Journal of General Microbiology (1979), **111**, 303–311. *Printed in Great Britain*

Ultrastructure of Septa in *Dimargaris cristalligena* R. K. Benjamin

By PETER JEFFRIES

Department of Microbiology, University of Edinburgh, Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG

AND TOM W. K. YOUNG

Department of Applied Biology, Chelsea College, University of London, Hortensia Road, London SW10 0QX

(*Received* 31 *August* 1978)

The fine structure of the septum in the vegetative hypha of the mycoparasite *Dimargaris cristalligena* is described. The cross-wall, continuous with the inner electron-lucent layer of the hyphal wall, bears a central pore with a flared margin occupied by a biumbonate electron-dense plug. A globose body, surrounded by a zone of ribosome-free cytoplasm, is situated next to the septal pore on each side of the cross-wall. In the neck of the haustorium, a globose body is normally observed only on that side of the septal pore next to the appressorium. Globose bodies appear structureless; they are electron-dense when fixed in potassium permanganate and of variable electron density in aldehyde–osmium fixations. Cytoplasmic continuity is maintained through the septal pore by the plasmalemma. In the sporangiophore and sporiferous branchlets, the septal plug bears an upper globose protuberance and a lower obconic one. The hyphal septum in *Tieghemiomyces californicus* is ultrastructurally similar to that of *D. cristalligena*. The possible functional and taxonomic significance of such septa is indicated.

INTRODUCTION

Dimargaris cristalligena, like other species in the genus, is parasitic on members of the Mucorales. Characteristically, it produces two-spored merosporangia and the hyphae are regularly septate. Liberated sporangiospores germinate on nutrient agar and the mycelium may make weak saprophytic growth in the absence of the host. Complex septa are produced in the mycelium, typically having a cross-wall with a flared central pore occluded by a plug, and usually with a spherical body on each side of the plug (Benjamin, 1959). Although the distinctive form of the septum found in the vegetative hyphae, as observed in the light microscope, is used as a diagnostic character of the Dimargaritaceae, the fine structure of such septa has not been described. Ultrastructural studies of septa (e.g. Moore & McAlear, 1962; Moore, 1965; Moss, 1975; Khan, 1976) have provided substantial information supporting taxonomic relationships. The aim of this paper is to describe the fine structure of the septum of the vegetative hypha of *D. cristalligena*, with notes on the sporangiophore septum and on a related species *Tieghemiomyces californicus*, for comparison with other known forms of septa.

METHODS

Organisms and culture procedures. Dimargaris cristalligena R. K. Benjamin, obtained from the Centraalbureau voor Schimmelcultures (CBS 219.59), was maintained in dual culture on the reference host *Cokeromyces recurvatus* Poitras (CBS 158.50) on yeast extract/peptone/soluble starch agar (YpSs;

0022-1287/79/0000-8346 $02.00

Benjamin, 1959) at 25 °C in the dark (for rationale, see Jeffries & Young, 1975*a*). *Tieghemiomyces californicus* R. K. Benjamin (CBS 448.59) was maintained similarly.

Electron microscopy. Specimens were prepared for electron microscopy according to Jeffries & Young (1975*b*, 1976*a*). Material was fixed either in 1 % (w/v) potassium permanganate in 0·1 M-phosphate buffer (pH 7·0) for 30 min at room temperature, or in a mixture of 0·5 % (v/v) formaldehyde and 1 % (v/v) glutaraldehyde in 0·1 M-cacodylate buffer (pH 7·2) for 30 min, followed by 1 % (w/v) osmium tetroxide in the same buffer for 3 h. During dehydration, preparations fixed by the latter technique were left overnight in 70 % (v/v) ethanol containing 2 % (w/v) uranyl acetate. Sectioned material fixed in aldehyde–osmium was post-stained in 2 % (w/v) aqueous uranyl acetate for 30 min, followed by 10 min in Reynold's lead citrate. Permanganate-fixed material was post-stained in lead citrate only (Jeffries & Young, 1976*a*). Scanning electron microscopy was carried out using critical point-dried, sputter-coated preparations (Jeffries & Young, 1975*a*).

To obtain material containing germ tubes or vegetative hyphae, fresh sporangiospore suspensions of *D. cristalligena* were spread over cellophane squares (6 × 6 cm, boiled in several changes of distilled water to remove plasticizers), placed on malt extract agar (MEA, Oxoid) or YpSs agar in Petri dishes and incubated at 25 °C for 2 to 4 d until sufficient germ tubes or hyphae had reached a suitable length. Sections of vegetative hyphae were also obtained from material prepared for infection studies in which yeast-phase cells of *C. recurvatus* were added and the dishes incubated for a further 24 h (Jeffries & Young, 1976*b*). For preparations of sporangiophores, freshly collected material was gently immersed in molten 2 % (w/v) tap water agar which was then allowed to solidify. Pieces of agar containing the sporangiophores were fixed, dehydrated and then flat embedded in rectangular moulds. After hardening, suitable areas of resin were excised, re-orientated and attached to conventional resin stubs for thin-sectioning.

