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Attachment studies of aquatic Hyphomycetes

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SUMMARY

The strength of attachment of conidia of eight species of freshwater Hyphomycetes (*Anguillospora crassa*, *Articulospora tetracladia*, *Dimorphospora foliicola*, *Lemonniera aquatica*, *Mycentrospora filiformis*, *Tetracladium marchalianum*, *Tumularia aquatica* and *Varicosporium elodeae*) was examined in a Fowler Radial Flow Chamber up to 24 hours after settlement. It was shown that conidium morphology, particularly the number of contact points made by the conidium to the substratum, had an effect on initial attachment and therefore that tetraradiate conidia were initially the most strongly attached. It was also shown that the subsequent formation of appressoria significantly increased the strength of attachment at the higher shear stresses examined.

1. INTRODUCTION

Aquatic Hyphomycetes colonize submerged plant material, particularly leaves, in the freshwater environment. Dispersal of these fungi is by conidiospores which are frequently large and either tetraradiate (figure 13) or sigmoid (figure 14). A prerequisite for gemination of dispersed fungal spores and subsequent colonization of substrata is their initial contact with, and attachment to, surfaces. These processes are particularly important for fungi which grow in turbulent, aquatic environments where high flow rates impose pressures unfavourable to contact and attachment. Webster (1987) suggested that the variously shaped conidia of aquatic Hyphomycetes were adapted to the aquatic environment and had evolved independently in unrelated taxa. He showed that diverse aquatic hyphomycete conidia attached to submerged collodion-coated rods at water speeds of up to 0.45 m s^{-1} and also that tetraradiate conidia were trapped the most efficiently (Webster 1959). Adhesion of such conidia was shown, at both scanning and transmission electron microscope levels, to be mediated by the production of mucilages at the tips of the conidial arms (Read 1990). No quantitative data on the attachment efficiency of differently shaped aquatic hyphomycete conidia are available to complement the entrapment results reported by Webster (1959). This paper addresses this aspect of their biology.

Determination of the strengths of attachment of aquatic hyphomycete conidia has not been attempted previously although attachment of bacteria (Duddridge *et al.* 1982), marine fungal spores (Hyde *et al.* 1989), algal zoospores (Gunn *et al.* 1984) and marine diatoms (Pyne *et al.* 1984) has been examined. The apparatus employed for these experiments was the Fowler Radial Flow Chamber (Fowler & McKay 1980).

Microorganisms attach to natural substrata, e.g. leaves, wood veneers (Rees & Jones 1984; Hyde & Jones 1989) (figures 13 and 15–19). However, these substrata do not lend themselves for experimental purposes because their topography gives rise to turbulence; they are often not sufficiently rigid (e.g. leaves) to withstand high shear stresses within flow chambers; counting conidia can be difficult; and experimental areas of sufficient dimensions are not possible. Natural substrata may already be subject to macromolecular fouling and lectins may be present which attract or repulse settling conidia. Perspex discs overcome many of these problems: they enable reproducibility of surfaces (dimensions, surface energy); easy counting of attached conidia and they are not preconditioned.

2. MATERIALS AND METHODS

(*a*) *Preparation for strength of attachment studies*

Use of the Fowler Radial Flow Chamber for investigation of the strength of attachment of conidia of freshwater Hyphomycetes follows the procedures outlined for the examination of marine ascospores detailed by Hyde *et al.* (1989). Where the flow in the chamber is laminar, the shear stress can be calculated using the following formula:

$$t_0 = 3Q/rh^2.$$

Where t_0 is the shear stress measured in N m^{-2}, Q is the volumetric flow rate in m^3 s^{-1}, the fluid viscosity = 1.002×10^{-3} N s m^{-2}, the radius (r) and disc separation (h) are measured in metres. Shear stress can only be calculated where flow is laminar and as imperfections in a surface create turbulence the substratum chosen for settlement of conidia was smooth Perspex and not a natural substratum.

Conidia of *Anguillospora crassa* Ingold, *Articulospora tetracladia* Ingold, *Dimorphospora foliicola* Tubaki, *Lemonniera aquatica* de Wild., *Mycocentrospora filiformis* (Petersen) Iqbal, *Tetracladium marchalianum* de Wild.,

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Tumularia aquatica (Ranzoni) Marvanova et Descals and *Varicosporium elodeae* Kegel were settled on surface sterilized, 100 mm diameter Perspex discs for 2, 6, 12 and 24 h. In addition, conidia of *D. foliicola*, *L. aquatica*, *M. filiformis* and *T. aquatica* were also settled for 5 and 30 min. At the end of each settlement period excess water was removed and counts of the conidia in the field of view of a Wild phase contrast microscope (× 15 eye piece and × 10 objective) were taken at 10 mm intervals along the disc radius. For each disc, four radii were sampled with ten replicate counts at each radius. This formed the control experiment, i.e. the number of conidia attached to the disc before exposure to any flow rate regime. The disc was then placed in the Fowler Radial Flow Chamber and water pumped through the chamber at 1, 2 or 4 l min^{-1} for 5 min. The range of shear stresses for which data were collected was 0.48–21.26 N m^{-2}. A second set of counts was then made as described for the control. For the purposes of comparison with other microorganisms the mean detachment values of the conidia were calculated. This value was coined for evaluation of small bacteria as the shear stress at which propagules began to be detached. It worked well for the aquatic Hyphomycetes before germination, however, it did not illustrate the differences observed at the higher shear stresses caused by the formation of attachment structures. Therefore, the mean detachment values in this paper were calculated as the shear stress at which 10 % of the conidia were detached.

(*b*) *Preparation for scanning electron microscopy*

Conidial suspensions were placed on to nucleopore membranes and veneers of European birch (*Betula verrucosa* Ehrh.) and the conidia were allowed to settle for 2, 6, 12 and 24 h. The specimens were then fixed in 2 % osmium tetroxide, dehydrated in an ethanol series, critical-point dried, mounted on to aluminium stubs, coated with gold and examined in a JEOL T20 scanning electron microscope.

