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THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 5. TISSUE ORGANISATION AND CYTOCHEMISTRY OF THE STIGMA ("SILK") OF ZEA MAYS L.

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SUMMARY

As originally proposed by RASPAIL in 1824 and endorsed by later workers, the "silk" of *Zea mays* constitutes a greatly extended stigma, which can be interpreted as being formed by the fusion of the two branches characteristic of the more usual grass stigma. After an early period of cell division in the basal zone, growth in length is mainly by cell extension. The principal pollen-receptive surface is contributed by two irregular marginal zones of trichomes. The trichomes extend throughout the length of the stigma except for a naked zone of c. 5 mm distal to the insertion into the ovary, and in the central part of an extended stigma they occur at a density of 120–140 per cm. The cells of the trichomes are adapted for both internal and external secretion in a manner precisely similar to those of other grasses, and in the receptive state the surface bears a continuous pellicle containing proteinaceous and pectic components. Each trichome arises from a single epidermal cell, and permeability mapping shows that this cell and all derivatives from it bear discontinuous cuticles in the manner of the receptive surfaces of other stigmas of the "dry" type. The morphology of the basal cell-complex of the trichome is such as to provide the initial guidance for the pollen tube in its entry into the principal axis of the stigma.

The total pollen receptive area contributed by trichomes of one stigma of the genotype investigated amounted to about 0.19 cm^2, an area only some 50% greater than that of the physically much smaller stigma of rye.

Two pollen-tube transmitting tracts extend the length of the stigma, adjacent to, but not part of, the two vascular bundles. The transmitting tissue is composed of elongated, fusiform cells, circular in cross section, with abundant intercellular secretion. As in other grass stigmas, this secretion, which forms the pollen-tube transmitting medium, contains proteins and acidic pectic polysaccharides. The tracts lie separated from the marginal trichomes by 5–8 files of cortical cells, with no intervening specialised transmitting tissue.

1. INTRODUCTION

Since the remarkable pioneering work of RASPAIL (1824), it has been known that the stigmas of the Gramineae, while all adapted for wind pollination, vary greatly not only in size but in morphology. RASPAIL recognised three major classes, (a) *stigmata disticha*, with "fibrilles" (the receptive hairs or trichomes)

arranged more or less in two ranks like the barbs of a feather, (b) *stigmata sparsa*, with the trichomes disposed around the axis of each stigma branch, and (c) *stigmata semi-sparsa*, with the trichomes aggregated towards the tip, forming a tuft surmounting a naked stretch of the axis of the branch. Type (a), which undoubtedly constitutes the most familiar form among the grasses, he further divided into three sub-groups, *toeniaeformia*, generally very long but with short trichomes with small or indistinct papillae; *plumosa*, with distinctly papillate but unbranched trichomes; and *plumoso-ramosa*, with branched, papillate trichomes. In earlier papers of the present series (J. & Y. HESLOP-HARRISON 1980, 1981, referred to hereafter as Papers 1 & 2) we have described various fine-structural and cytochemical features of stigmas of species of Hordeae conforming to RASPAIL's class *stigmata disticha plumosa*, and comparative studies of several genera of Andropogoneae, Paniceae, Phalarideae, Agrostideae, Aveneae and Festuceae have indicated that the findings are widely applicable to genera in other tribes. In this paper we consider the stigma of *Zea mays* (tribe Maydeae), certainly one of the most unusual of the manifold forms found in the family, and attempt to relate its organisation and function to those of a more orthodox facies.

RASPAIL (1824) referred the *Zea* stigma to his class *stigmata disticha toeniaeformia*, accepting that the whole length – the "silk" of common usage – constitutes a stigma. Subsequently, however, the terminology appropriate to the organ has been a matter of debate. Several later 19th Century authors, including BENTHAM & HOOKER (1862), referred to the silk as a style, and the same usage has been accepted more recently in other publications. WEATHERWAX (1916, 1917) noted that if the term "stigma" is taken to connote the pollen-receptive part of the pistil then the whole extended, trichome-bearing silk must logically be regarded as constituting a stigma in the functional sense. We accept this usage here. Regarding the lateral appendages, the fibrilles of RASPAIL (1824): as WEATHERWAX (1916) showed, these arise from single cells of the epidermis of the axis. They thus constitute trichomes (GUEGUEN 1901), and it is convenient to refer to their constituent cells, when recurved at the tip as they often are, as papillate cells or papillae, following RASPAIL (1824).

The general morphology of the stigma of *Zea* and many aspects of its anatomy and development have been described in greater or lesser detail in various earlier papers, notably those of WEATHERWAX (1916, 1917), MILLER (1919), RANDOLPH (1936), BONNET (1940, 1948) and KIESELBACH (1949). Receptive stigmas may vary in length from 2 to 70 cm, and they are composed for most of their length of a single axis traversed by two vascular bundles. The tips are however bifurcated, the vascular bundles separating, one to enter each branch. WEATHERWAX (1916) recognised that this organisation would be comprehensible were the silk to be regarded as comparable with a grass stigma of the more orthodox type in which the two branches ("stylodes" in the terminology suggested by PARKIN 1955) had become fused throughout much of their length to give a flattened, ribbon-like structure, the process being accompanied by great elongation.

WEATHERWAX (1917) illustrated a sectioned stigma axis, but was unable to

identify any defined pollen-tube pathway. MILLER (1919) recognised that the tubes traverse a specialised tissue associated with each of the vascular bundles: he concluded, however, that this tissue surrounded the conducting tissue of the bundles, and referred to it accordingly as forming a bundle sheath. This interpretation of the pollen-tube transmitting tissue was maintained by KIESELBACH (1949), although his illustration of a sectioned vascular bundle indicates that the tissue is confined to one side, the tissue in the corresponding position on the other being identified as phloem. This location of the transmitting tract has been confirmed recently by KROH *et al.* (1979) who noted that in each bundle the tract of transmitting tissue is separated from the xylem by a single layer of parenchymatous cells. These authors were unable, however, to identify the phloem.

The papillate trichomes extend in 6–10 irregular ranks along the margins of the narrow ribbon-like stigma, continuing along the separate axes of the unequally bifurcated tip. WEATHERWAX (1916) described the structure of the individual trichome, and gave an accurate account of its development from a single epidermal cell of the axis. He showed that asymmetrical growth of this initial inclines the tip towards the apex of the stigma, and that thereafter the division planes in the basal cell are oriented similarly so that the outgrowing trichome is tilted in the same direction. The earlier accounts are mostly in agreement that the trichomes form the main pollen-capturing sites of the stigma, and MILLER (1919) noted that an emerging tube may penetrate into the contiguous trichome immediately after germination, or may grow for a period over the surface before entering the axis at the base. RANDOLPH (1936) referred to the tube as entering into the central core of the trichome, and KIESELBACH (1949), who described the trichome as being composed of four ranks of cells with a continuous internal opening, stated that after penetrating the tube grows through this space, eventually to enter the axis. KROH *et al.* (1979) provided an electron micrograph showing tubes passing both through the central zone of a trichome and over the surface.

