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Neither compatible nor self-incompatible pollinations of *Brassica napus* involve reorganization of the papillar cytoskeleton

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SUMMARY

Compatible pollination of *Brassica napus* necessitates pollen hydration, pollen germination and growth of the pollen tube through the loosened walls of stigmatic papillar cells, whereas self-incompatible (SI) pollinations fail at one of these stages. Analyses of the early stages of pollination show that at high (but not low) relative humidities, both compatible and SI pollen hydrates, but SI germination is reduced and the rare pollen tubes generally fail to penetrate the papillar walls, although there is some wall loosening. Inside the papillae, both compatible and SI interactions may induce the formation of callose, but there is no evidence for a major accumulation of cytoplasm or secretory vesicles in the vicinity of the pollen tubes and neither microtubule nor F-actin patterns re-arrange in this zone. These observations indicate that the source of the wall-loosening enzymes is probably the pollen tube or pollen coat, and that the common cellular responses of plants to attempted invasions have become suppressed in the papilla–pollen tube interaction.

Key words: microtubules, actin microfilaments, self-incompatibility, *Brassica napus*, plant cell–cell interactions.

INTRODUCTION

In *Brassica* species, fertilization by self pollen can be prevented by the inhibition of pollen germination or growth on specialized stigmatic cells called papillae, due to a self-incompatibility system (Ockendon, 1972). After self-incompatible (SI) pollinations, pollen hydration and subsequent germination are blocked, possibly partly due to the activation of a papilla aquaporin-like channel protein which may direct water away from the pollen–papilla interface (Ikeda *et al.*, 1997). Under certain environmental conditions, e.g. high relative humidity, high temperature or raised CO_2 levels (Ockendon, 1973; Nakanishi & Hinata, 1975; Zuberi & Dickinson, 1985), incompatible pollen may succeed in germinating on self-stigmas. In such circumstances, the penetration of pollen tubes into papillae appears to be blocked by the failure of papillar outer wall loosening – a probable requisite for the entry of pollen tubes into the stigma (Elleman & Dickinson, 1990; Elleman *et al.*, 1992).

Previous research has suggested that papillar wall loosening during compatible pollinations (in both dry stigma plants such as the Cruciferae and the wet stigma species, which are beyond the scope of this paper) occurs via the release of a population of secretory vesicles from the papillar cytoplasm (Elleman & Dickinson, 1996). These vesicles were reportedly formed from endoplasmic reticulum (ER) and Golgi bodies which were apparently abundant below the site of wall loosening. Contact of compatible pollen tubes with the outer papillar wall presumably acts as a trigger for such a secretory process, whereas the interaction of the papillar wall with incompatible pollen tubes either fails to activate the required signalling pathway or inhibits it at some critical step.

The plant cytoskeleton plays a critical role in secretory processes such as wall formation (Derksen & Emons, 1990; Williamson, 1993) and the release of enzymes (discussed by Steer, 1988) and other extracellular products (Kristen & Lockhausen, 1983). Both actin microfilaments (MFs) and microtubules (MTs) are potentially involved in the movement of secretory vesicles from their point of

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formation, such as the ER and the Golgi bodies, to their site of exocytosis. The plant cytoskeleton can be induced to re-organize, at least in part, to direct secretion to precise cellular locations by external stimuli such as invading fungal hyphae (Bonfante-Fasolo, 1992; Kobayashi *et al*., 1992, 1994, 1997; Gross *et al*., 1993; Allen *et al*., 1994; Baluska *et al*., 1995; Dearnaley & McGee, 1996; Sanchez *et al*., 1997; Uetake *et al*., 1997), wounding (Hush & Overall, 1992) and plant growth substances (Shibaoka, 1991). These events are presumably controlled by a series of complex intracellular signalling pathways which are at present poorly understood (Baskin & Wilson, 1997).

Compatible pollinations in *Brassica* plants have been shown to be accompanied by signalling pathways that involve phosphorylation/dephosphorylation events (Kandasamy *et al*., 1993; Rundle *et al*., 1993) and raised levels of intracellular calcium (Dearnaley *et al*., 1997). Downstream targets of these signalling pathways have yet to be determined, but probably include the transfer of papillar cytoplasmic water to pollen during the hydration response, the initiation of wall loosening, and cytoskeletal rearrangements as outlined above.

We are investigating the cellular and molecular basis of self-incompatibility in *Brassica napus*. One approach to this problem has been to develop a fuller understanding of the processes which are important to successful pollinations. This study aims to elucidate components of the response pathway operating during compatible pollinations in *B. napus*. Actin and MT cytoskeletal organization were compared before and after compatible and SI pollinations to determine whether the papillar cytoskeleton is a downstream component of the compatible pollination pathway.

MATERIALS AND METHODS

Plant material

Self-compatible (cv. Westar, WS) and self-incompatible (SI, cv. W1) *Brassica napus* L. plants were grown in a growth chamber (16 h light, 22°C/8 h dark, 16°C, illuminated by a mix of incandescent and fluorescent sources producing approx. 980 μmol m^{-2} s^{-1}) for 2–3 months.

Pollen hydration and germination frequencies

Pistils were removed from 1–2 d post-anthesis W1 flowers and stuck to glass microscope slides with double-sided tape. To prevent dehydration, the cut end of the pistil was placed into distilled water in the removed end of a small Eppendorf tube. Pollen from dehisced anthers of either the WS cultivar (compatible cross-pollination) or the W1 cultivar (SI-pollination) were placed onto individual stigmatic papillae with a glass micropipette and each pollen grain was then monitored for hydration (see Dearnaley *et al*., 1997) and germination at 30, 60 and 90 min post-pollination. To ascertain that successful penetration of stigmatic papillae had occurred, after 90 min all pollen grains were mechanically agitated with the micropipette. Ungerminated pollen and pollen grains with pollen tubes blocked from entering the papilla wall were easily removed by this method, whereas pollen which had successfully penetrated papillae could not be dislodged. All experiments were conducted at 22–23°C and at 60–70% r.h. A total of 10 stigmas of each combination with an average of nine pollen–papillae interactions per stigma were used for these experiments.

