The role of mucilage in the attachment of conidia, germ tubes, and appressoria in the saprobic aquatic Hyphomycetes *Lemonniera aquatica* and *Mycocentrospora filiformis*

D.W.T. Au, E.B.G. Jones, S.T. Moss, and I.J. Hodgkiss

Abstract: Spore attachment of the saprobic aquatic Hyphomycetes *Lemonniera aquatica* and *Mycocentrospora filiformis* was compared on Thermanox cover slips at the scanning and transmission electron microscope levels. The strength of attachment to Perspex discs was measured with the Fowler cell adhesion measurement module. Data are presented on the initial attachment of conidia and subsequent attachment of germ tubes, hyphae, and appressoria. Attachment at all stages was mediated by mucilage. Both fungi exhibited a similar strength of initial conidial attachment, but attachment resulted from different strategies: (*i*) in *L. aquatica*, by active release of mucilage as a result of a thigmotropic response to the substratum; (*ii*) in *M. filiformis*, by a passive process involving attachment by pre-existing conidial mucilage prior to contact with the substratum. The strength of attachment increased with settlement time. A fast germination rate, concurrent with mucilage production, resulted in the firm attachment of *L. aquatica* conidia, while initial germination in *M. filiformis* was slow and this corresponded to a comparatively weaker conidial attachment. Further increase in the strength of attachment was the result of greater hyphal development, and most importantly, by appressorium formation. All these structures were enrobed in a mucilaginous sheath, although variations were observed in the morphology and texture of mucilages. This suggested differences in chemical composition of mucilage (*i*) between the two fungi, and (*ii*) among different structures of the same fungus.

Key words: aquatic Hyphomycetes, appressorium, attachment, germination, mucilage, ultrastructure.

Résumé : À l'aide de la microscopie électronique par balayage et par transmission, et en utilisant des lamelles de verre Thermanox, les auteurs ont comparé l'attachement des spores des hyphomycètes saprophytes aquatiques *Lemonniera aquatica* et *Mycocentrospora filiformis*. Ils ont mesuré la force d'attachement à des disques de perspex en utilisant le module de mesure d'adhésion des cellules selon Fowler. Ils présentent les données sur l'attachement initial des conidies et les attachements subséquents des tubes germinatifs, des hyphes et des appressoriums. A toutes les étapes, l'attachement implique du mucilage. Les deux champignons montrent des forces d'attachement initial des conidies comparables, mais l'attachement se fait selon des stratégies différentes : (*i*) le *L. aquatica*, implique le relâchement actif de mucilage suite à une réaction thigmotropique au substrat; (*ii*) le *M. filiformis* implique un processus passif faisant intervenir un attachement à partir de mucilage conidien pré-formé avant le contact avec le substrat. La force d'attachement augmente avec le temps. Un taux de germination rapide, lié à la production de mucilage, conduit à un attachement ferme chez les conidies du *L. aquatica*, alors que chez le *M. filiformis*, la germination initiale des conidies est lente, ce qui correspond à un attachement conidien comparativement plus faible. Une augmentation ultérieure de la force d'attachement fait suite à un développement plus important des hyphes et surtout à la formation des appressoriums. Toutes ces structures sont enrobées dans un manchon mucilagineux, bien qu'on observe des variations dans la morphologie et la structure des mucilages. Ceci suggère qu'il y aurait des différences dans la composition chimique du mucilage (*i*) entre les deux champignons, et (*ii*) entre les différentes structures du même champignon.

Mots clés : Hyphomycètes aquatiques, appressorium, attachement, germination, mucilage, ultrastructure.
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Introduction

Information on fungal attachment is not well documented compared with other microorganisms such as bacteria, algae, and diatoms (Jones 1994). Early observations of spore attachment in fungi were usually linked to studies on spore dispersal (Ingold 1965, 1968, 1971). Recently, aspects of spore adhesion were studied in depth, particularly in those fungi of economic and medical importance, such as pathogenic species of *Candida* (Douglas 1987*a*, 1987*b*; Kennedy 1990, 1991; Tronchin et al. 1991), nematophagous fungi (Dijksterhuis et al. 1990; Nordbring-Hertz 1988; Tunlid et al. 1992), and entomopathogenic fungi (Bidochka et al. 1995; Boucias and Pendland 1991). Studies on spore attachment of pathogens of plants include *Nectria haematococca* Berk. et Br. (Caesar-TonThat and Epstein 1991; Jones and Epstein 1989, 1990), *Magnaporthe grisea* Barr (Hamer et al. 1988; Bourett and Howard 1990; Howard and Ferrari 1989; Howard et al. 1991), and rust fungi (Clement et al. 1994; Epstein et al. 1987; Cole and Hoch 1991).

In all studies on fungal adhesion, mucilage secretion was ubiquitous (Jones 1994). However, other factors also play an important role in spore adhesion: electrostatic charges of the substratum (Jones and O'Shea 1994; Klotz et al. 1985); hydrophobicity (Clement et al. 1994; Hazen 1990; Latge et al. 1986); and chemical components of mucilage such as lectins and adhesins (Boucias and Pendland 1991; Douglas 1987*b*; Nordbring-Hertz 1988; Tronchin et al. 1991; Tunlid et al. 1992).

Saprobic aquatic Hyphomycetes play a major role in the decomposition of submerged plant material, e.g., leaf debris, twigs, and driftwood (Suberkropp and Klug 1980, 1981; Chamier and Dixon 1982; Singh 1982; Chamier et al. 1984; Au et al. 1992; Shearer 1992), and are regarded as intermediates of energy flow in freshwater ecosystems (Bärlocher 1992; Bärlocher and Kendrick 1974, 1976, 1981). Effective conidial attachment of aquatic Hyphomycetes contributes to their success in colonizing a wide range of substrata under highly turbulent conditions in streams and rivers. Thus, a series of studies of the adhesion of aquatic Hyphomycetes was embarked upon (Harrison et al. 1988; Read 1990; Read et al. 1988, 1991, 1992*a*, 1992*b*, 1992*c*). These studies indicated that the tetraradiate conidium of *Lemonniera aquatica* de Wild. was the most tenacious among the 10 species studied (Read 1990; Read et al. 1991). It secreted mucilage shortly after settlement and exhibited a fast germination response with subsequent appressorium formation. *Mycocentrospora filiformis* (Petersen) Iqbal was the most adherent among the species with sigmoid conidia (Read 1990; Read et al. 1991).

The aim of this paper was to investigate further the conidial attachment mechanisms of aquatic Hyphomycetes by comparing the different attachment strategies of *Lemonniera aquatica* and *Mycocentrospora filiformis* with respect to mucilage secretion, conidium morphology, germination, and appressorium production at the scanning and transmission electron microscope levels. The strength of conidial attachment was determined using the Fowler cell adhesion measurement module (CAMM).

Materials and methods

Fungal cultures and preparation of conidial suspension

Lemonniera aquatica and *Mycocentrospora filiformis* were isolated from submerged leaf litter from a stream in Hampshire, England and Pokfulam, Hong Kong, respectively. Isolates were subcultured weekly on to 2% (w/v) malt extract agar (MEA).

Sporulation of *Lemonniera aquatica* and *Mycocentrospora filiformis* was induced by submergence of two 9-mm diameter discs from a MEA culture in 50 mL sterilized distilled water (in a 100-mL conical flask) with a continuous supply of pumped filter-sterilized air. After aeration for 3–4 days at 20°C, a conidial density of 2–4 10^4/mL was obtained.

Germination and appressorium formation

Germination of conidia and formation of appressoria of *Lemonniera aquatica* and *Mycocentrospora filiformis* were compared from 9-day-old and 2-, 3-, 4-, 5-, and 6-week-old MEA cultures. Thermanox tissue culture cover slips (13 mm in diameter; Bio-Rad, Richmond, Calif.) were selected because microorganisms adhere to them readily, they are resistant to chemicals used in electron microscopy tissue processing, and they can be sectioned by diamond or glass knife for transmission electron microscopy.

Conidia in suspension were settled on Thermanox cover slips that had been previously sterilized in 95% ethanol for 2–3 h and then by UV radiation (Philips TUV 30 W) for 4–5 h, and incubated in sterilized moist chambers for 2, 6, 12, and 24 h at 20°C. The moist chamber consisted of a Petri dish containing filter papers dampened with sterile distilled water. At the end of the incubation period, the percentage of conidial germination was determined by counting the number of germinated conidia out of 100–200 observed (using a Leitz Dialux 22 EB light microscope at ×250 magnification). In addition, the number of germinated conidia with appressoria was recorded and the percentage was calculated. Each set of experiments was performed in triplicate and the percentages of each set were pooled and averaged (±SEM).

