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GERMINATION OF CORYLUS AVELLANA L. (HAZEL) POLLEN: HYDRATION AND THE FUNCTION OF THE ONCUS

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

[1] Welsh Plant Breeding Station, Plas Gogerddan, nr. Aberystwyth SY23 3EB, United Kingdom
[2] Cytogenetics Dept., Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, United Kingdom

SUMMARY

As reported by SCHOCH-BODMER fifty years ago, the onci play an important part in accommodating volume changes in the 3-porate pollen of *Corylus avellana* L. during cycles of dehydration and rehydration. The onci, which are rich in pectins judging from their cytochemical properties, possess a remarkable fine-structure, characterised by densely packed, convoluted lamellae. Staining responses suggest that the protein content of the body of the oncus is low, although esterase activity is distributed throughout.

During germination a granular sporopollenin seal at the preferred aperture is disrupted by the gelatinisation of underlying pectins, and the pollen tube emerges as an outgrowth from the inner cellulosic layer of the intine. This process is preceded by the release of proteins from each aperture site. The source of this outflow is a protein-rich umbonate cap, 1–3 μm in thickness, lying beneath the pore over the body of the oncus and extending under the encompassing exine annulus. The nexine is absent from the annulus, so that the apertural proteins are continuous with the interbacular material. This implies that in this pollen type there are no clearly separate "gametophytic" and "sporophytic" domains.

As in species of Compositae and Cruciferae with a similar sporophytic type of self-incompatibility (SI) system, incompatible pollen of *C. avellana* is inhibited on the stigma surface, the stigma papillae responding rapidly to the presence of the incompatible pollen by synthesis of internal callose. This suggests that the *S*-factors are held in the pollen wall, and it is possible that they form one component of the poral proteins. In the light of recent reports of sporophytic proteins held at the apertural sites in various angiosperm pollens, the possibility is considered that the poral proteins of *C. avellana* are also of sporophytic origin, at least in part. Should they contain tapetum-derived *S*-factors a ready explanation of the sporophytic control of the SI system is at hand.

1. INTRODUCTION

THOMPSON (1979a, b) reported that the self-incompatibility (SI) system of *Corylus avellana* L. (Betulaceae; Corylaceae, *apud* HUTCHINSON 1973) is of the sporophytic type, controlled by a single multiallelic *S*-locus, and the finding has been confirmed by GERMAIN et al. (1981). This discovery adds the first truly arboreal family to the small number in which a sporophytic system is known, namely Cruciferae, Compositae and possibly Convolvulaceae (DE NETTANCOURT 1977).

Notwithstanding the similarity of the genetical basis, the SI system of *C. avellana* does not conform in all respects with that of other families with sporophytic

control of pollen behaviour. Whereas in Cruciferae and Compositae the pollen is tricellular, *C. avellana* has bicellular pollen, a circumstance that breaks the correlation between pollen cytology and SI system first noted by BREWBAKER (1957). Moreover, the pollen is potentially long-lived as in other species with bicellular pollen (PISKORNIK et al. 1975), whereas the tricellular pollen of many species with the sporophytic type of SI system tends to be short-lived under normal storage conditions (HOEKSTRA & BRUINSMA 1975). In one notable feature, however, *C. avellana* is quite typical: as in other sporophytic SI systems, the inhibition of incompatible pollen occurs at or very near the stigma surface (THOMPSON 1971, 1979b, GERMAIN et al. 1981; CHO et al. 1982). In Cruciferae and Compositae, the blockage of self-pollen is generally accompanied by the deposition of callose in the contiguous cells of the stigma, whereas callose deposition is not induced in the stigma by compatible pollen (DICKINSON & LEWIS 1973a, b, KNOX 1973, J. HESLOP-HARRISON, et al. 1974, HOWLETT, et al. 1975, KERHOAS, et al. 1983). The speed and site of the stigma reaction suggest that it is induced by surface constituents of the pollen grain, and this interpretation receives support from the fact that callose deposition can be induced by eluates from incompatible pollen (DICKINSON & LEWIS 1973b, J. HESLOP-HARRISON et al. 1974; review, SHIVANNA & JOHRI 1985). In *C. avellana,* incompatible pollen produces a characteristic callose rejection reaction in the stigma, and in this species also pollen eluates induce the response (*figs. 1A* and *B; fig. 2*).

In Cruciferae and Compositae the cavities of the pollen exine convey substantial amounts of tapetum-derived (and thus sporophytically-synthesised) material in the cavities of the pollen exine, and this has been implicated in the surface rejection reaction of the SI system (J. HESLOP-HARRISON 1968, DUMAS & KNOX 1983, KNOX 1984). In the course of a study of the pollen-stigma interaction in *C. avellana* we have examined the structure of the pollen with the aim of determining the likely sources of the factors concerned in the SI response, and of tracing any parallels with the Compositae and Cruciferae. The present paper summarizes some aspects of the behaviour of the pollen during hydration and germination, and describes a form of protein storage in the pollen grain wall that has no obvious parallels in other families with a sporophytic SI system.

2. MATERIALS AND METHODS

The observations were made on pollen of plants from natural populations in west Wales during January to March in the years 1984, 1985 and 1986. Twigs bearing closed catkins were brought into the laboratory and kept in an ambient temperature of 19–22 °C in an atmosphere with 45–60% RH. Pollen was collected as required from freshly dehiscing anthers. For the various pre-treatments, pollen was transferred to polystyrene boxes with regulated RH, monitored by hair hygometers (Fischer) calibrated against a digital RH meter (Electrothermal Instruments, Surrey) with an error factor of $\pm$ 3%.

Satisfactory germination and pollen-tube growth was obtained both in liquid and on semi-solid media. The liquid medium contained 10^{-3} M H_3BO_3, 0.5×10^{-3}

