Conidium development in *Phialocephala dimorphospora* and a new pattern of wall thickening

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Phialocephala dimorphospora, the type species of *Phialocephala*, has long tubular collarettes and appears to produce conidia in chains. These characteristics are reminiscent of ring wall building development as found in the genus *Sporendocladia*. Scanning and transmission electron microscopy has reconfirmed that conidia in *P. dimorphospora* are formed by apical wall building and are thus fundamentally distinct from *Sporendocladia*. Furthermore, periclinal thickening in *P. dimorphospora* is significantly different from that found in other phialidic fungi. In this fungus, wall remnants remain after delayed secession and premature conidial proliferation. This is in contrast to the periclinal thickening in other phialidic fungi which is formed during the proliferation stage and not the delimitation and secession stage. New terminology is introduced to distinguish the unique mode of conidium development in *P. dimorphospora* from that found in other phialidic fungi.

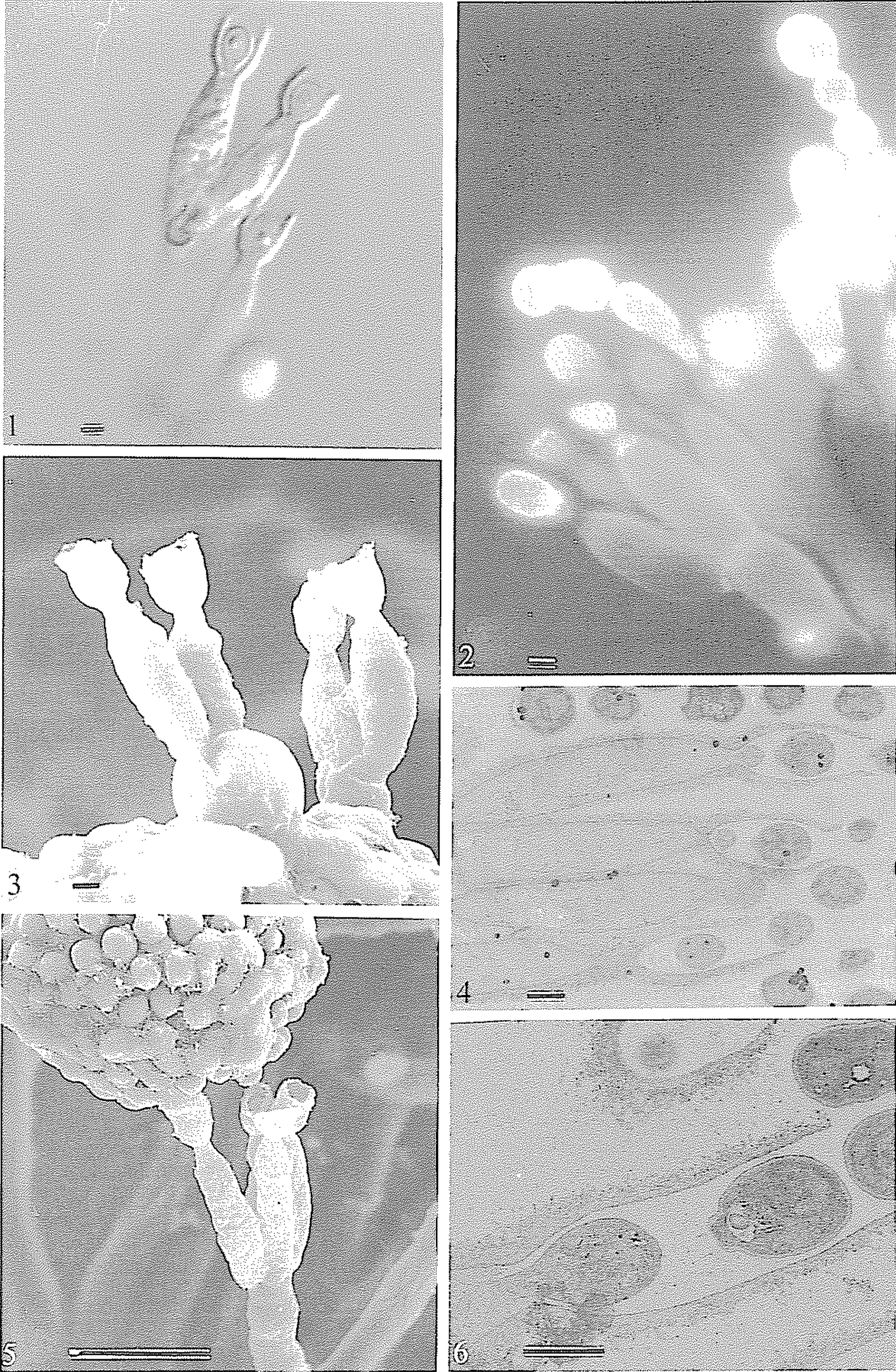
The *Leptographium* complex includes the genera *Leptographium* Lagerberg & Melin and *Phialocephala* Kendrick (Kendrick, 1961, 1963*b*). Species of *Leptographium* are characterized by annellidic conidial development, as opposed to *Phialocephala* spp., where conidia are produced from phialidic conidiogenous cells (Kendrick, 1961, 1963*a*,*b*). *Verticicladiella* Hughes, which was separated from *Leptographium* and *Phialocephala* solely on the basis of having sympodial conidial development (Hughes, 1953), was formerly a member of the *Leptographium* complex (Kendrick, 1963*b*, 1964). This genus was, however, reduced to synonymy with *Leptographium* by Wingfield (1985), who suggested that both annellidic and sympodial development occurred in *Verticicladiella*. Later, Van Wyk, Wingfield & Marasas (1988*a*) showed that an overlapping of the stages of secession and proliferation in conidium development leads to an illusion of sympodial conidiogenous cells that proliferate percurrently.

