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THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 6. THE STIGMA ('SILK') OF ZEA MAYS L. AS HOST TO THE POLLENS OF SORGHUM BICOLOR (L.) MOENCH AND PENNISETUM AMERICANUM (L.) LEEKE

Y. HESLOP-HARRISON[1], B. J. REGER[2] and J. HESLOP--HARRISON[1]

[1] Welsh Plant Breeding Station, Plas Gogerddan, nr Aberystwyth SY23 3EB, United Kingdom
[2] U.S. Department of Agriculture, Russell Research Center, Athens, Georgia 30613, U.S.A.

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SUMMARY

Comparison of the behaviour of *Zea*, *Sorghum* and *Pennisetum* pollens on the stigma of *Zea* shows that at normal growing temperatures atmospheric humidity is the principal factor controlling germination and pollen-tube penetration; greatest success for all three species is achieved with RH exceeding 70%. While at high humidity levels germination can occur on the stigma axis, it is the stigma trichomes, which have a thin, discontinuous cuticle and bear a surface secretion with protein and polysaccharide components, that provide the most favourable sites for pollen attachment, hydration, germination and penetration. In controlled, single-grain pollinations, the shortest period observed between attachment to a stigma trichome of *Zea* and germination was 9 min for the pollen of *Zea* itself, 1 min 25 sec for that of *Sorghum* and 1 min 42 sec for that of *Pennisetum* in RH 70%. The conformation of the basal cell complex of the trichome determines the direction in which the tubes grow into the stigma axis; it is highly effective with *Zea* tubes, less so with those of *Sorghum* and *Pennisetum*. In the stigma axis, *Zea* pollen tubes reach the transmitting tract by stepwise progression through the intercellular spaces of the cortex. *Sorghum* tubes are frequently disoriented in the stigma axis, failing to locate the transmitting tract; yet they are capable of high growth rates, and can achieve greater lengths in the *Zea* stigma than they do in the stigma of *Sorghum* itself. *Pennisetum* tubes enter the *Zea* stigma with greater difficulty, and in the axis tend to grow slowly through the cortical tissues without seeking the transmitting tract. With all three species the entry of the tubes promotes secretion of pectic polysaccharide and protein by the cells of the stigma axis.

The paper includes a brief discussion of the significance of the experimental results in relation to the growth physiology of the pollen tubes.

1. INTRODUCTION

Attempts to widen the range of variation available for breeding purposes in the Gramineae by interspecific and intergeneric hybridisation are often frustrated by the difficulty of obtaining an initial fertilisation. Pollen fails to germinate on the foreign stigma, or if it germinates, the emergent tube is unable to penetrate

the stigma surface; or if the tube does penetrate, it fails to complete the journey to micropyle of the ovule and discharge the male gametes into the embryo sac. In some instances, notably in interspecific combinations and in crosses between genera of the same tribe, the rejection shows a degree of specificity sufficient to suggest that a recognition event is involved, an interpretation supported by the fact that there is often an interaction with the self-incompatibility system (J. HESLOP-HARRISON 1982). Frequently, however, the failure of fertilisation results from physiological maladjustment of the partners, which lack the degree of co-adaptation required to enable the successful completion of each of the several steps between pollen capture and gamete transfer. In the latter case, it may be feasible to overcome the critical barrier or barriers by experimental manipulation; but this possibility cannot be exploited without an adequate understanding of the nature of the limiting factors. For this reason we have undertaken a detailed study of the physiology of the stigmas of *Zea*, *Sorghum* and *Pennisetum* and their interaction with foreign pollens as part of a programme on wide hybridisation of tropical cereals. In the previous paper of this series (Y. HESLOP-HARRISON, REGER & J. HESLOP-HARRISON 1984, hereafter referred to as Paper 5) we gave a first report of an investigation into the tissue organisation and cytochemistry of the stigma ('silk') of *Zea mays* (tribe Maydeae). The present paper is concerned with the behaviour of the pollens of *Sorghum bicolor* (L.) Moench (tribe Andropogoneae) and *Pennisetum americanum* (L.) Leeke (tribe Paniceae) on the *Zea* stigma. An earlier investigation of pollen germination and tube growth of *Sorghum* crossed to *Zea* led to the conclusion that the behaviour depends greatly upon environmental conditions (REGER & JAMES 1982), and it has been one of the objectives of the present work to investigate this in more detail.

2. MATERIALS AND METHODS

The stigmas of *Zea mays* employed for all experimental pollinations were from the stock used in the investigation of stigma organisation (Paper 5), Hybrid 304C from Pioneer Overseas Corporation, Des Moines, Iowa, USA. Plants were grown in a controlled environment chamber in 16 h days, with 45–50% RH and a temperature of 27–29 °C day and night. *Zea* pollen was obtained from greenhouse-grown plants of this stock, and also from a second, Pioneer Hybrid 3147.

Pollen of *Sorghum bicolor* was from an inbred line, Asgrow H674, and that of *Pennisetum americanum* from the line 23BE, from USDA, Tifton, Georgia, kindly supplied by Dr G. Burton. The plants of both species were greenhouse-grown. For the collection of pollen, inflorescences approaching anthesis were excised, transferred to water and brought into the laboratory. Pollen-shed was promoted as desired by exposure to a 300 W floodlamp. Samples were collected on microscope slides and held until required in petri dishes at laboratory temperature, c. 24 °C, in c. 70% RH. In general, pollen was used within 3 h from collection. Pollen quality was assessed before all trials by the FCR reaction (J. &

Y. HESLOP-HARRISON 1970; J. HESLOP-HARRISON et al. 1984).

The architecture of the maize plant is such that it is feasible neither to carry out precisely timed, single-grain pollinations on the intact plant, nor to control the environment of the individual female inflorescence or stigma with reasonable accuracy while it remains *in situ*. For this reason, all experiments were carried out with detached inflorescences, stigmas or stigma segments. The procedures were as follows.

(a) Single grain pollinations were performed on short (2–3 cm) segments taken from the central zone of virgin stigmas in the size range 15–25 cm. The segments were mounted in open glass cells on microscope slides, with the cut end held between saturated filter-paper strips. Pollinations were carried out under the dissecting microscope using single human-eyelash brushes, individual grains being placed in the position and posture required (J. HESLOP-HARRISON 1979; *fig. 3*). Except for the brief periods of observation when the pollinated segments were exposed to the laboratory atmosphere (*c.* 24 °C, 40% RH), the mounts were kept in plastic boxes at the same temperature with RH *c.* 70%.

(b) For the assessment of pollen germination and tube penetration on different zones of the stigma and in different environments, virgin stigma segments 5–10 cm in length were used, with the pollen applied in the sites and quantities required using a fine artist's brush. The preparations were transferred to plastic boxes and held in three humidity ranges, 5–10%, 70–75% and 90–95% RH, for the appropriate periods, after which the segments were cut with minimal disturbance into shorter lengths and mounted without prior fixation in decolourised aniline blue at pH 10. To soften the tissues slightly, the preparations were heated gently, and then pressed out under the coverslips, taking due care to avoid dispersing and losing loosely attached grains. Pollen tubes were detected by the fluorescence of the callosic inner layer of the tube wall (LINSKENS & ESSER 1957). For the localisation of points of tube entry, the stigma cuticle was stained with a second fluorochrome, auramine O (Y. HESLOP-HARRISON 1977).

(c) The rate and extent of pollen tube growth was assessed using excised female inflorescences bearing virgin stigmas. In each trial, the comparisons between the species were made on different, matched, stigmas of the same inflorescence, which was maintained with the base in water in a sealed chamber at *c.* 24 °C with 80–90% RH. In one trial, 20–25 cm stigmas were pollinated at the tips ('tip' pollination), and in a second, stigmas at a comparable stage of development were cut back to 5 cm before pollen was applied ('stump' pollination). Again, the decolourised-aniline blue method was used to detect the pollen tubes, in this case after thorough softening of the tissues in 5 N NaOH before gentle squashing.

Pollen-grain and -tube dimensions were measured directly *in situ* with a microscope fitted with a grid-graticule eyepiece, or in slide mounts using a camera lucida and Apple microcomputer with digitiser.

The preparation of sectioned material for optical and electron microscopy, and the cytochemical methods applied to resin-embedded preparations, were as described in Paper 5.

Table 1. Pollen and pollen tube dimensions. Measurements made on fully hydrated but ungerminated pollen grains (n = 25), and on free-growing tubes (n = 20) before penetration into the stigma.