3. STATISTICAL ANALYSIS OF THE ATTACHMENT DATA

A statistical package, GENSTAT V, was employed in an attempt to fit the observed data to given equations. There are three types of curve component which may be used. The first is the component which is independent of the variable (shear stress) and is therefore constant. The second type of component is a linear function which is dependent on the variable. The third type is an exponential function which is also dependent on the variable. The shapes of the graphs indicate that the fitted equation contains an exponential function. Therefore the equations examined were:

$$Y = A + B\exp^{CX}, \tag{1}$$

$$Y = A + B\exp^{CX} + DX, \tag{2}$$

$$Y = B\exp^{CX} + F\exp^{GX}. \tag{3}$$

Where Y is the percentage retention of conidia and X is the shear stress. A, B, D and F are constants that relate to the percentage of conidia acted on by each component. A is the percentage of conidia which are retained independent of the shear stress and which cannot be detached. B, D and F are the percentages of conidia available for detachment and which are acted upon by the shear stress. The total of the constants: $A+B$ in equation 1, $A+B+D$ in equation 2 and $B+F$ in equation (3), should equal 100 %. Further formulae were examined in which it was specified that the total must equal 100 %. This was shown to impose too many constraints on the fitting program. C and G are decay constants which define the slope of the exponential curve(s).

For evaluation of the results of the curve fitting, the mean square of the residuals (MS), given in table 1, was taken into consideration. This figure gives an indication of the amount of deviation of the observed values from those expected from the fitted curve, therefore, the smaller this number the closer the fit of the curve. The results of the curve fitting are presented in table 1 and show that the number of conidia available for detachment and the decay constant(s) are reduced with time.

4. RESULTS

The percentages of conidia retained at any given log shear stress were calculated and are presented as graphs (figures 1–8). In all of the species examined there was a trend towards increased strength of attachment with increased time and in the sigmoid and ovoid conidia there was an initial loss of settled conidia at low shear stresses owing to removal of non-viable, unattached or poorly attached conidia. After this initial loss the slopes of the curves became less acute.

Initially tetraradiate conidia were all strongly attached and a proportion were even retained at the highest shear stress of 21.26 N m^{-2}. It was also shown that the graph profiles were similar for each species. *Lemonniera aquatica* developed rapidly and once the majority of the conidia were attached, after settlement for 10–15 min, few were removed by a shear stress of less than 10.63 N m^{-2} (figure 2). This species germinated within 30 min and after settlement for only 5 min conidia of *L. aquatica* were strongly attached (figure 2), and 14 % of the conidia could not be detached by a shear stress of 21.26 N m^{-2}. After settlement for two hours shear stresses greater than 2.77 N m^{-2} were required to cause any conidial detachment in *Articulospora tetracladia* (figure 1). Also at two hours 12 % of the conidial population of *Tetracladium marchalianum* was weakly adherent (figure 3).

In *Lemonniera aquatica* the strength of attachment of conidia increased rapidly with time (figure 2) and at 24 h after settlement conidia were not detached by a shear stress of 10.63 N m^{-2} (figure 2). The greatest increase in conidial retention, and hence in the strength of attachment of *Tetracladium marchalianum* occurred between 2 h (26.9 %) and 6 h (9.1 %) after settlement. After 6 h little further increase in the strength of attachment was observed at 10.63 N m^{-2} (figure 3). However, there was an increase in the strength of attachment between 6 and 24 h at a shear stress

Table 1. *The results of fitting the observed values of retention of conidia to the curve* $Y = A + B\exp^{CX}$. *Fitted values of* A, B *and* C *after settlement periods of up to 24 h*

species	5 min	30 min	2 h	6 h	12 h	24 h
A. crassa						
A	not tested		14.77	44.80	50.19	57.83
B			84.39	61.93	49.81	42.17
C			−1.73	−0.3	−0.12	−0.11
MS			8.99	6.19	5.88	14.0
D. foliicola						
A	no attachment		−7.85	44.96	48.32	$F = 99.98$[a]
B			107.85	55.04	56.93	−0.06
C			−0.1	−0.14	−0.09	0.24
G						0.0006
MS			72.5	33.2	3.42	0.006
M. filiformis						
A	18.07	34.47	40.51	34.75	46.61	88.55
B	66.37	64.9	61.8	72.10	58.89	13.61
C	−0.26	−0.18	−0.2	−0.11	−0.10	−0.18
MS	4.56	7.19	5.06	12.9	5.38	0.95
T. aquatica						
A	no attachment		1.05	7.9	15.39	40.58
B			22.98	96.13	94.07	61.77
C			−0.64	−0.37	−0.23	−0.1
MS			20.8	68.7	59.1	4.89
V. elodeae						
A	not tested		47.8	65.78	72.32	78.98
B			69.98	33.79	30.14	23.77
C			−0.64	−0.16	−0.17	−0.19
MS			21.7	4.21	5.12	1.79