RESULTS

Sporangiospores of *D. cristalligena* germinate on YpSs and MEA, and septa develop in the germ tubes and the vegetative hyphae. At the limit of resolution of the light microscope, the central region of the cross-wall appears swollen and a central lenticular structure is discernible, bordered on each side of the septum by a globose body (Figs 1, 2). The sporangiophore cross-wall, first described by Benjamin (1959) and investigated histochemically by Benny (1972), differs structurally from that of the vegetative hyphae as only the upper lobe of the septal plug is globose, the lower being obconic (Benjamin, 1959; and Fig. 3).

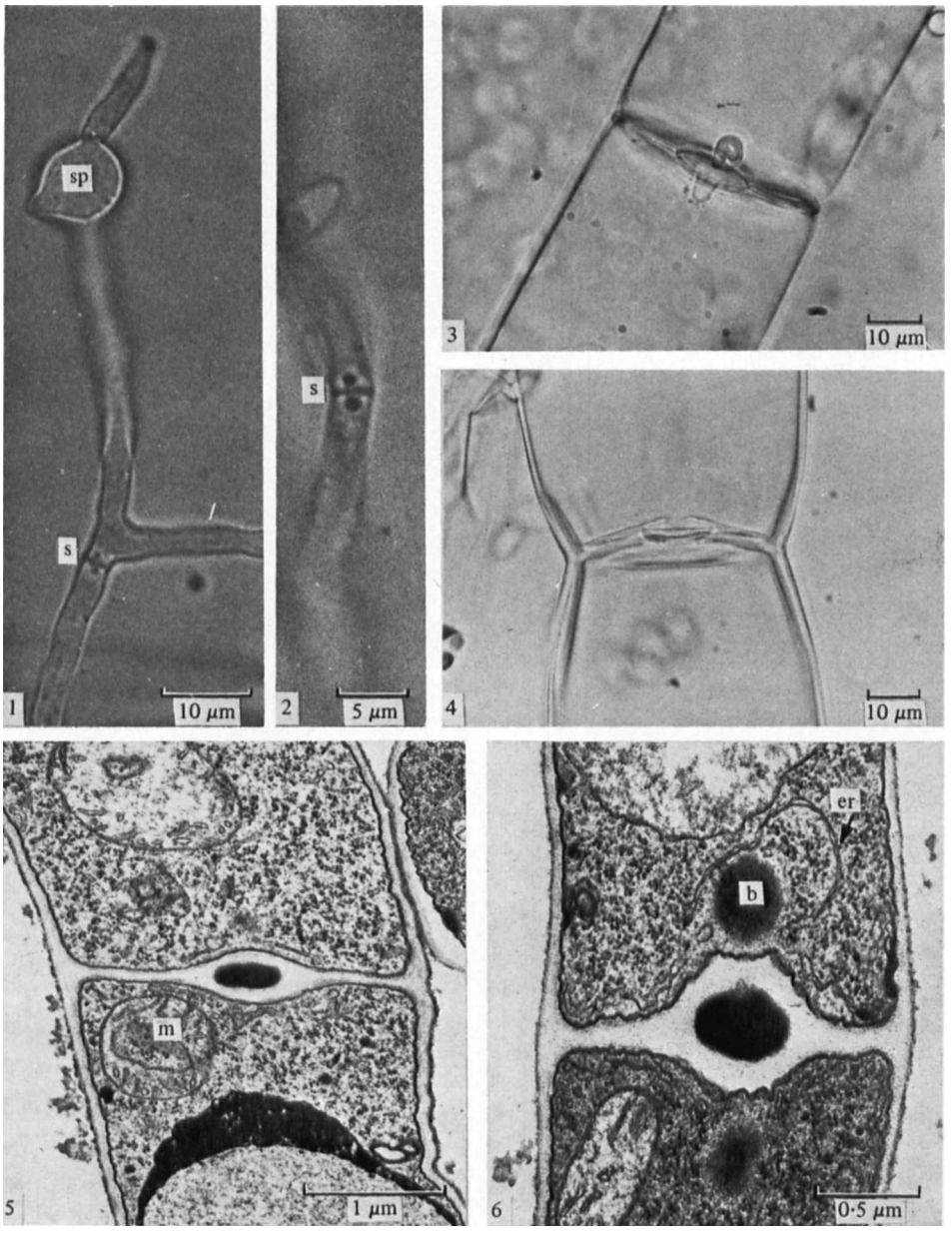
Ultrastructurally, the wall of the germ tube, infection peg and vegetative hypha is composed of an outer electron-dense (15 to 30 nm) and a thicker (40 to 80 nm) inner electron-lucent layer. The septal cross-wall appears similar to, and continuous with, the inner layer of the hyphal wall (e.g. Figs 5 to 11). The electron-lucent cross-wall flares in the pore region (e.g. Figs 5 to 7) which contains an electron-dense, often biconvex, plug. In the cross-wall of a vegetative hypha, a globose electron-dense body lies on each side of the septal pore. The plasmalemma lines the cross-wall and septal plug cavity and connects adjacent hyphal segments (e.g. Figs 7, 8). The cytoplasm on each side of a septum may vary markedly in electron density and organelle content (e.g. Figs 7, 8). The globose bodies are usually discrete and sometimes appear less dense than the septal plug (in aldehyde–osmium fixations, e.g. Fig. 7), although they are occasionally connected to the septal plug by material of equivalent density (Fig. 11). The cytoplasm around the plug and the globose bodies lacks ribosomes. Occasionally, the plug and globose bodies are much less dense and compact.