Species in the *Leptographium* complex are characterized by dark mononematous conidiophores that are penicillately branched. Ameroconidia accumulate in a slimy mass around the sporogenous apparatus (Hughes, 1953; Kendrick, 1961, 1963*b*). These fungi are well known as being associated with insects (Kendrick, 1961; Goheen & Cobb, 1978; Upadhyay, 1981; Wingfield, 1983) and may be important tree pathogens (Alexander, Horner & Lewis, 1988; Cobb, 1988; Hansen *et al.*, 1988; Morrison & Hunt, 1988; Wingfield, Capretti & MacKenzie, 1988).

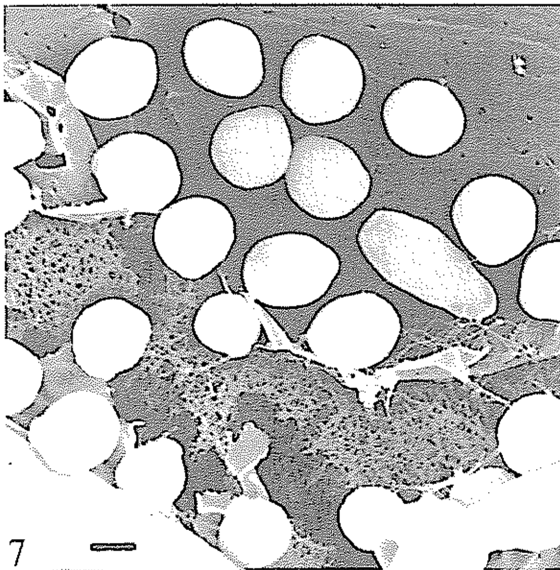
Leptographium spp. are generally accepted to be anamorphs of *Ophiostoma* H. & P. Sydow and related fungi (Upadhyay, 1981; De Hoog & Scheffer, 1984; Harrington, 1988). In contrast, no connection has thus far been found between *Phialocephala* and *Ophiostoma*. Only *O. francke-grosmanniae* Davidson has been suggested to have a *Phialocephala* anamorph (Davidson, 1971; Upadhyay, 1981). This view has, however, been questioned by various authors (Harrington, 1988; Wingfield, 1992). Based on a detailed study of conidium development, we have recently shown that *Leptographium* is a more appropriate genus for the anamorph of *O. francke-grosmanniae*.

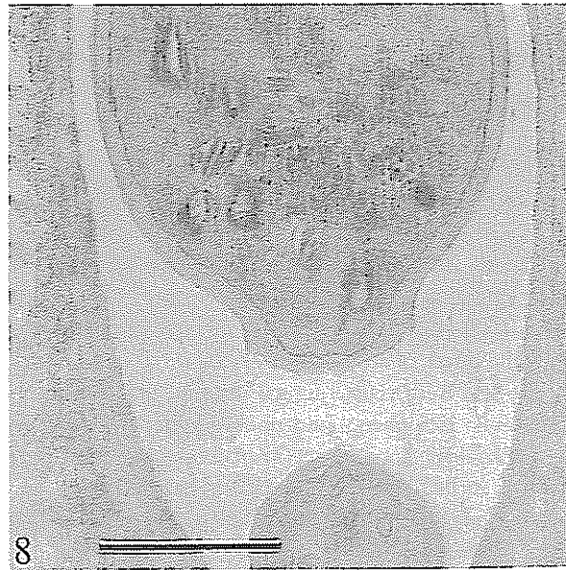
Wingfield, Van Wyk & Wingfield (1987) found that species of *Phialocephala* could be separated into two distinct groups with distinctly different conidial morphologies and, thus, modes of conidial development. One group has apical wall building development and oval conidia with single attachment points. Wingfield *et al.* (1987) retained these species in *Phialocephala*, whereas species with ring wall building development and cylindrical conidia having two attachment points were transferred to *Sporendocladia* Arnaud: Nag Raj & Kendrick. Despite these adjustments, species in *Phialocephala sensu stricto* still appear to represent a heterogeneous assemblage of fungi with phialides having various morphological forms. Based on this fact, Wingfield *et al.* (1987) suggested that the genus still requires characterization.

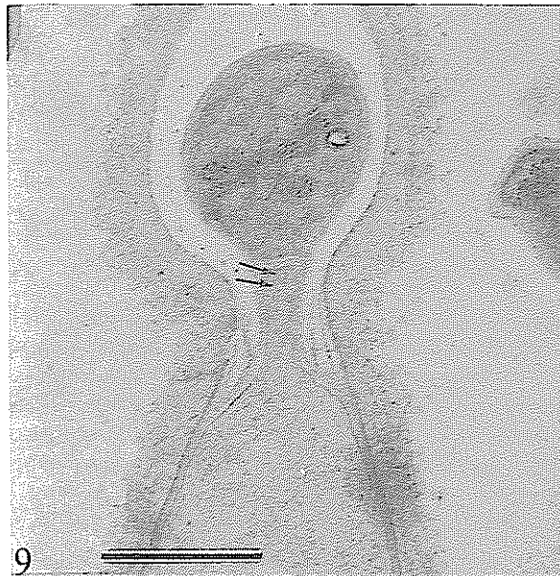
When establishing the genus *Phialocephala*, Kendrick (1961) introduced *P. dimorphospora* Kendrick as the type species because of its distinct phialidic conidial development and a well-differentiated conidiophore. In fact, this species is a remarkable member of the genus in having long tubular collarettes that in some ways resemble those of *Phialophora* Medlar. The fact that conidia are also produced in apparent chains is also unusual and might suggest that it has affinities with the *Chalara* (Corda) Rabenh.-like genus, *Sporendocladia*. Previous ultrastructural studies on this species (Carroll & Carroll, 1975; Wingfield *et al.*, 1987) have provided very little information on its mode of conidial development. The aim of this study was, therefore, to provide a detailed view on