Strength of attachment

An LH-Fowler CAMM, manufactured by Cell Adhesion Research and Development Limited, Bath, U.K., 1991 model, was used to determine strength of spore attachment. The operating principle of the CAMM is based on a defined hydrodynamic environment to create a continuum of known shear stresses across a test surface in a radial flow chamber. The hydrodynamic shear forces in the chamber are established between two stationary parallel discs (10 cm in diameter) that are 1 mm apart. One disc is made from Perspex onto which are settled the test conidia and the other is stainless steel. A water flow enters from the inlet at the centre of the stainless steel disc and flows centrifugally at decreasing velocity between the discs. The surface shear force (T, in $N \cdot m^{-2}$, where $N \cdot m^{-2}$ is the unit of surface shear force) on the Perspex at radius r is calculated by the formula (Fowler 1988)

$$[1] \quad T = \frac{3Q\mu}{\pi r h^2}$$

where Q is volumetric flow rate ($m^3 \cdot s^{-1}$), μ is dynamic viscosity, 1.002×10^{-3} $N \cdot s \cdot m^{-2}$ (where $N \cdot s \cdot m^{-2}$ is the unit of dynamic viscosity of fluid) for H_2O, r is radius (mm), h is disc separation, 1×10^{-3} m.

Settled conidia were subjected to shear forces of 3.2, 2.4, 1.9, 1.4, 1.1, 0.8, 0.6, and 0.5 $N \cdot m^{-2}$. The first four shear forces were those present along a radius at 15, 20, 25, and 35 mm from the centre of the disc when subjected to a water flow rate of 3 $L \cdot min^{-1}$. The second four shear forces were those at the same distances from the centre but with a flow rate of 1 $L \cdot min^{-1}$.

To measure strength of attachment conidia were allowed to settle and then incubated at 22°C on sterilized Perspex discs for 10 min, and 2, 6, 12, and 24 h. After incubation, the disc was tilted to remove excess water and the number of conidia at each selected distance from the centre along a radius was counted using a Leitz Wetzlar light microscope at ×100 magnification. After shear force treatment for 15 min (see above), the number of conidia that remained

attached were counted again, and the percentage of conidia that remained attached for each of the eight shear forces was calculated (two replicate Perspex discs were used for each time period). In this way, the strength of conidial attachment and the changes in the strength of conidial attachment with settlement time were studied.

Scanning electron microscopy

Conidial suspensions were settled for 2, 6, 12, and 24 h on sterilized Thermanox cover slips and incubated in a sterilized moist chamber. At the end of the settlement period, the Thermanox cover slips with attached conidia were fixed by immersion in 2% (w/v) aqueous osmium tetroxide for 12 h at 4°C. Fixed material was washed twice in distilled water for 5 min and then dehydrated in a graded ethanol series (10–90% in 10% steps), then to 95% ethanol followed by three changes in absolute ethanol (10 min for each change). Ethanol was substituted by acetone through a 3:1, 1:1, and 1:3 series (15 min each) and then three changes of pure acetone (20 min each). Dehydrated samples were critical point dried in a Polaron E3000 critical point drying apparatus using liquid carbon dioxide as the drying agent. Dried samples were mounted on aluminium stubs and gold-coated in a Polaron E5000 diode sputter coater before being examined at 20 kV in a JEOL T20 scanning electron microscope.

Transmission electron microscopy

Conidia settled on Thermanox cover slips (as described above) were fixed in either (*i*) 1% (w/v) aqueous potassium permanganate solution for 30 min at room temperature or (*ii*) 4% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 3 h at room temperature, washed in buffer followed by distilled water (each for 15 min) and then postfixed in 2% (w/v) aqueous osmium tetroxide for 12 h at 4°C. Fixed material was then dehydrated in a graded ethanol series to acetone as described for SEM. Samples were infiltrated and embedded in Möllenhauer's resin (Möllenhauer 1964). Ultrathin sections were stained with Reynolds' lead citrate (Reynolds 1963) and then poststained with a saturated solution of uranyl acetate in 50% ethanol (40 min each) before examination with a JEOL 100S transmission electron microscope at 60 kV.

Results

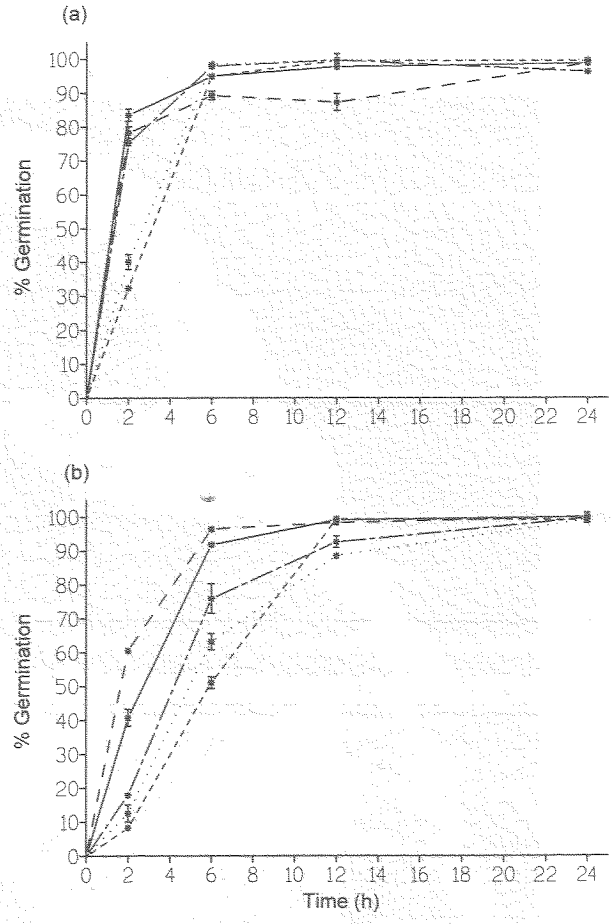
Germination of conidia

Germination of conidia of *Lemonniera aquatica* settled on Thermanox cover slips at 20°C showed an initial rapid rise with 80% germinated after 2 h for spores obtained from cultures incubated for 3–5 weeks, while for 8-day-old and 2-week-old cultures germination was 30–40% for the same time period. However, after 6 h of settlement, germination was over 90% irrespective of the age of the original culture (Fig. 1*a*). Conidia of *Mycocentrospora filiformis* showed between 10 and 60% germination after 2 h of settlement depending on the age of the cultures. After 12 h of settlement, germination reached about 90% irrespective of the age of the original culture (Fig. 1*b*). For both fungi, almost 100% germination was obtained after 24 h of settlement.

Formation of appressoria

Germinated conidia of both *Lemonniera aquatica* and *Mycocentrospora filiformis* showed no appressorium formation after 2 h of settlement (Fig. 2). Conidia of *Lemonniera aquatica* produced from 8-day-old to 5-week-old MEA cultures showed 10–90% appressorium formation after 6 h of settlement. However, all attained over 90% appressorial development after 24 h of settlement with the exception of the 4-week-old culture (Fig. 2*a*). For *Mycocentrospora filiformis*, the percent increase in appressorial development was more linear with time of settlement (Fig. 2*b*). In almost all circumstances, appressorium formation was lower than for *Lemonniera aquatica* and remained below 90% even after 24 h of settlement.

Fig. 1. Percentage germination of conidia from cultures of different ages on Thermanox cover slips over a 24-h period at 20°C. (*a*) *Lemonniera aquatica*. ---, 8 days; · · ·, 2 weeks; —, 3 weeks; – – –, 4 weeks; -—, 5 weeks. (*b*) *Mycocentrospora filiformis*. · · ·, 2 weeks; —, 3 weeks; – – –, 4 weeks; -—, 5 weeks; ---, 6 weeks.



Strength of attachment of conidia in the LH-Fowler cell adhesion measurement module (CAMM)

For both *Lemonniera aquatica* and *Mycocentrospora filiformis*, the percentage of conidia remaining attached to the Perspex disc decreased with increasing shear forces when settlement times were less than 6 h. After 12 and 24 h of settlement, 100% of conidia remained attached when subjected to weak shear forces ($< 1.5\ N \cdot m^{-2}$) (Fig. 3). The strength of conidial attachment increased with the length of time conidia were allowed to settle and this was most apparent at a shear force of $3.19\ N \cdot m^{-2}$. After 10 min of settlement, both species exhibited similar percentage conidial attachment (42–43%). After 2 h of settlement, the percentage attachment for *Lemonniera aquatica* conidia increased to 70% (Fig. 3*a*), while it took 6 h for *Mycocentrospora filiformis* conidia to attain the same level (Fig. 3*b*). Conidia of both fungi were difficult to dislodge after 12 h of settlement.