Endoplasmic reticulum adjacent to the cross-wall may partially enclose the globose enlargements (Fig. 6). Membranes continuous with the plasmalemma also occur in the region of the septum (Figs 7, 10).

Characteristically, a septum develops in the neck region of the penetration peg in infections of *C. recurvatus*. Usually, but not invariably, the globose body nearest the haustorium is either reduced in size or absent (Fig. 12).

Septa in the mycelium of *Tieghemiomyces californicus* (Fig. 17) are essentially similar to those of *D. cristalligena*.

Sporangiophore segments in *D. cristalligena* are vacuolate, although granular cytoplasm



Light micrographs (Figs 1 to 4) and electron micrographs (Figs 5, 6) of *Dimargaris cristalligena*.

Fig. 1. A germinating spore (sp) showing a germ tube with characteristic septum (s).

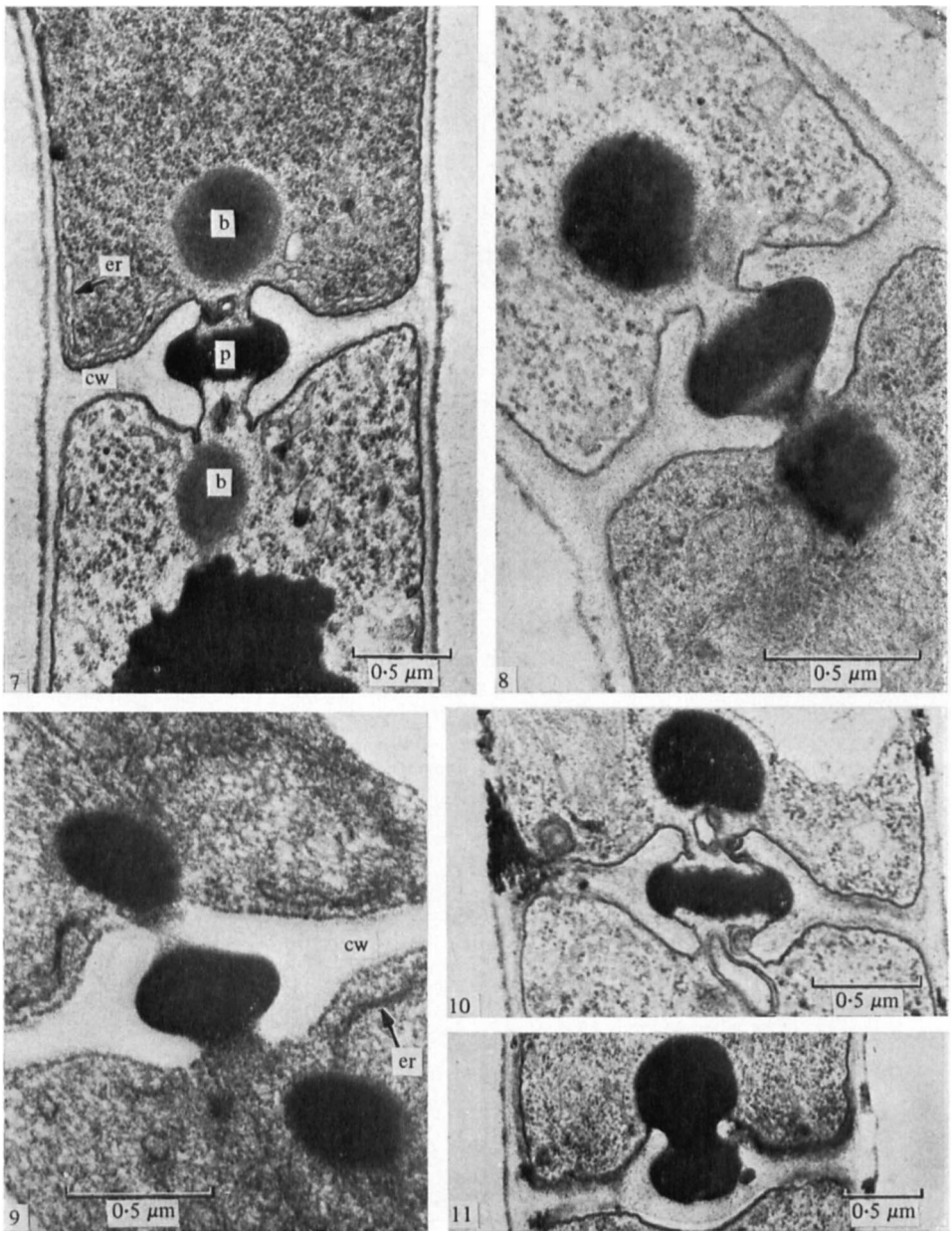
Fig. 2. A vegetative hypha containing a typical septum (s) with associated globose bodies (*Dimargaris*, Gk. two pearls).