The present paper is concerned principally with the organisation of the tissues in the *Zea* stigma. Details of the secretory systems in the stigmas of *Zea* and other grasses will be published in a further paper, and the behaviour of the pollen and pollen tube during germination and entry and passage through the stigma will be described elsewhere.

2. MATERIALS AND METHODS

The observations were made on *Zea mays* L. Hybrid 304C (Pioneer Overseas Corporation, Des Moines, Iowa, USA). The plants were grown in controlled environment chambers in 16 h days, under "dry" conditions with 25–30% RH, 32–33°C day temperature and 27–29°C night temperature, or "moist" conditions, with 45–50% RH and a temperature of 27–29°C day and night. Although the behaviour of pollen and pollen tubes differed in these environments, no structural differences were observed in the stigmas. Some samples were also taken

from greenhouse-grown plants of the same genotype.

The permeability of the cuticles of various zones of the stigma was investigated with neutral red (1% in 1% acetic acid), as described in Paper 1. This method is suitable for obtaining a rapid assessment of pathways of entry through the cuticle, but more detailed mapping can be achieved with calcofluor white (J. & Y. HESLOP-HARRISON 1982). This fluorescent dye, which has an affinity for β-1,4- and mixed 1,3-1,4-linked glucans (MAEDA & ISHIDA 1976), enters discontinuities in the cuticle and binds to the immediately underlying polysaccharide wall, providing an unambiguous map of the sites of entry. In the present application, segments of stigmas at the required stages of development were excised, rapidly rinsed, transferred to the dye (0.001% aqueous) for periods up to 30 min, rinsed again and mounted in dilute glycerol for observation. With longer immersion, the dye may diffuse away from the point of entry, and resolution is then lost. The method is also applicable to glutaraldehyde-fixed stigmas, with which immersion of 5 min is usually adequate to localise quite precisely the permeable areas of the epidermal cuticle.

Surface secretions were localised using ruthenium red (0.02% aqueous) and alcian blue 8GX (1% in 3% acetic acid) for pectic polysaccharides as described in Paper 1, and Coomassie blue (0.01% in 33% methanol with 2% acetic acid) for protein. Alcian blue at low pH we regard primarily as a means of localising polyanionic pectins, but the possibility that used cytochemically it will also stain acidic glycoproteins (SCOTT *et al.* 1964) is not of course excluded.

Surface esterase was detected on segments of freshly excised stigmas with α-naphthyl acetate as a substrate and tetrazotised o-anisidine as a coupler.

Various aspects of tissue organisation were investigated using thick, hand-cut sections, and also with macerates. The latter were prepared by warming segments of the stigmas in 5 N NaOH for 2–5 min, rinsing until the tissue was free of alkali, and dispersing the cells in calcofluor white (0.001% aqueous).

Fixation and preparation for optical- and electron-microscopy, and the cytochemical and staining methods applied to sectioned material, were as in Papers 1 and 2. Post-staining of EM sections with phosphotungstic acid (PTA), a procedure which imparts electron-opacity to pectins, was as described in J. & Y. HESLOP-HARRISON (1982).

3. OBSERVATIONS

3.1. General morphology

The gross structural features of the stigma of *Zea* have been described many times, and excellent accounts are given in several of the papers mentioned above. It is unnecessary therefore to discuss the general organisation here, and we wish merely to add quantitative data relating to the growth pattern and the distribution and dimensions of the receptive trichomes, the latter an important feature in relation to the pollen-receptivity of the stigma.

With the hybrid cultivar used in the present work, individual stigmas extended to maximum lengths of 65–70 cm in the course of 10 days of observation under

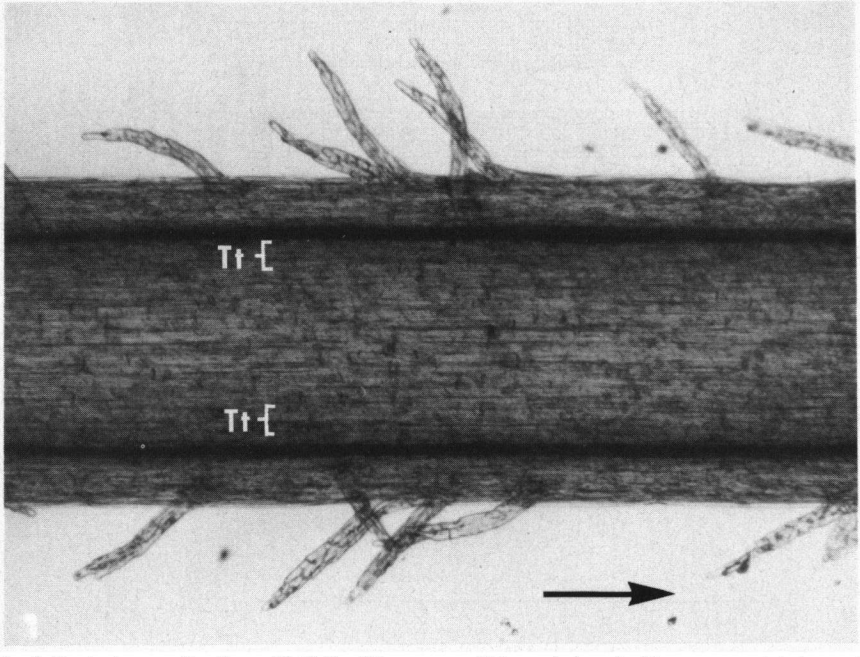

Fig. 1. Central zone of a stigma ("silk") of *Zea mays* c. 260 mm in length. The arrow points towards the base of the stigma.
Tt, location of transmitting tissues. × c. 105.

the "moist" regime. This growth is accompanied initially by cell division, which is maintained in the basal zone during the early extension of the stigma, but progressively declines. Thereafter growth in length is achieved principally by cell extension. The detail was followed by observation of epidermal cells in the central zone of the stigma between the marginal bands of trichomes. The distributions of cell lengths at the base, middle and apex (excluding the forked terminal part) in stigmas of three lengths, 3 mm, 20 mm and 260 mm, are shown in *fig. 2*. Evidently the epidermal cells in the upper part of the stigma increase in average length by some 20X during overall growth from 3 to 260 mm.

A central segment of a stigma c. 260 mm in length is illustrated in *fig. 1*, viewed from the adaxial side. The receptive trichomes are distributed in two marginal bands flanking the two vascular bundles. The distribution of the trichomes along stigmas of about this length is shown in *fig. 3*. The mean numbers per 5 mm length, counting those on both margins, rise from 0 in the first 5 mm to c. 60 in the 50–55 mm intercept. This frequency is maintained thereafter, with a slight decline towards the end of the entire zone of the stigma. The forked tips of stigmas of this length have a trichome density of c. 14 per mm.