Fig. 2. Formation of appressoria on germinated conidia from cultures of different ages on Thermanox cover slips over a 24-h period at 20°C. (*a*) *Lemonniera aquatica*. ---, 8 days; · · ·, 2 weeks; —, 3 weeks; – – –, 4 weeks; -—, 5 weeks. (*b*) *Mycocentrospora filiformis*. · · ·, 2 weeks; —, 3 weeks; – – –, 4 weeks; -—, 5 weeks; ---, 6 weeks.

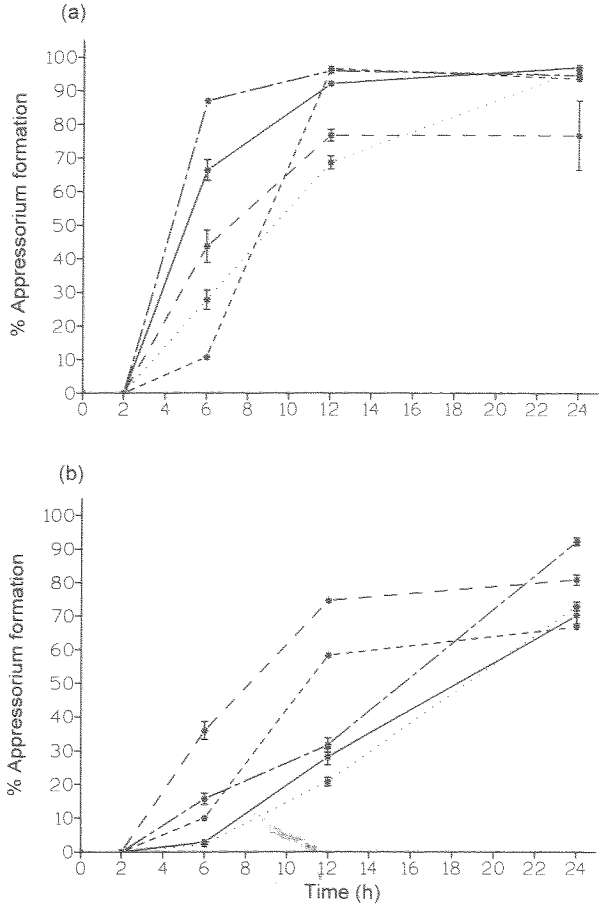
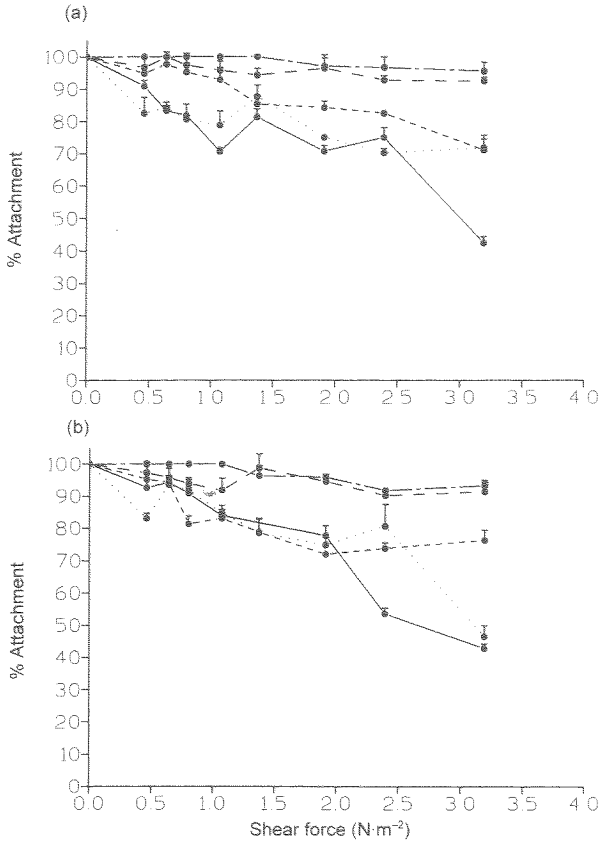


Fig. 3. Effect of surface shear forces ($N \cdot m^{-2}$) on percentage attachment after settlement of conidia and differentiation on Perspex discs for up to 24 h. (*a*) *Lemonniera aquatica*. (*b*) *Mycocentrospora filiformis*. —, 10 min; · · ·, 2 h; ---, 6 h; – – –, 12 h; -—, 24 h; (culture age 3 weeks).



Electron microscopy of conidial attachment in *Lemonniera aquatica*

Conidia of *Lemonniera aquatica* are tetraradiate with arms that are 70–80 μm long with rounded apices (Fig. 4). Upon germination, the apex of the attached conidial arm swelled and one or more germ tubes formed from the apical or subapical region (Fig. 5). Extracellular mucilage was present on the germ tube and it spread onto the substratum along the region of contact, but none was observed on the conidial arm (Fig. 5). The mucilaginous sheath was fibrillar, ca. 120 nm thick, and contiguous with the electron-transparent cell wall (Fig. 6). Within the germ tube many myelin-like bodies (vacuoles containing whorls of membranes) and small vesicles were associated with the plasma membrane (Fig. 6).

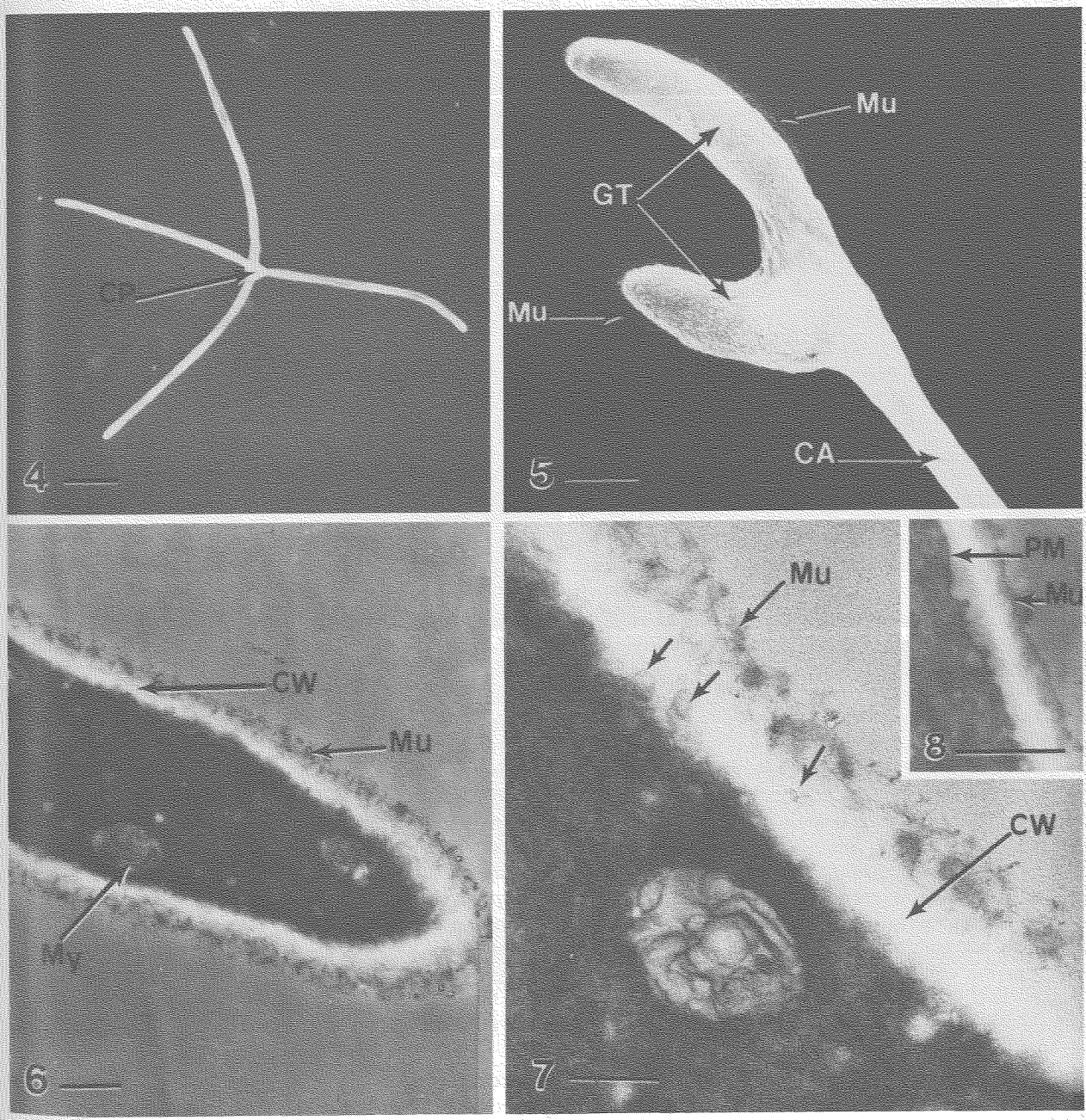
Following growth and septum formation, the germ tube became the germ hypha. The mucilaginous sheath on the germ hypha after settlement for 6 h (Fig. 7) was less homogeneous and uniform in thickness than after 12 h (Fig. 8), but the cytoplasm still contained myelin-like bodies associated with a convoluted plasma membrane. Inclusions in the cell wall were of similar texture to the extracellular mucilage, but their origin was not determined (Fig. 7). Lateral branches from the germ hypha were commonly present after 24 h of settlement and had similar morphology to the germ hypha, except that mucilage production was less extensive (Fig. 9). At the TEM level, the electron-dense mucilaginous sheath was fibrillar and dispersed, 300–420 nm thick (Fig. 10).