Specimen preparation for electron microscopy

W1 flowers were pollinated by gently brushing dehisced anthers of either the WS cultivar (compatible cross-pollination, three stigmas) or the W1 cultivar (SI-pollination, three stigmas) over the stigma surface. After 90 min at 22–23°C and at 60–70% r.h., the stigmas were removed from the flowers and prepared for electron microscopy following the methods of Kandasamy *et al*. (1994), with the following modifications. After 2.5% glutaraldehyde fixation in 100 mM phosphate buffer, stigmas were encased in low-melting-point agarose (Sigma, St Louis, MO, USA; Type VII low gelling temperature, 1% (w/v) in distilled water) to prevent dislodging of pollen grains, and post-fixed in 1% aqueous OsO_4 for 1 h at 4°C. Dehydration was in a graded acetone series, followed by embedding in Spurr's resin. Sections were post stained for 20 min in uranyl acetate (3% (w/v) in distilled water) and 8 min in Reynolds' lead citrate and examined with a transmission electron microscope.

Labelling of microtubules in papillae

Compatibly (WS pollen) and self-incompatibly (W1 pollen) hand-pollinated W1 stigmas were removed from flowers after 90 min (at 22–25°C and at 60–70% r.h.) and fixed at room temperature for 1.5 h in 3% paraformaldehyde in MT stabilizing buffer (MSB, Hause *et al*., 1993; 100 mM PIPES, 1 mM ethylenglycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM $MgCl_2$ 0.4% (w/v) polyethylene glycol 4000 and 0.05% Triton X-100, pH 6.9). After two rinsing steps in MSB (5 min each), stigmas were placed into Tissue-Tek embedding medium (Miles, Elkhart, IN, USA), frozen in liquid nitrogen and cryosectioned at 8 μm.

Sections were dried down onto glass slides (previously coated with 1 mg ml^{-1} poly-l-lysine in distilled water) and then incubated in mouse anti-human α tubulin (Cedar Lane, Hornby, ONT, Canada; 1:500 in phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.5 mM Na_2HPO_4, pH 7.0) containing 1% BSA) for 1 h at room temperature, rinsed briefly in PBS and then incubated in goat anti-mouse conjugated to fluorescein isothiocyanate (IgG, Cedar Lane, 1:30 in PBS/1% BSA) for 1 h at room temperature. After a rinse in PBS, the sections were stained for 5 min for callose with aniline blue (either 0.03 mg ml^{-1} Sirofluor (Biosupplies Australia, Parkville, VIC, Australia) or 0.1% aniline blue (Fisher Scientific, Toronto, ONT, Canada), both in PBS) to improve identification of pollen tubes. After a final rinse in PBS, the sections were mounted under a coverslip in Citifluor (Marivac, Halifax, NS, Canada). Labelled material was examined with a confocal microscope (BioRad, Mississauga, ONT, Canada) fitted with a krypton-argon laser and a BHS filter block (488 nm excitation filter, 510 nm dichroic mirror, 515 nm emission filter). A × 63 NA 1.4 oil immersion objective was used with a zoom factor of 2–3 and neutral density filters of two (3% transmission) or one (90% transmission) to obtain confocal images of MTs in papillae. Typically, 10–20 *z*-sections, 1 μm apart, were compiled to give the three-dimensional structure of the papilla cytoskeleton.

Labelling of actin microfilaments in papillae

W1 stigmas which had been compatibly (WS pollen) or SI (W1 pollen) hand-pollinated and then left for 90 min (at 22–25°C and 60–70% r.h.) were removed from flowers and pre-treated for 30 min at room temperature in 100 μM *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester, from a stock of 100 mM in dimethyl sulphoxide in stabilizing buffer (SB, Baluska *et al*., 1997; 50 mM PIPES, 5 mM EGTA, 5 mM $MgSO_4$, pH 6.9) to which was added 0.05% Triton X-100 to improve penetration (Sonobe & Shibaoka, 1989). After two brief rinses in SB, stigmas were then fixed in freshly prepared 3.7% paraformaldehyde in SB for 1 h at room temperature. Following this, stigmas were rinsed again in SB and incubated in 1 μM rhodamine phalloidin (RP, Sigma) in PBS for 3 h in the dark at room temperature. After two brief rinses in PBS the stigmas were stained for 5 min in aniline blue, then they were rinsed again in PBS, sliced into three to four sections with a razor blade, squashed flat under a coverslip on a glass microscope slide and mounted in Citifluor. Fluorescently labelled cells were examined with a confocal microscope as already described, except a YHS filter block (568 nm excitation filter, 585 nm dichroic mirror, 585 nm emission filter) was used.

RESULTS

In *B. napus*, inhibition of self-pollen occurs on the stigma surface, which is covered with large numbers of elongated epidermal cells known as papillae. At low relative humidity (normal growth conditions), SI pollen typically fails to hydrate on papillae (Carter & McNeilly, 1975, 1976; Ockendon, 1978; Zuberi & Dickinson, 1985; Dearnaley *et al*., 1997). By using high r.h. the number of SI pollen grains that germinated was increased, and because these cells adhered to papillae, the processes that occurred within papillae during the interaction would be examined.

Pollen hydration and germination frequencies

At 60–70% r.h., both compatible and SI pollen grains initially hydrated and germinated at similar frequencies, but by 90 min the germination rate for the SI interaction was substantially lower (Fig. 1). Most of the germinated compatible pollen tubes also penetrated the papillar walls, as shown by the fact that they were difficult to dislodge, but penetration was very rare for the SI combination (Fig. 1).

