Acta Bot. Neerl. 31 (4), August 1982, p. 307–319.

THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 3. FEATURES OF THE SELF-INCOMPATIBILITY RESPONSE

K. R. SHIVANNA[1], Y. HESLOP-HARRISON and J. HESLOP-HARRISON[2]

Welsh Plant Breeding Station, Plas Gogerddan, nr Aberystwyth SY 23, 3EB, United Kingdom

Key words: Rye, *Secale cereale*, *Gaudinia fragilis*, *Alopecurus pratensis*, pollination, self-incompatibility, pollen-tube inhibition

SUMMARY

In strongly self-incompatible genotypes of *Gaudinia fragilis* (L.) Beauv. and *Secale cereale* L., incompatible pollen grains germinate normally, but the tubes are arrested at or near the stigma surface. The response, which may occur within 30 sec of the emergence of the tube, seems to be contingent on contact between the tube tip and the surface secretions of the stigma. The suspension of tube growth is followed by the deposition of callose in the tube and the grain, but this is not the primary reaction. The earliest abnormality appears in the formation of the wall at the tube tip. The wall in the extending apex is composed of pectic microfibrils, contributed by the precursor particles ("P-particles") present in the ungerminated grain. In an incompatible tube these do not flow into the wall to form the normal thin apical sheath, but accumulate to form nodules or lamellate aggregates. It is suggested that this is the direct consequence of contact with the stigma-held incompatibility factors, which may inhibit growth by cross-linking the microfibrils in such a way as to prevent their re-distribution and re-orientation.

Individuals of *Alopecurus pratensis* vary in the strength of their self-incompatibility response. In some genotypes, self-pollen tubes penetrate the stigma surface and reach the transmitting tracts of the stylodia before they are inhibited. This behaviour provides a link with gametophytic self-incompatibility systems in other families, where the inhibition is normally in the transmitting tract of the style.

1. INTRODUCTION

In earlier papers in this series we have described the principal structural and cytochemical features of the grass stigma, and have given some account of the reaction of the stigma to pollination and the penetration of compatible pollen tubes (J. & Y. HESLOP-HARRISON 1980, 1981, referred to hereafter as Papers 1 & 2). The present paper deals with the responses associated with the operation of the self-incompatibility system, which have not hitherto been investigated in detail in any grass species. Some of our earlier findings have been summarised by DE NETTANCOURT (1977), who also provided a comprehensive account of the genetical work on self-incompatibility in the grasses. HAYMAN (1956) and

[1]Present address: Dept. of Botany, University of Delhi, Delhi 110007, India.
[2]All reprint requests to J. Heslop-Harrison, Welsh Plant Breeding Station.

LUNDQUIST (1956) showed the system to be of the gametophytic type, governed in the species they investigated by two polyallelic loci, *S* and *Z*. In this system, each combination of alleles determines a pollen specificity, and rejection occurs when that specificity is matched by one of the combinations present in the cells of the stigma. While the two-locus systems may well be the general one in the family (CORNISH, HAYWARD & LAWRENCE 1979), it is possible that the genetical control might be more complex in some species (SPOOR 1976; ØSTERBYE, LARSEN & LUNDQUIST 1980). The precise genetical basis of the control is, however, largely irrelevant for the experiments reported here, which are concerned with the mechanism of the response itself.

HAYMAN (1956) observed that in *Phalaris caerulescens* incompatible pollen tends to be inhibited at or near the stigma surface very soon after germination, an unusual feature in gametophytic self-incompatibility systems (J. HESLOP-HARRISON 1975). While a rapid response of this kind is found in many grass species, there is a considerable amount of variation in the timing and the site of the incompatibility reaction, both between and within species (J. HESLOP-HARRISON 1979b). The presence of this variation has some significance in relation to the evolution of the grass system, since it provides a link with other gametophytic self-incompatibility systems where the inhibition is in the pollen-tube transmitting tract of the style.