Protoappressoria formed as a result of swelling of the hyphal apex and became mature appressoria after delimitation by a septum (Fig. 11). Both protoappressoria and appressoria were attached to the substratum by conspicuous mucilage. Transmission electron micrographs of appressoria showed that the appressorial sheath was homogeneous, ca. 350 nm thick and with a well-defined outer margin (Fig. 12). Mitochondrial profiles and endoplasmic reticulum were abundant within the appressorium, indicating high metabolic activity (Fig. 12). Vesicles and electron-transparent regions (ETR) containing inclusions were fused or closely associated with the plasma membrane (Fig. 13). Fixation with $KMnO_4$ (Fig. 14) indicated that these electron-transparent regions were not glycogen material as no rosettes typical of fungal glycogen fixed in $KMnO_4$ were observed, the inclusions in the ETR were not distinct, and the mucilaginous sheath appeared more fibrillar.

Electron microscopy of conidial attachment in *Mycocentrospora filiformis*

Conidia of *Mycocentrospora filiformis* are filiform, curved, 250–300 μm long with a characteristic basal appendage that extends beyond the point of disarticulation from the conidio-

Figs. 4–8. *Lemonniera aquatica*. Fig. 4. SEM showing that the four arms of the tetraradiate conidium diverge from the swollen conidium primordium (CP) and are rounded at their apices. Scale bar = 20 μm. Fig. 5. SEM showing two germ tubes (GT) produced from the apical region of the conidial arm (CA). Mu, mucilage. Scale bar = 2 μm. Fig. 6. TEM showing the germ tube apex with a fibrillar mucilaginous sheath (Mu); the cell wall (CW) is electron-transparent and the adjacent plasma membrane convoluted with associated cytoplasmic vesicles. My, myelin-like body (membrane structure). Scale bar = 0.5 μm. Fig. 7. TEM of germ hypha after 6 h of settlement. The mucilaginous sheath (Mu) is fibrillar. Inclusions (arrows) are present in the cell wall (CW). Scale bar = 0.2 μm. Fig. 8. TEM of germ hypha after 12 h of settlement. The mucilaginous sheath (Mu) is more homogeneous and has a defined outer margin. The plasma membrane (PM) is convoluted. Scale bar = 0.5 μm.



phore (Fig. 15). A "fuzzy" mucilaginous sheath was observed on the conidial body (Fig. 16).

Germination takes place at any point along the length of the conidium rather than at the poles. At the SEM level, mucilage on the germ tube was wart-like and discrete at the subapical region (Fig. 17). This correlated with the TEM results that showed that the germ tube mucilage was granular, 50–100 nm thick, and intermittently arranged (Fig. 18).

Production of mucilage on germ hyphae was profuse, with the granular mucilage aggregated to form an undulating sheath (Fig. 19, lower hypha). At the TEM level, the germ hyphal sheath was irregular with a discrete outer margin (Fig. 20, lower hypha), whereas mucilage at the apex of the lateral hypha was dispersed (Fig. 20, upper hypha). Lateral hyphae were formed subapically on a germ hypha (Fig. 21) or behind the septum of an appressorium (Fig. 22). Changes in mucilage

Figs. 9–14. *Lemonniera aquatica*. Fig. 9. SEM showing a lateral hypha (from the germ hypha) after settlement for 24 h. The morphology of the lateral hypha is similar to a germ hypha except that the mucilaginous sheath (Mu) is less extensive. Scale bar = 1 μm. Fig. 10. TEM showing the lateral hypha after settlement for 24 h. The fibrillar mucilaginous sheath (*) on the hyphal tip is highly dispersed. Scale bar = 1 μm. Fig. 11. SEM showing a mature appressorium (A) that is covered in mucilage (arrows). S, septum; SH, hypha subtending the appressorium. Scale bar = 2 μm. Fig. 12. TEM of a mature appressorium that is nucleated (N) and has many mitochondrial profiles (*mi*), vacuoles (Va), lipid bodies (L), and a septum (S) delimiting the appressorium from the subtending hypha (SH). Mu, mucilage. Scale bar = 1 μm. Fig. 13. Higher magnification of the appressorial wall in Fig. 12. The mucilaginous sheath (Mu) is electron dense, ca. 330 nm thick, homogeneous with a well-defined outer margin. Electron-transparent regions (ETR) and vesicles (V) are fused or closely associated with the convoluted plasma membrane (PM). Scale bar = 0.5 μm. Fig. 14. TEM of a $KMnO_4$-fixed appressorium with fibrillar mucilaginous sheath. Electron-transparent regions (ETR) are closely associated with the plasma membrane (PM). Scale bar = 0.5 μm.

Figs. 15–20. *Mycocentrospora filiformis*. Fig. 15. SEM of the conidium that is filiform, curved, and characterizied by the basal appendage (BA) that forms beyond the region of disarticulation (arrow) from the conidiophore. GT, germ tube. Scale bar = 20 μm. Fig. 16. TEM of the conidial wall. The electron-transparent cell wall (CW) is covered with a fibrillar, dispersed mucilage (arrows). Scale bar = 0.2 μm. Fig. 17. SEM of a germ tube that was formed after settlement for 2 h. The wart-like appearance of the mucilage is more obvious at the subapical region (arrows). Scale bar = 1 μm. Fig. 18. TEM of a germ tube (GT) on which the mucilage is granular and intermittently arranged (long arrow); mucilage near or on the conidium is less discrete (short arrows). Scale bar = 1 μm. Fig. 19. SEM of two adjoining germ hyphae. The mucilaginous sheath is undulated, extensive, and granular. Strand-like mucilage extends on to the substratum (arrows) and also along the region of interhyphal contact (arrows). Scale bar = 1 μm. Fig. 20. TEM of two adjoining hyphae. The mucilaginous sheath (HS) on the germ hypha (GH) is irregular and with a discrete outer margin. Mucilage on the lateral hypha (LH) appears diffuse (arrows). Scale bar = 1 μm.

Figs. 21–26. *Mycocentrospora filiformis*. Fig. 21. SEM of germ hypha and lateral hypha formation. Note the warty nature of mucilage (arrow) on the germ hypha (GH) and the smoother nature of the mucilage on the lateral hypha (LH). Scale bar = 2 μm. Fig. 22. SEM of an appressorium (A) that is lobed and subtended by many lateral branches (LH), all of which possess a mucilaginous sheath. Scale bar = 10 μm. Fig. 23. SEM of a lateral hypha after 24 h of settlement. The mucilaginous sheath is warty except at the apex (arrow) where it is smooth. Scale bar = 1 μm. Fig. 24. TEM of an appressorium that is lobed and delimited from the subtending hypha (SH) by a septum (S). Mu, appressorial mucilage. Scale bar = 1 μm. Fig. 25. TEM of a lateral hypha after 24 h of settlement. The mucilaginous sheath is granular and forms an irregular layer except at the apex (arrow) where it is less discrete. Scale bar = 0.5 μm. Fig. 26. Higher magnification of the appressorial wall in Fig. 24 showing that the appressorial sheath (Mu) (400–650 nm) is heterogeneous in texture and consists of an electron-dense, fibrillar-like network (arrowheads) that extends from the cell wall (CW) to the surface in a less electron-dense matrix. *mi*, mitochondria. Scale bar = 0.5 μm.

morphology during different stages of hyphal development are illustrated in Fig. 21; the germ hyphal sheath was warty while the lateral hyphal sheath was smooth. However, the latter also became warty with a longer settlement time (Fig. 23) and the sheath was electron dense, granular, 100–150 nm thick, and less discrete at the apex (Fig. 25).