The mean length of the trichomes in the central zone of a stigma c. 200 mm in length was 151.2 ± 0.64 μm, with a range of 28 to 280 μm, and the mean central diameter of this population was 20.23 ± 0.32 μm. Treating the trichomes

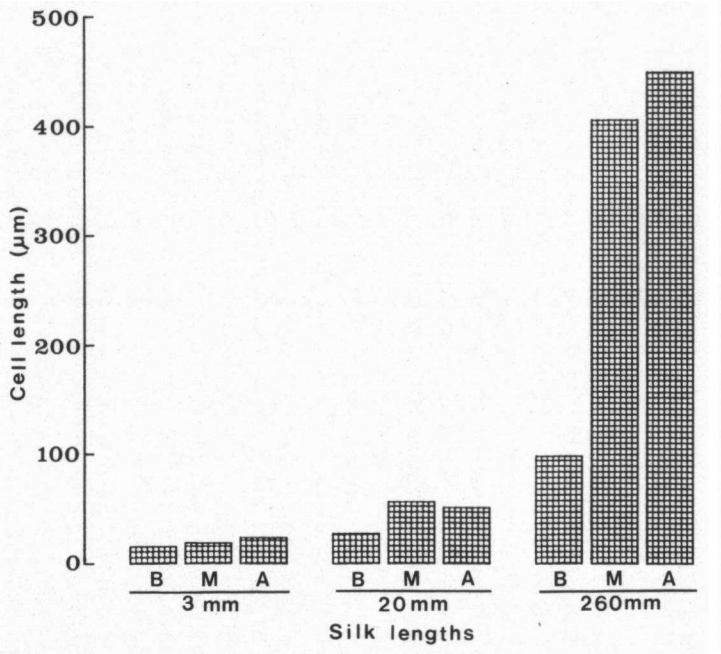

Fig. 2. Mean epidermal cell lengths at the base (B), central zone (M) and apex proximal to the forked part (A) in stigmas of *Zea mays* of three lengths, 3 mm, 20 mm and 260 mm.

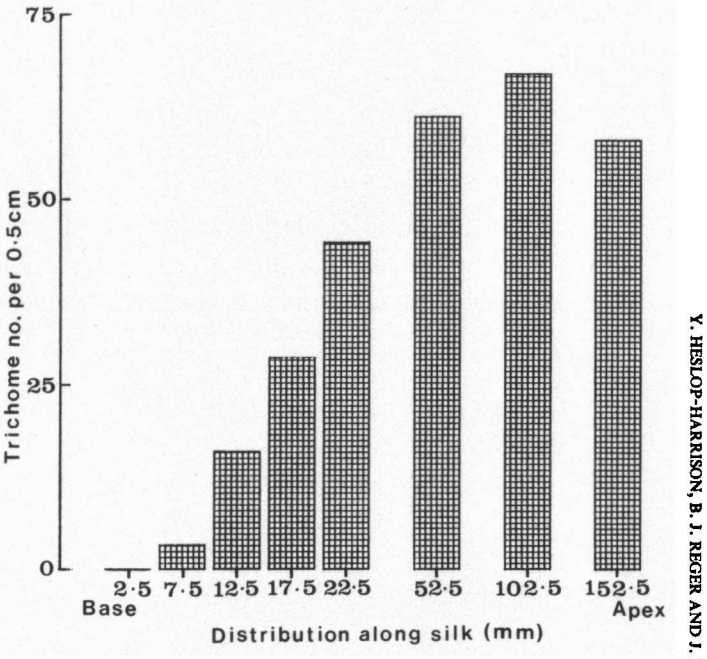

Fig. 3. Distribution of trichomes in 5 mm intercepts centred at the points shown along a stigma 240 mm in length.

as simple cylinders, it can be computed from these figures that a single stigma of 200 mm in length bearing c. 2000 trichomes would expose about 0.19 cm^2 of pollen-receptive surface, taking only the trichomes into account.

3.2. The principal axis

As WEATHERWAX (1916, 1917), MILLER (1919) and others showed, the stigma arises obliquely from the top of the ovary, adaxially to and separate from a short "stylar canal", which is not itself concerned at all with the conduction of pollen tubes. The axis is elliptical in section, and somewhat grooved between the vascular bundles, which lie towards the outer margins (*fig. 4*). For convenience, we will refer to the parenchymatous tissue lying between the bundles as the pith region, and the zone between the bundles and the marginal epidermis as the cortex, although it will be understood that these tissue regions are not sharply demarcated from each other.

As *fig. 4* shows, the vascular bundles are slightly displaced from, and inclined to, the mid plane of the stigma, lying somewhat towards the adaxial side. The phloem is well defined, lying external to the xylem and separated from the cortical parenchyma by an irregular layer of thin-walled cells (*fig. 5*). In one stigma examined, the bundles were compound, with inner and outer strands of phloem and xylem (*fig. 7*).

Fig. 9 presents a longitudinal view of a normal bundle viewed by phase contrast, and *fig. 10* is a fluorescence micrograph of the same field after staining with decolourised aniline blue for callose. A sieve tube, inconspicuous in *fig. 9*, is readily distinguished in *fig. 10* by the fluorescence of the callose plugs at the sieve plate and by scattered callose elsewhere. It will be appreciated that sieve tubes fluorescing after this staining procedure can readily be mistaken for pollen tubes with plugs; however, the latter can be distinguished not only by the location (pollen tubes rarely invade the vascular bundles) but by the absence of sieve plates traversing the plugs.

The cells of the cortical parenchyma are elongated, with a length: width ratio of up to 30:1 in the central zone of an extended stigma. They remain in communication during extension through pit-fields (*fig. 5*), clearly resolved in tissue macerates (*fig. 6*), and seen in electron micrographs to be traversed by numerous plasmodesmata (*fig. 8*). In the cortical tissue between the bundles and the trichome-bearing margins, intercellular spaces are conspicuous (*fig. 5*). The interstitial material stains for pectic polysaccharide and more lightly for protein (*table 1*), and also shows moderate electron-opacity in electron micrographs after PTA staining (*fig. 15*).

Structural details and cytochemical reactions of the epidermis of the trichome-bearing margin are illustrated in *figs. 11–13*. The outer walls are heavily thickened, and are seemingly of a normal pectocellulosic character, judging from the staining reactions (*figs. 11* and *12*). The cuticle is continuous over the surfaces of the cells (*figs. 12* and *17*, but deeply grooved between them (*Fig. 16*). In older fully extended stigmas the cuticle often appears to be disrupted in the furrows. The secretion held beneath the cuticle at the cell junctions and in the intercellular

Table 1. Staining reactions of the intercellular secretions of the principal axis and receptive trichomes of *Zea mays*.