Fig. 3. A sporangiophore septum. The septal plug bears an upper globose and a lower obconic lobe. Water mount.

Fig. 4. A sporangiophore septum treated with potassium hydroxide, for comparison with Fig. 3. Note the loss of the septal plug and associated bodies.

Fig. 5. Oblique section through a cross-wall of a vegetative hyphae showing the electron-lucent layer continuous with that of the hyphal wall. (m, mitochondrion.) Aldehyde–osmium fixation.

Fig. 6. Oblique section through a cross-wall of a vegetative hypha and associated septal-plug bodies (b) which are partly enclosed by endoplasmic reticulum (er). Aldehyde–osmium fixation.



Electron micrographs of *Dimargaris cristalligena*.

Fig. 7. Longitudinal section through a cross-wall (cw) of a vegetative hypha. The plug (p) appears electron-dense and the associated bodies (b) less dense. The plasmalemma connects hyphal segments via the pore. (er, endoplasmic reticulum.) Aldehyde–osmium fixation.

Fig. 8. Longitudinal section through a cross-wall of a vegetative hypha in which the plug and associated bodies are of similar electron density, for comparison with Fig. 7. Aldehyde–osmium fixation.

Fig. 9. Oblique section through a cross-wall (cw) of a vegetative hypha. The plug and associated bodies appear similar in electron density. (er, endoplasmic reticulum.) Potassium permanganate fixation.

Fig. 10. Oblique section through a septum of a vegetative hypha showing membranes associated with the septal pore. Aldehyde–osmium fixation.

Fig. 11. Oblique section through a hyphal septum. The globose body and septal plug appear to be connected by electron-dense material. Aldehyde–osmium fixation.