The protoappressorium of *Mycocentrospora filiformis* formed at the apex of the germ hypha and was covered with extensive mucilage that extended onto the substratum along the region of contact. The mature appressorium was lobed, and a number of lateral hyphae formed behind the septum; all structures were enrobed in a mucilaginous sheath (Fig. 22). At the TEM level, the appressorial sheath was ca. 850 nm thick, heterogeneous, and consisted of a fibrillar-like network that extended from the cell wall to the surface in a less electron-dense matrix (Fig. 26). Mitochondrial profiles were abundant within the appressorium, and electron-transparent regions were commonly found near the plasma membrane (Fig. 26).

Discussion

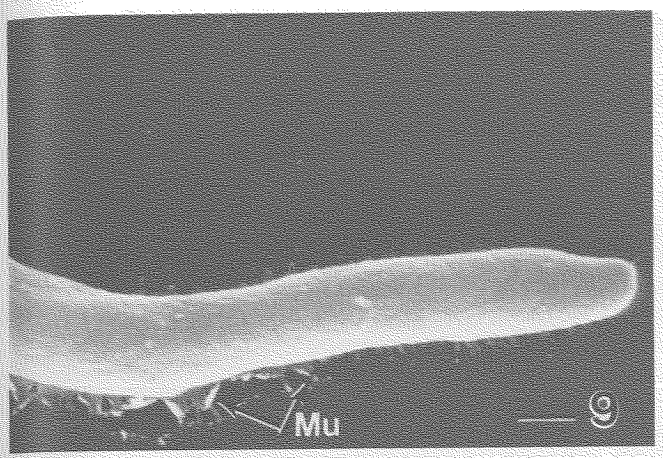
Five stages were recognized in spore attachment of *Lemonniera aquatica* and *Mycocentrospora filiformis*: (*i*) presettlement of conidia, (*ii*) initial contact, (*iii*) germ tube formation, (*iv*) hyphal mucilage secretion, and (*v*) appressorium formation.

Presettlement

Prior to settlement, released sigmoid conidia of *Mycocentrospora filiformis* were covered with fibrillar mucilage, whereas no extracellular mucilage was observed on the tetraradiate conidia of *Lemonniera aquatica*. Mucilage on released conidia has also been reported for the tetraradiate conidia of *Clavariopsis aquatica* de Wild. and the ovoid conidia of *Dimorphospora foliicola* Tubaki and *Tumularia aquatica* (Ranzoni) Descals et Marvanova (Read 1990). Ascospores of many marine fungi were also reported to be covered by a mucilaginous sheath e.g., *Leptosphaeria* spp., *Pleospora* spp., and *Astrosphaeriella striataspora* (Hyde) Hyde (Hyde 1985; Hyde and Jones 1989), whereas others lack mucilage but possess elaborate spore appendages that mediate in attachment to the substratum, e.g., *Carbosphaerella leptosphaerioides* Schmidt, *Dryosphaera navigans* Koch et Jones, *Appendichordella amicta* (Kohlm.) R.G. Johnson, Jones et Moss, and *Halosphaeria appendiculata* Linder (Hyde 1985; Hyde and Jones 1989). Appendaged spores that lack mucilage are also characteristic of many species of freshwater and marine Trichomycetes (Moss 1979).

Initial contact

Upon contact of *Lemonniera aquatica* conidia with a surface, fibrillar mucilage was secreted at the apex of the conidial arms in contact with the substratum. No mucilage was observed on other parts of the conidial arms or those not in contact with the substratum. Accordingly, mucilage secretion in *Lemonniera aquatica* is a thigmotropic response. Secretion of mucilage upon surface contact is common among various ecological groups of fungi, e.g., the ascospores of *Halosphaeria appendiculata* Linder (Hyde et al. 1986); zoospores of *Phytophthora palmivora* (Butler) Butler (Sing and Bartnicki-Garcia 1975),