	Axis				
	Epidermis[1]	Cortex[2]	Transmitting tissue	Pith	Trichome[3]
Toluidine blue	+++ (pink/purple)	+ (pink)	++ (pink)	0	+++ (pink/purple)
Alcian blue	+++	+	++	(+)	+++
Ruthenium red	+	0	++	0	+
Coomassie blue	++	(+)	+	0	++

[1] Sub-cuticular secretion between the cells, and between the epidermal cells and the outer cortical cells (*fig. 13*).
[2] Sector between the trichome-bearing margins and the vascular bundles.
[3] Sub-cuticular and intercellular secretion (*fig. 25*).
Key to symbols: +++, strong staining reaction; ++, moderate or localised staining; +, light staining only; (+), staining response very light or ambiguous; 0, no staining.

spaces between the epidermis and the outer cortical cells stains remarkably heavily with alcian blue (*fig. 13*), suggesting that it is rich in polyanionic polysaccharide, and it also shows a considerable affinity for PTA (*fig. 14*). There is evidence also of a protein component (*table 1*).

The pollen-tube transmitting tissue is seen in section in *figs. 4, 7, 18* and *20*, and the disposition of the tracts in relation to the bundles in the mid-zone of the stigma is indicated in *fig. 1*. The cells are elongated, fusiform, and roughly circular in cross section, linked by occasional pit-fields (*fig. 18*) and with relatively large intercellular spaces. In the young unextended stigma the cells have a dense protoplasmic content, but in the mature stigma the protoplast appears attenuated (*fig. 19*), or the cell content is dispersed around the walls, with no evident plasmalemma (*fig. 21*). The intercellular spaces contain a secretion staining for pectic polysaccharide and protein (*table 1, fig. 20*), which gains electron density with PTA (*fig. 21*).

3.3. The receptive trichomes

As shown in *fig. 1*, the receptive trichomes depart at an angle from the axis of the stigma, the inclination being determined by the sequence of early divisions (WEATHERWAX 1917). Each is derived from a single epidermal cell (*fig. 32*), in which the first anticlinal division is tilted towards the apex of the stigma. The complex of basal cells is illustrated in *figs. 22* and *23*. The structure of the base is critically important in determining the pathway of the pollen tube in the principal axis, since it is the geometry of the cells at the junction with the axis that ensures that the tube tip is directed towards the ovary on entering.

The organisation of the individual receptive trichome is virtually identical with that found in other grasses with a more conventional form of stigma, such

for example as *Secale cereale* (Paper 1). The external faces of the papillate cells are cuticularised, the cuticle, which has no outer lamellate layer, showing marked discontinuities (*fig. 26*). The adjacent wall layer exhibits staining responses suggestive of a considerable pectic content (*fig. 25*), and this grades into an inner zone which stains heavily with the fluorochrome, calcofluor white (*figs. 24* and *28*). The contents of the large intercellular spaces which provide the pollen-tube pathways stain for both pectins and protein (*fig. 25,* which may be compared with *fig. 11* of Paper 1, of the stigma of *Secale cereale*).

Permeability mapping of the intact stigma shows that the papillate tips of trichome cells are especially penetrable (*fig. 35*), but that the cuticle of the whole of the trichome cell-complex, including that of the extended basal cells tailing towards the ovary, is permeable (*fig. 34*). Remarkably enough, the individual epidermal cells which are the initials of the trichome complex are distinguishable by the possession of a highly permeable cuticle as soon as they are differentiated (*fig. 32*), and the character is shared by all the derivatives during the later development of the trichome (*fig. 33*).

3.4. Surface secretions

Characteristically, the stigmas of maize are slightly tacky to the touch from the earliest stages, becoming increasingly so as they extend. The property is attributable to suface secretions which accumulate during the functional life of the stigma.

In conformity with that of other grasses (Paper 1; Y. HESLOP-HARRISON 1976; J. HESLOP-HARRISON 1982), the secretion film borne by the receptive trichomes is layered, the outermost component being proteinaceous (*fig. 27*). As is seemingly invariable with such secreted pellicles of "dry" type stigmas (Y. HESLOP-HARRISON & SHIVANNA 1977), it shows esterase activity (*fig. 29*). In keeping with their origin as part of the trichome complex and the permeability of their cuticles as determined by dye penetration, the tapering cells at the base also show surface esterase activity (*fig. 30*). This micrograph strikingly illustrates the contrast with the neighbouring cells of the epidermis of the principal axis of the stigma, which are without surface proteins and have a continuous cuticle (*fig. 17*).

The surface secretion of the trichomes also includes a pectic component detectable with the standard staining procedures but nowhere voluminous. By analogy with *Secale* this is likely to form the inner layer of the extra-cuticular secretion over the whole of the receptive surface (see, e.g., *figs. 21* and *22* of Paper 1), although this has not been established with certainty in *Zea*.

No surface secretion, pectic or proteinaceous, could be identified by the present methods on the surface of the principal axis of extending stigmas. However, protein could readily be detected on fully extended and ageing stigmas in the depressions of the cuticle marking the junctions between the underlying files of epidermal cells (*fig. 31*). It is significant that the accumulations in this site lie over the grooves in the cuticle in which discontinuities develop during ageing (*fig. 16*), and that the secretion in the adjacent intercellular space is rich in protein

(*fig. 13*). Freshly collected stigmas from plants grown in the moist regime frequently showed rows of fluid droplets in these sites, a feature not seen in stigmas from the dry chamber.

Surface protein was also detected over cuticular cracks and fissures in old, wind-flexed stigmas from greenhouse-grown plants.

4. DISCUSSION

The organisation of the "silk" of *Zea mays* is clearly that of a stigma throughout its length except for the few millimetres immediately distal to point of attachment to the ovary, and the interpretation of RASPAIL (1824), endorsed almost a century later by WEATHERWAX (1916, 1917), is thus undoubtedly valid. As WEATHERWAX (1916) pointed out, the structure may be looked upon as conforming in the main with the more usual grass pattern, but vastly elongated, and with the two stylodia fused throughout most of their length. We have observed mutants in other genera in which the stylodia are similarly fused except at the extreme apex, so the condition is in no sense unique.

The structure of the receptive trichomes is virtually identical with that found in genera of other tribes, including *Secale* (Paper 1). The mean length of the trichomes in a stigma of *S. cereale* was found to be 269.9 + 1.89 μm, and the total area of the receptive surface of a stigma bearing c. 600 trichomes was approximately 0.12 cm^2. This provides an interesting comparison with *Zea*, in which the receptive area for the stigma examined was computed as being c. 0.19 cm^2. Notwithstanding the enormously greater total size of the *Zea* silk, the receptive surface may thus be greater by only some 50% than in the rye stigma. Leaving aside the possible role of the principal axis as a site for pollen capture, it would seem that any advantage of the *Zea* stigma as a pollen-collecting organ must lie in the greater spatial dispersal of the receptive trichomes.