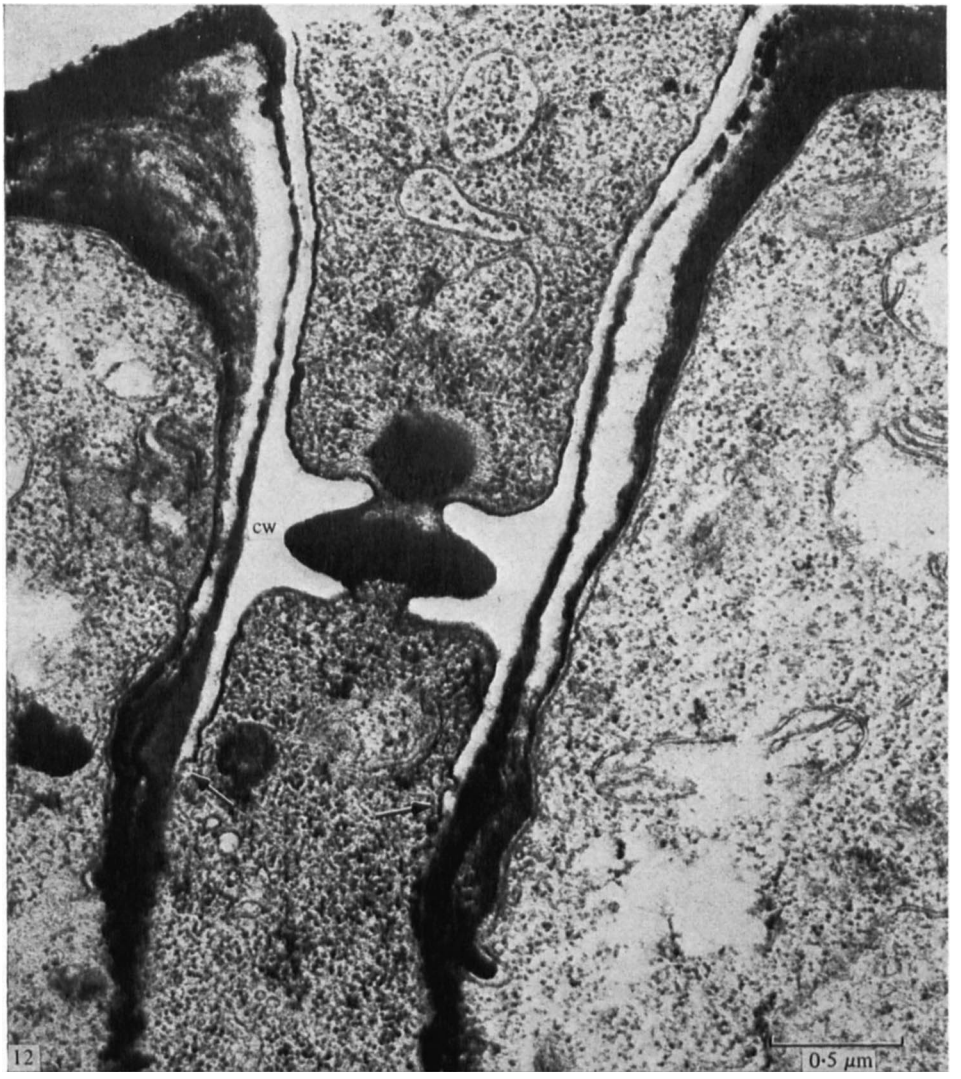
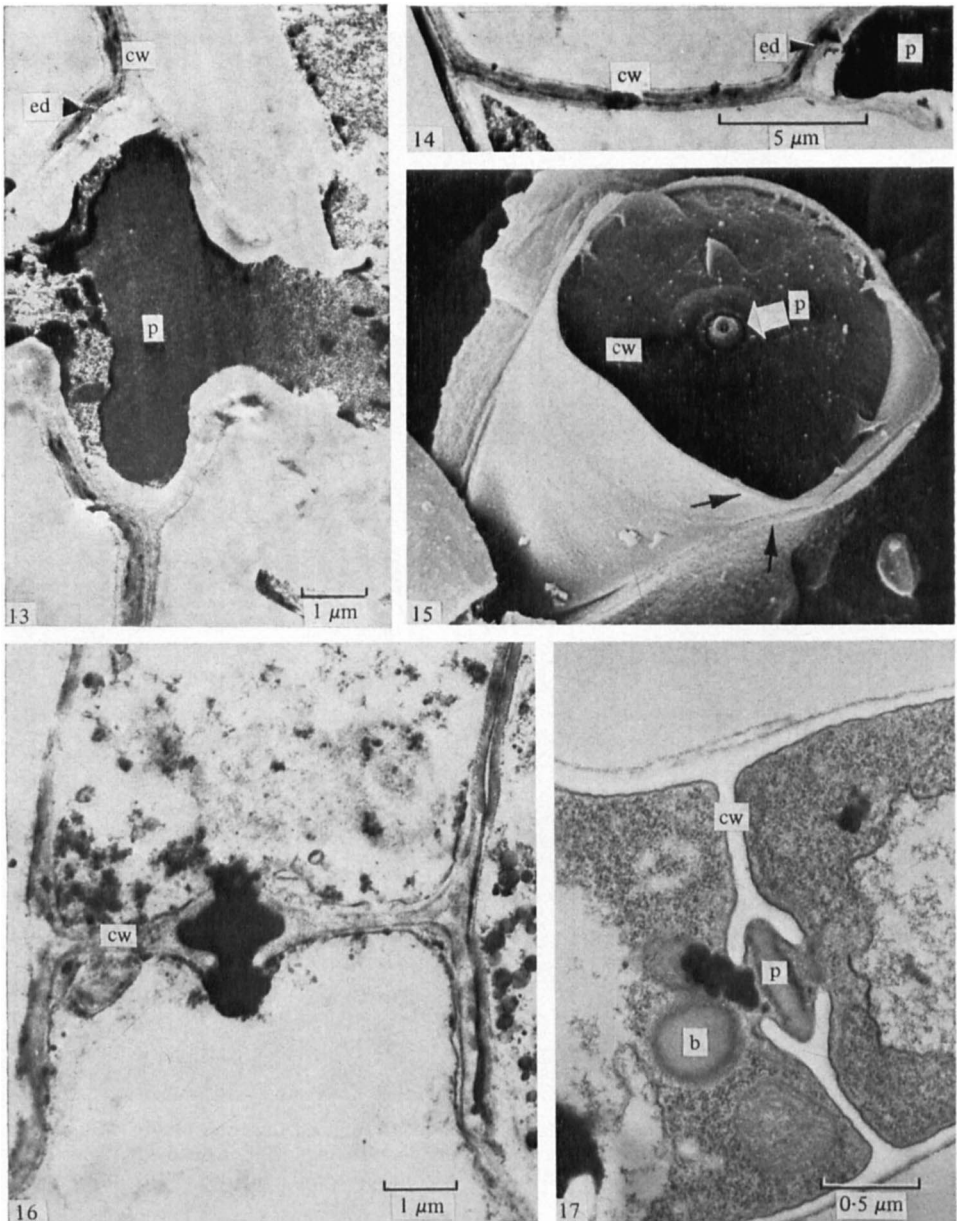