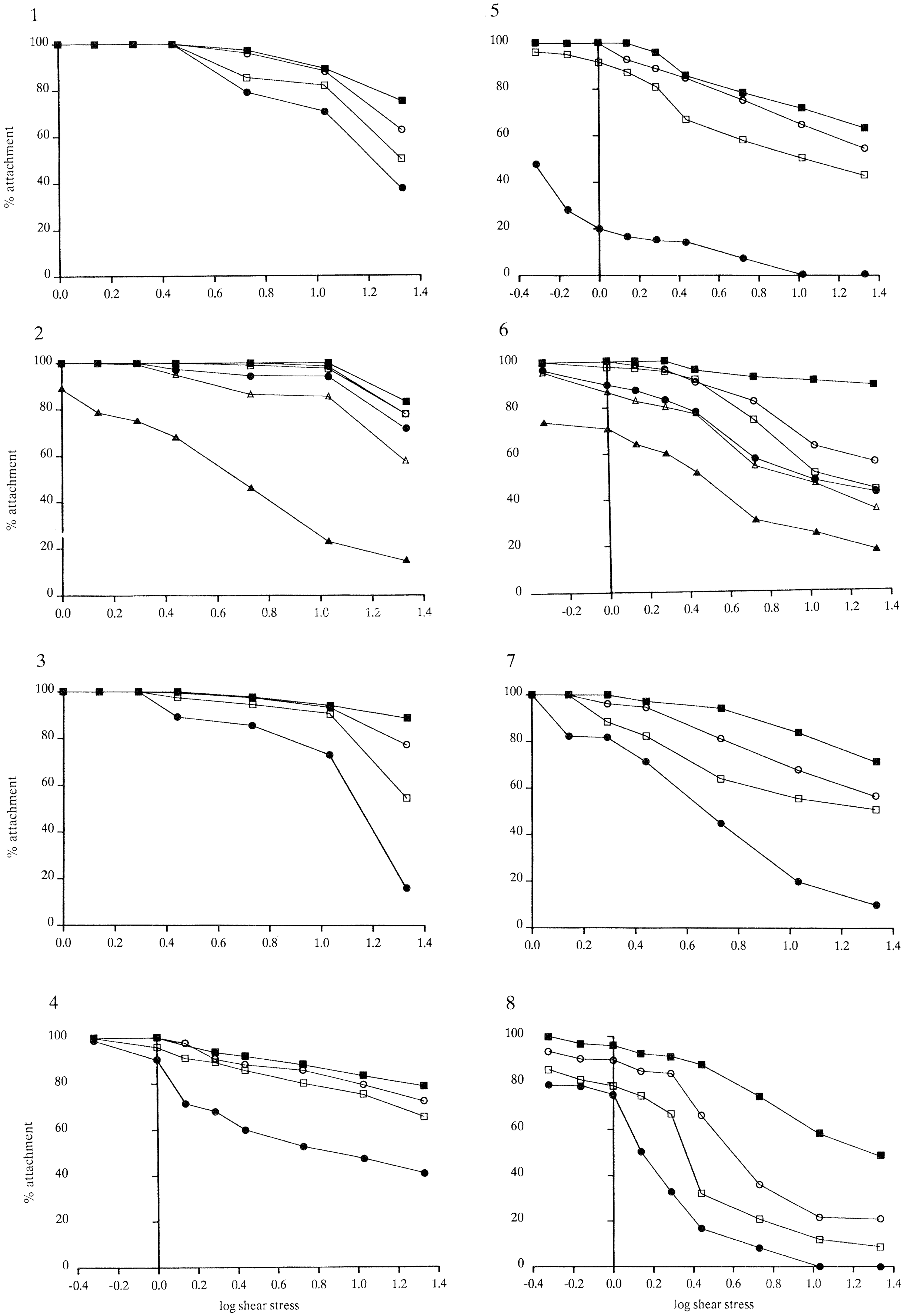
[a] Curve fitted was $Y = B\exp^{CX} + F\exp^{GX}$. Y = the total number of conidia retained at a given shear stress (100 %); A = the number of conidia retained independently of shear stress; B, F = the number of conidia retained dependent on the shear stress; C, G = the decay constants; X = the shear stress; MS = the mean square. Low MS values indicate a close fit.

of 21.26 N m^{-2}. These increases in the strength of attachment were not found to be proportional to the amount of time elapsed. Rather it was observed that the increases in conidial retention were concomitant with appressorium formation.

Varicosporium elodeae conidia became attached to the substratum within 10–15 min although adhesion was not strong and after two hours more than 50 % of the attached conidia were removed by a shear stress of 10.63 N m^{-2} (figure 4). At each time period a large proportion of the total number of conidia detached were removed at low shear stresses. The largest increase in the number of attached conidia which were retained and the corresponding reduction of the decay constant (C) (table 1) occurred between two and six hours after settlement. After 6 h there was only a small increase in retention with time and almost no change in the decay constant. The increase in the strength of attachment was given by the increase in the number of conidia (A) which could not be detached, this number rose sharply between 2 h (47.8 %) and 6 h (65.78 %) and then continued to increase steadily at 12 h (72.32 %) and 24 h (78.98 %). Appressoria were observed on a higher proportion of the conidia retained after being subjected to shear stresses greater than 10.63 N m^{-2} than at lower shear stresses after settlement for 12 h.

The profiles of attachment against shear stress shown by the two sigmoid conidial forms were similar and did not change appreciably with time (figures 5 and 6). During the first 6 h a large proportion of the conidia were detached by low shear stresses with *Mycocentrospora filiformis* the more firmly attached species. Settlement and initial attachment of *Anguillospora crassa* conidia occurred within 10–15 min although the initial strength of attachment was low (figure 5). At 2 h more than 52 % of the conidia were detached at a shear stress of 0.48 N m^{-2} and, 80 % of the conidia were lost at 0.93 N m^{-2}. After 6 h almost all of the conidia remained attached after exposure to a shear stress of 0.48 N m^{-2}.

Attachment of the majority of the conidia, therefore, took place after settlement for between 2 and 6 h. This is shown by an increase in A and a large decrease in C. The strength of attachment increased with time, fewer conidia were removed by the lower shear stresses and the decay constant continued to fall more slowly. Conidia of *Mycocentrospora filiformis* became attached within 5 min, for although large numbers were lost at low shear stresses, more than 17 % of the conidia were retained after exposure to a shear stress of 21.26 N m^{-2} (figure 6). A significant increase in the strength of attachment occurred between five and 30 min after settlement. The increase in attachment was slight between 30 min and 6 h after initial settlement. A further large increase in attachment was observed between 12 and 24 h after settlement and exposed at shear stresses of 10.63 N m^{-2}. Twelve hours after settlement many of the attached conidia were observed to possess mature appressoria. One of the data points at



Figures 1–8. Percentage of conidia retained on Perspex discs after subjection to shear stresses of up to 21.26 N m^{-2} for 5 min and after settlement for up to 24 h. Figure 1. *Articulospora tetracladia*. Figure 2. *Lemonniera aquatica*. Figure 3. *Tetracladium marchalianum*. Figure 4. *Varicosporium elodeae*. Figure 5. *Anguillospora crassa*. Figure 6. *Mycocentrospora filiformis*. Figure 7. *Dimorphospora foliicola*. Figure 8. *Tumularia aquatica*. ▲ = 5 min, △ = 30 min, ● = 2 h, □ = 6 h, ○ = 12 h, ■ = 24 h.

the 6 h stage was unusually low. This is not apparent on the graph (figure 6), however, it did produce a high value (12.91) for the MS and a low value (34.75) for A (table 1). In this case a better indication of the value of A was the point at which the curve levelled out, at 40%.