Mu
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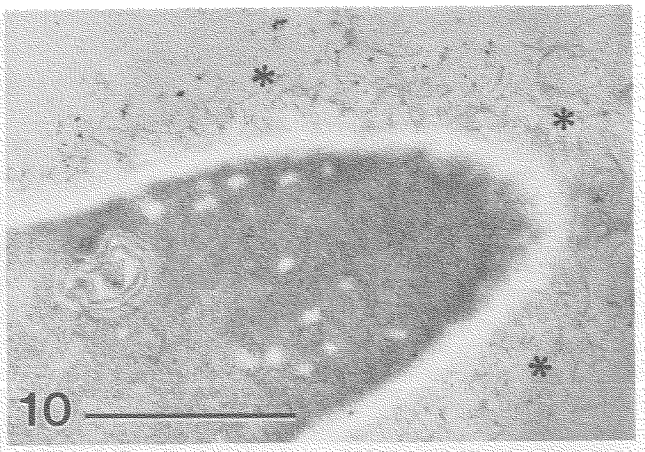
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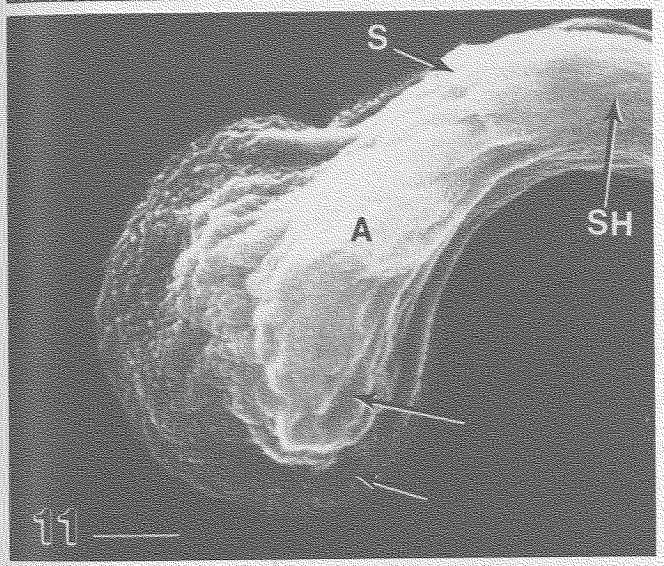
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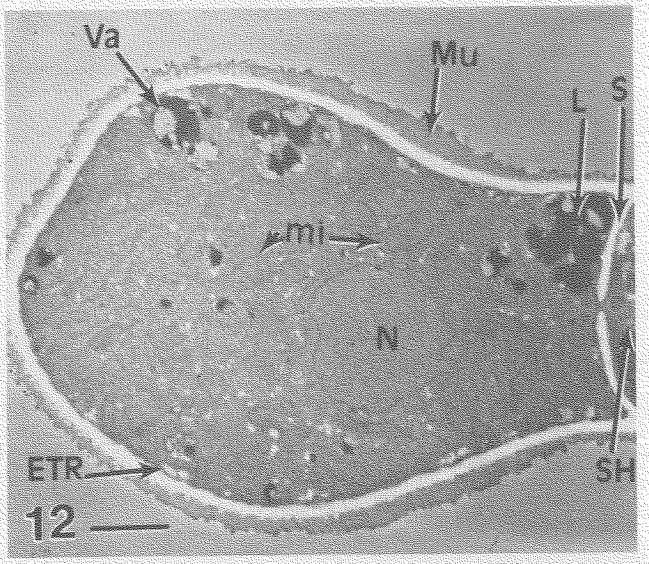
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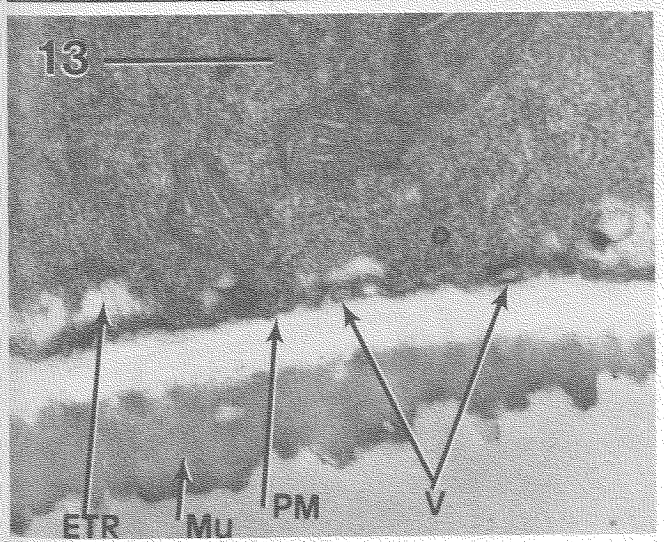
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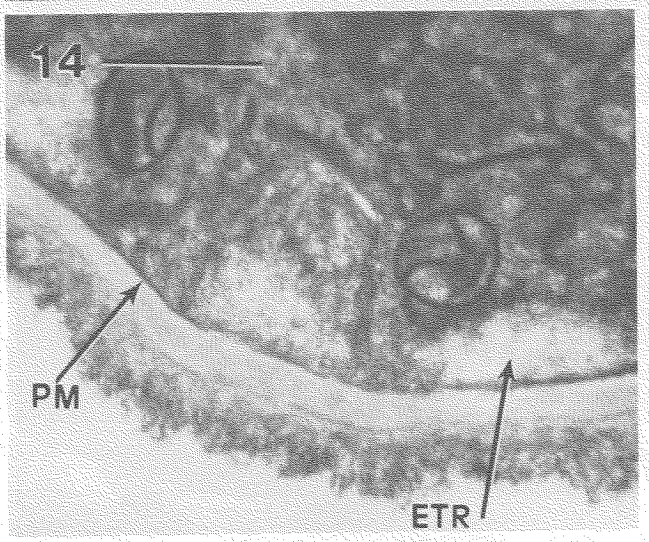
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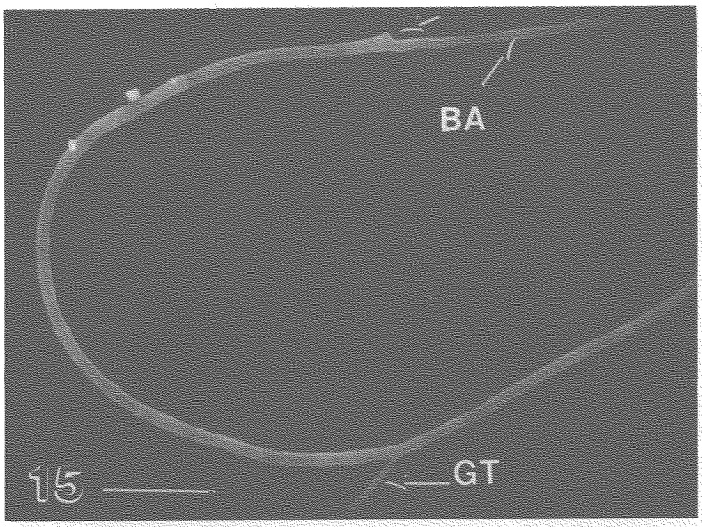
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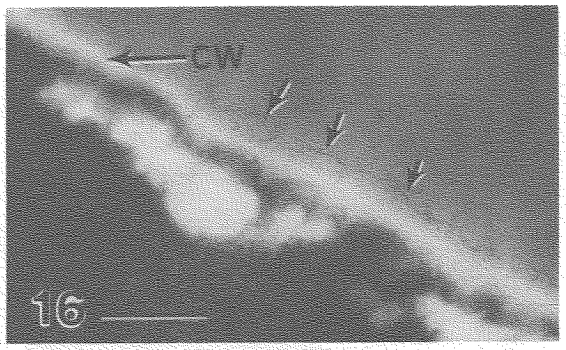
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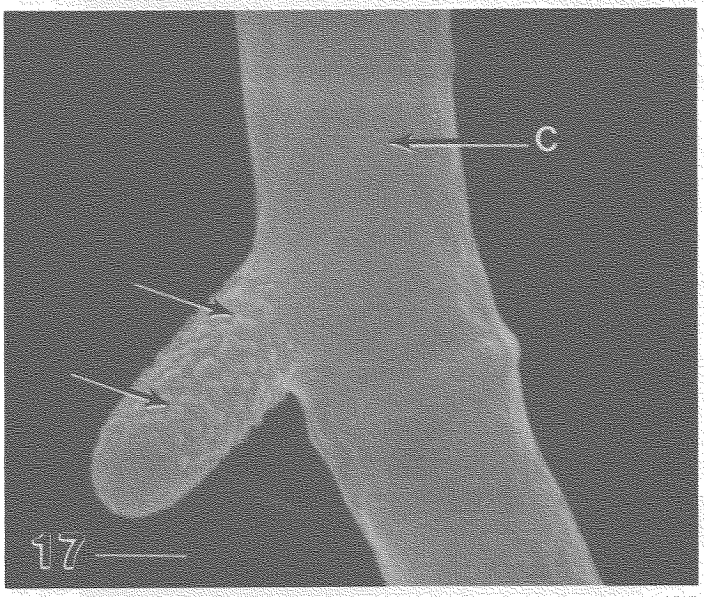
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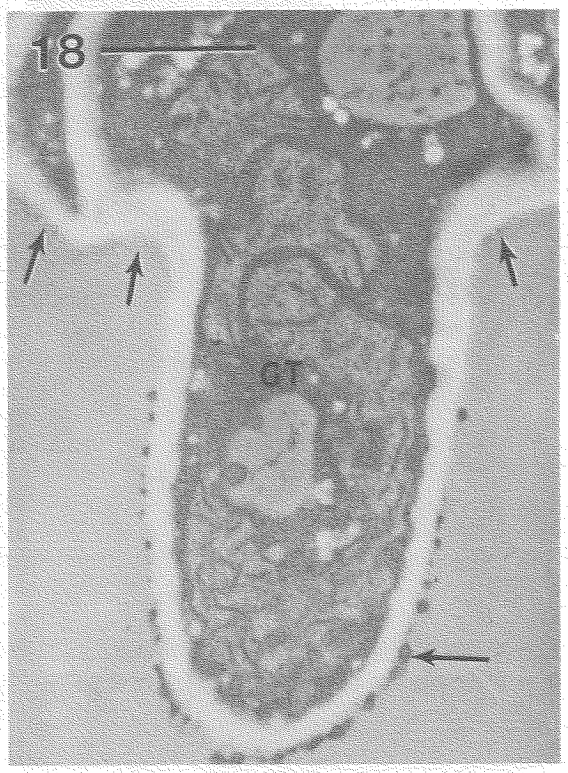
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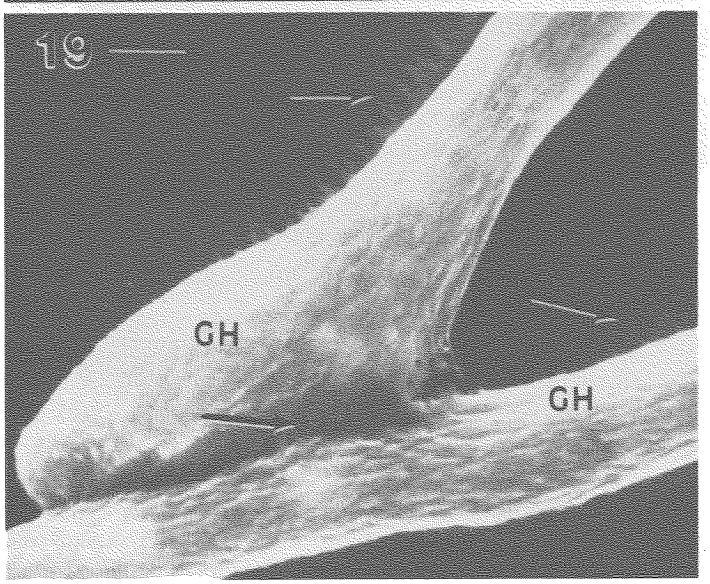
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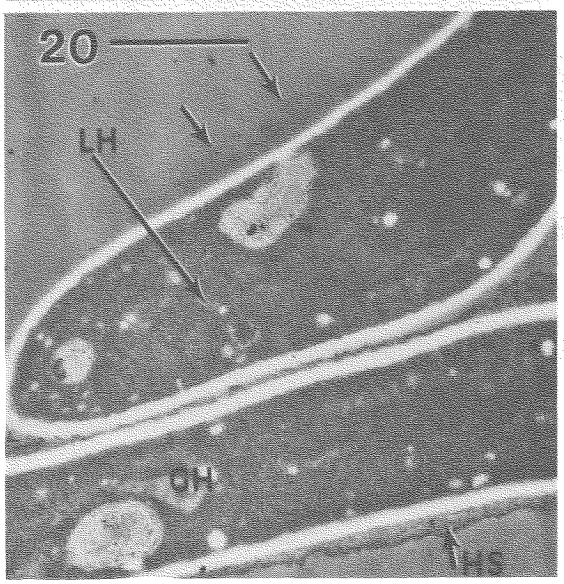