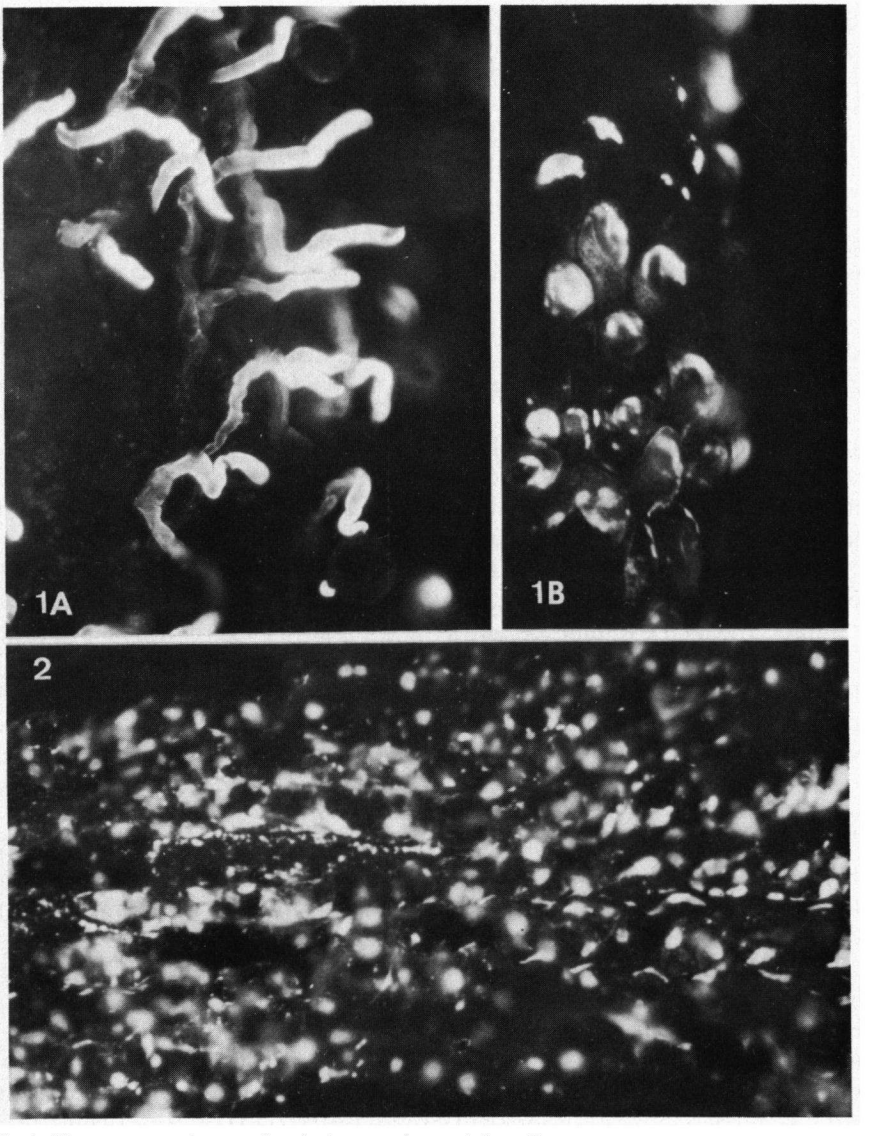


Fig. 1. Fluorescence micrographs of stigma surfaces of *C. avellana* following compatible and incompatible pollinations, DAB staining for callose. × *c.* 250. A. Compatible pollination. The pollen has germinated freely, and the tubes are penetrating the stigma surface. No callose is present in the stigma cells. B. Incompatible pollination. The applied pollen has failed to germinate, and most of the grains have become detached. The low papillae of the stigma surface have responded to the presence of the grains by the production of internal callose.

Fig. 2. Fluorescence micrograph of the surface of a stigma 3 h after immersion in an eluate from incompatible pollen, DAB staining. Essentially every papilla has responded by the production of internal callose. Control stigmas exposed to eluate from compatible pollen gave no comparable response. × *c.* 180.

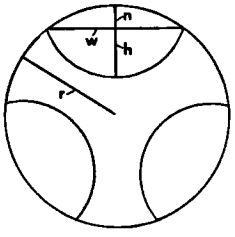


Fig. 3. Diagram of the pollen grain of *C. avellana* as seen in polar view, showing the dimensions measured for the calculation of grain, oncus and protoplast volume.

M $Ca(NO_3)_2$ and 10% sucrose. The pollen was dispersed in 0.5–1 ml medium in 3 ml vials, and incubated at the required temperature with constant aeration on a rotator at *c.* 80 rev/min. Samples were withdrawn as required to follow the course of germination and tube growth. The semi-solid medium was of the same composition as the liquid with the addition of 0.5% agar (BDH). The medium was cast in petri dishes at a thickness of 1–1.5 mm, and the pollen was sown on small segments cut out with coverslips and inverted onto microscope slides.

Pollen and pollen-tube dimensions were measured with a camera lucida system with microcomputer and digitiser. Because of the irregular shape of desiccated grains (*fig. 16A*), volume estimates could not be made for pollen in this state. For pollen in various stages of hydration, volumes were computed by treating the grain as a sphere and the three onci as each composed of paired, base-to-base spherical segments. For this purpose the measurements shown in *fig. 3* were made on grains in polar view. Since the grain is not a true sphere, and since the inner part of the oncus is not necessarily a true spherical segment, this method obviously involves some approximation.

The state of the membranes of the vegetative cell was assessed by the fluorochromatic (FCR) reaction. The medium was prepared by the semi-empirical method originally described (J. HESLOP-HARRISON & HESLOP-HARRISON 1970), with approximately 10^{-6} M fluorescein diacetate in 10% sucrose.

Emissions from hydrating and germinating pollen were followed by suspending samples in drops of germination medium on microscope slides at room temperature and infiltrating the required stains as appropriate. To trace the passage of gelatinising pectin from the germination apertures, a small amount of carbon black was added to the medium; emerging material displaces the carbon particles, rendering the diffusion shell visible (J. HESLOP-HARRISON 1979b).

Pollen samples for optical microscopy were fixed in 2% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2 with 10% sucrose for 2–4 h at room temperature, washed in buffer and then in water, dehydrated through an ethanol series, and embedded in HEMA resin (TAAB Laboratories, Reading). Sections were cut at 1–2 μm with glass knives. Continuous observation during the course of processing showed that the grains underwent some shrinkage. No satisfactory method has been found to preserve pollen in a fully hydrated state comparable to that in the sample of *fig. 20*.

The main staining procedures for intact and sectioned pollen were as follows. (a) Calcofluor white (0.001% aqueous) as a fluorochrome for β-1,4- and mixed β-1,3- and β-1,4-linked glucans (MAEDA & ISHIDA 1976); (b) alcian blue 8GX (1% in 3% acetic acid) for the broad class of polyanionic wall polysaccharides, referred to hereafter simply as pectins (SCOTT et al. 1964); (c) decolourised aniline blue (0.05% at pH 11) for callose (LINSKENS & ESSER 1957); (d) the fluorochrome auramine 0 (0.01% aqueous), and basic fuchsin (0.01% aqueous) for sporopollenin (Y. HESLOP-HARRISON 1977; FAEGRI & IVERSEN 1964), and (e) Coomassie blue R250 (0.01% in 7% acetic acid and 30% ethanol) for protein.

Pollen for electron microscopy was fixed for 2–3 h in 2% glutaraldehyde in 0.05 M phosphate buffer at pH 7.0 without subsequent osmication, dehydrated through an alcohol series and embedded in Spurr's resin. Sections were stained with uranyl acetate by the standard procedure, and also in phosphotungstic acid (PTA), 1% in 10% HCl, for periods of 5–15 min. This post-staining procedure imparts electron density to pectin-rich wall components (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1982b).

Esterase activity was detected in intact pollen with α-naphthyl acetate as a substrate and tetrazotised o-anisidine as a coupling agent (PEARSE 1972).

Intine "ghosts" were prepared from unhydrated and germinating pollen by the method described previously (J. HESLOP-HARRISON 1979b). Pollen samples were suspended in diethanolamine at approximately 90 °C for 30–90 min to remove the exine and wall pectins (BAILEY 1960, BOUVENG 1965). The digest was then diluted with several volumes of water and the grains recovered by centrifugation. They were then washed, suspended in N NaOH, and held just below boiling point until the cell contents had been removed. The sample was then recovered, washed, and suspended in calcofluor white (0.001% aqueous) for *c.* 5 min before washing again and concentration by centrifugation for observation by fluorescence microscopy. The procedure isolates and renders visible the microfibrillar cellulosic layers of the intine and the pollen tube wall (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1982a).