Fig. 12. Electron micrograph of a longitudinal section through an infection peg of *Dimargaris cristalligena* parasitizing *Cokeromyces recurvatus*. Only a proximal globose body is visible and the electron-lucent inner layer of the wall of the peg thins abruptly (arrows). (cw, cross-wall.) Aldehyde–osmium fixation.

tends to accumulate at the lower side of each septum. The terminal unit of the developing sporangiophore is delimited at the base by a septum. Cytoplasm initially moves through the pore into the terminal unit, but this flow ceases when the septum is presumed to be fully formed. In a water mount of a sporangiophore in which 3 % (w/v) aqueous potassium hydroxide is gradually drawn under the coverslip, the septal plug dissolves and cytoplasm flows rapidly through the septal pores (Fig. 4). In water mounts, rupture of the apex of a developing sporangiophore allows cytoplasm to flow from the broken unit, but the cytoplasm in the adjacent unit remains intact, presumably through the sealing action of the septal plug.

In thin sections, the sporangiophore wall is seen as two layers (Fig. 14), which are also apparent in broken sporangiophores viewed in the scanning electron microscope (Fig. 15).



Electron micrographs of *Dimargaris cristalligena* (Figs 13 to 16) and ***Tieghemiomyces californicus*** (Fig. 17).

Fig. 13. Longitudinal section through the septal pore region of a sporangiophore cross-wall (cw). Note the obconic lobe of the plug (p) and the zonate nature of the cross-wall (ed, electron-dense zone). Potassium permanganate fixation.

Fig. 14. Longitudinal section through a sporangiophore and septal cross-wall junction. The sporangiophore wall comprises an outer electron-dense layer and an inner electron-lucent layer. The septal cross-wall (cw) possesses at least two zones which are apparently continuous with the inner layer of the sporangiophore wall (ed, electron-dense zone). Potassium permanganate fixation.

Fig. 15. Scanning electron micrograph of a broken sporangiophore showing a septal cross-wall (cw) with a central pore complex (p, plug). Note the double nature of the sporangiophore wall (arrows). Magnification × 1350.

Fig. 16. Longitudinal section through a sporiferous branchlet showing a cross-wall (cw) and septal complex. Aldehyde–osmium fixation.

Fig. 17. *Tieghemiomyces californicus*: section through a hyphal cross-wall (cw) for comparison with *D. cristalligena* (b, globose body; p, septal plug). Aldehyde–osmium fixation.

The septal cross-wall consists of at least two zones (Figs 13, 14), and it is presumed that breakage of adjacent sporangiophore units (Benjamin, 1959, plate 15) occurs through separation of these zones. The septal plug is electron-dense, homogeneous and appears to be continuous with the protuberance on each side of the pore. Septa formed in and at the base of each sporiferous branchlet appear morphologically similar to those in the main axis of the sporangiophore (Fig. 16).

DISCUSSION

The light microscopical observations reported for the Dimargaritaceae (Benjamin, 1959, 1961, 1963, 1966; Benny, 1972), allied with the ultrastructural observations presented in this paper for *Dimargaris cristalligena* and *Tieghemiomyces californicus*, substantiate the idea that the complex septum, essentially comprising a pore with a plug and associated bodies, is characteristic of these fungi.