2. MATERIALS AND METHODS

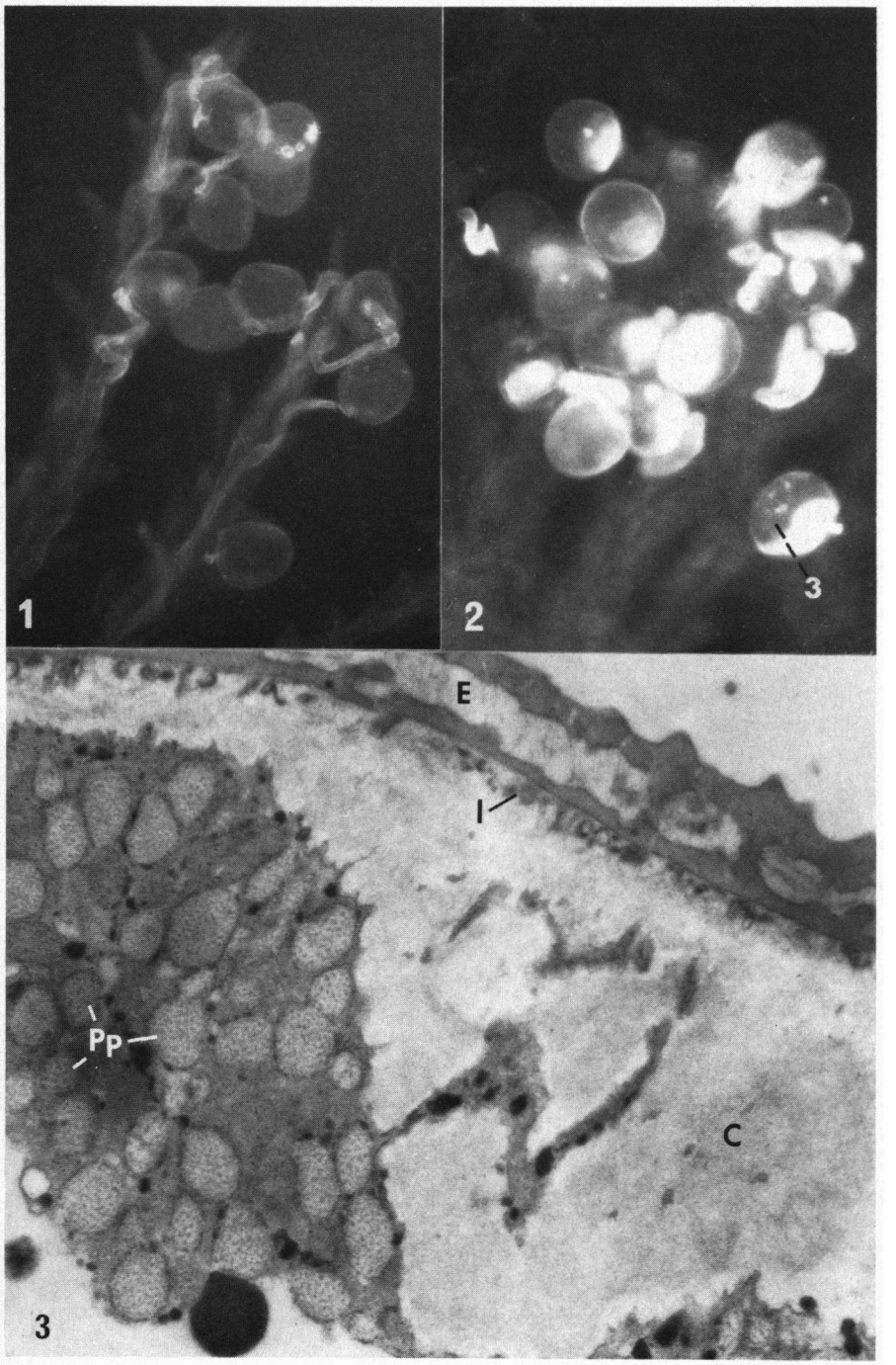
The principal observations were made on *Gaudinia fragilis* (L.) Beauv. (2n = 14) and *Secale cereale* L. cv. Rheidol (2n = 14) grown in experimental plots, and *Alopecurus pratensis* L. (2n = 28), taken from wild populations.

For the investigation of short-term responses, single-grain pollinations were carried out using freshly-emerged, virgin stigmas. The stigmas were excised and transferred to humid chambers constructed on microscope slides, where they were kept under continuous observation at magnifications up to 200× using

Fig. 1. Stigma of *Gaudinia fragilis*, 2 h after compatible pollination. Fluorescence micrograph, decolourised aniline blue staining for callose. The pollen tubes are visible because of their callosic inner walls; the grains contain no more than occasional callose deposits. × c. 190.

Fig. 2. As *Fig. 1*, self-pollination. Most of the grains have germinated normally, and short tubes have been produced. Tube growth has, however, been inhibited thereafter, and the tubes and grains have become occluded to a greater or lesser degree with callose. The dashed line "3" marks the approximate orientation of the electron micrograph of *fig. 3*, of a similarly blocked incompatible grain. × c. 190.

Fig. 3. Electron micrograph of an incompatible pollen grain of *G. fragilis* following the deposition of callose, sectioned in a plane corresponding to that indicated in *fig. 2*. The exine (E) lies to the top right; the intine (I) lying within it has not undergone dissolution. The callosic mass (C) is in this instance essentially amorphous, although it includes irregular trapped islets of cytoplasm. The cytoplasm of the vegetative cell retains a dense population of polysaccharide wall-precursor particles (Pp). × c. 20.000.


1
2
3
E
I
Pp
C
3

a Vickers epi-illumination system. Individual pollen grains from freshly dehisced anthers were placed on single papillae in the orientations required with a human eyelash hair, either manually, or with a Singer micromanipulator (J. HESLOP-HARRISON 1979a; Paper 2). Germination, tube growth and rejection responses were timed manually, or photographically using an automatic timer.