Electron microscopy

Loosening of the outer papilla wall, similar to that which has been previously reported for the Brassicaceae (Elleman *et al*., 1988; Doughty *et al*., 1992; Dickinson, 1995) was observed in all compatibly pollinated cells (11 of 11 cells examined). Wall loosening was more easily recognized in interactions where initial penetration of the papilla wall was occurring (Fig. 2a) but it could also be detected in regions in front of pollen tubes growing

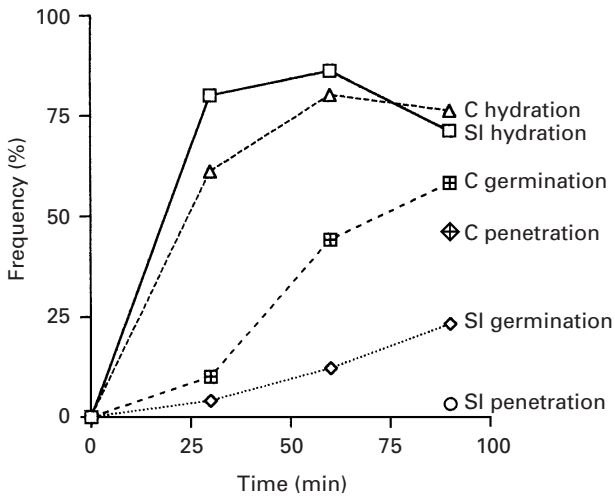

Figure 1. Pollen hydration, germination and penetration rates after compatible (C) and self-incompatible (SI) pollinations of *Brassica napus*. *n* = approx. 90 for each data point.

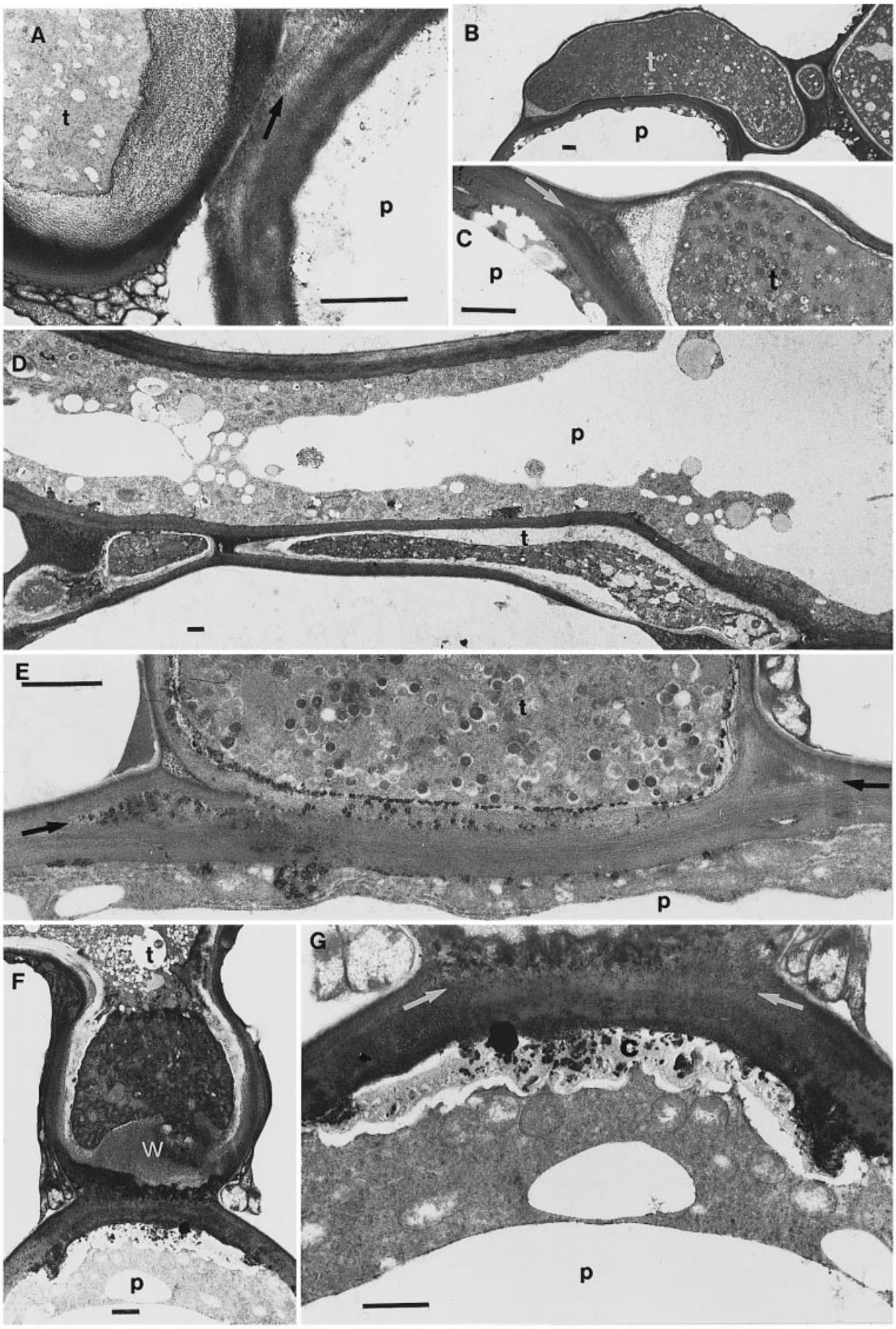

Figure 2. For legend see opposite.