The family Dimargaritaceae, classified initially in the Mucorales (Benjamin, 1959), was transferred together with the Kickxellaceae to the Kickxellales (Kreisel, 1969). Moss & Young (1978), however, would exclude the Dimargaritaceae from the Kickxellales on several grounds and Dr R. K. Benjamin currently plans to validate the Kickxellales for the Kickxellaceae and the Dimargaritales for the Dimargaritaceae, respectively (personal communication). In these families, although the morphology of the cross-wall is basically similar (Benjamin, 1959; Young, 1969; Benny & Aldrich, 1975), septal plugs of the Kickxellaceae lack associated globose bodies and they are insoluble in dilute alkali whereas those of the Dimargaritaceae are soluble. The kickxellaceous cross-wall (Young, 1969) is indistinguishable from that found in the Harpellales and Asellariales (Farr & Lichtwardt, 1967; Reichle & Lichtwardt, 1972; Moss, 1975; Moss & Young, 1978) and the indications are that the Kickxellaceae are more likely to be closely related to the Harpellales and Asellariales (Lichtwardt, 1973; Moss & Young, 1978) than to the Dimargaritaceae. Septum structure is a primary character in fungal taxonomy and that in the Dimargaritaceae appears to be unique. It is essentially dolipore in form but no distinctive septal pore cap is developed, although endoplasmic reticulum, reminiscent of the septal pore cap found in Basidiomycotina (e.g. Moore & Marchant, 1972), may partially surround the globose body on the side away from the pore. Again, the globose bodies are suggestive, by their position close to the cross-wall, of Woronin bodies of the Ascomycotina although, unlike Woronin bodies, they are not membrane-bound with the fixation methods used, and develop singly on each side of the cross-wall. The septal complex found in the hyphae of the ascosporogenous yeasts *Endomycopsis platypodis* Baker & Kreger-van Rij and *E. monospora* Saito (Kreger-van Rij & Veenhuis, 1969) resembles that of the vegetative hypha of *D. cristalligena* in having a globose electron-dense plug on each side of the cross-wall. These structures are membrane-bound, however, unlike the globose bodies in *D. cristalligena*. Further, no central lenticular plug is developed and the margin of the septal pore has a characteristic form (Kreger-van Rij & Veenhuis, 1969, Fig. 1) which more closely resembles the dolipore–parenthesome septum of Basidiomycotina in this respect. Benjamin's elegant demonstration of zygospore production in several members of the Dimargaritaceae (Benjamin, 1959) fully justifies their inclusion in the Zygomycotina although the complex nature of the septa does raise the question of the affinity of these fungi to other members of the Zygomycotina.

Septa in the sporangiophore of *D. cristalligena* differ from those of all other described species of the Dimargaritaceae as only the upper protuberance of the septal plug is globose, the lower being obconic (Benjamin, 1959). Benjamin's illustration of the liberation of virtually intact septal plugs from fragmented sporangiophores strongly suggests that the protuberances are an extension of the plug, an observation also supported by electron microscopy (Fig. 13).

The cytoplasm between the plug and the globose bodies in the mycelial septa lacks

ribosomes. Such an organelle-free zone might represent a region in which any connective elements are not preserved by the techniques employed. The apparent absence of connections between the plug and globose bodies, however, could indicate that connectives are transient in nature. Even if connectives could be shown to be regularly present in the vegetative hyphae, it is clear that *D. cristalligena* is polymorphic with respect to the septal pore apparatus.

In the absence of experimental evidence, it is only possible to speculate upon the possible functions of the complex dimargaritaceous septa. Benjamin (1959) suggested the possibility of the active association of the septal complex with cytoplasmic flow in which the movement could perhaps be regulated by the plugs. Although cytoplasmic continuity is maintained through the septal pore by the plasmalemma, the septal plug, which fully occludes the pore, would presumably restrict the passage of organelles between adjacent hyphal units but not necessarily the transport of nutrients. The free passage of cytoplasm through septal pores after removal of the plugs by treatment with dilute alkali suggests that the plug may prevent cytoplasmic flow. Sporangiophores of *D. cristalligena* frequently exceed 1·5 cm in length and can be fragmented at maturity. In *Tieghemiomyces* spp. the sporangiophore breaks naturally by a circumscissile rupture just below the zone of sporulation when the tip of the sporangiophore is touched by a solid object. A possibility is that septal plugs, in sporangiophores at least, could prevent excessive loss of cytoplasm from turgid cells following natural or accidental breakage. As a septum is regularly developed in the neck of the haustorium, and similar plugged septa have been demonstrated in the neck of the haustorium of several other parasitic fungi [e.g. *Colletotrichum graminicola* (Ces.) Wilson (Politis & Wheeler, 1973); *Erysiphe graminis* DC. ex Merat (Bracker, 1968)], it seems unlikely that the uptake of materials from the host would be prevented, although haustoria may function for a short time only, translocation ceasing or being severely curtailed when the septum in the neck is mature. Perhaps transport of nutrients via the plasmalemma, and possibly also through the walls, would be sufficient to sustain development of the parasite on the host.