Bulk pollinations were carried out using complete excised pistils or isolated stigmas planted in open petri dishes on 1% agar. Fresh pollen was transferred to the stigmas with a small sable brush, and dispersed evenly so as to ensure optimum contact of the grains with the stigma papillae. Where rapid assessments were required from living preparations, pollen tube growth was estimated directly in situ using mean pollen grain diameter as a reference standard.

For the observation of callose deposition, the pollinated stigmas were removed at the appropriate time intervals and either transferred directly to aniline blue (Merck or BDH, 0.05% at pH 11), or fixed in acetic-alcohol (1 part acetic acid, 3 parts ethanol) before washing and passage into aniline blue. Callose was detected by fluorescence microscopy (LINSKENS & ESSER 1957). Because of the irregular manner in which callose is laid down in blocked, incompatible grains and pollen tubes, it is not feasible to measure the volumes with a high degree of accuracy. Amounts were therefore scored visually against standards on a four-point scale, as detailed in the legend of *fig. 4A*.

Other cytochemical methods used in the present work have been described in earlier papers (J. HESLOP-HARRISON 1979a; Papers 1 & 2). The microfibrillar component of the polysaccharide particles associated with the growth of the grass pollen tube wall and also of the wall itself in the extreme tip zone has been identified as pectic in nature (J. & Y. HESLOP-HARRISON 1982, and in preparation). This is in agreement with the early observations of DASHEK & ROSEN (1966) for *Lilium longiflorum* (see also LINSKENS & KROH 1967).

3. OBSERVATIONS

3.1. Timing of the self-incompatibility response

Detailed timings were obtained from single-grain pollinations of *S. cereale*. Freshly shed grains appropriately placed on the stigma hydrate and germinate within 90 sec (J. HESLOP-HARRISON 1979a), and in a compatible combination the tube tip, in the absence of competition, will normally penetrate the stigma cuticle within the following 30–120 sec (Paper 2). The initial events associated with hydration, gelation of the Zwischenkörper, lifting of the operculum and emergence of the tube tip were found not to be affected in incompatible pollinations, nor was the early growth of the tube across the surface of the exine when the germination aperture faced away from the stigma surface (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978). This observation suggests that exine contact alone is inadequate to provoke the incompatibility response, notwithstanding the fact that stigma constituents are likely to be taken up freely into the grain during the initial hydration (J. HESLOP-HARRISON 1979c).