3. RESULTS

3.1. Germination: the hydration requirement

Earlier observations showed that the germinability of pollen of *C. avellana* in the partly desiccated state is low and variable, but that higher levels are consistently attained after pre-hydration in an atmosphere of 90–95% RH (J. HESLOP-HARRISON & Y. HESLOP-HARRISON 1985). The time course of pollen germination at two temperatures is shown in *fig. 4;* in each instance, the pollen sample was pre-hydrated for 150 min and sown on semi-solid medium. The sample at 27 °C achieved a higher initial germination rate, but in each culture over 95% had germinated after 2 h. This is near the upper end of the range encountered amongst the genotypes of the natural population tested, the minimum observed being 68%. The effect of pre-hydrating the pollen for varying times is seen in *fig. 5.* In this and other experiments it was established that with desiccated pollen

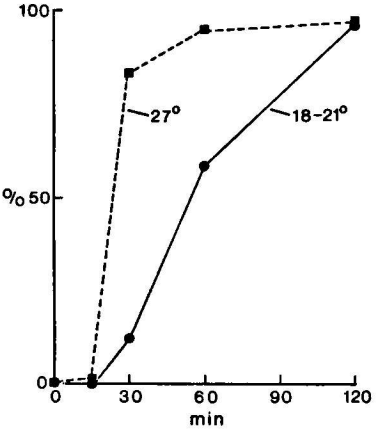


Fig. 4. Cumulative germination curves for *C. avellana* pollen in the standard liquid medium at 19–21 °C and at 27 °C. Pollen pre-hydrated for 150 min at *c.* 20 °C in an atmosphere with 90–95% RH.

the optimum period lies in the range 2–3 h in an ambient temperature of 19–21 °C, germinability falling with longer exposure to the humid atmosphere. No consistent differences were observed between the levels of germination obtained with pre-hydrated pollen on semi-solid and in the aerated liquid media.

Notwithstanding the simplicity of the standard liquid medium, the high values obtained suggested that the composition was near optimal at least for germination. All components of the medium were found to be necessary to sustain the maximal level. In a comparison in which 82% germination was obtained in the full liquid medium at a temperature of 19–20 °C, germination of 47.7% was observed with a medium lacking calcium, and 63.6% in the absence of boron. With sucrose alone (10%) the germination was 11.7%. No germination was obtained

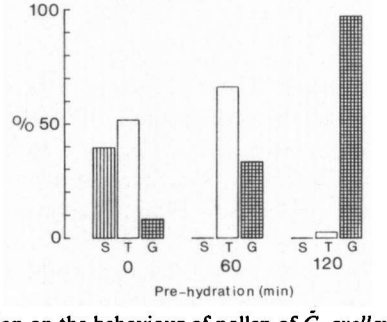
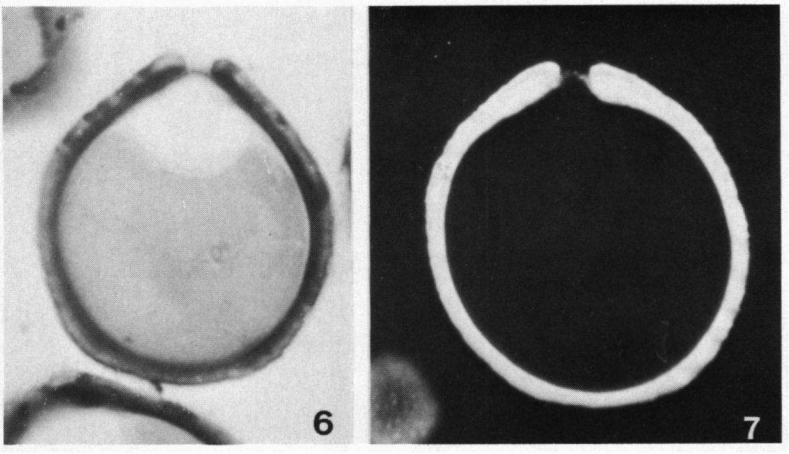


Fig. 5. Effect of pre-hydration on the behaviour of pollen of *C. avellana*. Pollen was held at 20 °C in an atmosphere with 90–95% RH for the periods shown, and then transferred to the standard liquid medium for 2 h.

Samples of 200–300 grains were recorded in three categories: S, shrunken, onci conspicuous (*fig. 19*); T, turgid, onci flattened (*fig. 20*); G, germinated.



Figs. 6 and 7. Optical micrographs of 1–1.5 μm sections of pollen of *C. avellana*, cut in the equatorial plane and showing one of the three onci. HEMA embedment, × *c.* 1500. Fig. 6. Acid fuchsin staining, differentiating the outer (sexine) and inner (nexine) strata of the exine. A thin granular cap of sporopollenin overlies the oncus. Fig. 7. As *fig. 6*, fluorescence micrograph, auramine 0 staining.

in the absence of sucrose.

3.2. Pollen-wall cytochemistry, and the effect of hydration

The pollen exine of *C. avellana* is of the common dicotyledonous 3-porate type. The diameter of the approximately circular apertures ranges between 2.7 and 3.2 μm, depending on the state of hydration of the grains. Sexine and nexine stain differentially with basic fuchsin (*fig. 6*). The sexine is baculate in the non-apertural exine, and the apertures are encompassed by a slightly raised annulus. Each aperture is initially closed by a granulate layer of sporopollenin, distinguishable light-microscopically with both basic fuchsin and auramine 0 staining (*figs. 6* and *7*).

Desiccated pollen transferred to the standard liquid medium expands initially, and then over a period of 60–90 min condenses into the state seen in *fig. 19*, in which the three onci are extremely conspicuous. Differential interference contrast (DIC) shows that in pollen fixed in this state the body of each oncus has a distinct substructure, with an inner zone distinguished by radially oriented striations and an outer more homogenous region, somewhat lenticular in form (*fig. 8*). In intact grains suspended in germination medium containing alcian blue, this outer pectic zone, and the thin layer lying beneath the nexine with which it is continuous, initially take up the dye more freely; but ultimately the whole body of the oncus stains (*fig. 18*).

The inner cellulosic layer of the intine is notably thickened under each oncus (*fig. 25*). Away from the aperture sites it extends as a thinner stratum beneath the pectic layer underlying the nexine. The continuity of the cellulosic stratum of the intine over the whole surface of the vegetative cell is well illustrated in