The authors wish to thank Mr A. Ginman for assistance in the preparation of the photographs.

REFERENCES

BENJAMIN, R. K. (1959). The merosporangiferous Mucorales. *Aliso* **4**, 321–433.

BENJAMIN, R. K. (1961). Addenda to the merosporangiferous Mucorales, I. *Aliso* **5**, 11–19.

BENJAMIN, R. K. (1963). Addenda to the merosporangiferous Mucorales, II. *Aliso* **5**, 273–288.

BENJAMIN, R. K. (1966). The merosporangium. *Mycologia* **58**, 1–42.

BENNY, G. L. (1972). Histochemistry of the cell wall and septum of vegetative cells of selected species of Dimargaritaceae and Kickxellaceae. *Mycologia* **64**, 854–862.

BENNY, G. L. & ALDRICH, H. C. (1975). Ultrastructural observations on septal and merosporangial ontogeny in *Linderina pennispora* (Kickxellales; Zygomycetes). *Canadian Journal of Botany* **53**, 2325–2335.

BRACKER, C. E. (1968). Ultrastructure of the haustorial apparatus of *Erysiphe graminis* and its relationship to the epidermal cell of barley. *Phytopathology* **58**, 12–30.

FARR, D. F. & LICHTWARDT, R. W. (1967). Some cultural and ultrastructural aspects of *Smittium culisetae* (Trichomycetes) from mosquito larvae. *Mycologia* **59**, 172–182.

JEFFRIES, P. & YOUNG, T. W. K. (1975*a*). Scanning electron and light microscopy of appressorium development in *Piptocephalis unispora* (Mucorales). *Archives of Microbiology* **103**, 293–296.

JEFFRIES, P. & YOUNG, T. W. K. (1975*b*). Ultrastructure of the sporangiospore of *Piptocephalis unispora* (Mucorales). *Archives of Microbiology* **105**, 329–333.

JEFFRIES, P. & YOUNG, T. W. K. (1976*a*). Physiology and fine structure of *Piptocephalis unispora* prior to infection. *Archives of Microbiology* **107**, 99–107.

JEFFRIES, P. & YOUNG, T. W. K. (1976*b*). Ultrastructure of infection of *Cokeromyces recurvatus* by *Piptocephalis unispora* (Mucorales). *Archives of Microbiology* **109**, 277–288.

KHAN, S. R. (1976). Ultrastructure of the septal pore apparatus in Tremella. *Journal of General Microbiology* **97**, 339–342.

KREGER-VAN RIJ, N. J. W. & VEENHUIS, M. (1969). Septal pores in *Endomycopsis platypodis* and *E. monospora*. *Journal of General Microbiology* **57**, 91–96.

KREISEL, H. (1969). *Grundzüge eines Natürlichen Systems der Pilze*. Lehre: J. Cramer.

LICHTWARDT, R. W. (1973). The Trichomycetes: what are their relationships? *Mycologia* **65**, 1–20.

MOORE, R. T. (1965). The ultrastructure of fungal cells. In *The Fungi*, vol. 1, pp. 95–118. Edited by G. C. Ainsworth & A. S. Sussman. London: Academic Press.

MOORE, R. T. & MARCHANT, R. (1972). Ultrastructural characterization of the basidiomycete septum of *Polyporus biennis*. *Canadian Journal of Botany* **50**, 2463–2469.

MOORE, R. T. & MCALEAR, J. H. (1962). Fine structure of Mycota. Observations on septa of Ascomycetes and Basidiomycetes. *American Journal of Botany* **49**, 84–94.

MOSS, S. T. (1975). Septal structure in the Trichomycetes with special reference to *Astreptonema gammari* (Eccrinales). *Transactions of the British Mycological Society* **65**, 115–127.

MOSS, S. T. & YOUNG, T. W. K. (1978). Phyletic considerations of the Harpellales and Asellariales (Trichomycetes, Zygomycotina) and the Kickxellales (Zygomycetes, Zygomycotina). *Mycologia* **70**, 944–963.

POLITIS, D. J. & WHEELER, H. (1973). Ultrastructural study of penetration of maize leaves by *Colletrotrichum graminicola*. *Physiological Plant Pathology* **3**, 465–471.

REICHLE, R. E. & LICHTWARDT, R. W. (1972). Fine structure of the trichomycete *Harpella melusinae* from black-fly guts. *Archiv für Mikrobiologie* **81**, 103–125.

YOUNG, T. W. K. (1969). Ultrastructure of aerial hyphae in *Linderina pennispora*. *Annals of Botany*, N.S. **33**, 211–216.