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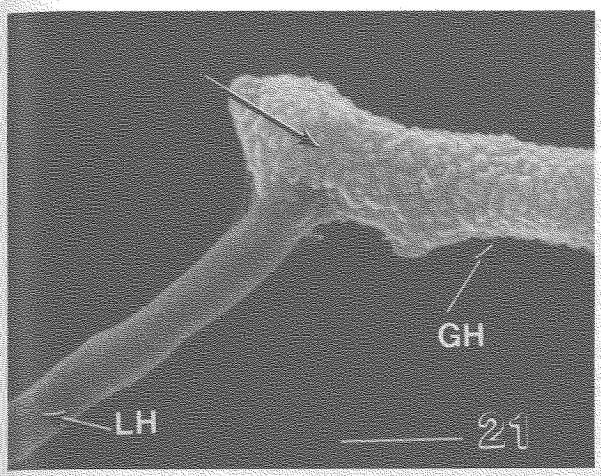
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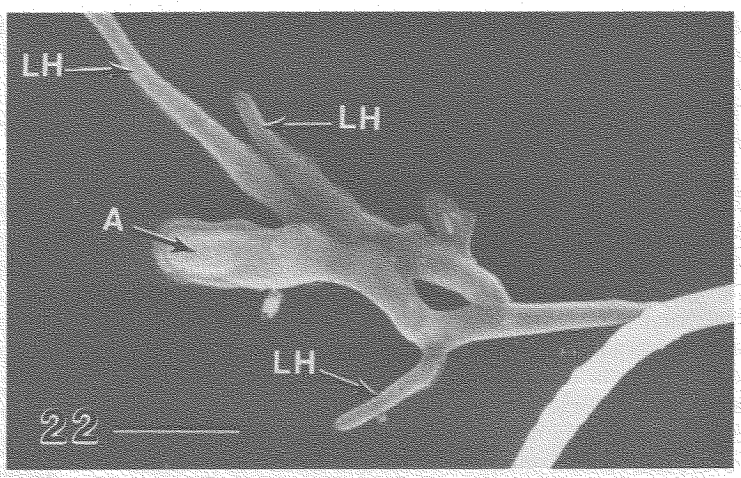
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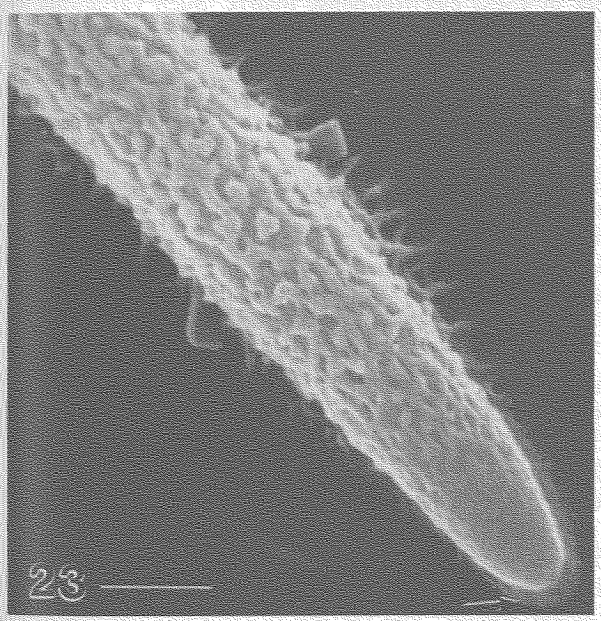
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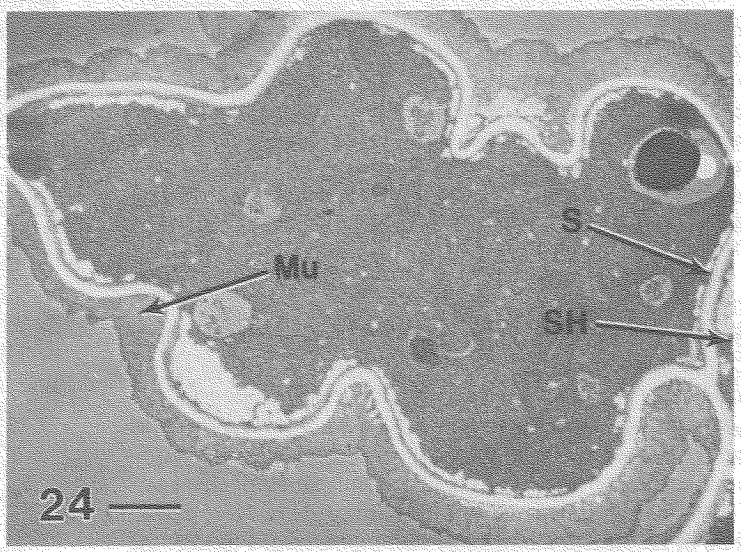
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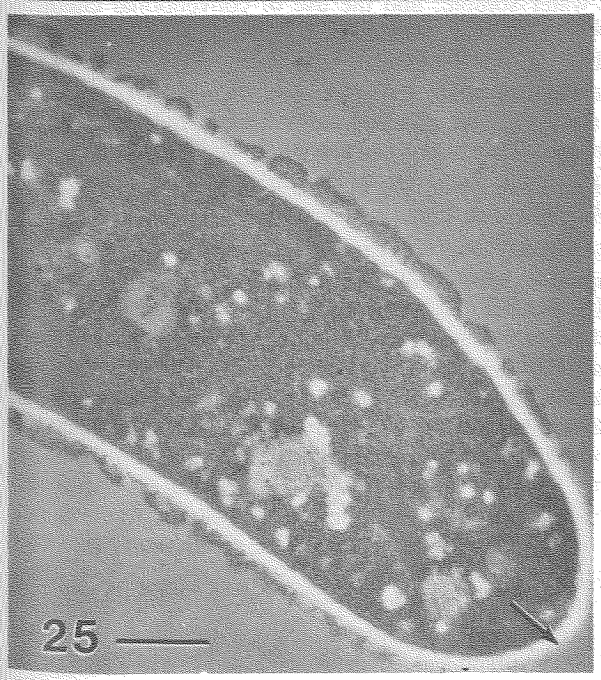
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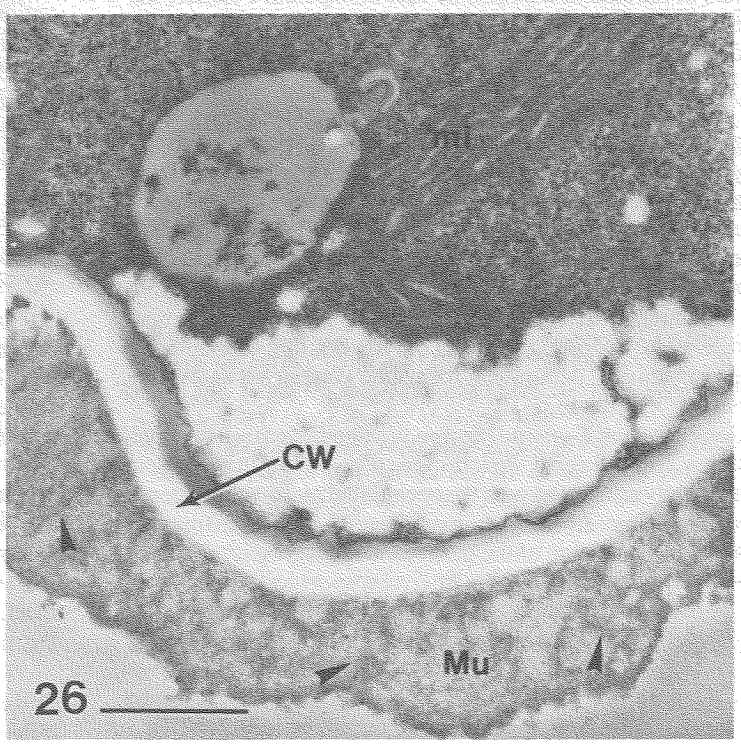
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Phytophthora cinnamomi Rands (Hardham and Gubler 1990), *Saprolegnia ferax* Gruit, *Saprolegnia parasitica* Coker, and *Saprolegnia diclina* Humphrey (Lehnen and Powell 1989; Burr 1991); zoospores of water moulds *Lagenidium*, *Coelomomyces*, *Leptolegnia*, and *Aphanomyces* spp. (Boucias and Pendland 1991); and the primary conidia of *Conidiobolus obscurus* (Hall et Dunn) Remaudière et Keller (Latge et al. 1986).