The ovoid conidia of *Dimorphospora filiicola* and *Tumularia aquatica* settled at similar rates to those with tetraradiate, branched or sigmoid conidia, however, initial attachment of ovoid conidia was poor. At time periods of 30 min conidia of these two species were not attached to the substratum (figures 7 and 8). After settlement for 2 h the strength of attachment of conidia of *Dimorphospora foliicola* showed a high value for the MS and a negative value for A (table 1). This was attributed to the clumping of unattached conidia around attached conidia. The point at which the curve levelled out was 0% and, this is a more valid value for A. At this stage the settled conidia were strongly attached to the substratum at the lower shear stresses. The minimum shear stress found to cause conidial detachment was 1.39 N m^{-2} when 18.2% of the conidia were removed and, the maximum shear stress (21.26 N m^{-2}) removed 90% of the settled conidia (figure 7). There was a significant increase in the strength of attachment between 2 and 6 h after settlement when a shear stress of 21.26 N m^{-2} removed 48.9% of the conidia. The increase in the strength of attachment which was observed between 6 h and 24 h after settlement appeared to be proportional to time. Twenty-four hours after settlement the majority of the conidia were securely attached and only 8.9% of the conidia were detached at a shear stress of 21.26 N m^{-2}.

Conidia of *Tumularia aquatica* did not attach strongly, and this was the most weakly attached species examined (figure 8) with no attachment in the first 30 min. After 2 h the minimum shear stress of 0.48 N m^{-2} detached 16% of the attached conidia and 2.77 N m^{-2} was sufficient to detach 82%. Although there was a marked increase in the strength of attachment between 12 and 24 h, the maximum percentage of conidia retained after settlement for 24 h was only 48.7% at a shear stress of 21.26 N m^{-2}. The increase in attachment and the total strength of adhesion appeared to be proportionally related to time. This was also supported by an increase in the shear stress required to detach a given number of conidia and a decrease in C (table 1).

5. RESULTS OF CURVE FITTING

Detachment of tetraradiate conidia was observed only at the highest shear stress examined, therefore, these data were not suitable for curve fitting equations. For the remainder of the species the equation $Y = A + B\exp^{CX}$ was found to fit the curves with the exception of *Dimorphospora foliicola* after settlement for 24 h. Where A was the proportion of conidia retained independently of shear stress and B the proportion retained dependent on the shear stress. When the shear stress (X) is high $B\exp^{CX}$ approaches 0 and, therefore, $A + B\exp^{CX}$ approaches A, thus A is the value at which the curve levels out. Because $B\exp^{CX}$ is the decay function of the curve, C the decay constant, is negative. For *D. foliicola* the data at 24 h fitted an equation where A was replaced by $F\exp^{GX}$ within the limits of X ($X =$ 0.48–21.26 N m^{-2}) and, where $F\exp^{GX}$ approaches F.

6. DISCUSSION

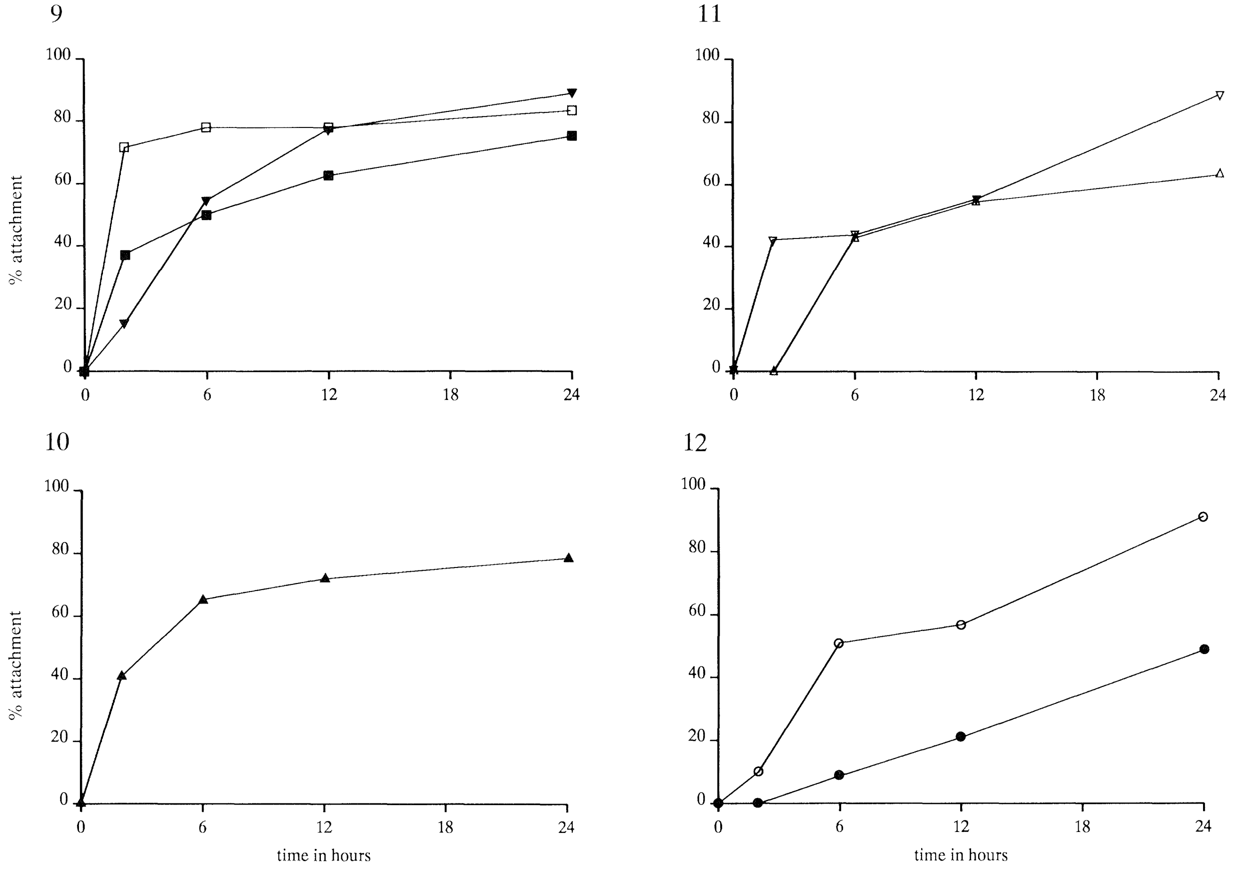
(*a*) *Conidium configuration and strength of attachment*