The trichomes of the *Zea* stigma arise as epidermal enations precisely as in other grasses. As we have already emphasised, the structure of the basal cell complex is important in that the shapes and orientations of the cells determine the direction in which the pollen tube will enter the principal axis, normally with the tip growing towards the ovary. The transmitting tissue is separated by 5–8 files of cortical cells from the points of entry, but there is no indication of any structural adaptation for the guidance of the tubes across the cortex. As we will describe in greater detail in a further paper, the passage is made by stepwise growth from file to file, following the secretion-containing intercellular spaces.

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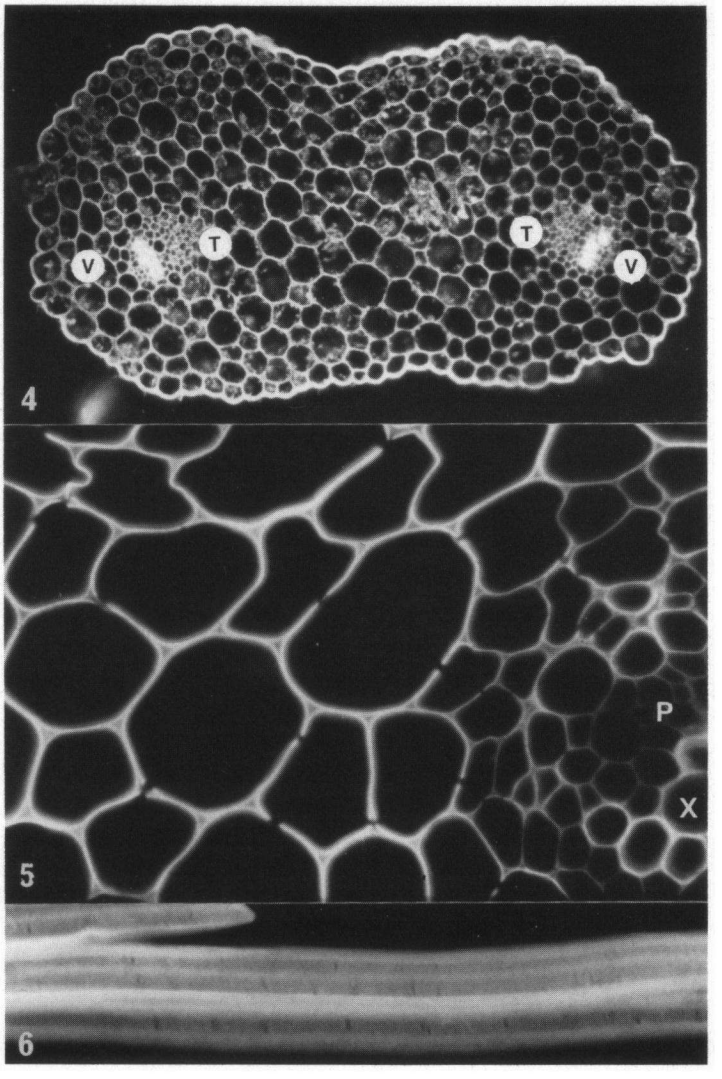

Plate I, Figs. 4–6.
Figs. 4–5. Fluorescence micrographs of the stigma of *Zea mays*, calcofluor white staining.

Fig. 4. Thick section of the principal axis in the mid zone of a stigma c. 200 mm in length. V, vascular bundles; T, pollen tube transmitting tracts. × c. 220.

Fig. 5 Semi-thin (1–1.5 μm) section of a resin-embedded, glutaraldehyde-fixed stigma comparable with that of *fig. 4*, showing the cortical tissue towards the left with conspicuous pit-fields, and a vascular bundle to the right. P, phloem; X, xylem. × c. 1300.

Fig. 6. Isolated cortical cells from a tissue macerate, showing the pit-fields in surface view. × c. 700.

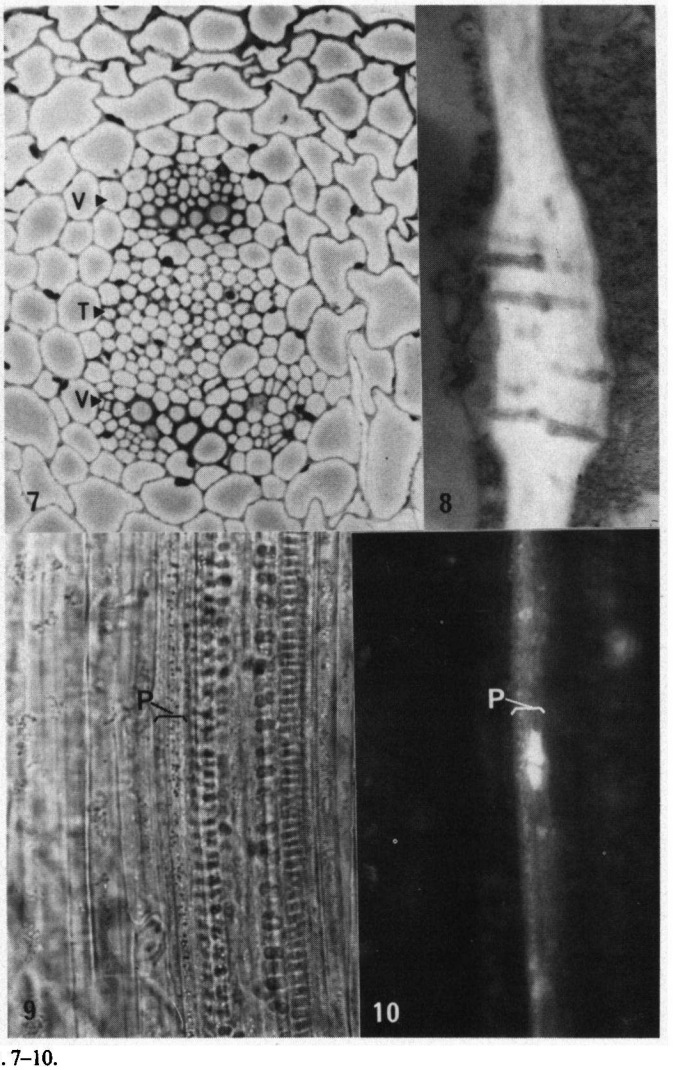

Plate II, figs. 7–10.

Fig. 7. Optical micrograph of a semi-thin section of a resin embedded, glutaraldehyde-fixed stigma of an anomalous type, with two vascular bundles (V) flanking the transmitting tissue (T). × c. 600.

Fig. 8. Electron micrograph of a pit-field with plasmodesmata linking adjacent cortical cells. × c. 28,000.

Fig. 9. Phase contrast micrograph of a vascular strand in the central region of a stigma in longitudinal section. P, site of the phloem. × c. 400.

Fig. 10. The same field as *fig. 9*, fluorescence micrograph, decolourised aniline blue staining. The phloem (P), with a callose plug, is clearly defined. × c. 400.

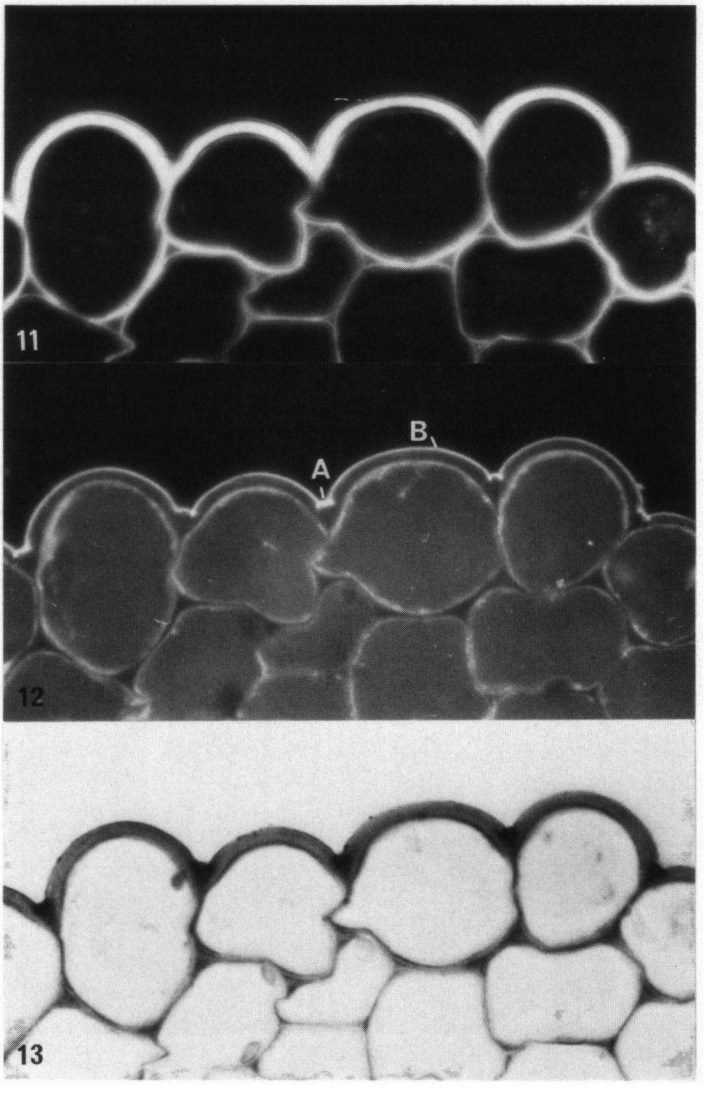

Plate III, figs. 11–13. Optical micrographs of a 1–1.5 μm section of resin-embedded, glutaraldehyde-fixed stigma. All show the same field, which includes the epidermis and cortical cells at the trichome-bearing margin. × c. 1200.

Fig. 11. Fluorescence micrograph, calcofluor white staining.

Fig. 12. Fluorescence micrograph, auramine O staining. The cuticle is clearly defined; the site approximately corresponding to the electron micrograph of *fig. 16* is indicated at 'A', and that corresponding to the micrograph of *fig. 17*, at 'B'.

Fig. 13. Bright field micrograph, alcian blue staining.

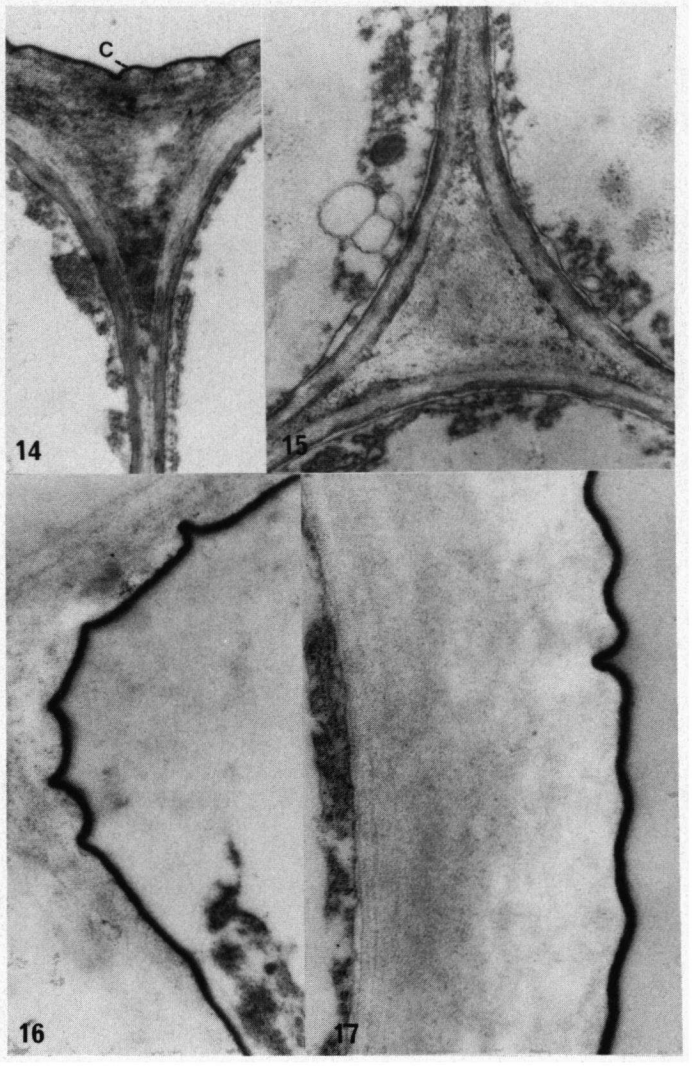

Plate IV, figs. 14–17. Electron micrographs of extended stigmas, transversely sectioned, standard fixation and embedding procedures.

Fig. 14. Junction of two epidermal cells at the trichome-bearing margin, showing the cuticle (C) and the interstitial material seen in the optical micrograph of *fig. 13*. PTA post-staining. × c. 14,000.

Fig. 15. As *fig. 14*, intercellular space in the cortical zone. PTA post-staining. The plasmalemmas are well defined, but no tonoplast is present in the cells at this late stage of development. × c. 18,000.

Fig. 16. Epidermal cuticle in the region 'A' of *fig. 12*. Uranyl acetate, lead citrate post-staining. × c. 31.000.

Fig. 17. As *fig. 16*, epidermal cuticle in the region 'B' of *fig. 12*. × c. 34,000.

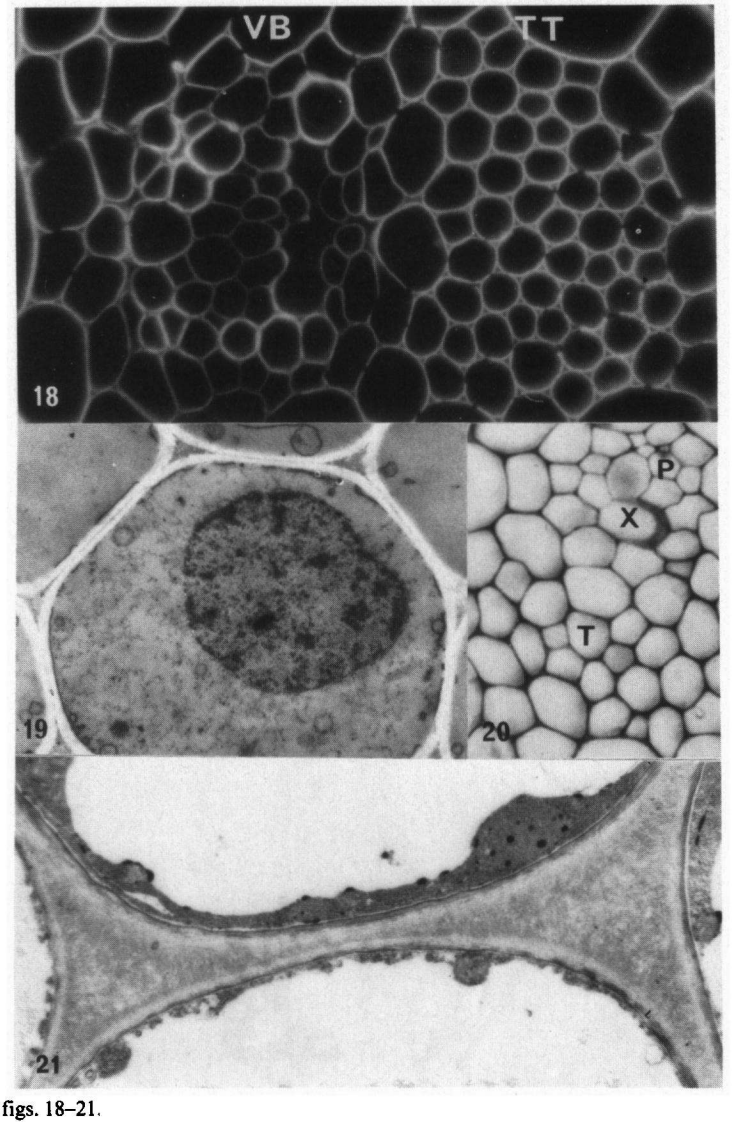

Plate V, figs. 18–21.

Fig. 18. Fluorescence micrograph of a 1–1.5 μm section of a glutaraldehyde-fixed, resin-embedded stigma, showing a vascular bundle (VB) with adjacent transmitting tissue (TT) separated from it by a layer of parenchymatous cells. × c. 1200.

Fig. 19. Electron micrograph of a cell of the transmitting tissue, standard post-staining. The section transects the nucleus, which lies in a diffuse protoplast. The pectocellulosic walls are electron-transparent with this preparation procedure. × c. 9000.

Fig. 20. As *fig. 18*; optical micrograph, toluidine blue staining. P, phloem; X, xylem; T, transmitting tissue. × c. 1100.

Fig. 21. Electron micrograph of adjacent cells of the transmitting tissue, showing the interstitial material. The plasmalemmas are well defined, but the cells lack continuous tonoplasts. × c. 18,500.

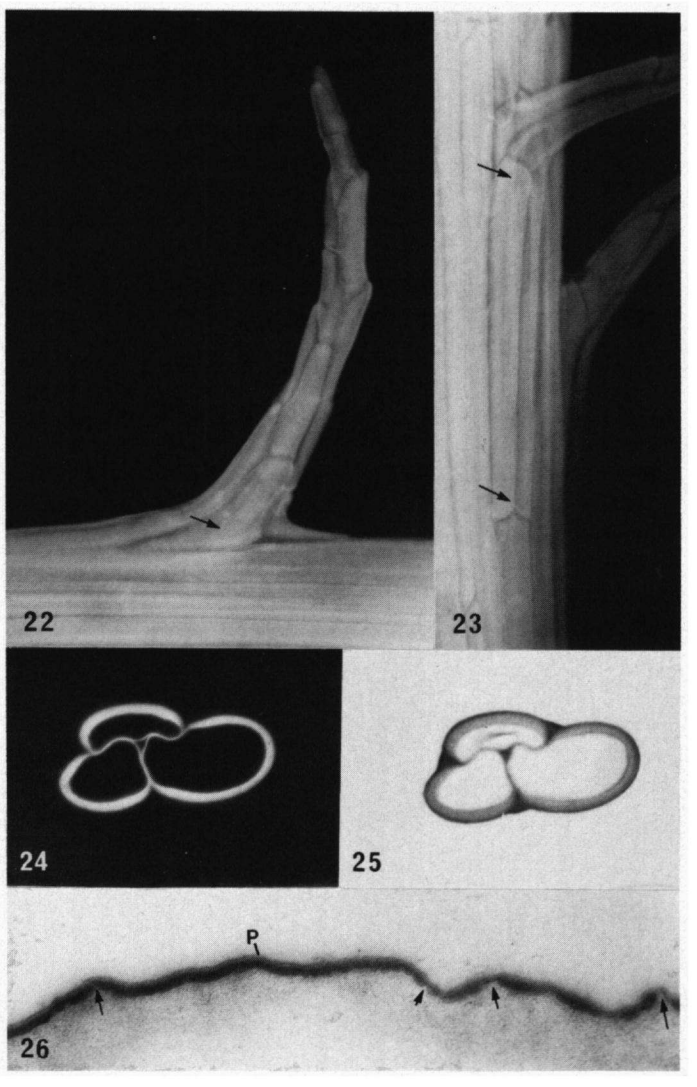

Plate VI, figs. 22–26.

Figs. 22 and 23. Fluorescence micrographs of partly macerated stigmas, calcofluor white staining.

Fig. 22. Single marginal trichome. The trichome is inclined towards the apex of the stigma, and is inserted into the epidermis by a basal cell complex of 5 cells. The arrow indicates a pedestal cell of which there are two, one on either side. To the left of it two elongated, tapering cells extend into the epidermis, and to the right the connection is made by a single angled cell. × c. 260.

Fig. 23. Basal cell complex of a trichome partly in surface view, showing two cells (arrows) inserted into the epidermis and tapering in the direction of the ovary. In another variant of the complex, only a single tapering cell is present (cf. *fig. 30*). × c. 280.

Figs. 24 and 25. Glutaraldehyde-fixed, resin-embedded trichome sectioned at 1–1.5 μm. × c. 1200.

Fig. 24. Fluorescence micrograph, calcofluor white staining, which mainly reveals the inner wall layer.

Fig. 25. Same field, bright-field micrograph, alcian blue staining. A pectic constituent is present through the thickness of the wall, but is most concentrated in the outer zone beneath the cuticle, and in the intercellular spaces. The zonation of the wall is identical with that seen in *Secale cereale* (Paper 1).

Fig. 26. Electron micrograph of the cuticle of a stigma papilla, showing numerous discontinuities (arrows), and the surface secretion layer (pellicle, P). × c. 44,000.

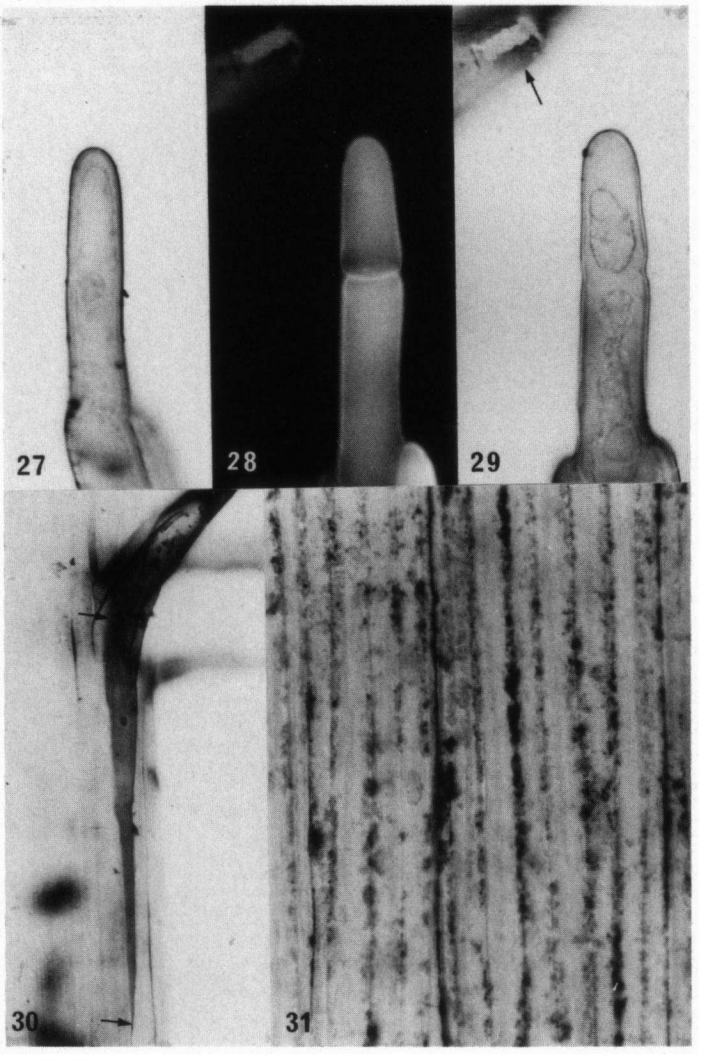

Plate VII. figs. 27–31.

Figs. 27–29. Optical micrographs of the intact terminal cells of stigmatic trichomes. × c. 800.

Fig. 27. Optical section showing the proteinaceous surface secretion (pellicle), Coomassie blue staining.

Fig. 28. Fluorescence micrograph, calcofluor white staining after esterase localisation; cf. *fig. 29.*

Fig. 29. Esterase localisation. The reaction product is seen in optical section, coating the surface. The arrow indicates a neighbouring trichome seen in surface view, with a fissured film of reaction product.

Fig. 30. Intact stigma, esterase localisation. The reaction product coats the surface of the tapering cell of the basal cell complex of the trichome (arrows), and is absent from the neighbouring epidermal cells. Cf. *fig. 34.* × c. 320.

Fig. 31. Surface of the epidermis of an ageing stigma, esterase localisation. Activity is confined to the furrows between the longitudinal files of cells (cf. *fig. 16*, showing the grooved cuticle in these sites). × c. 260.

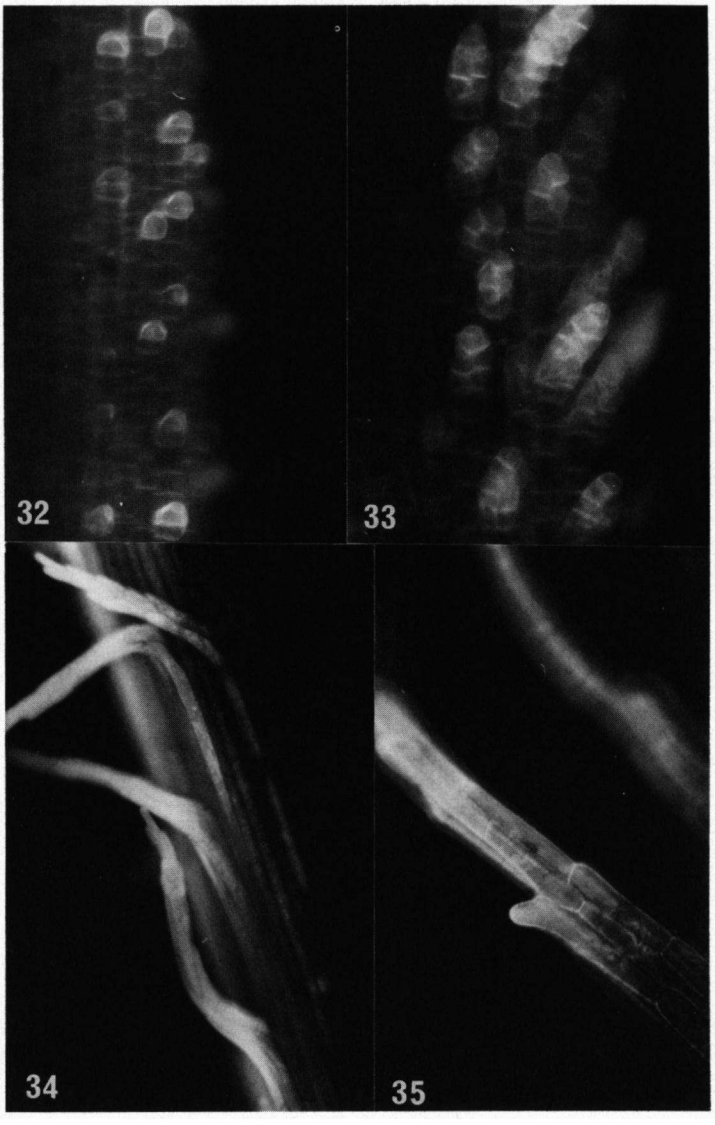

Plate VIII, figs. 32–35. Permeability mapping of the trichome-bearing margins of intact stigmas. Fluorescence micrographs, calcofluor white staining.

Fig. 32. Trichomes in uni- and bicellular stages. From the onset of differentiation the cuticles are permeable to the tracer. × c. 420.

Fig. 33. Trichomes in 6- to 9-cell stages. × c. 420.

Fig. 34. Intact mature stigma, illustrating the permeability of the cuticle of the tapering cell of the basal cell-complex of the trichome (arrow). Cf. *fig. 30*. × c. 160.

Fig. 35. Intact trichome during the early penetration of the tracer showing the high permeability of the papillate tips of the shaft cells. × c. 360.