A thigmotropic response may be an effective and economical approach for mucilage production. If mucilage secretion is activated too early, the effectiveness of the mucilage may be lost, or the mucilage may diffuse away from the cell, particularly in aquatic habitats. On the other hand, if the activation process is delayed, the effective attachment of the spore will be adversely affected. The mechanisms of signal transmission leading to mucilage release in fungal cells, however, still remain unknown (Cole and Hoch 1991). Since thigmotropic responses were not particularly substrate specific in our study, it is doubtful if a molecular recognition process was involved (e.g., lectin-like interactions). Cooksey and Cooksey (1995) reported that a calcium transmembrane flux was involved in signaling for adhesive release in the fouling diatom *Amphora coffeaeformis* (Agardh.). It is possible that transmembrane calcium channels are also involved in thigmotropic signalling for fungal mucilage secretion.

Germ tube formation

Apparent differences in texture and morphology of germ tube mucilage were observed in *Lemonniera aquatica* and *Mycocentrospora filiformis*. It was a fibrillar, electron-dense layer in *Lemonniera aquatica*, whereas in *Mycocentrospora filiformis*, it was wart-like and aggregated. Such wart-like mucilage has not been observed in other aquatic Hyphomycetes and is not commonly found in other fungi. However, on the conidia of *Hirsutella thompsonii* var. *thompsonii* Fischer, the water insoluble mucilaginous material forms warts around the conidial surface (Latge et al. 1988; Boucias and Pendland 1991). These wart-like, mucilaginous structures were 170–200 nm in diameter and more distinct compared with the warty material on the conidia of *Mycocentrospora filiformis*. Scanning (Fig. 17) and transmission (Fig. 18) electron microscope observations showed that the germ tube mucilage of *Mycocentrospora filiformis* was more compact at the apex, which is the site of active cell wall synthesis and presumably where mucilage is also simultaneously secreted. It is possible that immediately after secretion, rearrangement and chemical change, e.g., hydration and (or) polymerization of the newly formed mucilage, occurs and forms the condensed and warty secondary mucilage.

Germ tubes of *Lemonniera aquatica* and *Mycocentrospora filiformis* contain small vesicles (ca. 40–60 nm in diameter) found mainly associated with the convoluted plasma membrane. Apical vesicles (70–90 nm in diameter) were also present within the apical dome of the germ tube of *Magnaporthe grisea* (Bourett and Howard 1990). In *Gymnosporangium juniperi-virginianae* Schw., the apical vesicles (70–160 nm in diameter) were apparently Golgi-derived (Mims and Richardson 1989). In *Nomuraea rileyi* (Farlow) Samson, vesicular components were frequently found associated with the region of cell wall adjacent to that enrobed by germ tube mucilage (Pendland and Boucias 1984). Despite the fact that the exact function of these vesicles is not clear, they may be involved in cell wall formation and mucilage and (or) enzyme secretion.

Appressorial development

Most pathogenic fungi form appressoria on host surfaces. Appressoria in saprobic aquatic Hyphomycetes are required to prevent dislodgement of attached conidia and aid colonization (Read et al. 1992*a*). However, on natural substrata (e.g., leaves) the appressorium may aid in the penetration of the plant cuticle and cell wall. Appressoria of *Mycocentrospora filiformis* were lobed, whereas they were swollen in *Lemonniera aquatica*. The appressorial mucilage in *Lemonniera aquatica* was homogeneous, ca. 300 nm thick, with a discrete outer margin. In *Mycocentrospora filiformis*, the sheath was ca. 850 nm thick, heterogeneous, consisting of a fibrillar network in a less electron-dense matrix. Such variations in texture and morphology suggest further differences in chemical composition of mucilage between the two fungi. Dynamic changes in mucilage morphology and thickness were observed on the germ hypha, lateral hypha, subtending hypha, and appressorium of the same fungus. Such changes in morphology may indicate polymerizaton of fibrillar mucilage or production of chemically different mucilage types during different stages of fungal attachment.

The morphology of mucilage can be influenced by the chemical fixatives used, e.g., conidia of *Lemonniera aquatica* fixed in potassium permanganate exhibited fibrillar appressorial mucilage, whereas the mucilage was more homogeneous and lacked the fibrillar features when fixed in glutaraldehyde and postfixed in osmium tetroxide. Low temperature fixation of spores followed by freeze substitution for TEM (Howard and O'Donnell 1987) and observation of hydrated spores at low temperature SEM (Clement et al. 1994) were reported to be successful for cytochemical and morphological preservation, especially on those spores with water soluble mucilage. Mucilages on the spores of aquatic Hyphomycetes are unlikely to be water soluble, and the use of conventional chemical fixatives in the present study provides significant results with respect to spore attachment.

Electron-transparent regions with inclusions and vesicles were commonly found associated with the appressorial plasma membrane. These components might be involved in mucilage secretion, but their exact functions still need further investigation.

Strength of conidial attachment

The LH-Fowler CAMM is able to measure the ability of fungal conidia to adhere when subjected to increasing shear forces (Hyde and Jones 1989; Read et al. 1991). Although only artificial substrata can be used in this equipment, the results are quantitative and reproducible. Most importantly, the condition of water flow inside the chamber resembles the realistic situation for aquatic Hyphomycetes in rivers and streams. In addition, the disturbance introduced to the organisms, and the air entrapment problem, is mild compared with the disc shearing device (Weiss 1961).

Lemonniera aquatica and *Mycocentrospora filiformis* exhibited a similar degree of initial attachment despite the different strategies involved and discussed earlier. Tetraradiate conidia of *Lemonniera aquatica* with three conidial arms make con-

tact with the substratum and secrete mucilage immediately on contact. Sigmoid conidia of *Mycocentrospora filiformis*, with one or two contact points with the surface, possessed pre-existing conidial mucilage prior to contact. Thus, both the conidial mucilage and the surface area of contact made with a surface are influential to initial attachment of conidia.

The strength of conidial attachment increased with settlement time. Rapid germ tube formation, concurrent with mucilage production, is critical to establish stronger conidial adhesion as demonstrated by *Lemonniera aquatica*. A slower initial percent germination in *Mycocentrospora filiformis* corresponds with only a slight increase in percent conidial attachment. The strength of attachment was increased further with differentiation. Elongation of germ hyphae and formation of lateral hyphae increase the area of contact with the substratum, and all these were enrobed in a mucilaginous sheath. Appressorial development accompanied by copious mucilage production increased the strength of attachment to more than 90% for both fungi. Similar results were reported in the phytopathogenic fungus *Magnaporthe grisea* (Hamer et al. 1988).

Conidia of *Lemonniera aquatica* and *Mycocentrospora filiformis* are able to attach well on negatively charged glass surfaces or nonpolar nuclepore membranes, such as Perspex and Thermanox cover slips (Read 1990; present study). It seems that physical characteristics of the substrata, e.g., electrostatic interaction and hydrophobicity, are not of major significance for nonspecific, irreversible attachment of *Lemonniera aquatica* and *Mycocentrospora filiformis* conidia. Two pathways may be operating in the initial conidial attachment of aquatic Hyphomycetes: (*i*) active adhesion by contact stimuli leading to mucilage secretion; (*ii*) passive adhesion by the presence of pre-existing conidial mucilage on conidial branches. After initial attachment to the substratum is established, subsequent mucilage secretion and (or) production of "secondary" mucilage components at different stages of attachment occur, e.g., spore germination, germ tube formation, hyphal extension, and appressorium development. These increase the surface area of attachment, and concurrent production of adhesive results in a greater strength of attachment.

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