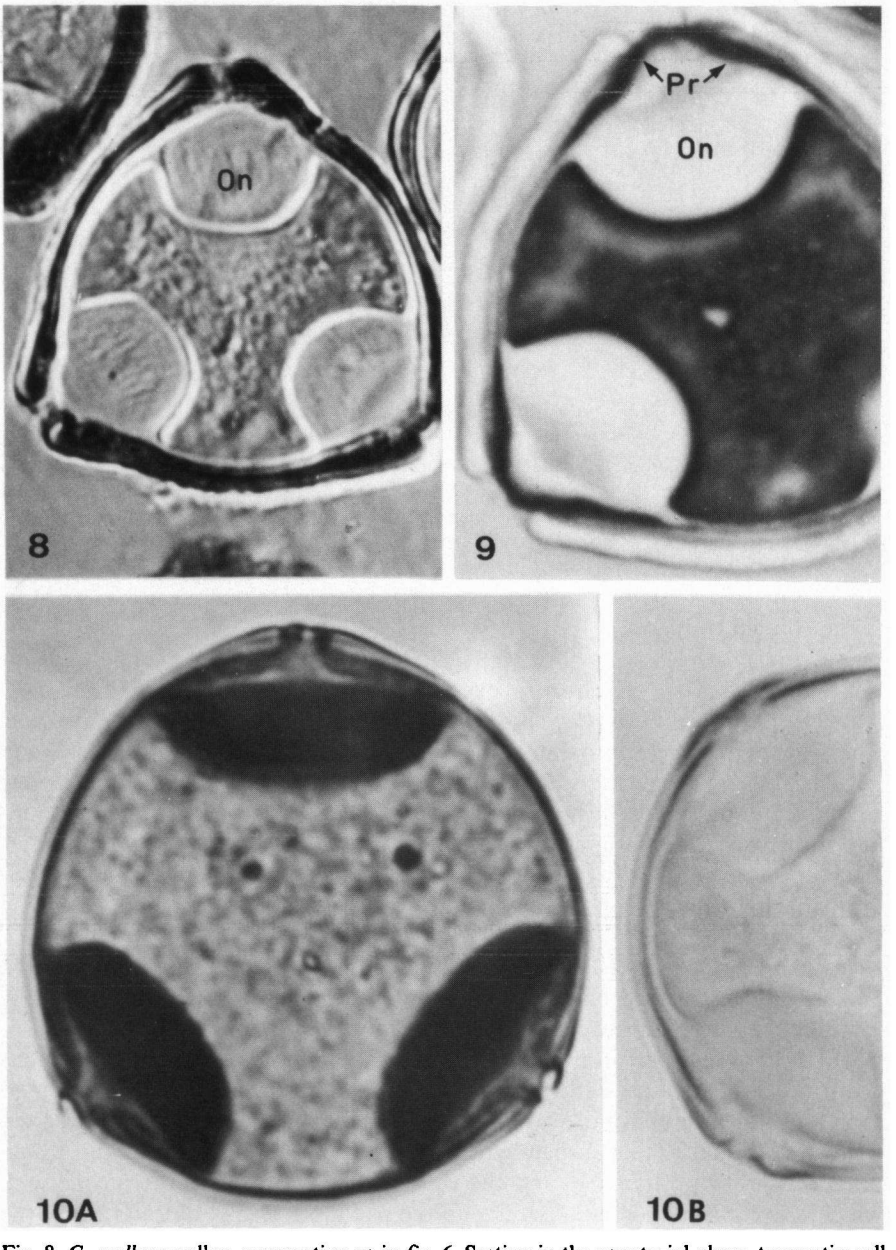


Fig. 8. *C. avellana* pollen, preparation as in *fig. 6*. Section in the equatorial plane, transecting all three onci. DIC, unstained. × *c.* 2100.

Fig. 9. As *fig. 8*, Coomassie blue staining. On, oncus; Pr, protein layer forming an umbonate cap below the aperture. × *c.* 2550.

Fig. 10A. Intact pollen grain, esterase localisation. The reaction product is distributed throughout the inner part of each oncus, but no activity is associated with the protein caps visible in *fig. 9*. × *c.* 2600.

Fig. 10B. Esterase control; reaction mixture less substrate. × *c.* 2600.

ghosts from which the exine, the pectic components of the wall and the cell contents have been removed (*fig. 26*).

The distribution of protein as observed in fixed and sectioned pollen is shown in *fig. 9*. The most significant feature is that the highest concentration, as judged by Coomassie blue staining, occurs in an umbonate cap, 1–3 μm in thickness, overlying the main body of each oncus. As may be seen in *fig. 9*, these protein-bearing caps are linked by a thin layer running beneath the nexine in the non-apertural parts of the wall. The body of the oncus shows far less affinity for the protein stain.

The location of non-specific esterase activity is seen in *fig. 10*. The reaction product is distributed throughout the inner part of each oncus, but little activity is associated with the protein-bearing caps clearly defined in *fig. 9*, nor with the homogeneous outer zone of the oncus.

3.3. Fine structure of the apertural exine and oncus.

The electron micrograph of *fig. 11* shows the lip of the exine annulus surrounding an aperture in profile, with the subadjacent protein cap and the underlying oncus. The striking feature is that the full stratification of the exine, with tectate sexine, baculate layer and nexine, which is present over the non-apertural parts of the grain and can be seen on the right of the micrograph, is reduced in the vicinity of the apertures by the elimination of the nexine. *Figs. 12* and *13* illustrate this feature in more detail. They reveal that the bacula, while linking to the nexine in the normal way in the non-apertural exine, end blindly in the protein cap near the apertures. The near-tangential section of *fig. 14* illustrates the point still more clearly, showing that the protein-reacting material under the aperture is continuous with that extending between the bacula over the rest of the grain.

The inner oncus and the adjacent cellulosic layer of the intine are seen in *fig. 15*. PTA-staining consistently reveals a highly distinctive fine-structure in the pectin of this zone of the oncus, with a dense and complex system of ramifying lamellae, or possibly tubules. Thin-sections do not show any structural feature that might contribute to the striate appearance so evident in DIC micrographs (*fig. 8*), and the zone does not show birefringence.

As *fig. 15* shows, the unique fine-structure of the pectins of the body of the oncus differentiates it quite clearly from the inner microfibrillar cellulosic layer, which is thickened at the apertural sites (cf. *fig. 25*).

3.4. Effects of pre-hydration

Figs. 16A, 16B and *16C* show the states of the pollen after 12 h desiccation, and at 30 min and 60 min following transfer to an atmosphere with 90–95% RH at 20–21 °C. The mean projected area of the desiccated grains observed in polar view was 431 μm; after 30 min pre-hydration, it had increased to 465 μm, and after 60 min, to 541 μm.

As indicated above, on transfer to the liquid germination medium grains in the state of *fig. 16A* hydrate initially to some extent, but eventually assume the conformation of *fig. 19*, with conspicuous onci and constricted vegetative-cell

