The ultrastructure of conidiogenesis in *Stilbella annulata*

Cecilia Roux,* C.F. van der Merwe[1] and K.T. van Warmelo[2]

*Mycology Research Unit, Plant Protection Research Institute, Private Bag X134, Pretoria, 0001 Republic of South Africa

[1]Electron Microscopy Unit, University of Pretoria, Pretoria, 0002 Republic of South Africa

[2]Department of Botany, Rand Afrikaans University, P.O. Box 524, Auckland Park, 2006 Republic of South Africa

Received 20 January 1995; revised 23 March 1995

Conidiogenesis in the synnematous hyphomycete *Stilbella annulata* (Berk. & M.A. Curtis) Seifert is initially enteroblastic with basipetally retrogressive secession and enteroblastic conidiogenous cell proliferation, where each conidiogenous cell apparently produces a maximum of seven conidia. The first conidium is produced enteroblastically. Chains of synnemata are formed as a result of two processes: (a) from conidia which may not normally be seceded and then germinate into hyphae, or be normally seceded and germinate above the conidiogenous locus with no physical attachment to the underlying structure, except the mucilage in which the conidia germinate; (b) the continuation of normal vegetative growth after the full complement of conidia has been produced. Ultrastructurally, the marginal hyphae contained no septa, which explained the non-abscission of these structures. Morphologically they are therefore modified hyphae and not analogous to conidiogenous cells.

Konidiogenese in die sinnematiese hifomiseet *Stilbella annulata* (Berk. & M.A. Curtis) Seifert is aanvanklik enteroblasties met basipetaal retrogressiewe afsnyding en enteroblastiese konidiogene selproliferasie waar elke konidiogene sel blykbaar 'n maksimum van sewe konidia produseer. Die eerste konidium word enteroblasties geproduseer. Kettings sinnemata word gevorm deur midddel van twee prosesse: (a) van konidia wat nie normaalweg vrygestel word nie en ontkiem en hifes vorm, of normaal vrygestel word en bokant die konidiogene lokus ontkiem, met geen fisiese aanhegting met die onderliggende struktuur nie, afgesien van die slym waarin die konidia ontkiem; (b) die voortgang van normale groei nadat die volle getal konidia geproduseer is. Ultrastruktureel bevat die marginale hifes geen septa nie wat verklaar waarom hierdie strukture nie vrygestel word nie. Morfologies is hulle dus gemodifiseerde hifes en nie analoog aan konidiogene selle nie.

Keywords: Chains of synnemata, conidiogenesis, false chains, retrogressive basipetal conidiogenesis.

*To whom correspondence should be addressed.

In the revision of the genus *Stilbella* Lindau a new combination *annulata* (Berk. & M.A. Curtis) Seifert was proposed (Seifert 1985). *S. annulata* is characterized by marginal hyphae, chains of synnemata and a conidial mass which is yellow at first, becoming red to red-brown when mature (Seifert 1985). Known distribution of this fungus is mainly in the tropics and sub-tropics (Seifert 1985). The term 'marginal hyphae' was coined by Tulloch (1972) to describe the ornamented hyphae on the conidiomata of species of myrothecium Tode ex Fr. and chosen by Seifert (1985) to describe the modified hyphae found in some species of *Stilbella*.

The morphology of the conidiogenous apparatus and marginal lobes was described by Roux *et al.* (1994) who used scanning electron microscopy to study the conidiogenous process and concluded that this was enteroblastic. They further noted that the marginal hyphae seemed to have limited growth in their apices and no abscission was observed.

The ultrastructural nature of the synnemata as they proliferate to form chains, the marginal hyphae as well as conidiogenesis in *Stilbella annulata*, are reported on in this paper.

Materials and Methods

A culture of *S. annulata* verified by Dr Keith A. Seifert PPRI 3750 (PREM 48983) was used in these studies. The isolate was grown on OMA and PDA (Hawksworth *et al.* 1983) at 24°C under near-UV and daylight/dark on a 12-h cycle until satisfactory growth had been obtained. Some cultures where stacked to observe the effect of oblique light on the development of the synnemata.