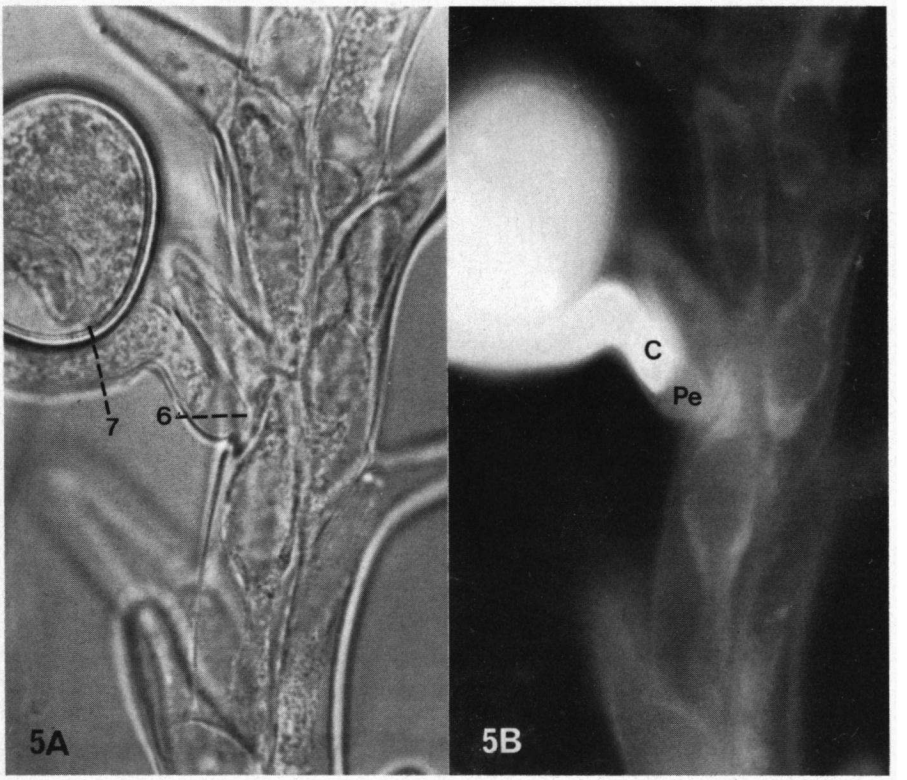
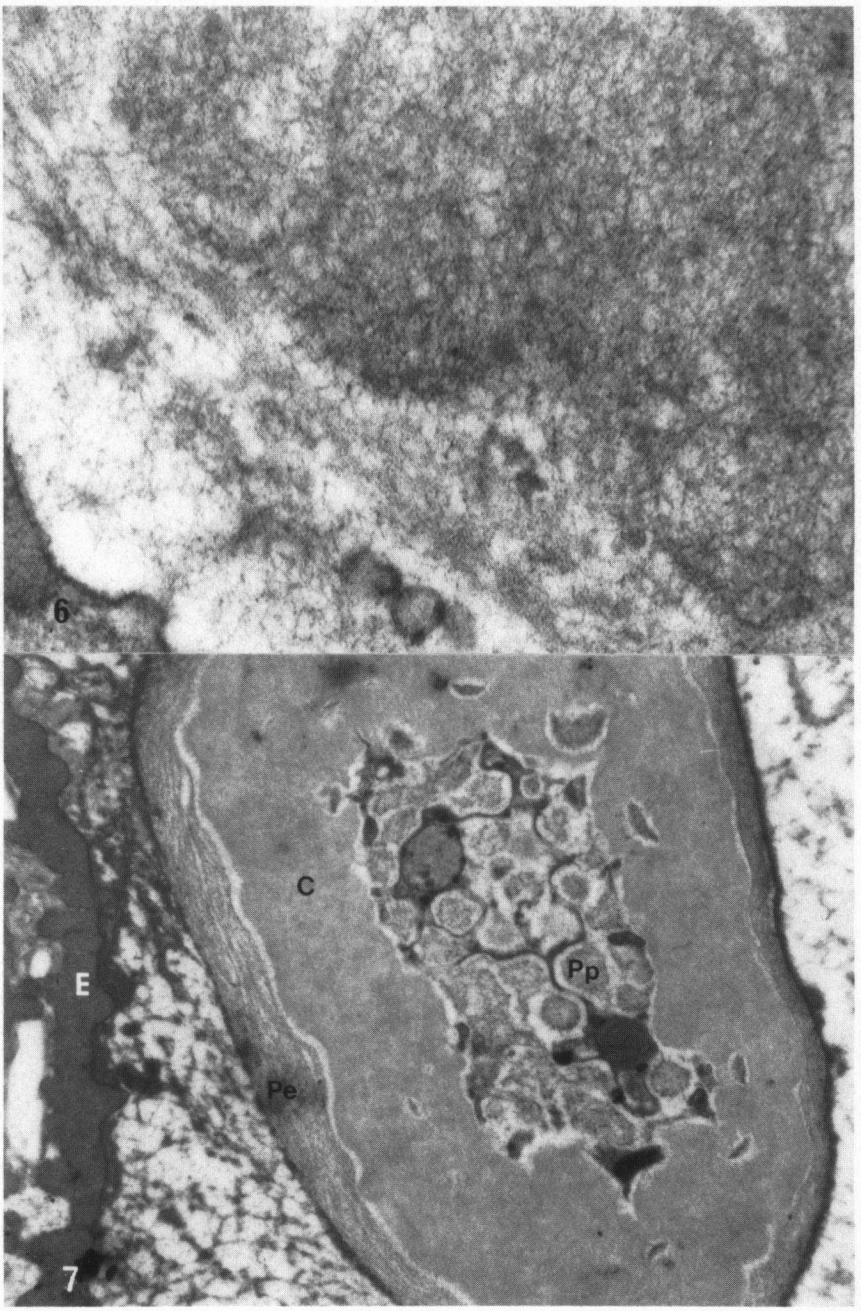


Fig. 5A. Bright-field optical micrograph of an inhibited pollen grain and tube of *G. fragilis* following an incompatible pollination. Germination has proceeded in a normal manner, and the tube has grown over the surface of the exine into contact with the stigma, after which growth has been arrested. The dashed line "6" marks the approximate plane of the electron micrograph of *fig. 6*, and that marked "7", the plane of the electron micrograph of *fig. 7*. × c. 700.
Fig. 5B. The same field as *fig. 5A*. fluorescence micrograph showing the distribution of callose. The apical 12 μm of the inhibited tube (Pe) is occluded, but the content does not react for callose. On the basis of cytochemical criteria the occluding material is identifiable as pectic in nature. The proximal part of the tube is lined thickly with callose, and there is a massive deposit in the grain itself. × c. 700.

In the more rigorously self-incompatible genotypes of *G. fragilis* and *S. cereale* tested, self-pollen tubes were, however, found to be inhibited soon after the contact of the tube with the stigma surface, and usually before the tip had penetrated the cuticle. The interval is determined by the posture of the grain and the amount of exploratory growth made before the tube reaches the papilla, but in both species it can be as short as 30 sec.

The arrest of growth is followed by an enhanced deposition of callose in the vicinity of the tube tip, and thereafter in the proximal parts of the tube and in the grain itself. The optical micrograph of *fig. 2* illustrates callose blocked


6
C
E
Pp
Pe
7

grains 2 h after a self-pollination; it may be compared with *fig. 1*, showing the appearance of the grains in a compatible cross after the same interval. The time sequences of callose deposition and tube growth in compatible and incompatible pollinations are compared in *figs. 4A* and *4B*.

While the deposition of callose can be used as an indicator of the self-incompatibility response, it is clear from the time course of the reaction that it is not the primary event, and certainly not the cause of arrested growth. Where the arrest follows immediately upon the emergence of the tube tip, the tip zone is quickly occluded and deposition then begins in the grain. However, if the tube passes through a period of normal growth before contact with the contiguous papilla, callose is not deposited at all in the immediate vicinity of the tip. This is clearly seen in the optical micrographs of *figs. 5A* and *5B*, of *G. fragilis*. The tip zone of some 12 μm is here occluded with pectic material; proximal to this, the tube is lined by a thick sheath of callose, and deposition has also begun in the grain itself.

The arrest of tube growth is not associated with any evident suspension of metabolic activity in incompatible pollen. In *S. cereale* the maximum length of the pollen tube pathway from stigma surface to micropyle is 3.5 mm, and in favourable conditions a tube may traverse this within 35 min, at which time the grain itself will be emptied of virtually all content (J. HESLOP-HARRISON 1979a). Following the suspension of growth in an incompatible pollination, callose deposition may continue for 2–3 h, and during this period the starch reserves of the grain are usually wholly depleted.

3.2. Fine-structural features

The electron micrograph of *fig. 3* is of an incompatible pollen grain sectioned approximately in the plane indicated by the dashed line in *fig. 2*. The section transects the exine and intine, and also a callose deposit within the intine. The callose in this instance is essentially amorphous, although there are irregularly trapped islets of cytoplasm within the mass. Occasionally in this species the callose laid down within the intine following the initial blockage of tube growth

Fig. 6. Electron micrograph of an incompatible pollen tube of *G. fragilis*, sectioned approximately in the plane "6" of *fig. 5A*. The pectic occluding material is seen to be microfibrillar, corresponding in its electron-staining and other properties with the microfibrillar content of the wall precursor particles (J. & Y. HESLOP-HARRISON 1982). × c. 80.000.

Fig. 7. As *fig. 6*, section plane "7" of *fig. 5A*. The exine of the grain (E) is seen towards the left, linked to the tube by flocculent material resulting from the fixation of constituents of the fluid film lying between grain, tube and stigma papilla. The wall of the tube shows a stratification similar to that of a normal tube at this distance behind the apex, with an outer microfibrillar layer (Pe) and a distinct and separate inner callosic layer (C). The latter is, however, much thicker than would normally be found in an actively growing tube. The tube cytoplasm contains wall-precursor particles (P_p), some of which are apposed to the inner surface of the callose layer. × c. 9.500.

is lamellated, the layers of amorphous material being interleaved with a fibrillar component (Fig. 3 in J. HESLOP-HARRISON 1982).

Fig. 7 is of an incompatible tube sectioned approximately in the plane marked "7" in *fig. 5A*. There is a clear structural distinction between the outer microfibrillar stratum of the wall and the inner, callosic part, and in this respect the stratification is comparable with that of the normal tube (J. HESLOP-HARRISON 1979a). However, the callosic layer is very greatly thickened, occupying some 70% of the cross-sectional area. The cytoplasm holds a considerable population of polysaccharide wall precursor particles (P-particles). Some of these are apposed to the wall, and ellipsoidal profiles of microfibrillar material within the callose layer are probably to be interpreted as residues of those that have recently been incorporated.

Fig. 6 is also of an incompatible tube, sectioned in this instance approximately in the plane marked "6" in *fig. 5A*. The whole volume of the tube in this zone is occupied by a mass of microfibrils. The structure and cytochemical properties of this material are indentical with those of the contents of the wall precursor particles, the principal polysaccharide of which has been shown to be pectic in nature in *Lilium* species and in the grasses (DASHEK & ROSEN 1966; VAN DER WOUDE, MORRÉ & BRACKER 1971; J. & Y. HESLOP-HARRISON 1982). Profiles adjacent to the tip during the early stages of the blockage of the tube *(fig. 8)* show that the build-up of this material results from the continued apposition of the precursor particles after the suspension of growth, without dissociation of the microfibrillar content or, seemingly, further metabolic conversion, the process beginning with the formation of pectic nodules in the wall at the tip itself.

3.3. The "weak" self-incompatibility reaction

The genotypes of *Secale cereale* and *Gaudinia fragilis* used in the foregoing studies were strongly self-incompatible, showing, characteristically, early inhibition of incompatible pollen on the stigma surface. Plants of *Alopecurus pratensis* taken from natural populations in the Royal Botanic Gardens, Kew and in west Wales were found to be very variable in their expression of self-incompatibility. Some individuals proved to be virtually wholly self-compatible, while others showed a partial or "weak" response. The callose reaction following selfing in plants of the latter type is illustrated in *figs. 9* and *10*. From 95 germinated grains on the stigma of *fig. 9* five tubes reached the base of the stylodia, the remaining tubes being arrested in the secondary stigma branches or in the transmitting tracts of the stylodia, usually with callose occlusion. Assuming that there were no blockage of the successful tubes in the upper ovary wall, there would evidently be some likelihood that this plant would be categorised as self-compatible on the basis of seed-set potential. Nevertheless, the abnormal nature of the wall in the region of the tips of the inhibited tubes proves the presence of a self-incompatility system, albeit one with low efficiency.

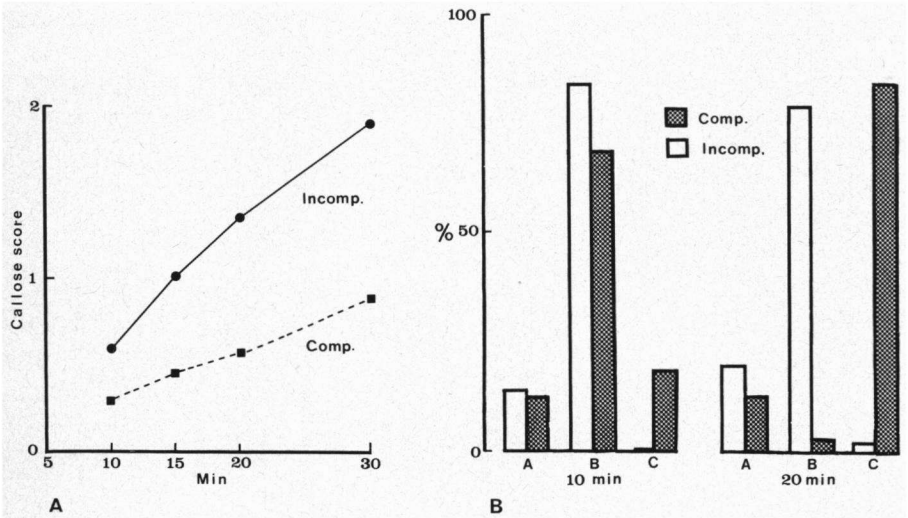


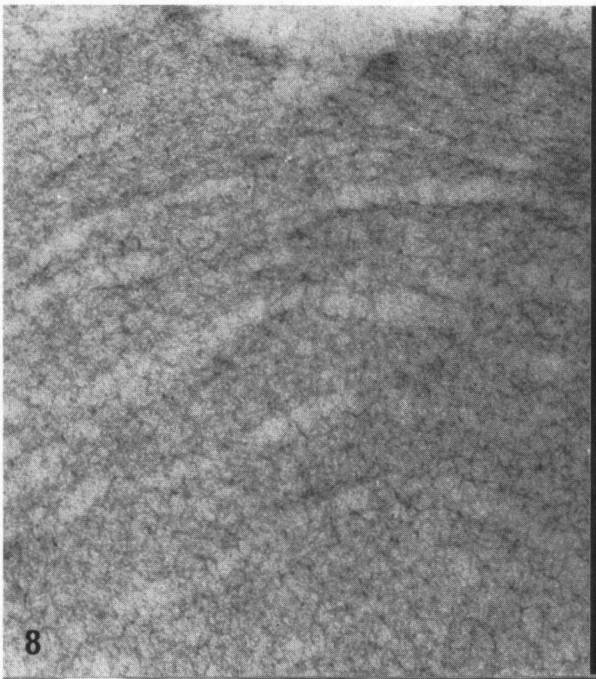
Fig. 4A. Callose accumulation in pollen grains and tubes over a period of 30 min following compatible and incompatible pollinations of *Gaudinia fragilis*. Individual grains and tubes were scored on a four-point scale: 0, no observable callose; 1, scattered light deposits; 2, medium deposit (callose occupying up to an estimated one quarter of the volume of tube and grain); 3, heavy deposit (callose occupying up to one half of the volume.) Seventy to 190 grains scored per series.

Fig. 4B. Pollen tube length distribution in compatible and incompatible pollinations of *G. fragilis* scored *in situ* at 10 min and 20 min after pollination. Length categories: A, no germination; B, tube up to 70 μm in length; C, tube over 70 μm. In the compatible pollination, most tubes had entered the proximate stigma branch and many had penetrated into the transmitting tracts of the stylodia in the 20 min series. In a similar study of pollen tube growth in *S. cereale*, rates in the range 1.35–1.55 μm sec^{-1} were measured during the initial growth of incompatible and compatible tubes; the former were arrested at the stigma surface, while the latter achieved rates of more than 2 μm sec^{-1} after penetrating into the stigma (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978).