The graphs of conidial attachment (figures 1–8) showed the percentage of conidia retained at each shear stress and settlement period. The graphs usually comprised an initial sharp reduction in retention followed by a less steep decrease. Because the conidia were evenly dispersed it is suggested that the time of settlement, after placement of the conidial suspension on the Perspex plates, is dependent on their original distance from the surface. Conidia may also form only a weak bond with the substratum on initial attachment. After longer settlement periods the number of newly settled conidia is progressively reduced. This resulted in a reduction in the number of conidia detached at the lower shear stresses with increased time.

Conidia allowed to settle for 5 min to 24 h showed increased development of attachment or germination structures. This produced increases in both the minimum shear stress which was required to cause conidial detachment and in the mean detachment value. The almost linear second part of the curve, therefore, was mainly due to the detachment of settled, attached, and in many cases germinated, conidia.

When the percentage of conidia which remained attached after subjection to a shear stress of 21.26 N m^{-2} for 5 min was plotted against time (figures 9–12) it was shown that conidium configuration had a significant influence on initial attachment. After settlement for only 2 h tetraradiate (figure 9) and branched conidia (figure 10) were attached more strongly than sigmoid conidia (figure 11) which in turn were attached more strongly than ovoid conidia (figure 12).

The two major physical factors which may have influenced these results are: (i) the number of contact points any given conidium is able to make with a substratum; (ii) the resistance presented by the attached conidium to the flow of water. With respect to the number of contact points formed by conidia of each species, tetraradiate conidia usually form three (figure 13), branched conidia form multiple contacts, sigmoid conidia form approximately two (figure 14) and ovoid conidia only one (figure 15). The second factor is more complex as it depends not only on the total surface area of the conidium but also on its orientation and the effect of conidium shape on microturbulence. Conidia of *Varicosporium elodeae* tend to lie flat against the surface forming multiple, extensive contacts and present little resistance to water flow almost independent of conidium orientation. In other species at least a part of the conidium projects out through the laminar boundary layer of water. In these instances it must be noted that the conidia settled evenly and at random over a smooth surface and were then subjected to a directional shear stress. This may not be the case in the natural



Figures 9–12. The effect of conidium morphology on the strength of conidial attachment. The percentage of conidia retained after subjection to a shear stress of 21.26 N m^{-2} for 5 min after settlement on Perspex discs for up to 24 h. Figure 9. Tetraradiate conidia. ■ = *Articulospora tetracladia*, □ = *Lemonniera aquatica*, ▼ = *Tetracladium marchalianum*. Figure 10. Branched conidium. ▲ = *Varicosporium elodeae*. Figure 11. Sigmoid conidia. △ = *Anguillospora crassa*, ▽ = *Mycocentrospora filiformis*. Figure 12. Ovoid conidia. ○ = *Dimorphospora foliicola*, ● = *Tumularia aquatica*.

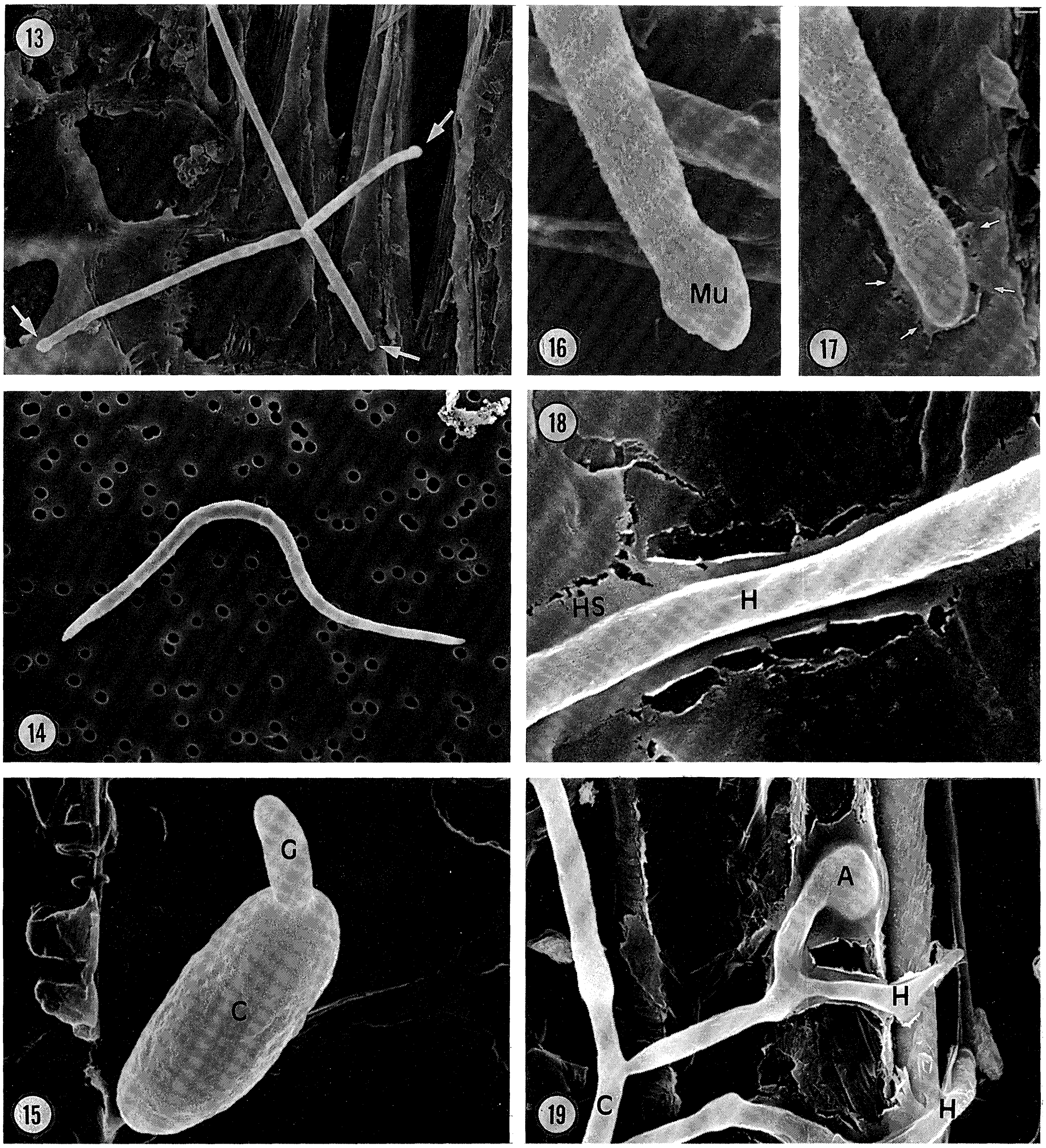
environment where conidia settle onto a substratum with uneven topography while subjected to variable water currents. In tetraradiate and ovoid conidia the influence of this effect may be limited by their symmetrical appearance when viewed from a number of different angles. Sigmoid conidia however, appear markedly different when observed from polar and lateral viewpoints.