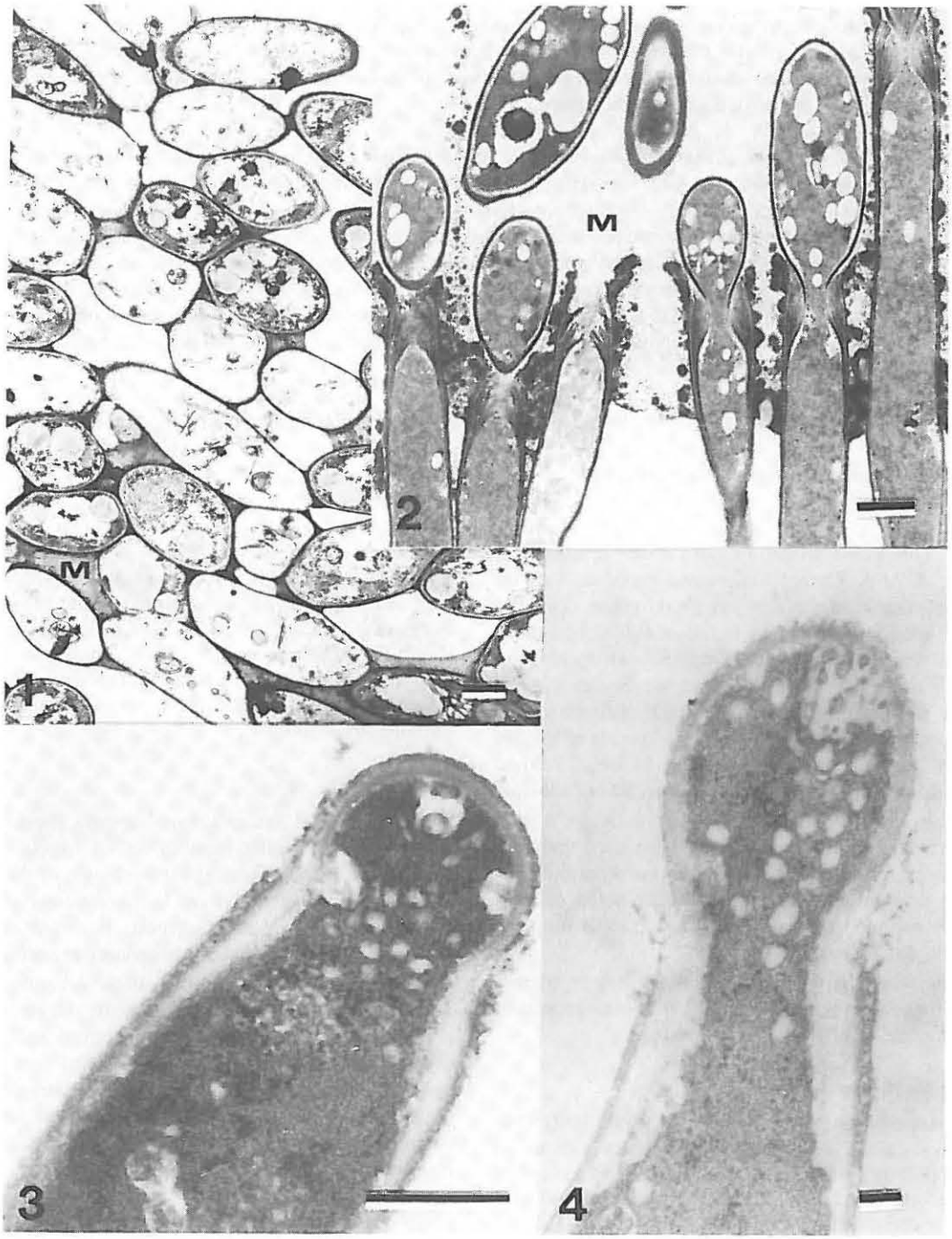
Chains of synnemata were removed from the culture and embedded in molten agar to keep their structure intact. Synnemata bearing marginal hyphae were embedded separately. The material was fixed at ambient temperature for 1 h in 2.5% glutaraldehyde-buffered 0.75 M sodium phosphate, post-fixed in 1% aqueous osmium tetroxide for 1 h, dehydrated in an acetone series, and embedded in Quetol 651 resin (Kushida 1974) as modified by van der Merwe & Coetzee (1992). Silver interference sections (20-nm) were cut using a diamond knife, then contrasted with uranyl acetate (10 M) and lead citrate (2 M) (Reynolds 1963). Electron micrographs were taken using a Philips 301 at 60 kV.

Results

The synnemata of *Stilbella annulata* show positive phototropism and consist of septate smooth-walled hyphae with mucilage deposited at random throughout the length of the structure (Figure 1). No difference is noted between young and older synnemata. In the region of conidiogenesis, however, a distinct layer of mucilage is present in older proliferating synnemata (Figure 2).

Conidiogenesis starts when globular structures (polar bodies) start to migrate into the neck area while the electron-translucent layer thickens apically on the inside of the conidiogenous cells to form the region of the periclinal thickened area (Figure 3). The developing conidium is delimited by a constriction at the conidiogenous locus (Figure 3). The outer electron-dense layer of the conidiogenous cell disintegrates at the conidiogenous locus resulting in the formation of a colarette (Figure 3). A vacuole containing circular bodies is present in the apical region of the developing conidium (Figure 4). Polar bodies move into the conidium as the neck of the conidiogenous cell is defined and the periclinal thickening becomes more pronounced (Figure 5). The electron-translucent layer in the conidium and the conidiogenous cell are not continuous, thus the first conidium is formed enteroblastically

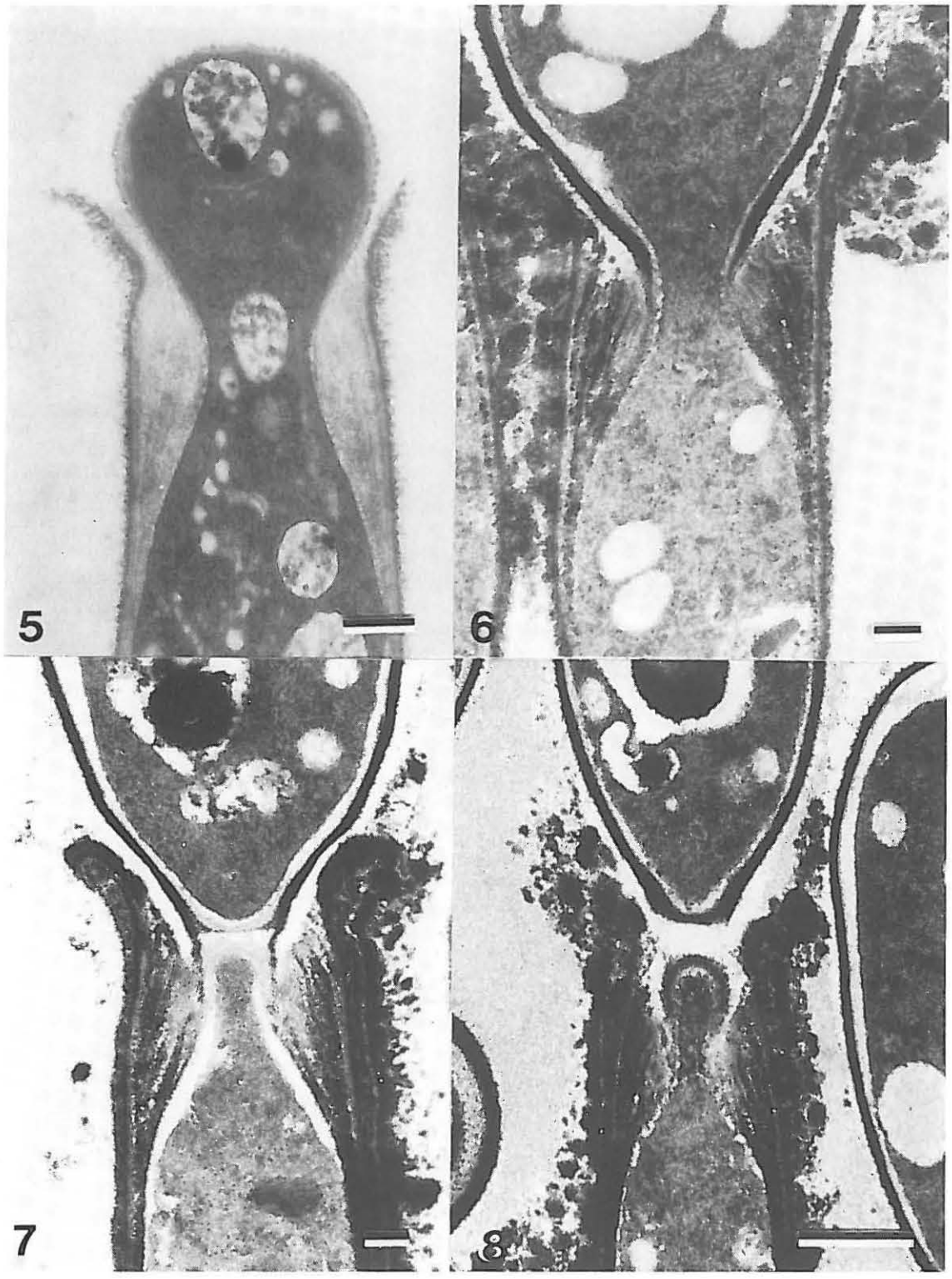
and leaves a frayed edge on the outer electron-dense cell wall layer (the collarette) (Figure 4). The outer electron-dense layer of the conidium becomes more pronounced and ragged as conidiogenesis proceeds (Figure 5). After the secession of several conidia, distinct electron-dense layers, representing the various conidia produced, can be distinguished in the neck area of the conidiogenous cell (Figures 6–8). The frayed edges become very pronounced and exude mucilage in which the conidia are embedded at a more advanced stage of conidiogenesis (Figure 7). Secession of conidia is schizolytic, the septa forming at successively lower levels than the first conidiogenous locus (Figure 7). Subsequent conidia are formed from successively lower levels in the conidiogenous cell, indicating that secession is retrogressive, proliferation enteroblastic. Up to seven layers of electron-dense material representing seceded conidia are produced in single conidiogenous cells (Figures 6–8). Formation of the next conidium moves the preceding conidium into the surrounding mucilage after secession (Figures 2, 6–8). Although the new conidia are formed at a lower level than their predecessors, the actual growth of developing conidia takes place well above the point of secession (conidiogenous locus) (Figure 8) i.e. in the apex of the developing conidium. The apices of the actively sporulating synnemata are enveloped in mucilage which increases in volume and changes in appearance as conidiogenesis progresses (Figures 5–8). Senescent conidiogenous cells show degeneration of the cytoplasm resulting in an open-ended conidiogenous cell with only the



Figures 1–4 Electron micrographs of *Stilbella annulata*. All bars = 1 µm. **1.** Section of a synnema where mucilage is deposited at random. (M – mucus). **2.** Area of proliferation in a chain of synnemata. (M – mucus). **3.** Apical area of a conidiogenous cell about to produce its first conidium. Delimitation of the conidium is evident at the constriction. **4.** Circular bodies in the apical region of the developing conidium.

cell wall and cytoplasmic remnants remaining (Figure 12).

The level on which proliferation takes place is not distinct but submicroscopically uneven with longer and shorter conidiogenous cells (Figure 2). Chain formation of synnemata is a result of two different processes: (1) by germinating conidia borne on live conidiogenous cells, evidently when all conidia have been produced (Figures 10 & 11). The conidium at the base of the developing synnema can be distinguished by its position at the apex of the conidiogenous cell and the cell wall structure which has a very distinct electron-dense layer (Figures 11 & 12). The conidium forming the base of the next synnema may still be intact (Figure 10) or be seceded (Figure 11). (2) After cessation of conidiogenesis, vegetative growth of the conidiogenous cells continues. A septum is seen in the necks of some proliferating cells where prominent periclinal thickenings are evident (Figure 10). However, in older conidiogenous cells this septum is partially dis-



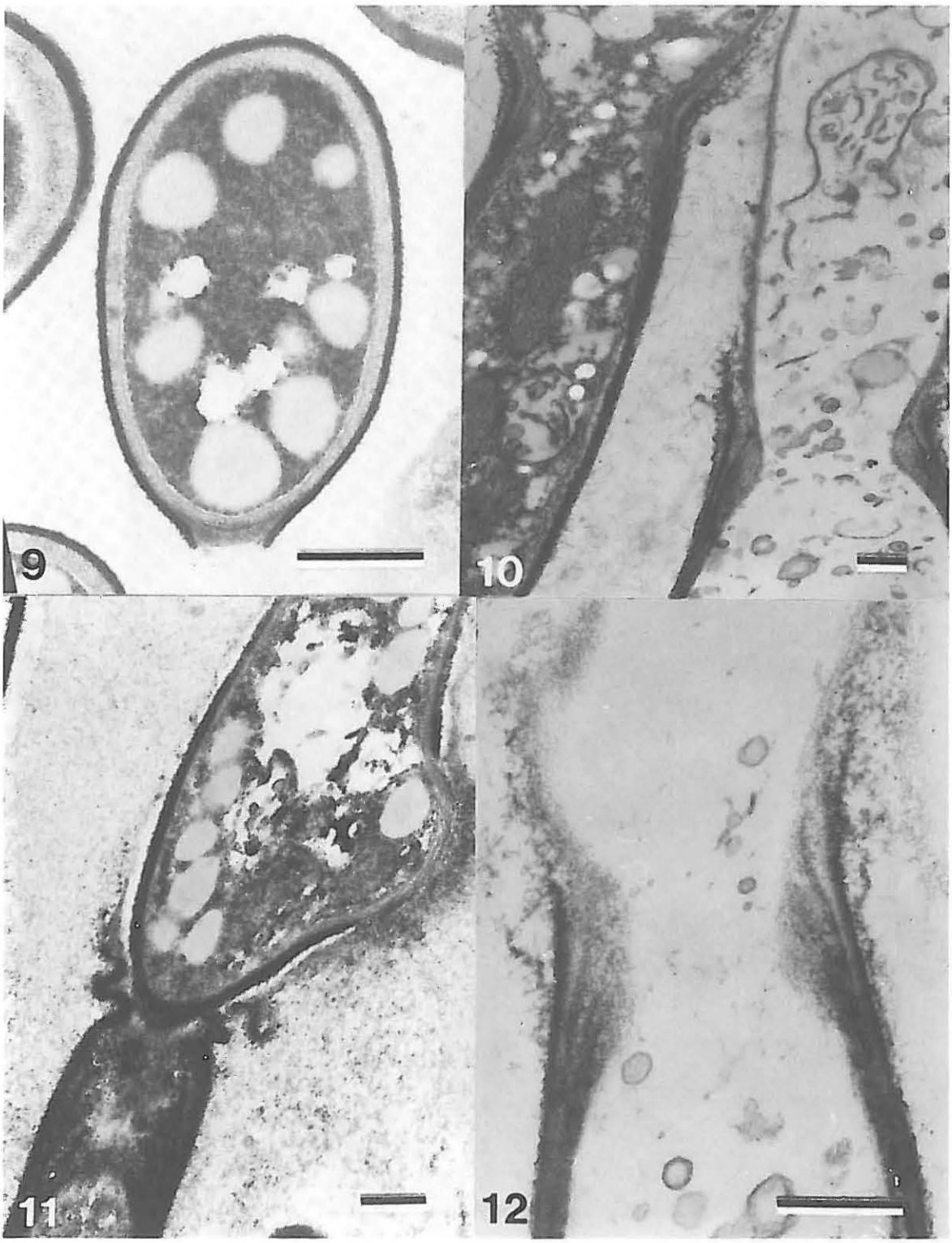
Figures 5–8 Electron micrographs of *Stilbella annulata*. All bars = 1 µm. **5.** Periclinal thickening becomes more pronounced and the cell wall differentiates further. **6.** Electron-dense layers in the neck region of the conidiogenous cells. **7.** Collarette on the neck area of the conidiogenous cell and septum between conidium and conidio-genous cell become evident. **8.** Conidium is seceded.

solved (Figure 12). These two processes of proliferation occur side by side (Figure 10). The apex of the dead proliferating conidiogenous cells remains open (Figure 10).

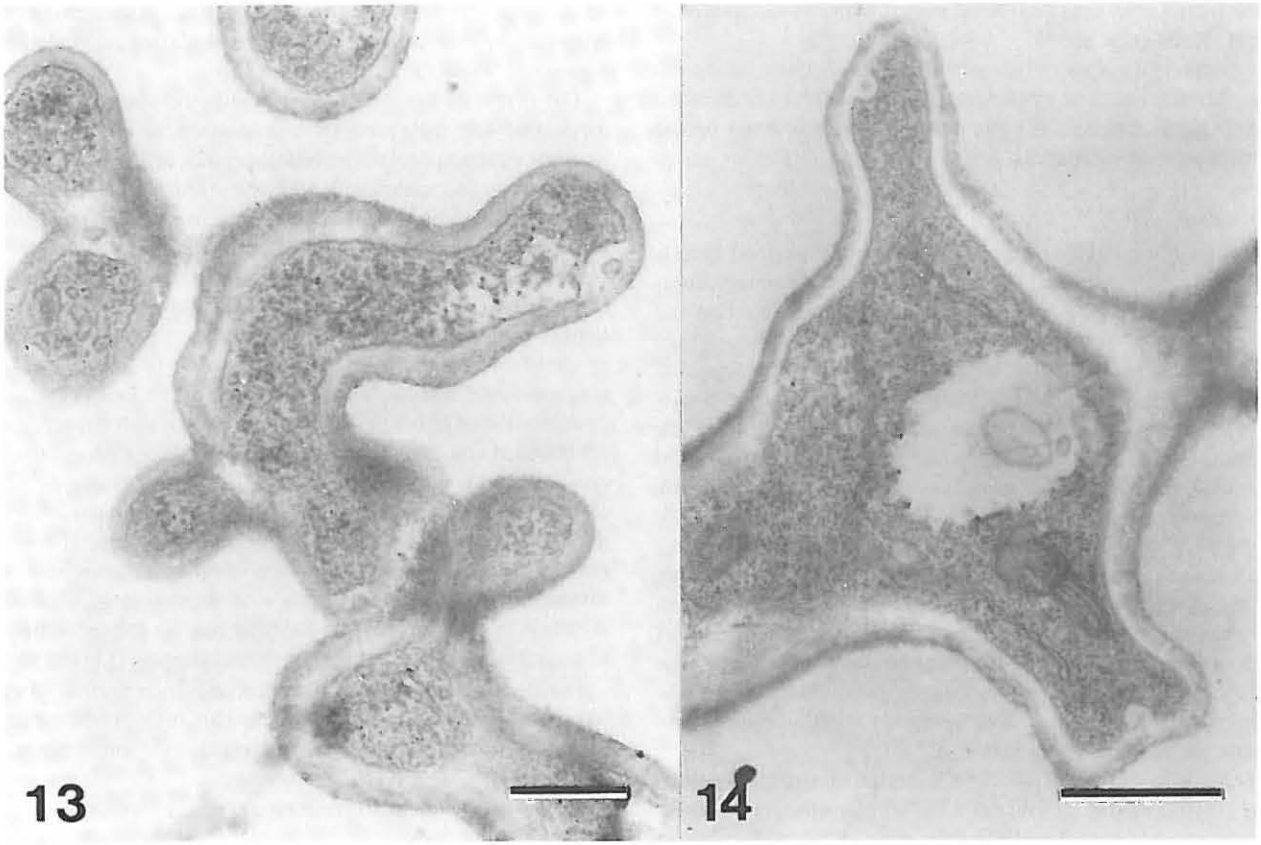
Growth of germ tubes shows negative geotropism resulting in upright chains of synnemata which are not influenced by illumination. The base of the next synnema is embedded in the mucilage resulting from conidiogenesis on the previous synnema (Figures 2 & 11). No degeneration of conidia is noted.

Germination of the conidia takes place by extension of the inner less electron dense layer in the conidial cell wall (Figure 11).

The electron-dense outer conidial cell wall layer is present from the initial conidial formation in the periclinal thickened region (Figure 6) and continuous with the inner electron-dense layer of the conidiogenous cell after the formation of the separating septum (Figure 7). The electron-dense layer at the base of the conidium forms at the same time as maturation (Figure 8) and maturation of the conidia takes place after they have seceded. The conidial cell wall ultimately consists of three distinct cell wall



Figures 9–12 Electron micrographs of *Stilbella annulata*. All bars = 1 µm. **9.** Mature conidium. **10.** Conidiogenous cells proliferating by resuming vegetative growth. **11.** A conidium germinates on top of a conidiogenous cell. **12.** A senescent conidiogenous cell.



Figures 13–14 Electron micrographs of *Stilbella annulata*. All bars = 1 μm. **13.** Circular bodies at the apices where growth occurs in the marginal hyphae. **14.** Section through a marginal hypha showing the circular bodies at the apices and branching in the structure.

layers: (1) an outer electron-dense layer which is open at the base; (2) a thicker partially electron dense layer which surrounds the cytoplasm entirely and has a more fibrillous structure under a more electron dense substance in the basal pore area, and (3) an electron-translucent layer adjacent to the cytoplasm (Figure 9).

No septal pores are seen in median aligned sections of the conidia but the outer electron-dense layer is not completely continuous (Figure 9).

The marginal hyphae differ from vegetative hyphae in that the cell walls are thicker and the electron-translucent layer is more prominent than in the vegetative hyphae (Figures 13 & 14). At the apices of the marginal hyphae, globular bodies similar to those seen in the developing conidia, are observed in the live cells (Figure 14), which are always associated with areas of growth. No septa are observed in these marginal hyphae (Figures 13 & 14). What may seem to be septa are tangential sections through the convoluted cell walls resulting from frequent branching by intermittent growth of hyphae as they grow (Figure 14). The plasmalemma of the cell forming the marginal hypha is quite distinct and can be followed throughout correctly aligned sections (Figures 13 & 14). Although globular bodies are engulfed by the plasmalemma, no connection between them was observed (Figure 14). The globular bodies in the electron-translucent wall layer may be the point of growth where branching occurs (Figure 14). Apices

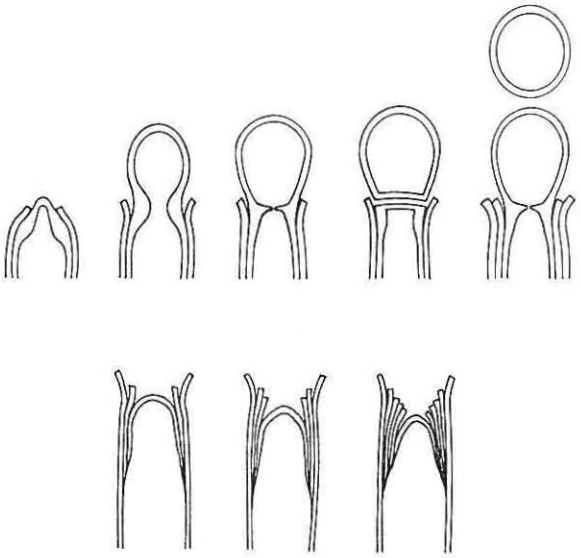


Figure 15 Schematic presentation of conidiogenesis in *S. annulata*.

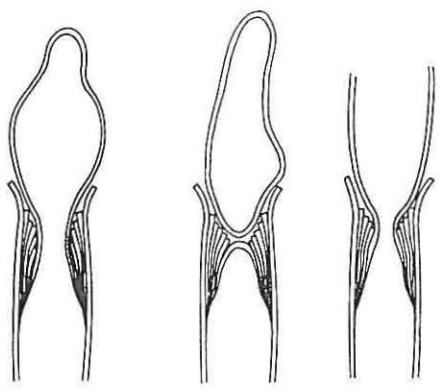


Figure 16 Schematic presentation of proliferation of synnemata in S. *annulata*: conidium not seceded, conidium seceded and vegetative growth resumed.

without lomasomes are considered determinate, as substantiated by SEM (Roux *et al.* 1994).

No marginal hyphae occur within the synnemata. Marginal hyphae develop better on synnemata grown on OMA and die soon after becoming mature. Only the cell wall remains intact on the outer perimeter of synnemata.

Discussion

The mitospore ontogeny of the first conidium described here is enteroblastic, contrary to what has been accepted for phialoconidial formation (Cole & Samson 1979). The further delimitation and secession of conidia described here is retrogressive although proliferation of the locus is enteroblastic. Progressive phialoconidial formation in *Termitaria snyderi* Thaxter and retrogressive proliferation for *Septofusidium berolinense* has been depicted by Cole & Samson (1979). The layered electron-dense deposits represent each newly formed conidium initial (Minter *et al.* 1983) where the initial growth occurs by ring wall building (Minter *et al.* 1982). The ring wall building is, however, initiated from a slightly lower region for each conidium, i.e. enteroblastic. Most growth occurs in the apical region of the conidiogenous cell, whereas maturation takes place through diffuse wall building. Exact pinpointing of growth cannot be determined by standard TEM techniques. The morphology of the conidiogenous cells is phialidic where periclinal thickening of the apex of the condigenous cell, a channel and collarette are present, sensu Sutton (1980).

According to Minter *et al.* (1983) secretory organelles, also termed 'spitzenkörper' (Gribandt 1957), polar clusters of secretory vesicles, or lomasomes (Cole & Samson 1979) move towards the point where growth occurs. The first conidium is initiated from electron-translucent material not continuous with the same layer in the conidiogenous cell. This conidium wall is thus produced enteroblastically, similar to what Tiedt & Jooste (1988) reported for *Fusarium* sect. *Liseola.* This was also found by Jones (1976, see his Figure 14) in *Microsphaeropsis olivaceum* (Bon.) Höhn. who, however, did not describe it as such. Rupture of the outer electron-dense material of the conidiogenous cell delimits the conidium and pinpoints the conidiogenous locus where schizolytic abscission occurs. The initial growth starts well below the apex of the conidiogenous cell and proliferation of the electron-translucent material, which will become the periclinal thickening, starts to form in the process of ring wall building, as proposed by Minter *et al.* (1983). This electron-translucent layer is, however, of much greater thickness in the initial conidiogenous cells than in the mature and senescent conidiogenous cells where the electron-dense layers representative of each conidium produced have been assimilated into the periclinal thickening. An electron-dense layer, possibly immediately adjacent to the plasmalemma, is evident on the inner periphery of the cytoplasm and is possibly continuous with similar zones in the periclinal thickening.

The growth of the conidia is initially apical and later diffuse (Minter *et al.* 1983) in the upper region of the conidia. If translocation of the conidiogenous locus is basipetal (Hawksworth *et al.* 1983) or retrogressive or progressive (Cole & Samson 1979), it follows that the outer electron-dense layer of conidiogenous cells may appear to elongate. Extremely long and elaborate collarettes may be formed on senescent conidiogenous cells in this way.

In terms of the definitions given by Sutton (1993) to be incorporated into the 8th edition of the Dictionary of Fungi, the description of conidiogenesis in *S. annulata* can be formulated as follows (Figure 15): mitospore ontogeny enteroblastic, maturation by diffuse wall building, delimitation by one septum, secession schizolytic, enteroblastic percurrent mitosporogenous cell proliferation with retrogressive delimitation of next mitospore, producing false chains of mitospores, periclinal thickened area in neck of the mitosporogenous cell enlarges and moves downward with each mitospore. This denotes an additional method of conidiogenesis; 'event 27/2'.

The origin of the mucilage in which the conidia are borne is associated with their successive production, as no trace is evident on very young sporulating conidiogenous cells. The structure of the mucilage associated with the hyphae of the synnemata and that formed around the conidiogenous cells is similar. The base of a proliferating synnema is immersed in mucilage which protects and envelops the apex of the preceding synnema. Where seceded conidia germinate to give rise to a new synnema, the link is sustained by the mucilage only. However, when non-seceded conidia or proliferation of the conidiogenous cell is found, the link is much stronger and such chains are of a more permanent nature. Proliferation of phialidic conidiogenous cells to form percurrently proliferated conidiogenous cells is depicted for *Chloridium virescens* (Pers. ex Pers.) W. Gams & Hol.-Jech. (Cole & Samson 1979). The level of proliferation is not absolute, as was expected in Seifert's (1985) description of 'determinate parallel synnema', but can best be described as a region of proliferation where growth resumes by means of the reversion to vegetative growth and germination of seceded and non-seceded conidia lodged in the necks of conidiogenous cells of the previous synnema (Figure 16).

Proliferation of the conidiogenous cells by reversion to vegetative growth has many implications. This may indicate that very little difference exists between vegetative and reproductive growth.

The better developed synnematal structure which is seen here, however, must inevitably result in the prolongation of conidiogenesis and therefore enhances the chances of survival of this species. This proliferation of the synnema may be an adaptation to its environment which is mainly confined to tropical and sub-tropical regions (Wicklow 1981, 1985). One can also consider it 'regeneration' (Minter *et al.* 1982) where the senescent conidiogenous cells are replaced by hyphae which will in due course produce a new generation of conidiogenous cells to effect the continuation of conidiogenesis.

The mucilage in which the conidia is extruded is reminiscent of the mucilage encountered in the conidiogenous layer of *Bartalinia robillardoides* (Roux & van Warmelo 1990) and *Tiarosporella graminis* var. *karoo* (Roux *et al.* 1990). The mucilage may protect the conidiogenous cells from dehydration so that conidiogenesis 'regeneration' (Minter *et al.* 1982) can continue undisturbed and may facilitate distribution of conidia by droplet dispersal (Ingold 1971).

According to Cole & Samson (1979) globular bodies which they termed 'lomasomes', were found associated with possible points of growth in the marginal hyphae. The only similarity in cell wall structure between conidiogenous cells and marginal hyphae was the more prominent electron-translucent layer found in the young periclinal thickened region and throughout the cell walls of the marginal hyphae. However, no septa were observed and consequently no secession was possible.

Tulloch (1972) did not define her concept of 'marginal hyphae' and, as applied to the genus *Myrothecium,* it overlaps with 'ornamenting cells' as used by Sutton (1973). The term 'marginal hyphae' as used by Seifert (1985) is not clear, as he followed Tulloch's description and applied it to morphologically different structures. The ornamenting cells in *Tubercularia lateritia* (Berk.) Seifert and marginal hyphae in *S. annulata* (Seifert 1985) seem to be very similar, as branching occurs in both, although there are no septa in marginal hyphae of *S. annulata.* As 'marginal hyphae' and 'ornamenting cells', as used above, cannot be readily distinguished or described unambiguously, the term 'ornamenting cells' appears to be a better choice. 'Ornamenting cells' therefore, describes vegetative peripheral simple or proliferated cellular

structures with determinate or intermittent growth. Marginal hyphae (Seifert 1985) as observed in this study conform to a larger degree with ornamenting cells (Sutton 1973) although they occur on the same whorl as conidiogenous cells in the vegetative hyphae.

Acknowledgements

Thanks to Elsa van Niekerk for the schematic representations.

References

COLE, G.T. & SAMSON, R.A. 1979. Patterns of development in conidial fungi. Pitman, London.

GIRBARDT, M. 1957. Der Spitzenkörper von *Polystictus versicolor* (L.). *Planta* 50: 47–59.

HAWKSWORTH, D.L., SUTTON, B.C. & AINSWORTH, G.C. 1983. Ainsworth & Bisby's Dictionary of the fungi, 7th edn. CAB International Mycological Institute, Kew, United Kingdom.

INGOLD, C.T. 1971. Fungal spores. Their liberation and dispersal. Oxford Univ. Press, London and New York.

JONES, J.P. 1976. Ultrastructure of conidium ontogeny in *Phoma pomorum, Microsphaeropsis olivaceum* and *Coniothyrium fuckelii*. *Can. J. Bot.* 54: 831–851.

KUSHIDA, H. 1974. A new method for embedding with a low viscosity epoxy resin 'Quetol 651'. *J. Electron Microsc.* 23: 197.

MINTER, D.W., Kirk, P.M. & SUTTON,B.C. 1982. Holoblastic phialides. *Trans. Br. mycol. Soc.* 79: 75–93.

MINTER, D.W., KIRK, P.M. & SUTTON, B.C. 1983. Thallic phialides. *Mycol. Res.* 80: 39–66.

REYNOLDS, E.S. (1963). The use of lead citrate at high pH as an electron-opague stain in electron microscopy. *J. Cell Biol.* 17: 208–212.

ROUX, C. & VAN WARMELO, K.T. 1990. Conidiomatal and conidial ontogeny in *Urohendersonia platensis*. *Mycol. Res.* 92: 223–229.

ROUX, C., BOTHA, A.J. & VAN WARMELO, K.T. 1994. Scanning electron microscopy of *Stilbella annulata*. *Mycologia* 86(5): 602–606.

ROUX, C., COETZEE, J. & VAN WARMELO, K.T. 1990. Ontogeny and conidiogenesis in *Tiarosporella graminis* var. *karoo*. *Mycol. Res.* 94: 254–262.

SEIFERT, K.A. 1985. Monograph of the genus *Stilbella* and allied Hyphomycetes. *Stud. Mycol.* 27: 1–235.

SUTTON, B.C. 1973. Hyphomycetes from Manitoba and Saskatachewan (Canada). *Mycol. Pap.* 132: 16–20.

SUTTON, B.C. (1980). The Coelomycetes. CAB International Mycological Institute, Kew, United Kingdom.

SUTTON, B.C. 1993. Mitosporic fungi (Deuteromycetes) in the dictionary of fungi, pp. 27–55. In: The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, eds. Reynolds, D.R. & Taylor, J.W. CAB International, Wallingford, United Kingdom.

TIEDT, L.R. & JOOSTE, W.J. 1988. Ultrastructure of collarette formation in *Fusarium* Sect. *Liseola* and some taxonomic implications. *Trans. Br. mycol. Soc.* 90: 531–536.

TULLOCH, M. 1972. The genus *Myrothecium* Tode ex Fr. *Mycol. Pap.* 130: 1–42.

VAN DER MERWE, C.F. & COETZEE, J. 1992. Quetol 651: A revision of the formula. *Proc. Electron Microsc. Soc. Sth. Afr.* 21: 31–32.

WICKLOW, D.T. 1981. Biogeography and conidial fungi. In: Biology of conidial fungi, eds. Cole, G.T. & Kendrick, B., Vol. 1, pp. 417– 442. Academic Press, New York.

WICKLOW, D.T. 1985. Ecological adaptation and classification in *Aspergillus* and *Penicillium*. In: Advances in *Penicillium* and *Aspergillus* systematics, eds. Samson, R.A. & Pitt, J.I., pp. 255–266. Plenum, New York.