	Pollen diameter (μm)		Rel. volume	Tube diam. (μm)
	max.	min.		
Zea mays	92.6±1.11	73.4±0.62	1.000	10.08±0.43
Sorghum bicolor	46.2±0.85	37.9±0.64	0.130	6.69±0.44
Pennisetum americanum	41.2±0.67	32.3±0.71	0.087	5.09±0.38

3. RESULTS

3.1. Stigma organisation

The principal structural features of the axis and lateral trichomes of the stigma of *Zea* are shown in the diagrams of *fig. 1*, based upon the observations reported in Paper 5. The tissues of the axis are drawn to scale in *fig. 1*, from which the comparatively small cross-sectional area of the pollen-tube transmitting tracts, no more than 2% of the whole, may be judged. No specialised tissue lies between the insertion sites of the lateral trichomes and the transmitting tracts.

The individual trichomes vary considerably in size and cell number, but all possess a similar structural organisation at the site of insertion into the axis (*fig. 1*). The disposition and shapes of the basal cells and the inclination of their walls is such as to determine that the trichome is inserted at an angle, with its axis tilted towards the apex of the stigma (*fig. 3A*). This architecture will necessarily tend to ensure that pollen tubes growing down through the intercellular spaces of the trichome, whether in central or superficial sites, will be fed into the cortical tissue of the axis in a polarised manner, with the extending tube tips directed towards the ovary.

3.2. Pollen and pollen-tube dimensions

Grain and tube diameters, the latter measured in free-growing tubes before entry into the stigma, are set out in *table 1*. The substantial difference in the volumes of the pollens of *Zea* and *Pennisetum* is illustrated in *figs. 3B* and *3C*, which show pollen grains of the two species placed successively in the same sites on the same two stigma trichomes.

3.3. Stigma-surface secretions and pollen adhesion

In Paper 5, it was shown that the external faces of the cells of the marginal trichomes of the stigma of *Zea* bear a permeable cuticle, and that a thin surface secretion with polysaccharide and protein components is present when the stigma is in the receptive state, the familiar surface pellicle of dry-stigma species. The stratification of the secretions and the underlying wall is essentially identical with that previously described from *Secale cereale* (J. & Y. HESLOP-HARRISON 1982). The axis of the *Zea* stigma carries a thicker and less permeable cuticle

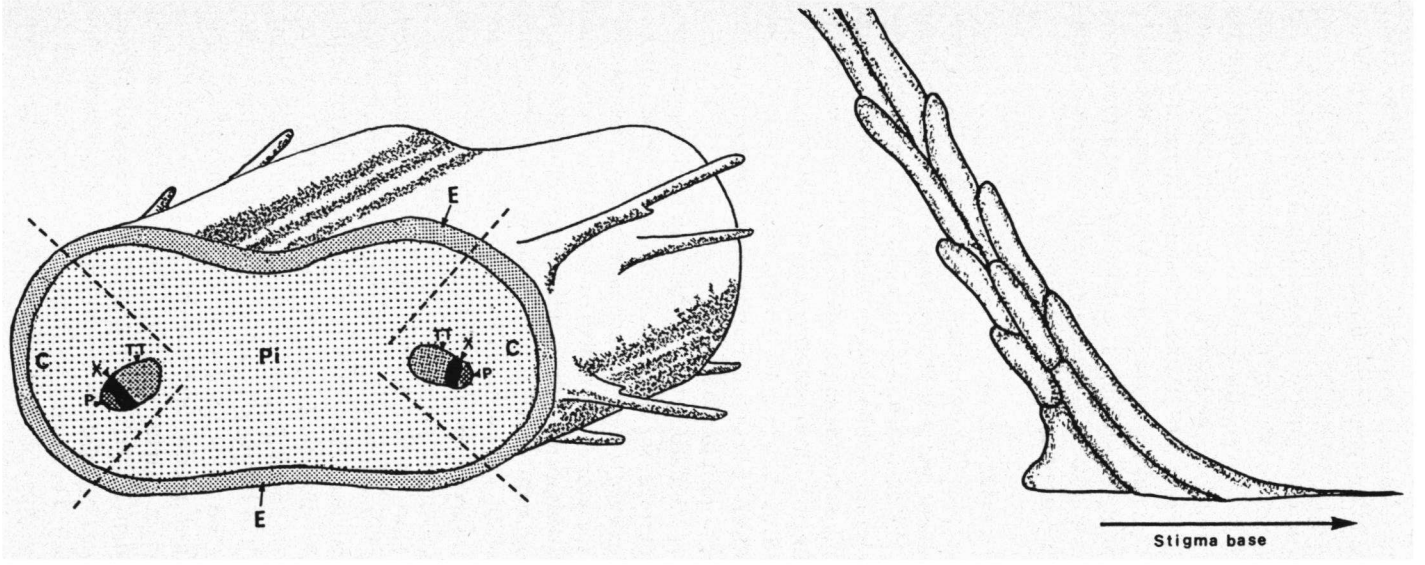

Fig. 1. Diagrams of the stigma of *Zea mays*, showing tissue distribution.
Left, Segment of the stigma axis. E, epidermis; Pi, pith; TT, transmitting tracts; X, xylem; P, phloem. The sectors 'C' mark the areas of the cortex penetrated by *Zea* pollen tubes traversing towards the transmitting tract. × c. 170.
Right, Base of a stigma trichome, showing the disposition of the cells at the site of insertion into the stigma axis. One of the tapering cells is seen to the right of the basal cell complex (cf. fig. 23 in Paper 5), and to the left, the single wedge-shaped 'pedestal' cell. While the inclination of the former tends to guide the extending pollen-tube tip into the axis in the direction of the stigma base and the ovule, the latter serves to deflect the tip and prevent it from entering the axis in the wrong sense. × c. 350.

between the insertion sites of the trichomes. No continuous superficial secretion pellicle is present during early development, and the secretions that accumulate as the stigma becomes receptive are mainly restricted to the longitudinal grooves between the epidermal cells, where the cuticle is flexed (fig. 16 in Paper 5). In the conditions of atmospheric humidity prevailing in the laboratory during the tests (*c.* 40% RH at 24 °C), the adhesivity of the pollens of all three of the species tested on the *Zea* stigma was found to be related in the predictable manner to the distribution of the surface secretions. Pollen transferred manually to the trichomes adhered well, while that placed on the stigma axis was held insecurely and could often be detached simply by shaking. In higher levels of atmospheric humidity (70%+ RH), pollen could be attached more readily to the axis, seemingly because a linking meniscus was formed more rapidly.

3.4. Pollen hydration and germination

In single-grain pollinations of *Zea* × *Zea* using freshly shed pollen, the shortest interval between pollen attachment and germination was 9 min in an ambient humidity of *c.* 70% at 24 °C. The early events progressed much more rapidly with *Sorghum* pollen. In single-grain pollinations on segments of matched stigmas, again with fresh pollen and under the same conditions, the minimum time for hydration and the beginning of exine exudation (J. HESLOP-HARRISON 1979) was 38 sec, and for germination, as evidenced by the first emergence of the tube tip, 85 sec. With *Pennisetum* pollen the minimum times were somewhat longer: 85 sec to exudation and 102 sec to germination.

Notwithstanding attempts to standardise the technique in these experiments by using pre-hydrated pollen derived, for each species, from the same sources, the results with individual grains were somewhat inconsistent. Apart from possible differences in the state of the grains themselves, much of the diversity can no doubt be attributed to small disparities in the handling procedure, the factors determining the variation being mainly the posture of the grain on the stigma trichome and the speed with which a linking meniscus is formed. However, the results do show that with both species the interval between initial contact and germination is actually shorter than with the pollen of *Zea* itself.