A third difference which may have influenced conidial attachment is the composition and efficiency of the conidial (figures 16 and 17), hyphal (figures 18 and 19) and appressorial (figure 19) mucilages. It has been shown (Read 1990) that although the majority of the mucilages produced by these species of aquatic Hyphomycetes are polysaccharide based, they do possess different functional groups.

A comparison of the graphs of attached tetraradiate conidia showed similar profiles (figure 9). The majority of conidia settled for only two hours were securely attached. This is due to the presence of a discrete mucilage at the apices of the conidial arms (figure 16) which adheres the conidium to a substratum on contact (figure 17). Initial attachment of *Lemonniera aquatica* was stronger than the other tetraradiate conidia owing to its more rapid formation of appressoria. However, the interspecific differences were greatly reduced 24 h after settlement when appressoria were present in all tetraradiate species (figure 9). The greater proportion of conidia with appressoria retained at high shear stress further indicates that the appressorium may be responsible for the increase in the strength of attachment.

Conidial attachment and the final strength of adhesion of conidia of *Varicosporium elodeae* (figure 10) were similar to those of tetraradiate conidia (figure 9). However, the graph of attachment of *V. elodeae* with respect to shear stress (figure 4) showed a similar pattern to those of the sigmoid conidia (figures 5 and 6). It is possible that the short, cylindrical fragments of *V. elodeae* conidia are acted upon by similar forces to those on the sigmoid conidia. However, these fragments are attached along their whole length and are, therefore, attached more strongly.

Of the two species which formed sigmoid conidia, those of *Mycocentrospora filiformis* (figure 6) were initially more strongly attached than those of *Anguillospora crassa* (figure 5). This was particularly apparent when the strength of conidial attachment was plotted against time (figure 11). It is suggested that one of the factors responsible for this initial difference is conidium orientation. Conidia of each species when orientated parallel to the water current present similar resistances, however, conidia orientated at a tangent to the current will show differing resistances as conidia of *A. crassa* are wider and, thus, present a larger surface area to the flow of water. Consequently this may have resulted in



Figures 13–19. Scanning electron micrographs of conidia of aquatic Hyphomycetes after settlement for up to 12 h. Figure 13. *Articulospora tetracladia*. Conidium settled for 2 h on a birch veneer. Note the swollen apices of the conidial arms (arrowed). × 1000. Figure 14. *Anguillospora crassa*. Conidium settled for 2 h on a nucleopore membrane. × 500. Figure 15. *Dimorphospora foliicola*. Conidium (C) with a short germ tube (G) after settlement for 2 h on a birch veneer. × 5000. Figure 16. Discrete mucilage (Mu) at the apex of a conidial arm of *Articulospora tetracladia* which has not formed a substratum contact after settlement for 2 h on a birch veneer. × 7500. Figure 17. Conidial arm of *Articulospora tetracladia* which has formed a contact point within 2 h of settlement. The mucilage at the apex of the arm (arrowed) adheres it to the substratum. × 7500. Figure 18. Hypha (H) with hyphal sheath (HS) formed by a conidium of *Articulospora tetracladia* after settlement for 12 h on a birch veneer. × 7500. Figure 19. Conidium (C) of *Varicosporium elodeae* which has formed both hyphae (H) with sheaths and also an appressorium (A) after settlement for 12 h on a birch veneer. × 2000.

the detachment of a greater proportion of the conidia of *A. crassa*. Differences in the conidial adhesive may also be a contributory factor.

Differences in adhesion between *A. crassa* and *M. filiformis* subsequent to their initial attachment illustrate very clearly the influence of appressorium formation on attachment. Once the sigmoid conidia became established on the substratum between 6 and 12 h after settlement there was little difference between their strengths of attachment. However, when appressoria were formed by *M. filiformis* between 12 and 24 h after settlement there was a marked increase in the attachment of *M. filiformis* over that of *A. crassa* which lacked appressoria (figure 11).