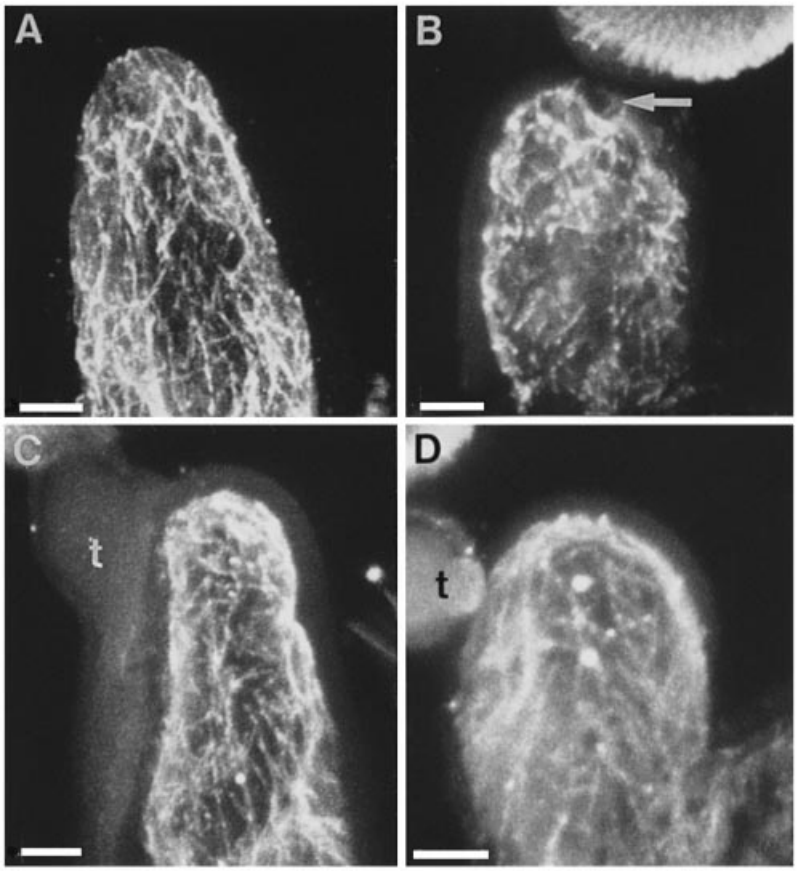

Figure 3. Microtubule (MT) organization in unpollinated (a), compatibly (b,c) and self-incompatibly (d) pollinated papillae of *Brassica napus*. In unpollinated papillae, the MTs were orientated predominantly longitudinally (a). There were no major changes in the organization of MTs in any of the interactions (b–d). The pollen tubes (t) or their position (arrow in b, identified by aniline blue staining, not shown) are indicated. Bars, 5 μm. Depth of cytoplasm included in z-series: a, 12 μm; b, 15 μm; c, 14 μm; d, 15 μm.

within the papilla walls (Fig. 2b,c). In most SI interactions where pollen tubes were in contact with papillae, outer papillar wall loosening was also detected (13 of 14 cells; Fig. 2e,g). The extent of loosening seen in both interactions was less than shown by Elleman *et al*. (1988), possibly due to our liquid fixations which may have caused shrinkage in the loosened walls.

In neither interaction was there any evidence for accumulation of cytoplasm or organelles such as vesicles, ER or Golgi bodies in the papillae adjacent to the pollen tubes (zero of 25 cells; Fig. 2d–g). This observation applied to interactions between both the tips of the pollen tubes (as shown by the accumulation of vesicles characteristic of growing tips) which were at the forefront of the interaction (e.g. Fig. 2a–c, e–g) and sub-apical regions which would have been showing a later stage of interaction (e.g. Fig. 2d).

Callose deposition was seen inside the papillar walls in the region of contact with the pollen tubes in both compatible (six of 11 cells) and SI pollinations (10 of 14 cells, Fig 2f,g). However, the interactions differed in the formation of unusual thickenings of

Figure 2. Electron microscopy of compatible (a–d) and self-incompatible (e–g) interactions between papillae (p) and pollen tubes (t) of *Brassica napus*. a. Wall loosening (arrow) preceding initial penetration of the papillar wall by a pollen tube. b. A pollen tube growing within the papillar wall. c. Higher magnification of the growing tip of the pollen tube seen in (b), showing the loosening of the outer papillar wall (arrow). d. Pollen tube (with artefactually plasmolyzed cytoplasm) growing through the lateral wall of a papilla. The cytoplasm in the papilla is of similar thickness and contains similar populations of organelles both adjacent to the pollen tube and on the opposite (upper) side of the cell. e. The tip of a pollen tube in contact with a papilla, showing wall loosening in the papillar wall (arrows) and the absence of either accumulated cytoplasm or any specific accumulation of organelles and vesicles in the papilla adjacent to the tube tip. f. Tip of a pollen tube containing an abnormal accumulation of wall material (w) and adjacent vesicle-rich cytoplasm which is unusually electron-opaque and thus possibly moribund. g. Detail of (f) showing papillar wall loosening (arrows), callose-like material in the papilla (c) and the absence of any unusual accumulation of vesicles or organelles in the adjacent papillar cytoplasm. Bars, 1 μm.

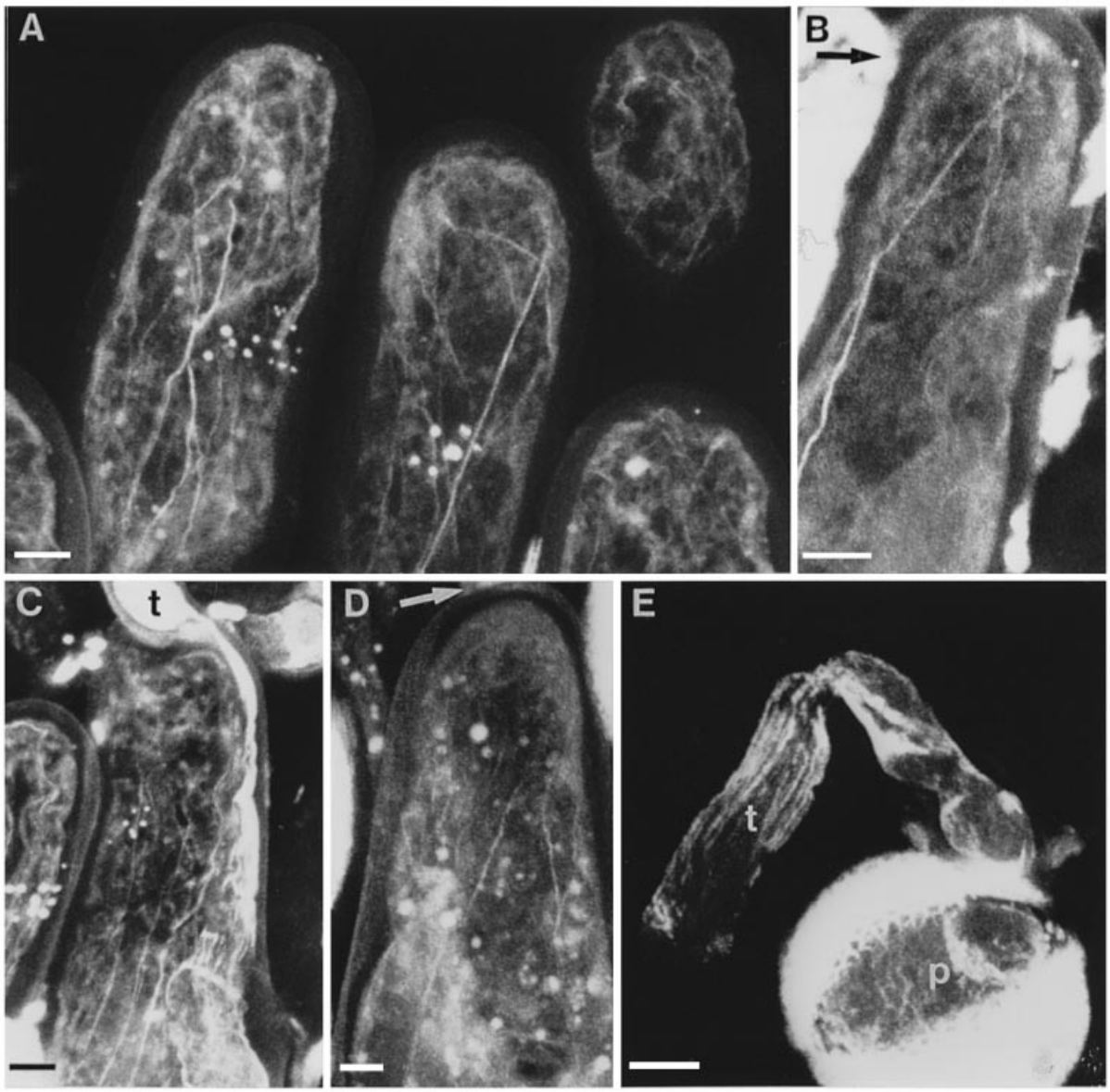

Figure 4. Actin microfilament (MF) organization in unpollinated (a), compatibility (b,c) and self-incompatibly (d) pollinated papillae of *Brassica napus*. In unpollinated papillae the actin cytoskeleton existed as primarily long, longitudinally orientated MFs which were less obvious and more randomly arranged at the tips of the papillae (a). There were no major changes in the organization of MFs in the vicinity of the pollen tubes (arrows in b and d and t in c) in any of the interactions. e. The staining procedure preserved longitudinal MFs in pollen tubes (t) and a more random pattern of finer peripheral filaments in the pollen grains (p). Bars, 5 μm. Depth of cytoplasm included in z-series: a, 9 μm; b, 6 μm; c, 7 μm; d, 12 μm; e, 15 μm.

the pollen tube wall in the tips of some SI pollen tubes (four of 14 cells, Fig. 2f,g). Such deposits were not seen in any compatible pollen tubes.

Microtubule cytoskeleton organization in papillae

In unpollinated papillae, MT arrays were somewhat variable but typically orientated predominantly parallel to the long axes of the cells (Fig. 3a). In the hemispherical tips of the papillae, the MT profiles appeared shorter, no doubt in part because they curve over the tips of the cells. These patterns were too complex to measure, but subjective analysis of the pictures showed no change in the MT arrays in the regions of contact with the pollen grains or tubes in either the compatible or SI interactions at any of the time points analysed (Fig. 3b–d). In these experiments 65 unpollinated papillae (three stigmas), 32 compatibly pollinated papillae (three stigmas) and 17 self-incompatibly pollinated papillae (two stigmas) were examined.

Actin microfilament organization in papillae

Unpollinated papillae contained a variable number of MF bundles which were orientated primarily parallel to the long axis of the cells (Fig. 4a). As with the MTs, these MFs appeared to be less abundant

and more variably orientated in the hemispherical tips of the papillae, where they probably curved over the tips. Again, subjective observations of the pictures showed no detectable changes in either the abundance or orientation of the MFs in the majority of papillae with either compatible or SI pollen grains or tubes associated with them at any of the time points analysed (Fig. 4b–d). The only possible change was that in both compatible (18%) and SI (17%) pollinations, there was a slight increase, relative to the unpollinated papillae (5%), in the number of papillae in which no actin MFs were visible. In addition to the MFs revealed by the RP staining, papillae also contained diffuse staining which may indicate the presence of finer populations of actin which were not resolved into distinct bundles. In these experiments 150 unpollinated papillae (three stigmas, the first 50 papillae observed on each stigma), 57 compatibly pollinated papillae (eight stigmas, all papillae with adherent pollen), and 23 self-incompatibly pollinated papillae (seven stigmas, all papillae with adherent pollen) were examined.

The ability of the RP staining procedure to reveal faithfully MFs with the protocol used was indicated by the revelation of longitudinal MF bundles in pollen tubes, similar to those reported in other plant pollen tubes by Tang *et al*. (1989) and a fine mesh of MFs within pollen grains (Fig. 4e).

In both the MT and MF staining results, it is important to emphasize that the examined and presented images are compiled *z*-series representing approximately half the total thickness of the papillae. Thus it is unlikely that hypothetical changes in organization or abundance of MTs and MFs would have been missed due to the limited depth of field of the confocal microscope. However, because of this depth of displayed cytoplasm, the MFs and MTs do not stand out as clearly as they would in thinner optical sections.

DISCUSSION

These results demonstrate that the papillar MTs and MFs do not show any rearrangements during either compatible or SI interactions, and thus apparently do not play any detectable role in the early stages of interactions of pollen tubes with *B. napus* stigmas. Thus the downstream cellular target of the signalling pathway activated by compatible pollinations (Kandasamy *et al*., 1993; Rundle *et al*., 1993; Dearnaley *et al*., 1997) remains undetermined, but clearly does not involve substantive changes to the papillar cytoskeleton.

A number of interpretations follow from these results. The first is that if wall loosening does involve the secretion of enzymes from the papillae via secretory vesicles, as previously suggested (Elleman & Dickinson, 1996; see also e.g. Dickinson & Lewis, 1975; Roberts *et al*., 1984 for observations of secretory vesicles in papillar cytoplasm in the vicinity of crucifer pollen tubes), such vesicles must presumably already be peripherally located, so that extensive cytoskeleton-based transport from diverse points of origin is unnecessary. This explanation is supported by data suggesting that wall loosening is independent of protein synthesis (Elleman & Dickinson, 1996). However, the lack of obvious residual secretory organelle accumulation under areas of wall loosening in any of the interactions observed in this study is contrary to this hypothesis, but is comparable to previously encountered difficulties in identifying the source of papillar callose-secreting vesicles (Dickinson & Lewis, 1975).

A second, and more likely, explanation for our results is that wall loosening is caused by enzymes derived from the pollen coat or the advancing tube. *Brassica* pollen has been shown to contain a number of potential wall-softening enzyme activities (Albani *et al*., 1991; Hiscock *et al*., 1994). Thus it is possible (a) that SI pollen tubes are deficient in these enzymes; (b) that their activity is reduced; or (c) that they are not activated to the same level as in compatible interactions.

The growth of the pollen tubes within the cell walls of the papillae is initially comparable with interactions between plant cells and fungi or bacteria, but the absence of changes in the cytoskeleton is very different. In both plant pathogen and symbiont invasions, penetration of plant cells by fungal hyphae or bacteria can lead to a range of cytoskeletal responses (Kobayashi *et al*., 1992, 1994; Gross *et al*., 1993; Allen *et al*., 1994; Baluska *et al*., 1995; Sanchez *et al*., 1997; Uetake *et al*., 1997), among which a major theme appears to be that cytoskeletal rearrangements are part of resistance responses through the localized deposition of defence-related materials (Kobayashi *et al*., 1997). A fundamental difference between pollen tube penetration of the papillar wall and plant–microbe interactions is that the pollen tube makes no contact with the papillar plasma membrane. Since the plasma membrane is undoubtedly involved in sensing extracellular activity and is thought to be important in regulating cytoskeletal changes (Zandomeni & Schopfer, 1993), cytoskeletal rearrangements and the initiation of defence responses may have been avoided by the evolution of a system whereby pollen tubes grow only within the papilla wall. Support for this idea comes from the fact that compatible pollen tubes in immature papillae, which grow between the papillar wall and the plasma membrane, are prevented from passing further into the stigma (Elleman & Dickinson, 1990). However, since mechanical damage (Goodbody & Lloyd, 1990; Hush & Overall, 1992) and compressive stress on the cell wall (Zandomeni & Schopfer, 1994) may also control cytoskeletal reorganization in plant cells, it is evident that a

critical part of compatible pollinations may be the bypassing of defence-related mechanisms which lie in wait for pathogens and incompatible and foreign pollen. Further exploration of these mechanisms may explain the cellular and molecular basis of self-incompatibility in *Brassica* species.

In addition to our observations related to the cytoskeleton, our results make a number of other contributions to the compatibility issue. For example, our observation that raised humidity overcomes the SI hydration block previously reported for this (Dearnaley *et al*., 1997) and other (Carter & McNeilly, 1975, 1976; Ockendon, 1978; Zuberi & Dickinson, 1985) species is further evidence that self-incompatibility in *Brassica* species has numerous checkpoints (Heslop-Harrison, 1975). It also suggests that the involvement of the aquaporin-like protein in self-incompatibility in *Brassica* species (Ikeda *et al*., 1997) may only operate at low relative humidities and that other control mechanisms function in other environmental conditions. Blocking incompatible pollen tubes from penetrating papillae may be the next step in this process. However, our data showing that some SI pollinations overcome this barrier by eliciting wall loosening indicate that further checkpoints are also involved. These could include inhibition of tube growth in the style, similar to gametophytic self-incompatibility systems (Dzelzkalns *et al*., 1992).

The papilla callose-like depositions observed in this study after both pollination types are consistent with our recent observations at the light microscope level (Dearnaley *et al*., 1997), and reinforce the concept that callose formation and *Brassica* self-incompatibility are independent phenomena (Elleman & Dickinson, 1996). However, to our knowledge, this is the first report of apical pollen tube wall thickening during SI interactions. Picton & Steer (1983) present strikingly similar images in *Tradescantia* pollen tubes which have altered wall vesicle transport when grown at abnormally high Ca^{2+} levels. Although SI pollinations typically do not involve increases in papillar Ca^{2+} (Dearnaley *et al*., 1997), Singh *et al*. (1989) have shown that the level of pollen Ca^{2+} increases during SI interactions. It is probable than, that these pollen wall thickenings represent an impairment of normal wall deposition caused by an elevation in pollen tube Ca^{2+}, the source of which remains unclear.

Despite equivalent hydration frequencies in the two pollen–stigma interactions used, the germination frequency of SI pollen is considerably lower than the compatible interaction. Since it is unlikely that SI pollen fails to hydrate fully under these conditions of high relative humidity, blockage of incompatible pollen germination supports the existence of a papilla-derived germination inhibitor as previously suggested (Ferrari & Wallace, 1976; Hodgkin & Lyon, 1986; Sarker *et al*., 1988). However, since Dickinson & Elleman (1994) present contrary evidence, the basis of the lower germination rates awaits further study.

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REFERENCES

Albani D, Altosaar I, Arnison PG, Fabijanski SE. 1991. A gene showing sequence similarity to pectin esterase is specifically expressed in developing pollen of *Brassica napus*. Sequences in its 5′ flanking region are conserved in other pollen-specific promoters. *Plant Molecular Biology* **16**: 501–513.

Allen NS, Bennett MN, Cox DN, Shipley A, Ehrhardt DW, Long SR. 1994. Effects of Nod factors on alfalfa root hair Ca^{2+} and H^+ currents and on cytoskeletal behaviour. In: Daniels MJ, Downie JA, Osborne AE, eds. *Advances in molecular genetics of plant–microbe interactions*, *Vol. 3*. Dordrecht, The Netherlands: Kluwer Academic, 107–113.

Baluska F, Bacigalova K, Oud JL, Hauskrecht M, Kubica S. 1995. Rapid reorganization of microtubular cytoskeleton accompanies early changes in nuclear ploidy and chromatin structure in postmitotic cells of barley leaves infected with powdery mildew. *Protoplasma* **185**: 140–151.

Baluska F, Vitha S, Barlow PW, Volkmann D. 1997. Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: a major developmental switch occurs in the postmitotic transition region. *European Journal of Cell Biology* **72**: 113–121.

Baskin TI, Wilson JE. 1997. Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules. *Plant Physiology* **113**: 493–502.

Bonfante-Fasolo P. 1992. Plant–fungal interface in VA mycorrhizas: a structural point of view. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, eds. *Mycorrhizas in ecosystems*. Wallingford, UK: CAB International, 340–347.

Carter AL, McNeilly T. 1975. Effects of increased humidity on pollen growth and seed set following self-pollination in Brussels sprout (*Brassica oleracea* var. *gemmifera*). *Euphytica* **24**: 805–813.

Carter AL, McNeilly T. 1976. Increased atmospheric humidity post pollination: a possible aid to the production of inbred line seed from mature flowers in Brussels sprout (*Brassica oleracea* var. *gemmifera*). *Euphytica* **25**: 531–538.

Dearnaley JDW, McGee PA. 1996. An intact microtubule cytoskeleton is not necessary for interfacial matrix formation in orchid protocorm mycorrhizas. *Mycorrhiza* **6**: 175–180.

Dearnaley JDW, Levina NN, Lew RR, Heath IB, Goring DR. 1997. Interrelationships between cytoplasmic Ca^{2+} peaks, pollen hydration and plasma membrane conductances during compatible and incompatible pollinations of *Brassica napus* papillae. *Plant and Cell Physiology* **38**: 985–999.

Derksen J, Emons AMC. 1990. Microtubules in tip growth systems. In: Heath IB, ed. *Tip growth in plant and fungal cells*. Sand Diego, CA, USA: Academic Press, 147–181.

Dickinson HG. 1995. Dry stigmas, water and self-incompatibility in *Brassica*. *Sexual Plant Reproduction* **8**: 1–10.

Dickinson HG, Elleman CJ. 1994. Pollen hydrodynamics and self-incompatibility in *Brassica oleracea*. In: Stephenson AG, Kao T-H, eds. *Pollen–pistil interactions and pollen tube growth*. Rockville: American Society of Plant Physiologists, 45–61.

Dickinson HG, Lewis D. 1975. Interaction between the pollen grain coating and the stigmatic surface during compatible and incompatible intraspecific pollinations in *Raphanus*. In: Duckett JG, Racey PA, eds. *The biology of the male gamete*. London, UK: Academic Press, 165–175.

Doughty J, McCubbin A, Hedderson F, Elleman CJ, Dickinson HG. 1992. The role of the pollen grain coating in pollination and self-incompatibility in *Brassica oleracea*. In: Ottoviano E, Mulcahy D, Sari Gorla M, Bergamini Mulcahy G, eds. *Angiosperm pollen and ovules*. Berlin, Germany: Springer, 55–65.

Dzelzkalns VA, Nasrallah JB, Nasrallah ME. 1992. Cell–cell communication in plants: self-incompatibility in flower development. *Developmental Biology* **153**: 70–82.

Elleman CJ, Dickinson HG. 1990. The role of the exine coating in pollen–stigma interactions in *Brassica oleracea* L. *New Phytologist* **114**: 511–518.

Elleman CJ, Dickinson HG. 1996. Identification of pollen components regulating pollination-specific responses in the stigmatic papillae of *Brassica oleracea*. *New Phytologist* **133**: 1297–205.

Elleman CJ, Willson CE, Sarker RH, Dickinson HG. 1988. Interaction between the pollen tube and the stigmatic cell wall following pollination in *Brassica oleracea*. *New Phytologist* **109**: 111–117.

Elleman CJ, Franklin-Tong V, Dickinson HG. 1992. Pollination in species with dry stigmas: the nature of the early stigmatic response and the pathway taken by pollen tubes. *New Phytologist* **121**: 413–424.

Ferrari TE, Wallace DH. 1976. Pollen protein synthesis and control of incompatibility in *Brassica*. *Theoretical and Applied Genetics* **48**: 243–249.

Goodbody KC, Lloyd CW. 1900. Actin filaments line up across *Tradescantia* epidermal cells, anticipating wound-induced division planes. *Protoplasma* **157**: 92–101.

Gross P, Julius C, Schmelzer E, Hahlbrock K. 1993. Translocation of cytoplasm and nucleus to fungal penetration sites is associated with depolymerization of microtubules and defence gene activation in infected, cultured parsley cells. *EMBO Journal* **12**: 1735–1744.

Hause B, Hause G, Pechan P, Van Lammeren AAM. 1993. Cytoskeletal changes and induction of embryogenesis in microspore and pollen cultures of *Brassica napus* L. *Cell Biology International Reports* **17**: 153–168.

Heslop-Harrison J. 1975. Incompatibility and the pollen–stigma interaction. *Annual Review of Plant Physiology* **26**: 403–425.

Hiscock SJ, Dewey FM, Coleman JOD, Dickinson HG. 1994. Identification and localisation of an active cutinase in the pollen of *Brassica napus* L. *Planta* **193**: 377–384.

Hodgkin T, Lyon GD. 1986. The effect of *Brassica oleracea* stigma extracts on the germination of *B. oleracea* pollen in a thin layer chromatographic bioassay. *Journal of Experimental Botany* **37**: 406–411.

Hush JM, Overall RL. 1992. Re-orientation of cortical F-actin is not necessary for wound-induced microtubule re-orientation and cell polarity establishment. *Protoplasma* **169**: 97–106.

Ikeda S, Nasrallah JB, Dixit R, Preiss S, Nasrallah ME. 1997. An aquaporin-like gene required for the *Brassica* self-incompatibility response. *Science* **276**: 1564–1566.

Kandasamy MK, Thorness MK, Rundle SJ, Goldberg ML, Nasrallah JB, Nasrallah ME. 1993. Ablation of papilla cell function in *Brassica* flowers results in the loss of stigma receptivity to pollination. *Plant Cell* **5**: 263–275.

Kandasamy MK, Nasrallah JB, Nasrallah ME. 1994. Pollen–pistil interactions and developmental regulation of pollen tube growth in *Arabidopsis*. *Development* **120**: 3405–3418.

Kobayashi I, Kobayashi Y, Yamaoka N, Kunoh H. 1992. Recognition of a pathogen and a non pathogen by barley coleoptile cells. III. Responses of microtubules and actin filaments in barley coleoptile cells to penetration attempts. *Canadian Journal of Botany* **70**: 1815–1823.

Kobayashi I, Kobayashi Y, Hardham AR. 1994. Dynamic reorganisation of microtubules and microfilaments in flax cells during the resistance response to flax rust infection. *Planta* **195**: 237–247.

Kobayashi Y, Kobayashi I, Funaki Y, Fujimoto S, Takemoto T, Kunoh H. 1997. Dynamic reorganisation of microfilaments and microtubules is necessary for the expression of non-host resistance in barley coleoptile cells. *Plant Journal* **11**: 525–537.

Kristen U, Lockhausen J. 1983. Estimation of golgi membrane flow rates in ovary glands of *Aptenia cordifolia* using cytochalasin B. *European Journal of Cell Biology* **29**: 262–267.

Nakanishi T, Hinata K. 1975. Self-seed production by CO_2 gas treatment in self-incompatible cabbage. *Euphytica* **24**: 117–121.

Ockendon DJ. 1972. Pollen tube growth and the site of incompatibility reaction in *Brassica olereacea*. *New Phytologist* **71**: 519–522.

Ockendon DJ. 1973. Selection for high self-incompatibility in inbred lines of Brussels sprouts. *Euphytica* **22**: 503–509.

Ockendon DJ. 1978. Effect of hexane and humidity on self-incompatibility in *Brassica oleracea*. *Theoretical and Applied Genetics* **52**: 113–117.

Picton JM, Steer MW. 1983. Evidence for the role of Ca^{2+} ions in tip extension in pollen tubes. *Protoplasma* **115**: 11–17.

Roberts IN, Harrod G, Dickinson HG. 1984. Pollen–stigma interactions in *Brassica oleracea*. I. Ultrastructure and physiology of the stigmatic papillar cells. *Journal of Cell Science* **66**: 241–253.

Rundle SJ, Nasrallah ME, Nasrallah JB. 1993. Effects of inhibitors of protein serine/threonine phosphatases on pollination in *Brassica*. *Plant Physiology* **103**: 1165–1171.

Sanchez F, Quinto C, Cardenas L, Guillen G, Villanueva M, Hepler PK, Vidali L. 1997. Intracellular calcium changes, actin microfilament rearrangements and differential expression of actin cytoskeleton genes in the *Phaseolus vulgaris–Rhizobium* symbiotic interaction. *Plant Physiology* (Suppl.) **114**: 38.

Sarker RH, Elleman CJ, Dickinson HG. 1988. Control of pollen hydration in *Brassica* requires continued protein synthesis, and glycosylation is necessary for intraspecific incompatibility. *Proceedings of the National Academy of Sciences, USA* **85**: 4340–4344.

Shibaoka H. 1991. Microtubules and the regulation of cell morphogenesis by plant hormones. In: Lloyd CW, ed. *The cytoskeletal basis of plant growth and form*. London, UK: Academic Press, 159–168.

Singh A, Perdue TD, Paolillo DJ Jr. 1989. Pollen–pistil interactions in *Brassica oleracea*: cell calcium in self and cross pollen grains. *Protoplasma* **151**: 57–61.

Sonobe S, Shibaoka H. 1989. Cortical fine actin filaments in higher plant cells visualized by rhodamine–phalloidin after pretreatment with m-maleimidobenzoyl *N*-hydroxysuccinimide ester. *Protoplasma* **148**: 80–86.

Steer MW. 1988. The role of calcium in exocytosis and endocytosis in plant cells. *Physiologia Plantarum* **72**: 213–220.

Tang X, Lancelle SA, Hepler PK. 1989. Fluorescence microscopic localization of actin in pollen tubes: comparison of actin antibody and phalloidin staining. *Cell Motility and Cytoskeleton* **12**: 216–224.

Uetake Y, Farquhar ML, Peterson RL. 1997. Changes in microtubule arrays in symbiotic orchid protocorms during fungal colonization and senescence. *New Phytologist* **135**: 701–709.

Williamson RE. 1993. Organelle movements. *Annual Reviews of Plant Physiology and Plant Molecular Biology* **44**: 181–202.

Zandomeni K, Schopfer P. 1993. Reorientation of microtubules at the outer epidermal wall of maize coleoptiles by phytochrome, blue-light photoreceptor, and auxin. *Protoplasma* **173**: 103–112.

Zandomeni K, Schopfer P. 1994. Mechanosensory microtubule reorientation in the epidermis of maize coleoptiles subjected to bending stress. *Protoplasma* **182**: 96–101.

Zuberi MI, Dickinson HG. 1985. Pollen–stigma interactions in *Brassica*. III. Hydration of the pollen grains. *Journal of Cell Science* **76**: 321–336.