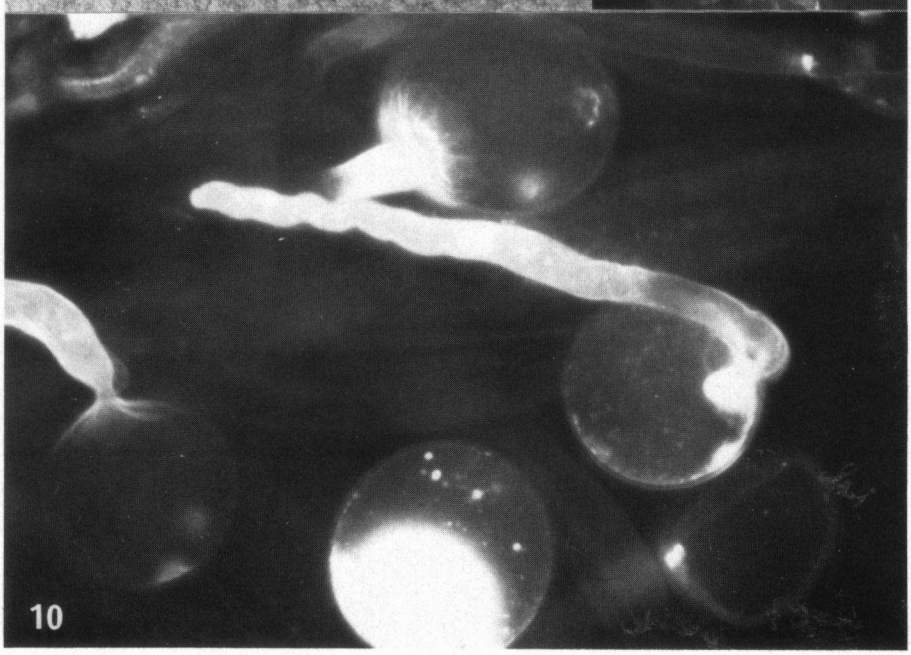
4. DISCUSSION

The principal conclusion to be drawn from the foregoing evidence is that the self-incompatibility response in the grasses involves a retardation or arrest of tube extension resulting from a disturbance of wall growth in the tip region (SHIVANNA, Y. HESLOP-HARRISON & J. S. HESLOP-HARRISON 1978). This disturbance of apical growth follows upon normal germination and is initiated after the contact of the pollen tube with the stigma surface. In this "strong" form, the response may be expressed rapidly in the early arrest of the tube at the surface, or, in the "weak" form, in the retardation and ultimate arrest of the tubes after the penetration of the cuticle and entry into the transmitting tissues.

The arrest of apical growth is evidently not brought about by the general metabolic inhibition in the male gametophyte, nor is it attributable to the deposition of callose; the latter is evidently a secondary response. The primary effect


8


9


10

is upon the deposition of wall material at the apex. Tip growth in the grass pollen tube occurs through the apposition on the wall of cell wall material from precursor bodies, the principal constituent of which is a microfibrillar pectin (J. HESLOP-HARRISON 1979a; J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1982). The wall itself in the extreme apex has a similar composition, and, as *fig. 8* may be taken to indicate, the disruption of normal growth in an incompatible tube results from the formation of banks or nodules of the microfibrillar content of the precursor particles, which does not flow into the extending wall to form the thin apical sheath characteristic of the normal tube tip. Such a dislocation of the normal pattern of wall growth might be expected were the function of the stigma-held incompatibility factors to cross-link the wall microfibrils in such a manner as to prevent their progressive re-orientation and re-distribution in the course of growth (J. HESLOP-HARRISON 1982).

This interpretation of the morphological aspects of the self-incompatibility response in the grasses can probably be extended to the systems in other families. It has long been known that the response is very generally associated with abnormalities in wall formation in the vicinity of the tube tip (see the reviews of earlier literature given by LINSKENS & KROH 1967, and DE NETTANCOURT 1977). Most commonly these abnormalities take the form of distorted or retarded growth, and sometimes with branching of the tube. The arrest of the tube is often followed by the accumulation of substantial amounts of callose, but the deposition of callose in the tip zone is by no means a universal associate of the incompatibility response. DE NETTANCOURT, DEVREUX, BOZZINI, CRESTI, PACINI & SARFATTI (1973), for example, found that imcompatible pollen tubes in *Lycopersicum peruvianum* generally become inflated at the tips in the pollen tube transmitting tract of the style without any appreciable accumulation of callose. The inflated tubes were often then seen to burst, with the release of numerous wall precursor particles (the polysaccharide particles of the present account) into the intercellular spaces of the transmitting tract. Electron micrographs published by these authors show that the wall at the apex, although free of callose, is somewhat thickened by the accumulation of finely microfibrillar material, corresponding, presumably, to the pectic microfibrillar material described here from the grasses.

Fig. 8. Electron micrograph of the wall of an incompatible tube early during the development of the wall abnormality. The section plane is approximately at right angles to the long axis of the tube, and is estimated to be immediately proximal to the apex itself. The wall is thickening by the apposition of microfibrillar pectic material, and the stratification, in the form of flattened ellipsoidal inclusions, indicates that these are derived from apposition of wall-precursor particles. × c. 85.000.