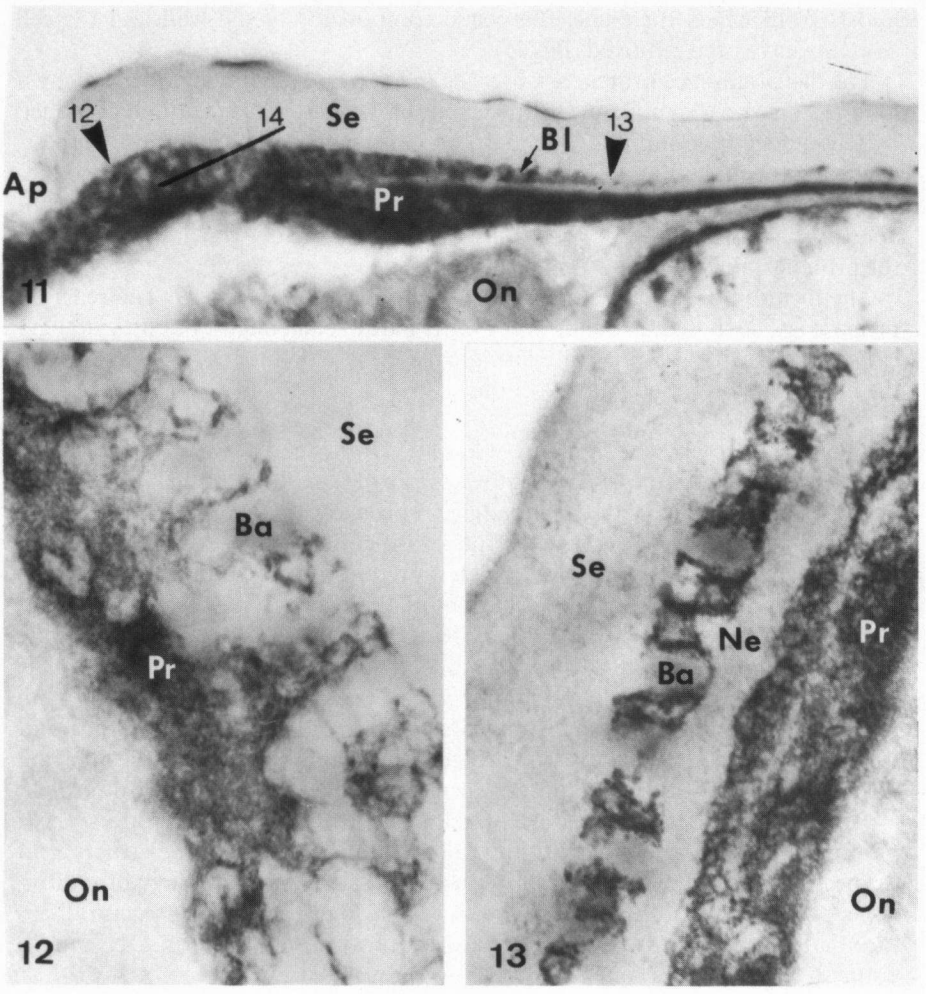


Fig. 11. Electron micrograph of the wall of a pollen grain of *C. avellana* sectioned through the exine lip at the edge of an aperture (Ap). Se, sexine; Bl, baculate layer of the sexine; Pr, protein layer underlying the aperture (*cf. fig. 9*); On, oncus. The arrow heads 12 and 13 indicate the approximate sites of the sections of *figs. 12* and *13*, and the line shows the approximate plane of the section of *fig. 14*. × *c.* 7500.

Fig. 12. Wall sectioned in the radial plane in the site indicated in *fig. 11*. Ba, bacula; other labelling as in *fig. 11*. The nexine is absent in this site, and the bacula extend into the protein layer. × *c.* 25.000.

Fig. 13. As *fig. 12*, site as indicated in *fig. 11*. Here, as elsewhere over the non-apertural surface, the nexine (Ne) forms a continuous layer linking the bacula (Ba). × *c.* 18.000.

protoplasts. In sharp contrast, those in the state of *fig. 16C* become fully hydrated in the liquid medium, retaining turgid protoplasts and flattened onci as seen in *fig. 20* until the onset of germination. Partly pre-hydrated grains comparable with the sample of *fig. 16B* produce a mixed population after 1 h, as in *fig.*

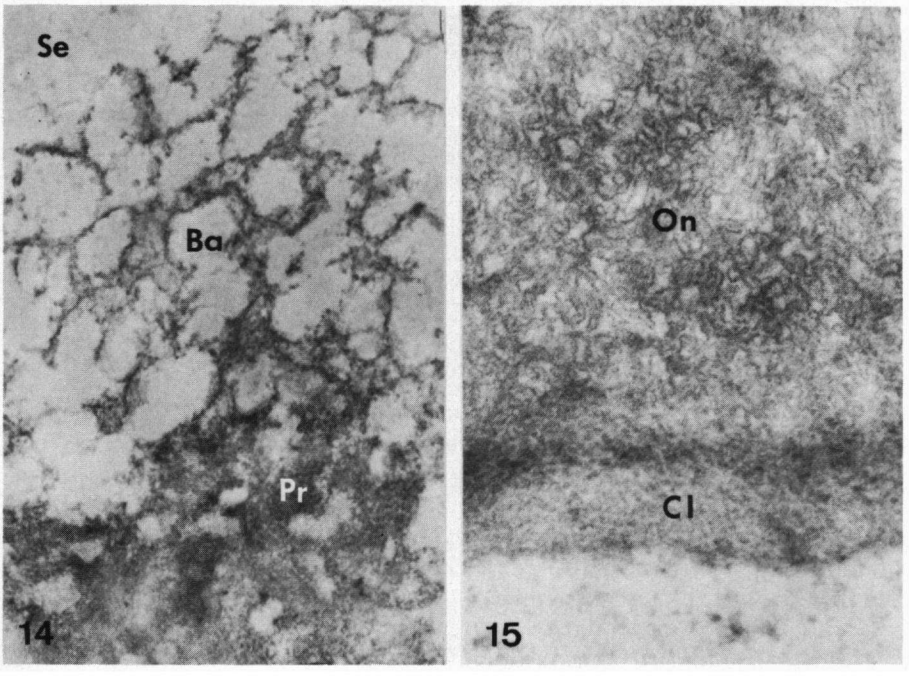


Fig. 14. As *fig. 11*, section as indicated in that micrograph, in an approximately tangential plane transecting the sexine (Se), bacula (Ba) and protein layer (Pr). This section clearly shows the continuity between the material of the proteinaceous layer within the aperture with that held in the cavities of the exine. × *c.* 17.500.

Fig. 15. Electron micrograph of the inner part of an oncus (On) and inner cellulosic layer of the intine (C1), sectioned radially, PTA post-staining. With this staining the oncus is seen to have a distinctive fine-structure, with a prominent lamellate meshwork. × *c.* 29.000.

17. The volumes of grain and protoplast in pollen samples comparable with those of *figs. 19* and *20* are set out in *table 1*. While the simplifications inherent in the computational method employed mean that the values can be no more than estimates, it is evident that the volume of the protoplast is greater by some 90% in fully hydrated grains. The onci also increase slightly in volume on hydration, although the fact that they become flattened against the exine makes them less conspicuous.

Pollen in the state of the sample of *fig. 19* shows very low germinability, while that subject to optimal pre-hydration and expanding in the germination medium to the extent of the sample in *fig. 20* will give up to 98%, depending upon the genotype (*fig. 4*).

The level of germinability is matched by the fluorochromatic reaction. In one trial, pollen desiccated for *c.* 12 h gave an FCR score of 0%; after 30 min pre-hydration, the score rose to 56.76%, and after 60 min to 91.62%. The implications of these observations are discussed further below.

Table 1. Volumes of grain, oncus and vegetative cell protoplast in pollen of *Corylus avellana* in two states of hydration, minimal, as in *fig. 19*, and maximal, as in *fig. 20*. Measurements from pollen observed in polar view (*fig. 3*); 50 grains per sample.

	Mean volume (μm^3)	
	Minimal (grain condensed)	Maximal (grain turgid)
Grain	9.16×10^3	17.48×10^3
Single oncus	2.05×10^3	2.99×10^3
Protoplast	3.01×10^3	8.53×10^3

3.5. Protein emission, and the fate of the intine during germination

Fig. 21 shows the release of protein from the apertures of partly hydrated grains suspended in the standard liquid medium; similar rapid emissions occur from fully pre-hydrated grains. The protein release is followed by the dispersal of gelatinising pectins from one or more apertures (*figs. 22* and *23*). The fate of the granular sporopollenin layer overlying the aperture in the ungerminated grain (*figs. 6* and *7*) has not been determined, but presumably it is dispersed during the course of the emissions.

Germination of appropriately pre-treated pollen begins within 10–15 min of transfer to the standard liquid medium. The tube tip emerges from the preferred aperture into a halo of dispersing pectins, evidently derived by the dissolution of the oncus at that site (*fig. 24*). As is evident from *fig. 27*, the tip is initiated as a small papilla, derived from the inner cellulosic layer of the intine, which extends into the shaft of the exine pore. The aperture is not enlarged during germination, so that the emerging tube is severely constricted where it passes through the exine (*figs. 28* and *29*). A mass of pectic material, evidently representing the residuum of the oncus, often remains associated with one flank of the tube base as cylindrical growth is assumed (*fig. 30*).

4. DISCUSSION

Half-a-century ago SCHOCH-BODMER (1936), in one of the earliest critical investigations of pollen hydration, showed that the equilibrium volume of the pollen

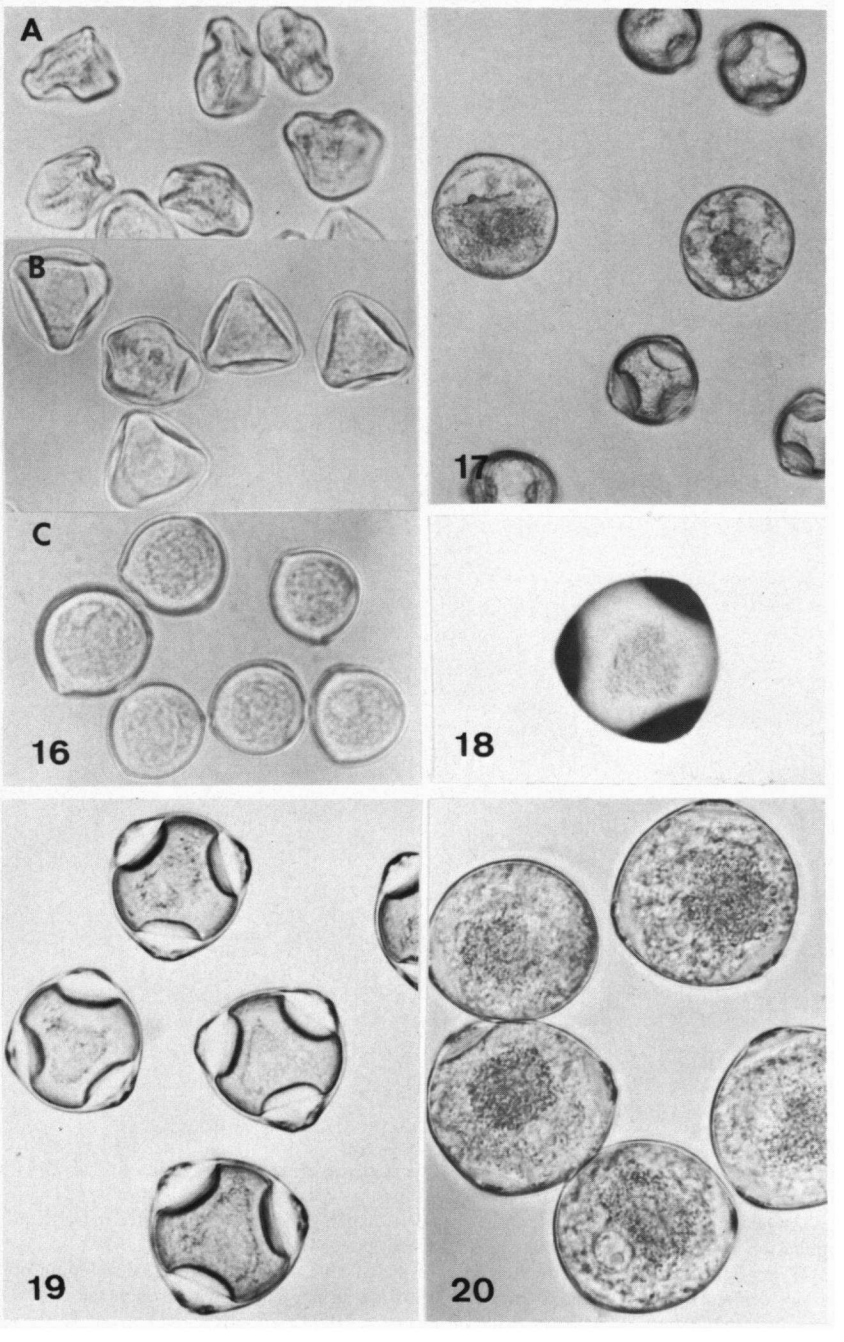
Fig. 16. Intact pollen of *C. avellana* in three states of hydration following desiccation for 12 h in an atmosphere with 5–10% RH at 20–21 °C. A, no re-hydration; B, 30 min after transfer to an atmosphere with 90–95% RH at 20–21 °C, and C, 60 min after transfer. Grains suspended in Whitemor oil; no staining. × *c.* 400.

Fig. 17. Pollen comparable with that in *fig. 16B*, *c.* 1 h after transfer to germination medium. × *c.* 560.

Fig. 18. Pre-hydrated pollen suspended in germination medium, alcian blue staining for wall pectins. × *c.* 750.

Fig. 19. Pollen comparable with that of *fig. 10A c.* 1 h after transfer to germination medium. × *c.* 830.

Fig. 20. Pollen comparable with that of *fig. 16C* on the verge of germination after transfer to the standard medium. × *c.* 830.


A
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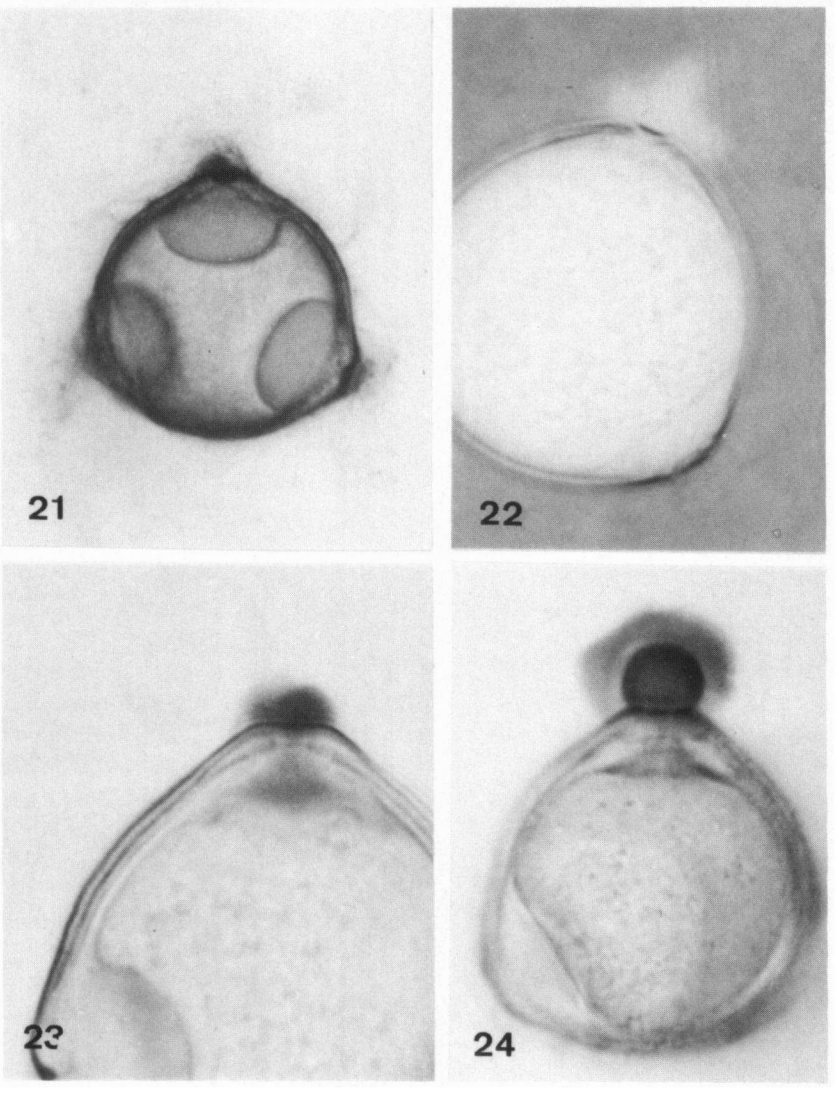
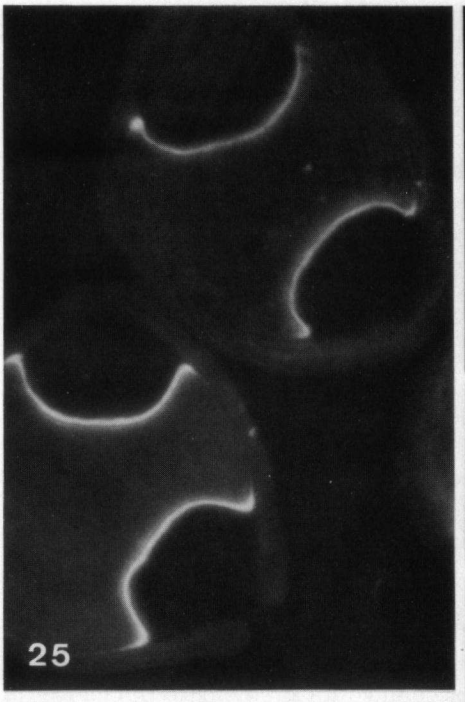


Fig. 21. Release of protein from the apertures of a partly hydrated pollen grain of *C. avellana* suspended in germination medium. Coomassie blue staining. × *c.* 1300.
Fig. 22. Gelatinising pectin emerging from one aperture of a pollen grain suspended in the standard germination medium to which carbon black has been added. × *c.* 1600.
Fig. 23. As *fig. 22,* diffusing pectin stained with alcian blue. At this stage the developing pollen tube tip, derived from the inner cellulosic layer of the intine, has grown through the oncus and is pressing into the shaft of the aperture (*cf. fig. 27*). × *c.* 2100.
Fig. 24. As *fig. 23;* later stage of germination, with the tube tip emerging into the cloud of gelatinised pectin. × *c.* 1500.

of *C. avellana* attained in humid atmospheres was closely related to water vapour pressure, and further, that under conditions approaching saturation a high proportion of the grains would actually reach the point of germination and form normal tubes. She found that pollen immersed directly in water enlarged at first, only to shrink again in the course of time. Her observations led her to stress the importance of the elasticity of the exine in accommodating the volume changes associated with hydration and water loss, and she concluded that the intine below the germination pores (the site of the onci) also plays a significant part in volume regulation. Pollen immersed in sucrose solutions of high tonicity (1.8 M) did not collapse or undergo plasmolysis, but remained spherical, "the swelling intine taking the place of the shrinking cell contents". This perceptive interpretation of the role of the onci in osmotic regulation retains some of its validity, but it is not wholly acceptable. The present observations do not support the contention that regulation after the initial hydration depends on 'a very intense hemispherical swelling" of the onci in solutions of high tonicity. The absolute volume occupied by the onci appears actually to be slightly greater in the fully enlarged grain in solutions with balancing tonicity. It is true, as SCHOCH-BODMER observed, that the severe shrinkage of the protoplast of the vegetative cell in solutions of higher tonicity does not lead to plasmolysis; but this results from the fact that the onci dehydrate to a lesser extent as the grain contracts, so that they come to occupy a greater proportion of the internal volume, undergoing a concomitant change in shape as the volume diminishes.

The effect of pre-hydration can be accounted for on the basis of changes in the properties of the membranes of the vegetative cell. The failure of partly desiccated pollen to show fluorochromasia implies that in this state the plasmalemma does not provide an effective barrier to the passage of fluorescein, while the increase in the proportion of grains giving a positive FCR reaction during pre-hydration must reflect a progressive recovery of normal membrane properties. In this respect the pollen of *C. avellana* responds in essentially the same way as that of many other angiosperms in which fluorochromasia and germination are low at the time of dispersal but recover with pre-hydration in humid air (SHIVANNA & J. HESLOP-HARRISON 1981, J. HESLOP-HARRISON et al. (1984).

The observation of SCHOCH-BODMER (1936) that in water or solutions of low tonicity which ultimately disrupt the vegetative cell the pollen of *C. avellana* first enlarges and then shrinks again is also readily explicable. Initially the solute potential of the vegetative cell and the matric potential related to the cytoplasmic colloids hold the water potential of the grain below that of the medium so inducing a rapid influx of water; but if the plasmalemma is not effective as an osmotic barrier, slow leakage will ultimately reduce the solute potential of the grain until the water potential gradient is reversed. A progressive efflux of water will then follow, leading to shrinkage. The spherical shape is retained as this happens because of the elastic recovery of the wall and change in the shape of the onci. Fixation fails to preserve pollen in the fully dilated condition presumably because the membrane changes induced by the fixative also lead to leakiness and a consequent fall in solute potential, again accompanied by an elastic contrac-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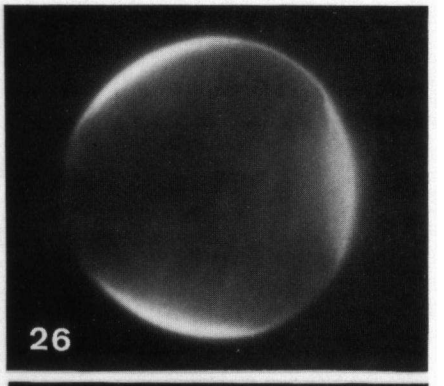
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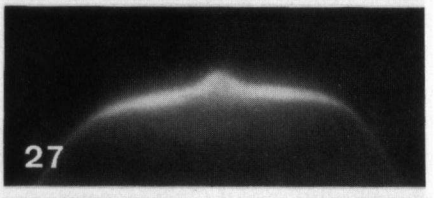
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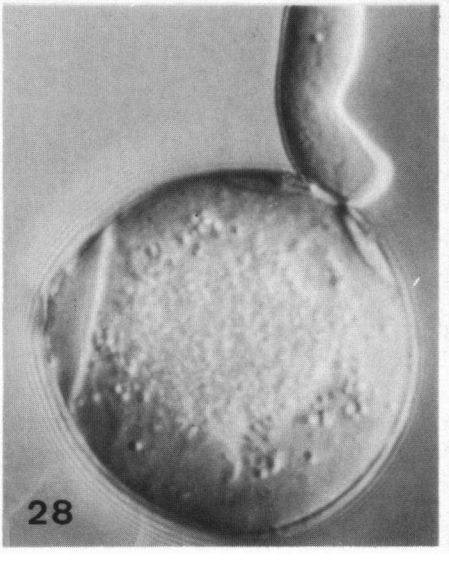
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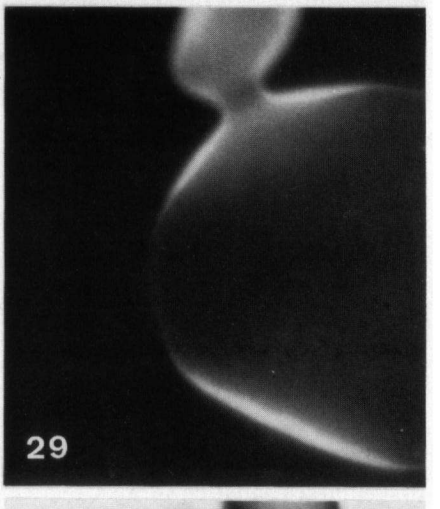
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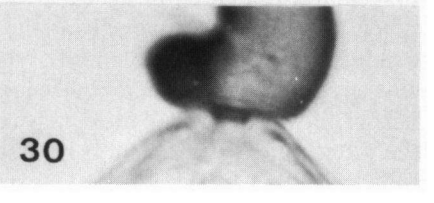
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tion of the wall. Predictably, and in the accordance with this interpretation, glutaraldehyde fixation, while having little initial effect on the esterase activity responsible for cleaving the fluorogenic ester, eliminates fluorochromasia because the retentivity of the cell membranes is impaired.

In the standard medium, suitably pre-hydrated pollen retains its fluorochromatic properties throughout the period of germination and tube growth, indicating that the plasmalemma remains an effective barrier. The grains retain their dilated state initially, but the emergence of the tube evidently relaxes the hydrostatic pressure within, leading ultimately to shrinkage and associated shape changes in the onci.

From the present results and those of SCHOCH-BODMER (1936) we may conclude that onci do play a significant part in osmoregulation in the pollen of *C. avellana*, mainly in providing a device which in effect enhances the capacity of the wall to accommodate the volume changes related to hydration and dehydration. It is to be expected that this component of the intine will have a corresponding function in other pollens of similar morphology.

Little can be said as yet about the remarkable fine-structure of the inner oncus, since the nature of the convoluted lamellae seen in *fig. 15* is quite obscure. The intense esterase activity throughout this zone (*fig. 10A*) implies that it is permeated with enzymic protein; yet the total protein content must be low, judging from the poor affinity for protein stains (*fig. 9*). Localisation of enzyme activity at the electron-microscopic level should throw light on the matter.

One of the objectives of the present study was to discover the source of the proteins released from the apertures of the pollen grain during hydration, because of the likelihood that these could include the S-specific factors responsible for inducing the stigma rejection reaction observed following incompatible pollination. A striking feature of the pollen-grain wall of *C. avellana* is the presence of the protein-bearing stratum just within each exine aperture, readily revealed

Fig. 25. Fluorescence micrograph of a semi-thin section (1–1.5 μm) of pollen grains of *C. avellana*. Glutaraldehyde fixation; HEMA embedment; calcofluor white staining. The cellulosic inner stratum of the intine is seen to be thickened under each oncus. × *c.* 1800.

Fig. 26. Intine "ghost" from an ungerminated pollen grain, prepared as described in the text. The thickened cellulosic layer underlying each oncus seen in section in *fig. 25* has expanded outwards after the removal of the exine and oncus pectins to give the spherical shape. × *c.* 1200.

Fig. 27. As *fig. 26*, ghost prepared from a germinating grain. The cellulosic layer of the intine is seen in profile, with the pollen tube tip forming as a slight papilla which, in the intact grain, pushes into the shaft of the germination aperture (cf. *fig. 23*). × *c.* 1200.

Fig. 28. DIC micrograph of an intact germinated grain, showing how the oncus has dissipated at the aperture with the emergence of the tube. While callose cannot be detected in mature, viable pollen of *C. avellana*, this wall polysaccharide appears as an inner lining to the tube immediately cylindrical growth is assumed (*cf. fig. 1*). × *c.* 1750.

Fig. 29. Intine ghost prepared from a germinated grain in the state of that of *fig. 28*. The tube has developed from a papilla formed on the inner cellulosic layer of the intine like that seen in *fig. 27*. The aperture does not enlarge during germination, so that the tube remains permanently constricted where it passes through the exine. × *c.* 1700.

Fig. 30. Germinated grain in the state of that of *fig. 28*, alcian blue staining. Residual oncus pectin forms a persistent boss at the base of the tube. × *c.* 2200.

by the appropriate staining procedures (*fig. 9*). Proteins from these sites must certainly be amongst the first emissions from the apertures during hydration and germination. At each aperture, the proteinaceous stratum lies adjacent to the main body of the oncus, and the fact that the layer extends – albeit in attenuated form – between the onci suggests that it should be assigned to the intine. Yet the distinction between "sporophytic" and "gametophytic" domains of the pollen wall, so clear in many other angiosperm pollens (J. HESLOP-HARRISON, 1975), cannot be made in *C. avellana*, since the proteinaceous material of the outer intine is continuous with the inter-bacular material of the exine at the aperture sites. The origin of this material during the development of the pollen of *C. avellana* remains to be established, but it is evidently now a matter of some importance that this should be investigated. Proteins incorporated in the intine during its deposition are the products of the gametophyte (KNOX & J. HESLOP-HARRISON 1970), but PACINI et al. (1981) have shown that proteins of sporophytic origin may also accumulate at, or actually within, the apertures in many angiosperm pollens. In the light of the work of these authors we consider it entirely possible that the apertural proteins of *C. avellana*, if not wholly sporophytic, contain at least a contribution from this source. If so, they are obvious candidates for involvement in the SI reaction, which must depend in this species on the conveyance of sporophytic information by the pollen. The apertural sites would then be serving as repositories for the storage of tapetal products in substitution for the sexine cavities that fulfil the role in the Cruciferae and Compositae.

The onci are involved in pollen germination in much the same manner as in other angiosperm species (J. HESLOP-HARRISON & Y. HESLOP-HARRISON, 1980, 1985, J. HESLOP-HARRISON 1986). The unsealing of the apertures by the dispersal of the sporopollenin cap first requires the gelatinisation and exudation of the underlying pectins. Thereafter, a tube tip is defined as a papilla on the inner cellulosic lay of the intine, in register with the aperture. As the tip extends a funnel-shaped shaft is formed through the material of the oncus, and the pectic residue is ultimately dispersed. As SCHOCH-BODMER (1936) showed, tubes frequently emerge from more than one aperture, depending on the conditions of pre-hydration and, in culture, on the composition of the medium; but normally one quickly assumes dominance. The cellulosic part of the pollen-tube wall retains its continuity with the inner layer of the intine after the final dispersal of the apertural pectins. As cylindrical growth is assumed, the characteristic stratification of the tube wall is established (J. HESLOP-HARRISON 1983), with an outer pectic layer extending over the tip, a middle cellulosic stratum and an inner callosic sheath.

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