Table 2. *Mean detachment values (in N m*$^{-2}$*) of algae, diatoms, bacteria, marine fungi and freshwater fungi observed in the Fowler radial flow chamber*

Species	Propagules settled for up to 24 h 1 h	2 h	8 h	24 h
algae[a]				
Enteromorpha intestinalis (L.) Link	51.8	—	59.25	59.25
bacteria[b]				
Pseudomonas fluorescens (NCIB 9046)	—	—	11.0	11.0
diatoms[c]				
Achnanthes longipes Agardh.	0.96	—	1.5	2.29
Licmophora flabellata (Carm.) Agardh.	0.75	—	2.12	5.64
Amphora coffeaeformis (Agardh.) Kutz.	—	—	2.98	—
marine fungi[d]				
Amylocarpus encephaloides Currey	1.17	1.3	1.85	1.85
Ceriosporopsis circumvestita (Kohlm.) Kohlm.	2.33	2.33	2.33	2.33
Eiona tunicata Kohlm.	5.01	6.38	7.75	7.75
Groenhiella bivestia Koch, Jones et Moss	1.43	1.64	2.6	2.6
Halosphaeria appendiculata Linder	—	—	—	2.86
Iwilsoniella rotunda Jones	3.5	3.5	3.5	3.5
freshwater fungi				
Anguillospora crassa Ingold	—	< 0.48	1.18	2.45
Articulospora tetracladia Ingold	—	4.24	6.13	10.17
Dimorphospora filiicola Tubaki	—	1.32	2.73	8.56
Lemonniera aquatica de Wild.	7.39	12.61	14.82	17.00
Mycocentrospora filiformis (Petersen) Iqbal	0.87	0.97	3.02	14.98
Tetracladium marchalianum de Wild.	—	2.78	11.56	18.38
Tumularia aquatica (Ranzoni) Marvanova et Descals	—	< 0.48	0.62	2.56
Varicosporium elodeae Kegel	—	0.98	1.98	4.10

Data after Duddridge *et al.* (1982)[b]; Gunn *et al.* (1984)[a]; Hyde *et al.* (1989)[d] and Pyne *et al.* (1984)[c].

The relation between conidial attachment and shear stress in *Dimorphospora foliicola* and *Tumularia aquatica* (figures 7 and 8) appeared similar to that shown by the sigmoid conidia (figures 5 and 6). However, initial attachment in the ovoid conidia was far weaker and the relation between conidial detachment and shear stress was exponential. Furthermore, the increase in strength of conidial attachment was shown to be proportional to time (figure 12).

After settlement for 24 h the majority of the conidia were securely attached, however, *A. crassa* and *T. aquatica* were exceptions. The similarities in the strengths of attachment of conidia with different configurations after extended periods of settlement suggests that once conidia have become attached their subsequent germination and the formation of attachment structures reduces the initial advantage gained by the multiple contact points of the tetraradiate conidia.

(*b*) *Strength of attachment of propagules of aquatic algae, bacteria, diatoms and fungi*

In previous studies (Duddridge *et al.* 1982; Gunn *et al.* 1984; Pyne *et al.* 1984; Hyde *et al.* 1989) the adhesion of propagules was expressed in terms of the mean detachment values, shear stresses at which significant numbers were detached. To discuss the strength of attachment of aquatic hyphomycete conidia in relation to the propagules of other aquatic microorganisms their detachment values were calculated and are presented for comparison in table 2. All of the comparable propagules are from marine species, with the exception of *Pseudomonas fluorescens*, as much of these data come from studies of marine fouling organisms which are of commercial importance. Four important aspects arise from examination of table 2: (i) spores of the alga *Enteromorpha intestinalis* are the most strongly attached followed by cells of *Pseudomonas fluorescens*; (ii) spores of the marine fungi showed either no increase in attachment with respect to time: *Ceriosporopsis circumvestita*, *Iwilsoniella rotunda*; or, no further increase in attachment after settlement for 8 h: *Amylocarpus encephaloides*, *Eiona tunicata*, *Groenhiella bivestia*; (iii) strength of attachment of diatoms, and conidia of aquatic Hyphomycetes, increases with respect to time; (iv) spores of the marine fungi are initially attached more strongly than conidia of the aquatic Hyphomycetes. However, after settlement for 8 h the two groups are approximately equal but at 24 h the aquatic Hyphomycetes are more strongly attached.

Great care must be exercised when comparing the data for the strength of attachment of various propagules of marine organisms. The size and morphology of the structures involved vary considerably and this presents a difference to the water flow within the chamber. Eddy currents will be set up by the larger propagules while these will be less pronounced with the smaller bacterial cells and *Enteromorpha intestinalis* zoospores. This may, in part, explain the strong attachment exhibited by the smaller propagules.

If the conidia of the aquatic Hyphomycetes in table

2 are separated by their conidium morphology the initial strengths of attachment of the tetraradiate conidia are comparable to those of the much smaller marine fungal spores examined by Hyde *et al.* (1989). The similar adhesive strengths of tetraradiate conidia and marine fungal spores after settlement for only 2 h may indicate that the arms of the former have a similar function to the appendages of the latter. Non-tetraradiate species of aquatic Hyphomycetes showed lower values for initial attachment. After settlement for 8 h the strengths of attachment of aquatic hyphomycete conidia, except for *T. aquatica*, were similar to those of the marine fungal spores. At this stage attachment was stronger than that of the fouling diatoms but weaker than *E. intestinalis*. Between 8 and 24 h after settlement attachment of the marine fungal spores did not increase appreciably, however, notable increases were observed for each species of aquatic hyphomycete. Hyde (1985) reported that with the exception of *G. bivestia* the marine fungal spores tested did not germinate on the perspex plates. Therefore increases in attachment of these marine fungal spores were due solely to appendage maturation or secretion of secondary mucilage(s) (Hyde *et al.* 1989).

A comparison of the results obtained for conidia of aquatic Hyphomycetes with those of other aquatic spores or propagules confirms that increases in the strength of attachment of aquatic hyphomycete conidia with respect to time are dependent on the formation of germ tubes and further attachment structures.