Fig. 9. Fluorescence micrograph of a segment of a stylodium of *Alopecurus pratensis*, 3 h after self-pollination, decolourised aniline blue staining for callose. The grains have germinated freely, and many tubes have penetrated into the stigma before arrest. This "weak" self-incompatibility reaction may be compared with that illustrated in *fig. 2* for a strongly self-incompatible genotype of *G. fragilis*. × c. 105.

Fig. 10. As *fig. 9*, detail showing different patterns of callose deposition in pollen grains and tubes. As in the tube of *G. fragilis* illustrated in *Fig. 5B*, the callose occlusion does not extend into the extreme apex in the short tube at the top centre. × c. 550.

Their evidence may thus be interpreted as indicating that in this dicotyledon the abnormal tube growth associated with the incompatibility response is brought about, as in the grasses, by the premature stabilisation of the microfibrillar component of the wall in the tip region.

As we have noted, the existence among the grasses of "weak" self-incompatibility systems such as that of *Alopecurus pratensis* marks a link with the gametophytic systems in other families, like that of the Solanaceae, where incompatible pollen tubes are inhibited while traversing the transmitting tracts of the style. Evidently in weakly responding genotypes of *A. pratensis* the pistil-side incompatibility factors are not encountered – or not encountered in effective concentrations – on the stigma surface, adequate levels being present only in the transmitting tracts. The variation observed in the strength of the response within and between species may simply reflect differences in the rate of secretion of the incompatibility products (J. HESLOP-HARRISON 1982). While there are indications of the source of the superficial and interstitial secretions in the grasses stigma (Paper 1 & 2), there is obviously now a challenge to work out the details of the secretory system and to establish which specific components of the secretions are concerned in governing pollen tube behaviour.

ACKNOWLEDGEMENTS

We thank the Curator of the Royal Botanic Gardens, Kew, for the provision of some of the plant material used in this work, and Professor J. P. Cooper, Director of the Welsh Plant Breeding Station, for the use of the Station's facilities. The participation of KRS was made possible by the award of a Royal Society Commonwealth Bursary, and of YHH by the award of a Research Fellowship by the Leverhulme Trust.

REFERENCES

CORNISH, M. A., M. D. HAYWARD & M. J. LAWRENCE (1979): Self-incompatibility in ryegrass. I. Genetic control in diploid Lolium perenne L. *Heredity* **43:** 95–106.

DASHEK, W. V. & W. G. ROSEN (1966): Electron microscopic localisation of chemical components in the growth zone of lily pollen tubes. *Protoplasma* **61**: 192–204.

HAYMAN, D. L. (1956): The genetical control of incompatibility in Phalaris caerulescens. *Aust. J. Biol. Sci.* **9: 32–36.**

HESLOP-HARRISON, J. (1975): Incompatibility and the pollen-stigma interaction. *Ann Rev. Plant Physiol.* **26:** 403–425.

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

— (1979c): An interpretation of the hydrodynamics of pollen. *Amer. J. Bot.* **66: 537–546.**

— (1982): Pollen-stigma interaction and cross-incompatibility in the grasses. *Science* **215:** 1358–1364.

— & Y. HESLOP-HARRISON (1980): 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.

— & — (1981): The pollen-stigma interaction in the grasses. 2. Pollen-tube penetration and the stigma response in Secale. *Acta Bot. Neerl.* **30:** 289–307.

— & — (1982): The growth of the grass pollen tube. 1. Characteristics of the polysaccharide particles ("P-particles") associated with apical growth. *Protoplasma* (In press).

LINSKENS, H. F. & ESSER, K. (1957): Uber eine spezifische Anfärbung der Pollenschläuche im Griffel und die Zahl der Kallosepfropfen nach Selbstung und Fremdung. *Naturwissensch.* **44:** 1–2.

— & KROH (1967): Incompatibilität der Phanerogamen. In *Encyclopedia of Plant Physiology*, ed. W. RUHLAND, Vol. **18,** pp. 506–530. Springer-Verlag, Berlin, Heidelberg, New York.

LUNDQUIST, A. (1956): Self-incompatibility in rye. I. Genetic control in the diploid. *Hereditas* **42: 293–348.**

NETTANCOURT, D. DE (1977): *Incompatibility in Angiosperms.* pp. 230. Springer-Verlag: Berlin, Heidelberg, New York.

— , M. DEVREUX, A. BOZZINI, M. CRESTI, E. PACINI & G. SARFATTI (1973): Ultrastructural aspects of the self-incompatibility mechanism in Lycoperiscum peruvianum Mill. *J. Cell Sci.* **12:** 403–419.

ØSTERBYE, U., K. LARSEN & A. LUNDQUIST (1980): Comments on self-incompatibility in the Gramineae. *Incompat. Newslett.* **12,** 45–49.

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

SPOOR, W. (1976): Self-incompatibility in Lolium perenne L. *Heredity* **37:** 417–421.

VAN DER WOUDE, W. J., D. J. MORRÉ & C. E. BRACKER (1971): Isolation and characterisation of secretory vesicles in germinated pollen of Lilium longiflorum. *J. Cell Sci.* **8:** 331–351.