An attempt was made to explore the interaction with ambient humidity with single-grain pollinations using *Pennisetum* pollen. Success with this method was limited because of the considerable technical difficulties involved, but the course of one informative comparison is illustrated in *fig. 20*. Two matched grains were placed in the same posture on one trichome, one on a cell of the trichome shaft, and the other on a tapering cell of the basal complex (*fig. 20A*), and the stigma segment was then exposed to the relatively dry laboratory atmosphere, with 40% RH at 24 °C. In 90 sec both grains were showing a net loss of water, as evidenced by local collapse of the exines (*fig. 20B*). During the succeeding 3 min, the grain on the basal cell dehydrated further, while that on the shaft formed a linking meniscus and showed some evidence of rehydration; neither had achieved germination (*fig. 20C*). The stigma segment was then transferred to a chamber with RH 90–95% at the same temperature. After a further 6 min,

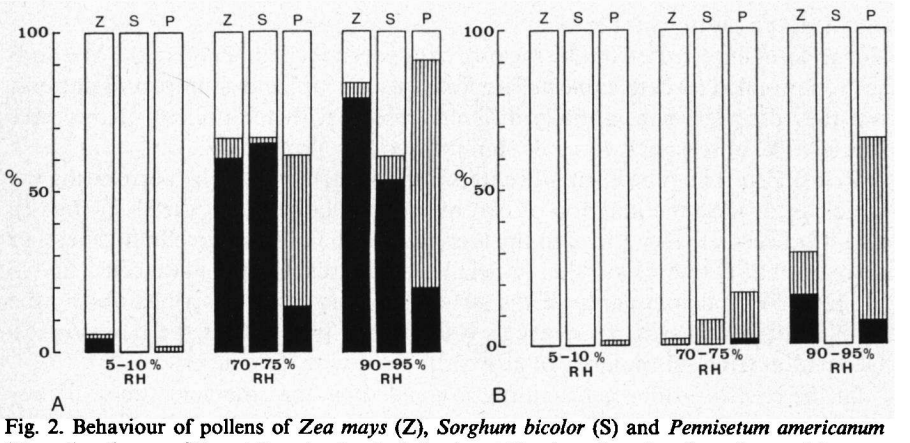

Fig. 2. Behaviour of pollens of *Zea mays* (Z), *Sorghum bicolor* (S) and *Pennisetum americanum* (P) on the stigmas of *Zea* at three levels of relative humidity. A, pollen placed on stigma trichomes; B, pollen placed on stigma axis. Key: open columns, pollen not germinating; vertically hatched columns, pollen germinating, but tubes failing to enter the stigma; filled columns, pollen germinating and penetrating the stigma.

both grains were fully hydrated, and that attached to the shaft had germinated (*fig, 20D*).

3.5. Germination sites and atmospheric humidity

The behaviour of the pollens of *Zea*, *Sorghum* and *Pennisetum* applied to trichome and stigma-axis sites and held at three different levels of relative humidity according to procedure (b) in the Methods section is recorded in *fig. 2*.

The comparisons in *fig. 2A* dramatically illustrate the effects of atmospheric humidity on germination and tube penetration with grains applied to the stigma trichomes. With *Zea* and *Pennisetum* pollens, the germination rate was progressively enhanced with increasing humidity, as was the proportion of tubes effecting entry into the stigma. The effect was essentially similar with *Sorghum* pollen, the reduction in percentages germinating and achieving penetration at the highest level being accounted for in part by an increase in the number of grains bursting on the stigmas.

Fig. 2B shows that in all humidity conditions and with all three species, the proportions of pollen applied to the stigma axis hydrating to the point of germination were extremely low. No penetration at all was observed at the lowest humidity level. At the highest, an appreciable proportion of the applied grains of *Zea* germinated, and of these, rather more than one-half achieved penetration. An even higher proportion of *Pennisetum* grains germinated on the stigma axis, and a fraction of the tubes achieved entry. This latter result was scarcely unexpected, since *Pennisetum* pollen showed sporadic germination even on the inhospitable surface of a microscope slide in an atmosphere of 90–95% RH. In this particular experiment *Sorghum* pollen germinated on the axis only in the intermediate humidity range, and no penetration was achieved. However, the penetration of *Sorghum* tubes in axial sites was observed occasionally in other experiments (*fig. 17*).

3.6. Pollen-tube pathways

The experiments carried out by the procedure described under (c) in the Methods section revealed no detectable differences between 'tip' and 'stump' pollinations, but they did yield substantially different results with the pollens of the three species. The observations may be summarised as follows.

Zea × *Zea*. The tube normally enters a stigmatic trichome by perforating the cuticle at or near the junction of two of the papillate cells of the shaft (*fig. 4*), and it proceeds thence through the secretion in the axial intercellular space, or through that held beneath the cuticle between adjacent papillate cells (fig. 25 in paper 5). In consequence of the structural adaptation described above, the tip is directed towards the ovary as it leaves the trichome at the base (*fig. 6*). Departures from this polarity of growth are rare with *Zea* tubes.

In the case of grains germinating in axial sites, the emergent tubes, if they penetrate at all, do so in the grooves between the epidermal cells (*fig. 5*). The tubes entering the axis in these sites show no preferred direction of growth, and many proceed towards the stigma tip – that is, away from the ovary.

Tubes entering the axis from trichome sites are confined to the sectors labelled 'C' in *fig. 1*. They may grow for one or two mm through the intercellular spaces of the cortex, but ultimately they make their way into the transmitting tissue, passing in a stepwise manner from file to file of the cortical cells (*fig. 8*). They do not invade the vascular tissue, but skirt it, again by a stepwise manoeuvre, to enter the transmitting tract from the sides (*figs. 7* and *14*). Once in the tract, the tubes continue growth in the intercellular secretion (*fig. 13*).

An immediate effect of the entry of a pollen tube into the axis of the stigma, whether from a trichome or directly, is to promote the accumulation of further secretory product in the intercellular spaces in the cortex. The secretion is rich in acid pectic polysaccharide (*fig. 10*) and notably so in protein (*fig. 11*). The cortical cells of the mature receptive stigma are already partly disorganised, lacking a continuous plasmalemma (*fig. 12*, and fig. 15 in Paper 5), and it seems likely that the passage of further material into the intercellular spaces results simply from the pressure of the entering tube, which distorts adjacent walls (*fig. 11*), and disrupts the plasmodesmata at the pitfield (*fig. 12*).

Tubes traversing the transmitting tract form callose plugs at somewhat variable intervals. The progressive withdrawal of the protoplast from one segment into the next as extension proceeds allows the older segments to collapse. The walls are retained, however, so that the course of the tube can readily be followed (*figs. 9, 13* and *14*).

Notwithstanding earlier reports (e.g., MILLER 1919), the tube-tip is not itself enlarged, nor is the diameter of the tube greater in the zone temporarily accommodating the gametes. Very occasionally tubes burst at the apex while traversing the transmitting tract, releasing the contents and presenting the appearance illustrated in fig. 8 in the paper by KROH et al. (1979).

Zea × *Sorghum*. Pollen tubes from grains germinating on stigma trichomes penetrate by perforating the cuticle at cell junctions. As with *Zea*, the tubes continue growth through the intercellular spaces in internal or sub-cuticular sur-

Table 2. Maximum pollen-tube growth rates, allowing for hydration and germination times, and tube lengths attained, in matched stigmas of *Zea mays* of the same excised inflorescence held at 24°C in 80–90% RH.

Pollen source	Site	Max. growth rate (μm sec^{-1})	Max. length (mm)
Zea mays	Trans. tract	1.14	7.6 (2 h)
Sorghum bicolor	Trans. tract	0.82	8,8 (3 h)
Pennisetum americanum			
(1)	Cortex	0.22	2.31 (3 h)
(2)	Cortex for 246 μm; then trans. tract	0.21	2.26 (3 h)

face locations to the trichome base. However, the basal cell complex is evidently less effective in controlling the direction of entry into the axis with *Sorghum* tubes. An example of basipetally directed growth on entry into the axis is illustrated in *fig. 15*; the tube is here following precisely the pathway defined by the configurations of the basal cells, as might be expected with a *Zea* tube. A tube which has escaped from the constraint of the basal cell complex is seen in *fig. 16*; it has traversed one rank of trichome cells, by-passed the wedge-shaped pedestal cell (cf. *fig. 6*), and entered the cortex growing in the direction of the stigma tip. The ratio of basipetally oriented to acropetally oriented tubes, *c.* 1:1, suggests that the direction of growth is essentially a matter of chance.

The occasional tubes emerging from *Sorghum* pollen that has germinated on the stigma axis managing to enter do so by perforating the cuticle between the files of epidermal cells (*fig. 17*). Again there is no indication of a preferred direction of growth.

After entry into the stigma axis, *Sorghum* tubes may continue growth in the cortex, following the intercellular spaces (*fig. 18*), or they may ultimately reach the transmitting tract by a stepwise progression from file to file of the cortical cells, much in the manner of *Zea* tubes (*fig. 19*). The pathway of tubes in the cortex is often tortuous, and the walls somewhat irregular, as may be seen in *fig. 18*; in contrast those in the transmitting tract tend to be straight and smooth-walled. This suggests that tubes meet greater resistance in the cortex, presumably because of the narrower intercellular spaces and the numbers of pitfields with interlinking plasmodesmata (figs. 5 and 6 in Paper 5).

Zea × *Pennisetum*. As is apparent from *fig. 2A*, *Pennisetum* pollen germinates quite freely on the stigmatic trichomes of *Zea*, but only a small proportion of tubes is able to effect entry through the cuticles of the papillate shaft cells (*fig. 21*). Those that are successful may proceed either towards the tip or base of the trichome, once again growing through the sub-cuticular secretion, or entering the central intercellular space. Tubes growing basipetally (*fig. 22*) enter the axis without any preferred orientation, indicating that, as with *Sorghum* tubes, the basal cell complex is largely ineffective in imposing directionality.

Once in the cortex, *Pennisetum* tubes begin growth along the longitudinal axis (*figs. 23* and *24*), indifferently in the basipetal and acropetal directions.

They seemingly pass more readily between the cortical cells than do *Sorghum* tubes, showing fewer abrupt directional changes and wall irregularities. *Pennisetum* tubes were only occasionally observed in the transmitting tracts.

Pennisetum pollen germinates freely in sites on the stigma axis in conditions of high ambient humidity (*fig. 2B*), and a proportion of the tubes does effect entry (*fig. 25*). Many are frustrated, however (*fig. 26*).

3.7. Growth rates

The maximum growth rates achieved by the pollen tubes of the three species in the experiments carried out by the procedure (c) of the Methods section are recorded in *table 2*, together with estimates of the maximum tube lengths attained during the period of observation.

4. DISCUSSION

Few direct observations have been made hitherto of the early stages of pollen hydration and germination in *Zea*, and the existing reports vary widely in their estimations of the timing. MILLER (1919) referred to the emergence of the tube as taking place 'a few hours after the pollen grains lodge on the hairs of the silk', and similar statements suggesting that a quite protracted interval elapses between the capture of pollen and germination occur in other accounts. In contrast, RANDOLPH (1936), in the most circumstantial of the earlier reports, recorded that the pollen germinated 'almost immediately' after it reached the stigma, and that within 5 min after pollination the tubes were seen to have emerged and to be entering trichomes. The diversity of the reports may in part reflect deficiencies in techniques of observation, but a factor contributing to the variability is undoubtedly the state of hydration of the applied pollen, itself strongly dependent upon the water status of the parent plant, the ambient humidity, and the period of pollen storage (BARNABAS & RAJKI 1976). The results reported here show that with pollen from the same sources and subjected to the same pre-treatments, the ambient humidity at the time of application to the stigma and in the interval thereafter is critically important in determining the success of pollination. This is true when *Zea* is itself the source of pollen as well as when *Sorghum* and *Pennisetum* are the pollen donors. The first potential barrier to hybridisation with the latter genera as pollen sources is of course at the stigma surface, and the lesson to be learned from the present findings is that to maximise the chances of germination and tube penetration it is desirable that atmospheric relative humidity in the immediate vicinity of the stigmas be maintained above 70%.

One may surmise that even the first, stigma-surface, barrier may not have been overcome in some reported unsuccessful attempts to hybridise *Zea* and *Sorghum* in field conditions with unregulated humidity levels. However, it remains difficult to explain the failure reported by MOCK & LOESCHER (1973a) in laboratory experiments with *Zea* and *Sorghum*. These authors were unable to obtain *Zea* × *Sorghum* hybrids in field experiments, a result that could have

been due to the failure of pollination or several other subsequent processes, but in a second paper (MOCK & LOESCHER 1973b), they reported that they were unable to obtain growth of *Sorghum* tubes on excised *Zea* stigmas *in vitro*, and attributed the failure of their hybridisation attempts to this. The conditions of the experiment were such as to suggest that neither temperature nor humidity would be limiting, although if, as described, the preparations were covered with cover glasses before incubation, oxygen deprivation could have been a factor. In the experiments of DHALIWAL & KING (1978), germination of *Sorghum* pollen on the *Zea* stigma was obtained *in vitro* using excised segments of the female inflorescence held in enclosed – and presumably therefore humid – chambers at 28 °C, and tubes were observed to penetrate within 30 min. The subsequent tube growth was limited, however. These authors observed that '*Sorghum*... pollen tubes invariably penetrated corn silks via stigmatic hairs', and they attributed the observed failure of tube penetration in the basal 5–10 mm of the stigma to the absence of trichomes in this region. The present results confirm the observations of DHALIWAL & KING (1978); it is evident that *Sorghum* tubes do not readily penetrate the axis of the *Zea* stigma, even under the most favourable of conditions.

Various features of pollen-tube growth in the *Zea* stigma have been described in the papers by MILLER (1919) and KIESSELBACH (1949) and more recently in a brief report, accompanied by electron micrographs, by KROH et al. (1979), but there have been no detailed accounts of the pollen-tube pathway nor of the relationship with the secretory systems of the stigma. As we have shown in Paper 5, the organisation of the receptive trichomes of *Zea* is virtually identical with that of the trichomes of the stigma of *Secale cereale* (J. & Y. HESLOP-HARRISON 1980), and the superficial and intercellular secretions, which form the sites of pollen capture and initial tube growth respectively, are evidently broadly similar so far as can be judged from their cytochemical properties. *Zea* tubes, which tend to pass through the cuticle in the angles between the papillate tips of the shaft cells and the trichome axis, are generally oriented towards the trichome base. They normally enter the stigma axis inclined towards the ovary, and we have emphasized that this orientation is determined primarily by the architecture of the basal cell complex of the trichome. *Sorghum* and *Pennisetum* tubes escape from this constraint, so that they may end by growing either towards the base or the apex of the stigma. This, together with the fact that *Zea* tubes entering the stigma axis directly show no preferred orientation, indicates that the directionality of growth in the axis can hardly depend upon chemotropic control.

There remains the question of how the entering *Zea* tubes ultimately locate the transmitting tracts. No specialised tissue intervenes between the trichomes and the tracts, and as *figs. 7* and *9* show, the cortex may be crossed in a steep stepwise traverse, or more gently after a period of longitudinal growth. Nothing in the present observations rules out the possibility that the tubes seek the transmitting tract in consequence of a chemotropic stimulus originating from the cells of the transmitting tissue; but it is also feasible that mechanical factors

determine the progression, perhaps related to the sizes of the cortical cells and the distribution of intercellular spaces.

If the passage of *Zea* tubes across the cortex is indeed controlled chemotropically, the stimulus must have little or no influence on the tubes of *Sorghum* and *Pennisetum*, which are evidently capable of persistent growth through the intercellular spaces of the cortex without any special compulsion to seek the transmitting tracts. On the other hand, if it is the mechanical circumstances of growth through the cortex which compels the *Zea* tubes ultimately to pass into the transmitting tracts, the persistent growth of tubes of *Sorghum* and *Pennisetum* in the cortex might simply mean that they are less hindered there, being substantially narrower than those of *Zea* (*table 1*).

In the cortex, the tubes grow through the intercellular secretions, which, as we have seen, are supplemented in the vicinity of each tube as it progresses, probably by displacement of additional product from the contiguous cells by mechanical compression. The protein content of the intercellular material in the neighbourhood of the tubes of all three species is notably enhanced, so far as can be judged from cytochemical criteria (*fig. 11*). The cortical tissues of mature, virgin stigmas readily release fluid with slight pressure and without maceration, and if this exudate represents principally the intercellular secretion, it should be feasible to gain a more detailed appreciation of the composition of both protein (including glycoprotein) and carbohydrate components by microfractionation techniques.

Presumably additional secretion product is also released into the intercellular spaces of the transmitting tracts during the passage of the pollen tubes. The leading part of the *Zea* tube – the segment which contains the protoplast, between the tip and the first callose plug – may have a cross-sectional area of more than 100 μm^2 (*fig. 13*), so that the pressure of the entering tip must compress the neighbouring transmitting tract cells, which have a smaller cross-sectional diameter than those of the cortex, quite substantially.

The overall path of the pollen tube in *Zea* can be up to several decimeters. Although the protoplast evacuates the older parts of the tube as growth progresses, the pollen tube wall is left *in situ* and its material is not re-cycled. Wall synthesis must therefore be a major metabolic load. Taking a mean tube diameter of 10.08 μm (*table 1*) and a mean wall thickness of 0.5 μm, the volume of the hydrated wall is *c.* $15 \times 10^4 \mu m^3 cm^{-1}$. The initial volume of the hydrated *Zea* pollen grain, less exine, is *c.* $28 \times 10^4 \mu m^3$, and from the analogy with other grass pollens, it may be accepted that the greater part of this volume is accounted for by potential wall precursor material (J. HESLOP-HARRISON 1979). The relationship of dry weight and hydrated volume is not known for the reserve polysaccharides of the grain and the pollen tube wall; but making the reasonable assumption that the conversion of grain reserves (mainly held in the pectin-rich P-particles and starch, J. & Y. HESLOP-HARRISON 1982) into wall material involves no drastic change in volume, it would seem from this calculation that without recourse to exogenous metabolites the *Zea* pollen grain could sustain no more than 2 cm of wall growth. The corresponding figures for *Sorghum* and

Pennisetum pollens are 0.5 and 0.4 cm respectively.

Although certain approximations enter into the foregoing calculations, the errors cannot be of great magnitude. The results lead to the conclusion that the pollen tube of *Zea* in traversing 10 cm of the stigma from the point of entry must take up at least 80% of the required wall metabolites, and that for the tubes of *Sorghum* and *Pennisetum* to accomplish the same journey, the uptake would need to be 95% or more.

For the *Zea* pollen tube, a possible interpretation of the course of events is that growth through the trichome, across the cortex and initially in the transmitting tract is accomplished at the expense of the reserves in the grain, and that thereafter the tube becomes dependent increasingly upon exogenous metabolites in the secretion transferred into the pollen-tube pathway by the cells of the transmitting tract.

Even although *Sorghum* tubes may be disoriented and grow abnormally during passage into the stigma and in the cortex, those that do successfully enter the tract are evidently capable of making the transition to dependence on exogenous substrate. The comparisons in *table 2* show that a *Sorghum* tube entering through a trichome and locating the transmitting tract can attain an overall growth rate in the *Zea* stigma, namely 0.81 μm sec^{-1}, approaching that of a *Zea* tube, and in 3 h achieve a wall length, 8.8 mm, greater than could be accounted for simply by transfer of precursor material from the grain itself. In other experiments over longer periods, lengths of up to 15 mm have been measured (REGER & JAMES 1982), far longer than would ever be seen in the stigma of *Sorghum* itself. Whether eventually a limit is reached remains to be determined, but it would seem that under appropriate conditions the *Zea* stigma can be a satisfactory host for *Sorghum* tubes, supporting their growth at least for distances that should be adequate to allow fertilisation from a suitably truncated stigma.

The situation may be different with *Pennisetum* tubes. As the measurements in *table 2* show, both in the cortex and the transmitting tract the maximum growth rates were relatively low, and the maximum lengths achieved were in fact less than would have been attained in the *Pennisetum* stigma in the same interval. This may indicate that all of the growth is achieved at the expense of endogenous reserves, and that the transition to dependent growth is never made.

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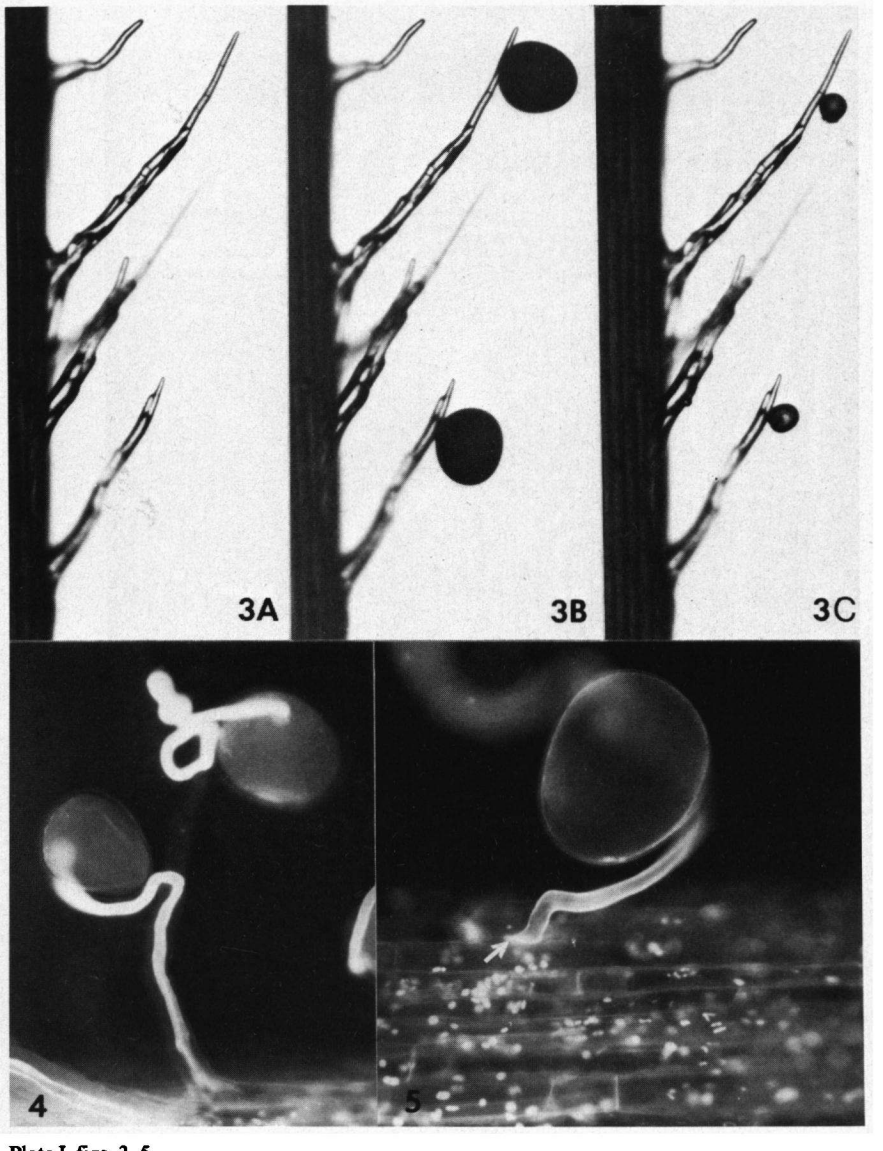

Plate I, figs. 3–5.

Fig. 3. Stigma trichomes of *Zea mays*, illustrating the single-grain pollination technique. A, unpollinated; B, manual pollination of two trichomes with *Zea* pollen; C, *Zea* pollen removed and replaced with *Pennisetum* pollen. Note the diversity of trichome size and the uniform inclination to the stigma axis. × c. 105.

Fig. 4. Germination of *Zea* pollen. The upper tube has failed to enter the trichome; the lower has entered and passed into the stigma axis. Fluorescence micrograph; decolourised aniline blue staining. × c. 172.

Fig. 5. Germination of *Zea* pollen on the stigma axis. The arrow marks the point of entry of the tube tip between the files of epidermal cells. Preparation as for *fig. 4*. × c. 280.

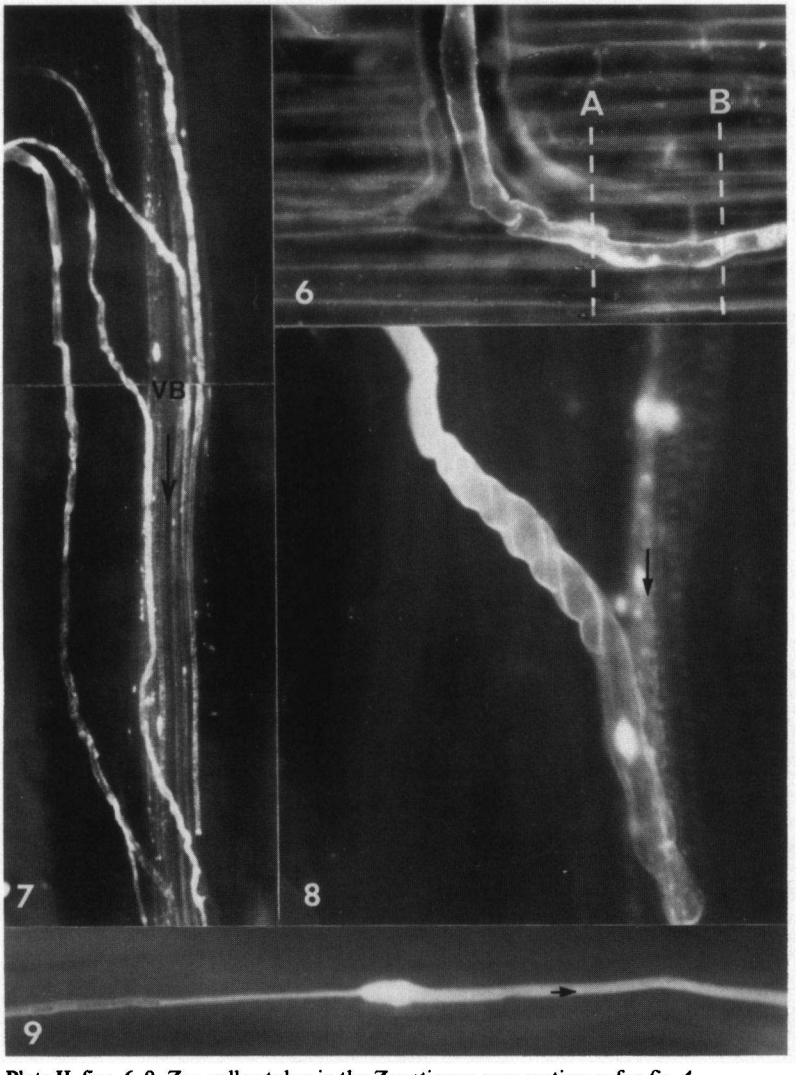

Plate II, figs. 6–9. *Zea* pollen tubes in the *Zea* stigma; preparation as for *fig. 4*.

Fig. 6. Basal cell complex of a stigma trichome, showing the passage of a tube into the axis. The line 'A' marks the approximate location of the section illustrated in *fig. 10*, and line 'B', the position of the section of *fig. 11*. × c. 345.

Fig. 7. Passage of tubes from trichome entry points across the cortex and into the transmitting tract. The tubes grow for a short distance between the cells of the cortex, and then traverse in a stepwise manner into the tract, skirting the vascular tissue. The arrow indicates the direction of the stigma base. × c. 120.

Fig. 8. As *fig. 7*, showing a detail of the tube as it passes from cell-file to cell-file across the cortex. × c. 390.

Fig. 9. Tube growing through the transmitting tract. The arrow, which indicates the direction of growth, lies on the leading segment of the tube containing the protoplast. This is separated by a callose plug from the evacuated and collapsed older segment to the left. × c. 140.

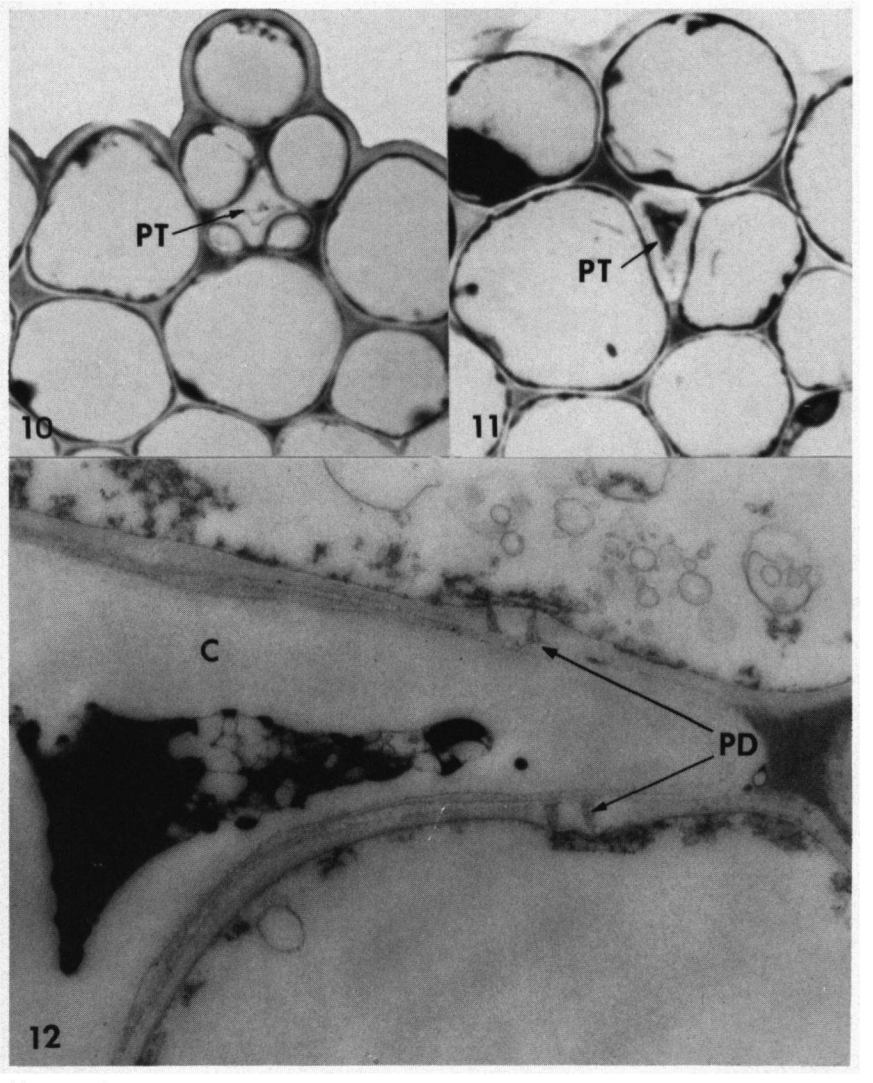

Plate III, figs. 10–12.

Fig. 10. Semi-thin (1.5–2.0 μm) transverse section of the stigma axis, transecting the tapering cell of a trichome base in a position corresponding to the plane A in *fig. 6*. A pollen tube (PT) has entered the axis directed towards the stigma base by the inclination of the walls of the basal cell complex. JB4 resin embedment, alcian blue staining for polyanionic polysaccharide. The protoplast has already evacuated the tube at this site, leaving the walls and a sparse residue of cytoplasm. Note the enhanced staining of the intercellular secretory material in the vicinity of the tube. × c. 1250.

Fig. 11. As *fig. 10*, section along the plane B of *fig. 6*. Coomassie blue staining for protein. The protein content of the intercellular secretion is also greatly enhanced in the immediate vicinity of the tube. × c. 1300.

Fig. 12. Electron micrograph of a pollen tube of *Zea* in a cortical site corresponding to that of *fig. 11*. Preparation as described in Paper 5; phosphotungstic acid post-staining. The protoplast has left this segment of the tube, leaving a pycnotic residue enclosed in the persistent, double-layered, pollen tube wall. A disrupted pitfield with plasmodesmata is seen at PD, and also the deeply staining intercellular secretory material. C, callosic inner wall of the tube. × c. 9500.

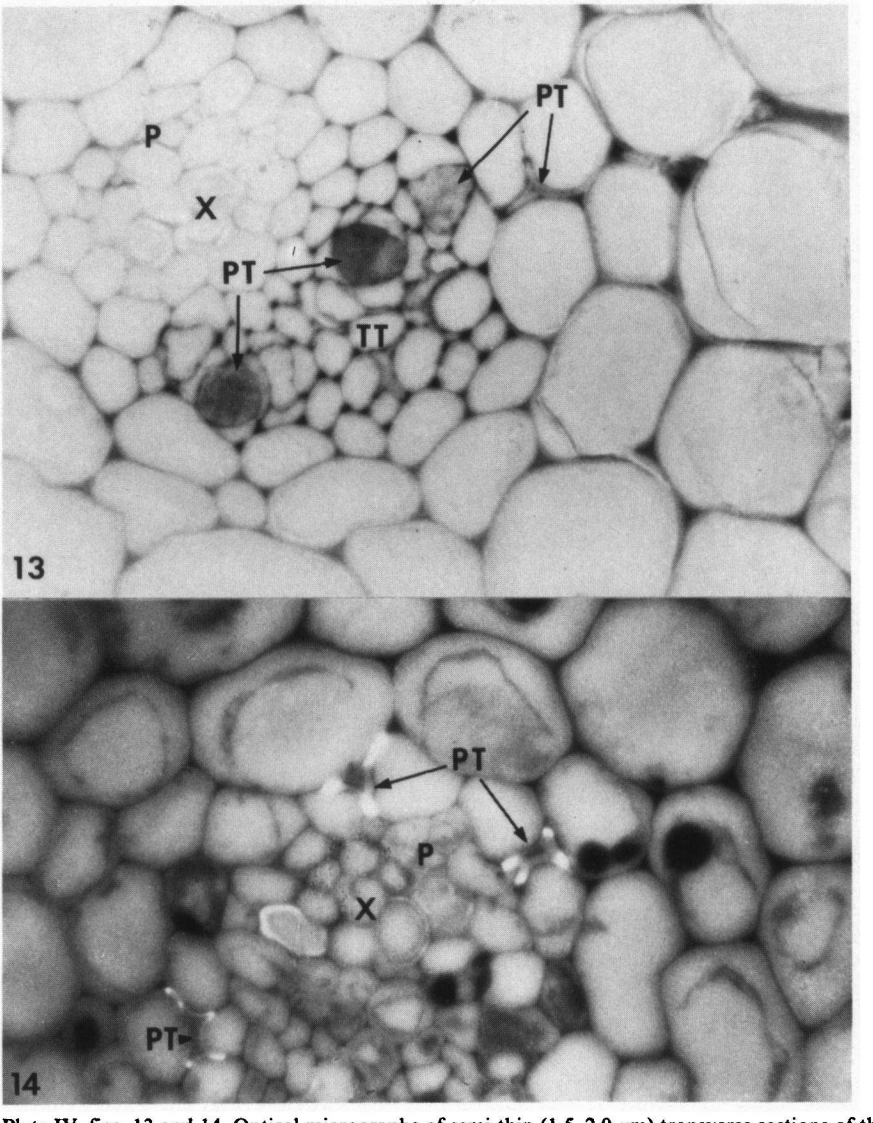

Plate IV, figs. 13 and 14. Optical micrographs of semi-thin (1.5–2.0 μm) transverse sections of the vascular tissue and transmitting tract of a *Zea* stigma, with pollen tubes in section (PT). JB4 embedment.

Fig. 13. Alcian blue staining. P, phloem; X, xylem; TT, transmitting tract cells with dense intercellular secretion. In the tract, the section has intersected the leading segment of three tubes, which retain dense protoplasmic contents. In the cortex, the section plane has intersected older segments of the tubes from which the protoplast has withdrawn. × c. 1100.

Fig. 14. Double staining with decolourised aniline blue and toluidine blue, viewed with dual incident UV and transmitted visible-light illumination. The walls of the tubes in the cortex from which the protoplasts have withdrawn (PT) are clearly revealed by callose fluorescence. × c. 1100.

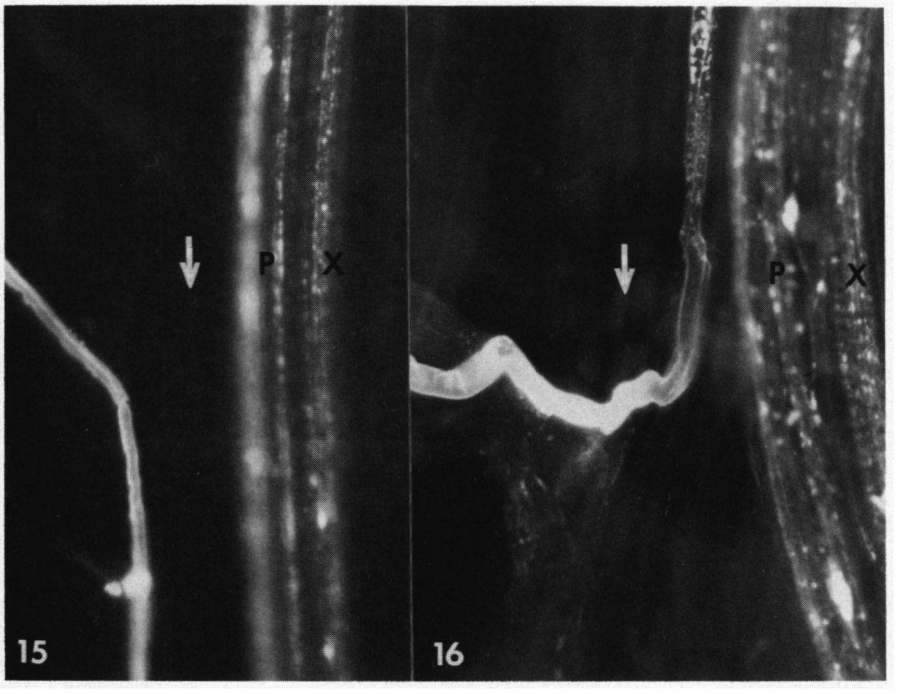

Plate V, figs. 15 and 16. *Sorghum bicolor* pollen tubes in *Zea* stigmas. The arrows indicate the direction of the ovule. Preparations as for *fig. 4*. P, phloem; X, xylem.

Fig. 15. Entry of a tube through a trichome site. In this instance, the tube has followed the conformation of the cells of the basal complex and begun growth in the cortex of the stigma axis oriented towards the ovule (arrow). P, phloem, X, xylem. The transmitting tract lies to the right of the vascular strand. × c. 280.

Fig. 16. As *fig. 15*, but with the *Sorghum* tube entering the cortex oriented away from the ovule, having escaped from the constraint of the basal cell complex of the trichome. × c. 420.

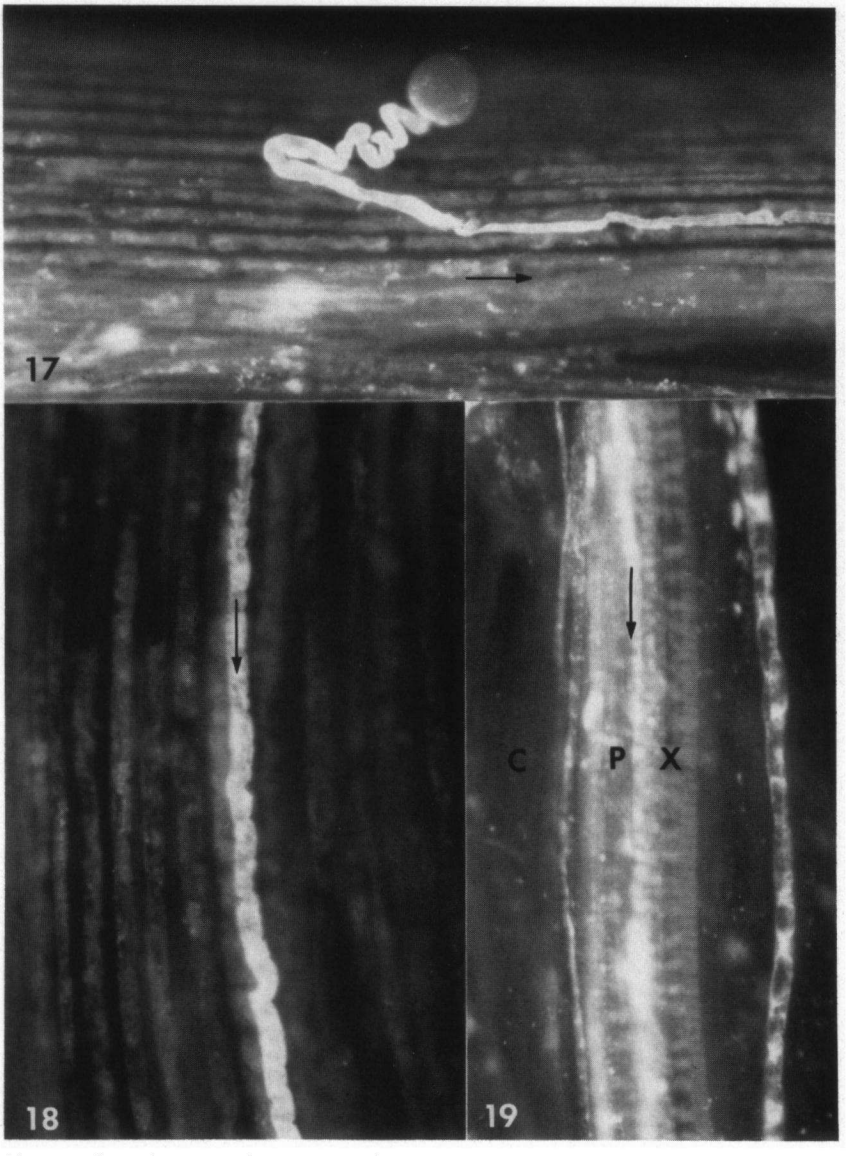

Plate VI, figs. 17–19. *Sorghum* pollen tubes in *Zea* stigmas. The arrows indicate the direction of the ovule. Preparations as for *fig. 4*.

Fig. 17. Pollen tube entering the stigma after germinating on the axis. The tube has made several attempts to breach the cuticle, and has finally succeeded in the cleft between two files of epidermal cells. × c. 250.

Fig. 18. Tube growing through the intercellular spaces of the cortex, oriented towards the ovule. The irregularity of the wall is characteristic of *Sorghum* tubes in this site. × c. 410.

Fig. 19. Tube growing through the transmitting tissue (TT), oriented towards the ovule. C, cortex; P, phloem; X, xylem. × c. 410.

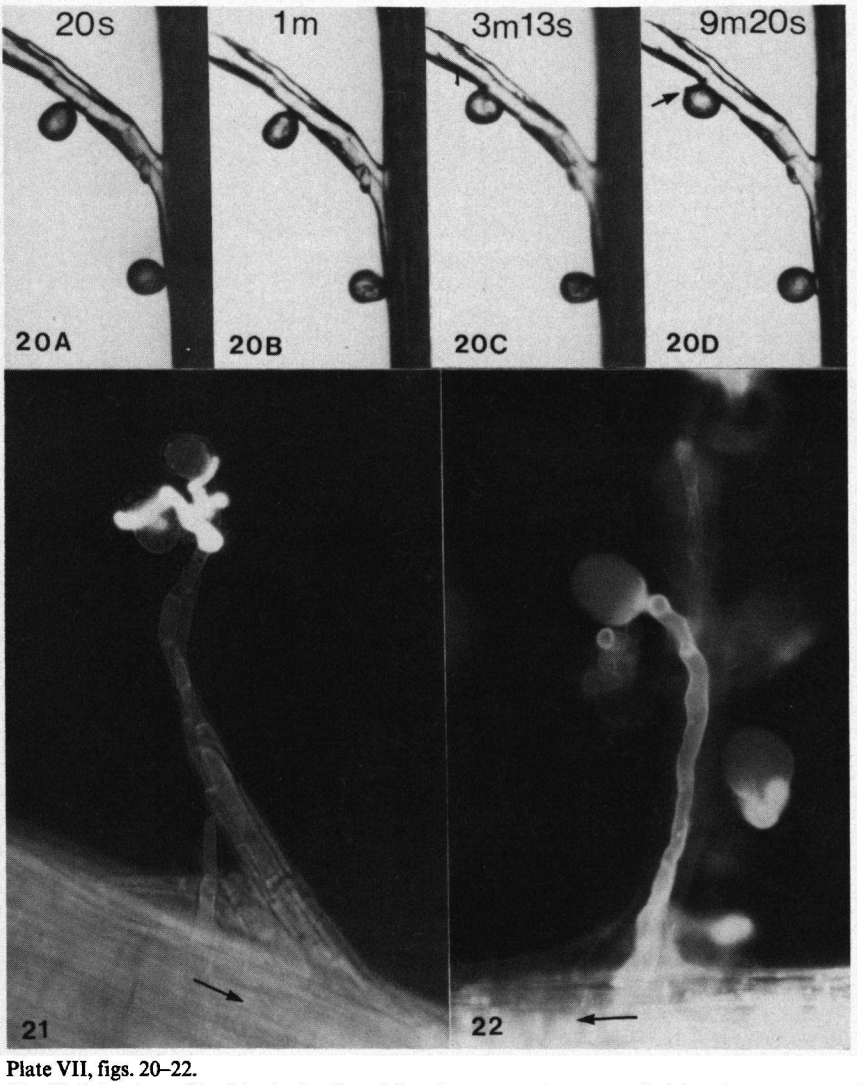

Plate VII, figs. 20–22.

Fig. 20. Behaviour of freshly-shed pollen of *Pennisetum americanum* on the *Zea* stigma over a period of c. 10 min. The upper grain has been placed on the shaft of a trichome, and the lower on a tapering cell of the basal complex. A–B, RH of 40% at 24°C; in these conditions, both grains lose water, as shown by shrinkage and the pleating of the exine. D, after 6 min at RH 70%+; both grains have rehydrated. The upper is germinating (arrow), and a meniscus has formed at the contact face of the lower. × c. 180.

Figs. 21 and 22. *Pennisetum* pollen germinating on stigma trichomes of *Zea*, preparation as for *fig. 4*.

Fig. 21. Two grains have germinated, but neither tube has penetrated. × c. 200.

Fig. 22. An example of successful germination and penetration; but the tube enters the axis by growing across the basal cell complex without following the conformation of the cells. × c. 260.

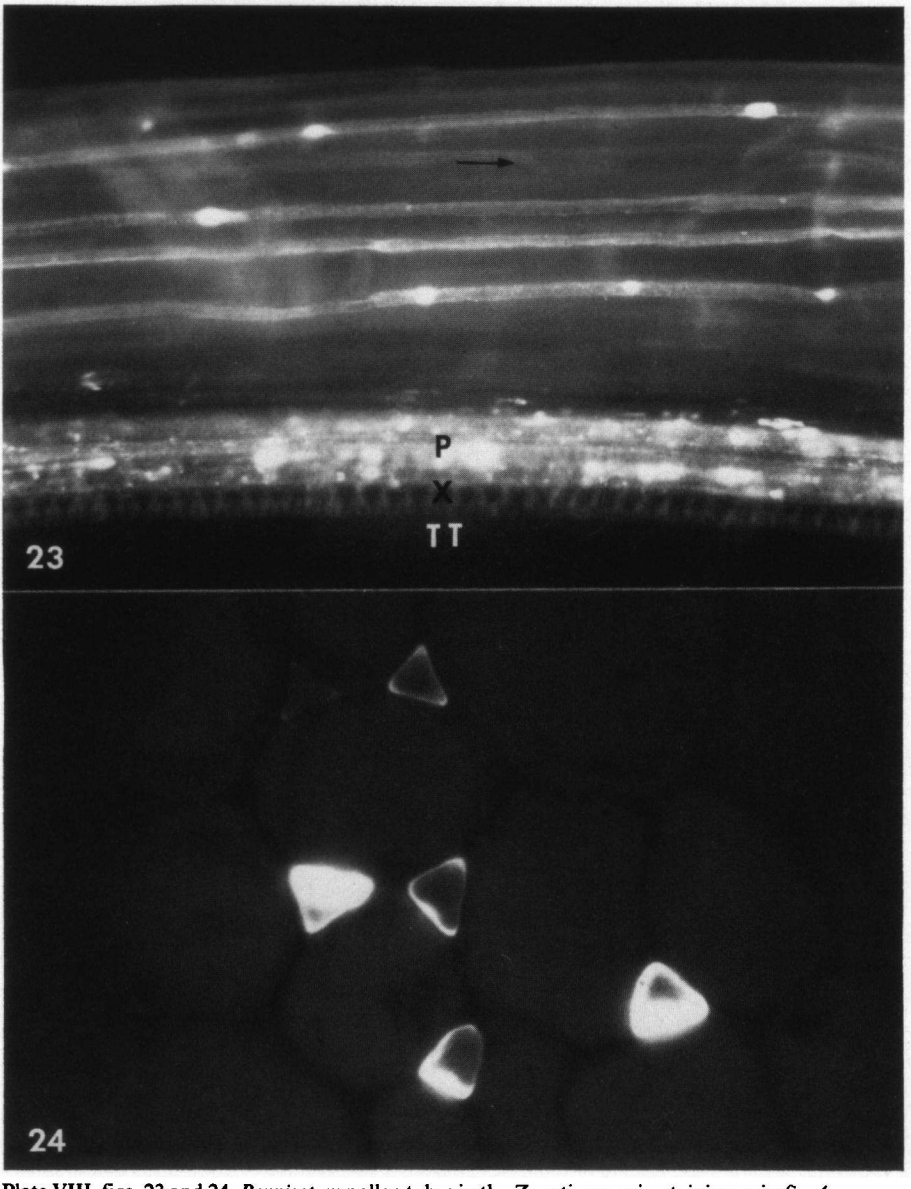

Plate VIII, figs. 23 and 24. *Pennisetum* pollen tubes in the *Zea* stigma axis, staining as in *fig. 4*.
Fig. 23. Whole mount, tubes in the intercellular spaces of the cortex. P, phloem; X, xylem; TT, transmitting tract. The arrow shows the direction of the ovule. × c. 280.
Fig. 24. Fluorescence micrograph of a semi-thin (1.5–2.0 μm) transverse section of the stigma axis, showing tube walls in the intercellular spaces of the cortex. The protoplasts have evacuated all of the sectioned segments, and the walls have partly collapsed. × c. 1400.

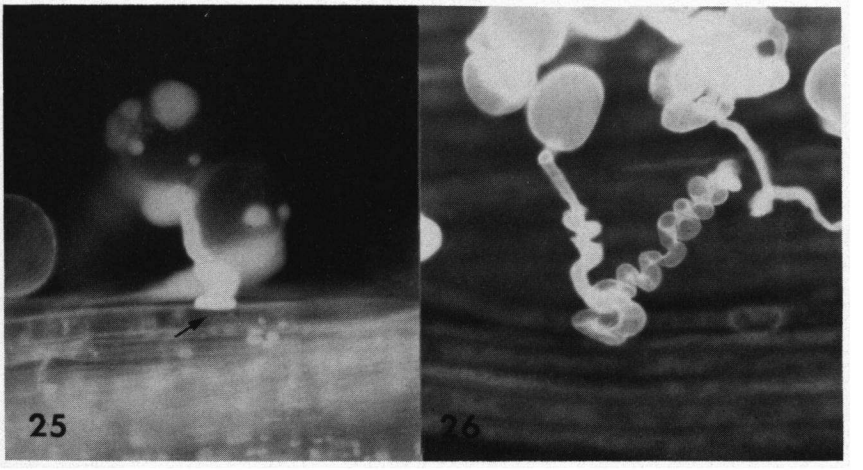

Plate IX, figs. 25 and 26. Germinating *Pennisetum* pollen on the axis of *Zea* stigmas, preparation as for *fig. 4*. High humidity.

Fig. 25. The tube tip (arrow) has penetrated into the cortex by perforating the cuticle between two files of epidermal cells. × c. 330.

Fig. 26. Tubes showing random, often spiralised, growth after failing to penetrate the cuticle of the epidermis. × c. 280.