The conclusions drawn from this study are that initial attachment in tetraradiate conidia is stronger than other conidium types owing to the possession of multiple contact points with the substratum. The initial adhesion of aquatic hyphomycete conidia is comparable to that of marine ascospores. This indicates that the strength of attachment in diverse aquatic fungi has evolved to approximately the same levels albeit by slightly different methods. Mucilage produced by conidia subsequent to settlement and then by their germ tubes also served to increase attachment. Appressoria, produced by all of the tetraradiate conidia and by some of the other species some 12 h after the initial settlement were correlated with increased strength of attachment at the higher shear stresses. Therefore it is suggested that one of the functions of the appressorium in aquatic Hyphomycetes is adhesion to a substratum with the object of aiding colonization.

Although the results presented in this paper were obtained from an artificial, inert Perspex substratum, observations of the development of germ tubes, hyphae and appressoria on Perspex paralleled their development on wood veneers (figures 13 and 15–19). Consequently it is suggested that the structures which were found to have such a profound effect on attachment to a Perspex substratum will have similar functions in the natural environment.

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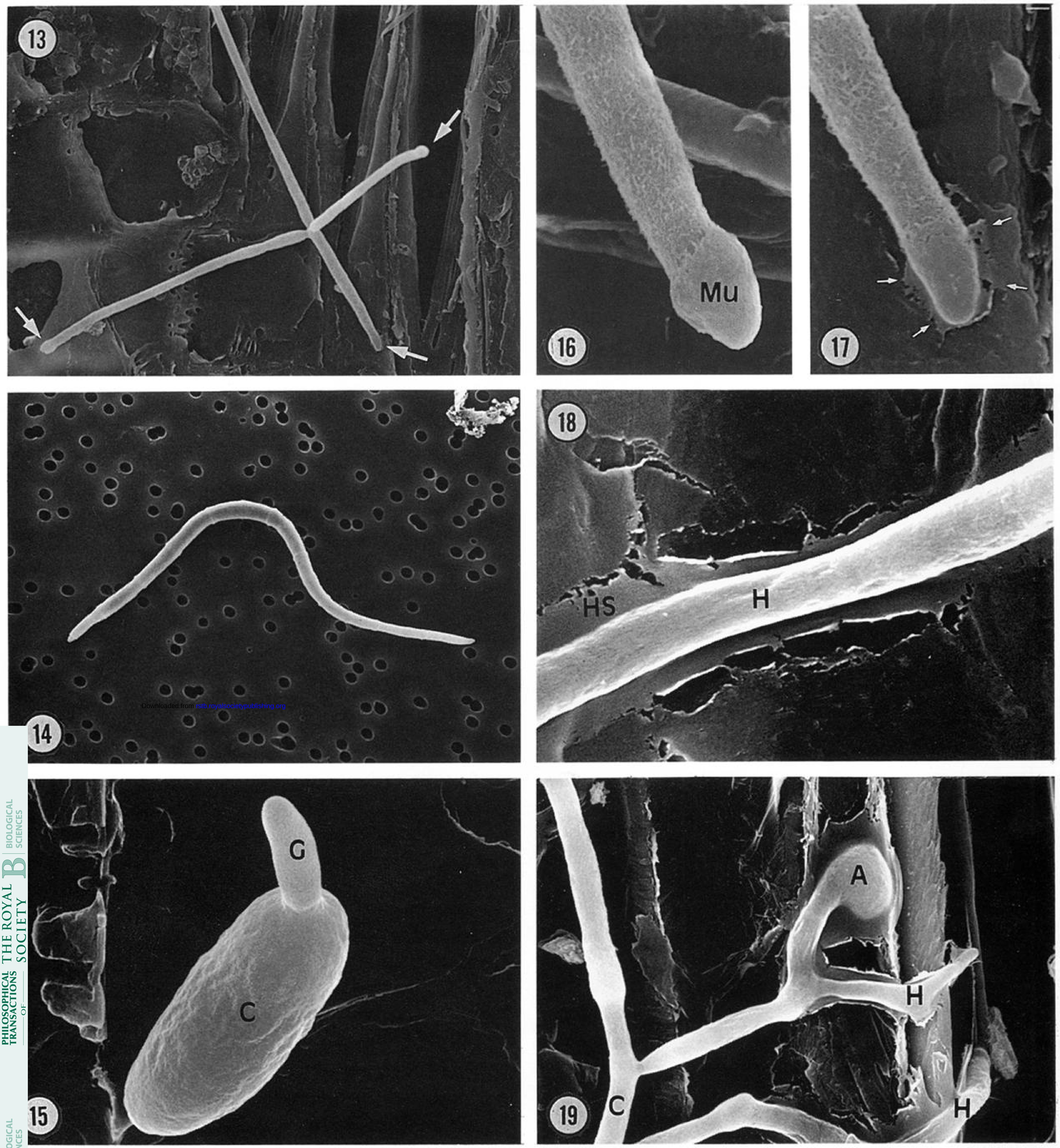

Figures 13–19. Scanning electron micrographs of conidia of aquatic Hyphomycetes after settlement for up to 12 h. Figure 13. *Articulospora tetracladia*. Conidium settled for 2 h on a birch veneer. Note the swollen apices of the conidial arms (arrowed). × 1000. Figure 14. *Anguillospora crassa*. Conidium settled for 2 h on a nucleopore membrane. × 500. Figure 15. *Dimorphospora foliicola*. Conidium (C) with a short germ tube (G) after settlement for 2 h on a birch veneer. × 5000. Figure 16. Discrete mucilage (Mu) at the apex of a conidial arm of *Articulospora tetracladia* which has not formed a substratum contact after settlement for 2 h on a birch veneer. × 7500. Figure 17. Conidial arm of *Articulospora tetracladia* which has formed a contact point within 2 h of settlement. The mucilage at the apex of the arm (arrowed) adheres it to the substratum. × 7500. Figure 18. Hypha (H) with hyphal sheath (HS) formed by a conidium of *Articulospora tetracladia* after settlement for 12 h on a birch veneer. × 7500. Figure 19. Conidium (C) of *Varicosporium elodeae* which has formed both hyphae (H) with sheaths and also an appressorium (A) after settlement for 12 h on a birch veneer. × 2000.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES