertural site and the development of a wall-extension mechanism at the outgrowing tube tip, and these changes evidently require the re-establishment of metabolic machinery in the vegetative cell after the period of enforced dormancy during dispersal.

The emerging tube tip releases a variety of enzymes, and as already noted these include a pectinase which is presumably responsible for the dissolution of the stigma wall layer D in the vicinity of the contact face. The erosion of the cuticle indicates that a cutinase is also released, but it seems likely that this is initially inactive, being activated only on contact with the stigma surface. As with various other "dry" stigma species, the enzymic removal of the proteinaceous part of the stigma surface pellicle, while not affecting the germination of the pollen, does prevent the tube tip from dissolving away the cuticle (J. & Y. HESLOP-HARRISON 1977, Y. HESLOP-HARRISON 1977, SHIVANNA, Y & J. HESLOP-HARRISON 1978). As an explanation for this effect, we have suggested that the pollen emissions are themselves inadequate to bring about the penetration of the cuticles of stigma papillae, and that some activating factor on the stigma surface is needed, a factor that is either bound to the proteins of the pellicle, or is itself a peptide. Binding of the lectin concanavalin A to the pellicle also interferes with tube penetration in the grasses, a similar result to that obtained by KNOX et al. (1976) with *Gladiolus*. The enzyme neuraminidase was included in the present experiments because of the part sialic acid has been said to play in animal cell adhesion as a component of cell surface glycoproteins (LLOYD 1975). The results suggest that sialic acid has no function in this plant system since neuraminidase is without effect on pollen adhesion, germination or penetration. The present study has clarified certain aspects of the stigma reaction to pollination, first described for grasses by KATO & WATANABE (1957). Evidently the advent of pollen rapidly increases the per-

meability of the stigma papillae, and this is correlated with a progressive dissolution of the plasmalemma, visible in electron micrographs. According to KATO & WATANABE, the response is elicited by grass pollens but few others. This suggests that the effect is not due simply to a rupture of the membranes by the rapid movement of water occasioned by the hydration of pollen on the papilla, but is induced by the passage of some specific factor or factors from the pollen into the stigma cells, probably in the first instance during the early period of exudation through the exine. The subsequent reaction of the contiguous stigma cells is often to block the pit fields with callose; the cells directly affected by the entry of the tube thereafter become necrotic, and the vacuolar content fills the whole lumen. In rye, the passage of one tube through a stigma branch seems to be sufficient eventually to induce necrosis in all of the adjacent cells, although these are never entered by the tube, which is confined throughout to an intercellular route, passing through the internal secretion comprising the layer D. The pervasive nature of the effect may be judged from *fig. 18*.

The stigma reaction, together with the limited capacity of an individual stigma branch to support the hydration of captured pollen, may provide the means whereby the numbers of tubes competing in the transmitting tract of the stylodia are regulated (J. HESLOP-HARRISON 1979c). An investigation of the implications of this and of the characteristics of the transmitting tracts of the stylodia and ovary wall will be reported upon in a further paper.

ACKNOWLEDGEMENTS

It is a pleasure to thank Professor J. P. Cooper, Director the Welsh Plant Breeding Station, for the use of the Station's facilities. The participation of YHH was made possible by the award of a Research Fellowship by the Leverhulme Trust.

REFERENCES

HESLOP-HARRISON, J. (1975): The physiology of the pollen grain surface. *Proc. Roy. Soc. Lond.* **B180**: 275–299.

— (1978): Recognition and response in the pollen-stigma interaction. *S.E.B. Symposium No.* **32**: 121–138.

— (1979a): Aspects of the structure, cytochemistry and germination of the pollen of rye (Secale cereale L.). *Ann. Bot. Vol.* **44** *Suppl.* No. 1, 1–47.

— (1979b): An interpretation of the hydrodynamics of pollen. *Amer. J. Bot.* **66**: 737–743.

— (1979c): Pollen-stigma interaction in grasses: a brief review. *New Zeal. J. Bot.* **17**: 537–546.

— & Y. HESLOP-HARRISON (1977): Enzymic removal of the proteinaceous pellicle of the stigma papilla prevents pollen tube entry in the Caryophyllaceae. *Ann. Bot.* **39**: 163–165.

— & — (1980a): The pollen-stigma interaction in the grasses. 1. Fine-structure and cytochemistry of the stigmas of Hordeum and Secale. *Acta Bot. Neerl.* **29**: 261–276.

— & — (1980b): Cytochemistry and function of the Zwischenkörper in grass pollens. *Pollen Spores* **22**: 5–10.

HESLOP-HARRISON, Y. (1976): Localisation of Concanavalin A binding sites on the stigma surface of a grass species. *Micron* **7**: 33–36.

— (1977): The pollen-stigma interaction: pollen-tube penetration in Crocus. *Ann. Bot.* **41**: 913–922.

KATO, K. & K. WATANABE (1957): The stigma reaction II. The presence of the stigma reaction in interspecific and intergeneric pollinations in the Gramineae. *Bot. Mag. (Tokyo)* **70**: 85–100.

KNOX, R. B., A. E. CLARKE, S. HARRISON, P. SMITH & J. J. MARCHALLONIS (1976): Cell recognition in plants: determinants of the stigma surface and their pollen interactions. *Proc. Nat. Acad. Sci. USA* **73**: 2788–2793.

LLOYD, C. W. (1975): Sialic acid and the social behaviour of cells. *Biol. Rev.* **50**: 325–350.

SHIVANNA, K. R. Y. HESLOP-HARRISON & J. HESLOP-HARRISON (1978): The pollen-stigma interaction: bud pollination in the Cruciferae. *Acta Bot. Neerl.* **27**: 107–119.

—, — & J. S. HESLOP-HARRISON (1978): Inhibition of the pollen tube in the self-incompatibility response of grasses. *Incomp. Newslett.* **10**: 5–7.

WATANABE, K. (1955): Studies on the germination of grass pollen. 1. Liquid exudation of the pollen on the stigma before germination. *Bot. Mag. (Tokyo)* **74**: 131–137.