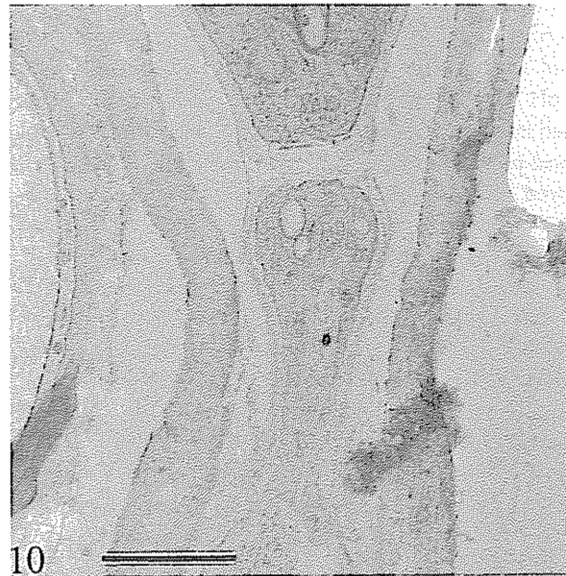


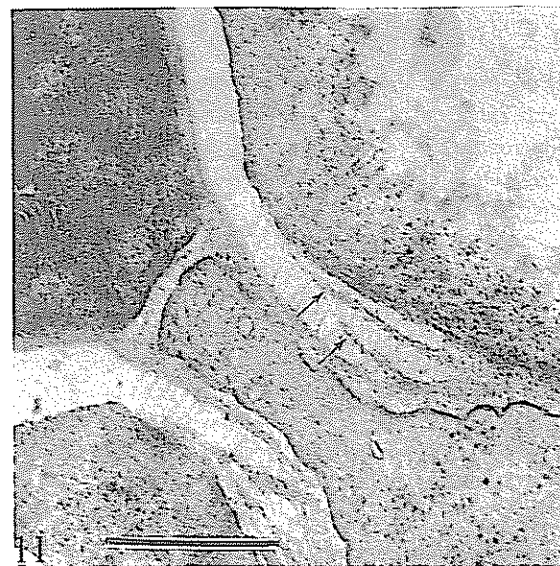
Figs 1–6. For captions see p. 102

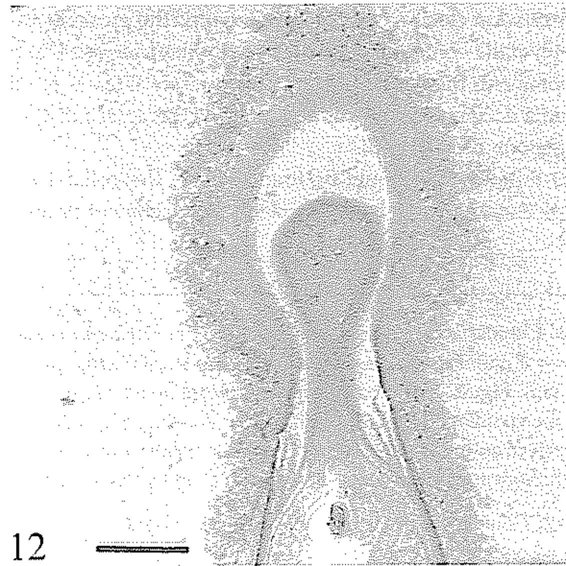












Figs 7–12. For captions see p. 102.

conidial development in *P. dimorphospora* and to explore the possibilities of finding relationships within anamorphic fungi having ring wall building conidial development.

MATERIALS AND METHODS

The isolate of *P. dimorphospora* examined was collected by Dr S. J. Hughes and obtained from the Biosystematics Research Institute, Canada (DAOM 165556). The culture was grown on 2% malt extract agar (20 g Difco malt extract; 20 g Difco Bacto agar; 1000 ml H_2O) in Petri dishes and incubated at 25 °C until the onset of sporulation.

For bright-field microscopy, fungal material was mounted on glass slides in lactophenol and photographed with Ilford FP4 film. Material for fluorescence microscopy was mounted on glass slides in buffered cellifluor optical brightener (0·1 M phosphate buffer). Photography was with a Zeiss Axioskop fluorescence microscope, uv light and a dark background.

Specimens for scanning electron microscopy (SEM) were cut from cultures in Petri dishes and fixed in 3% glutaraldehyde (24 h) and 1% osmium tetroxide (2 h) in a sodium phosphate buffer (pH = 7) at room temperature. Dehydration was performed in a graded acetone series, after which the material was critical-point dried, mounted, coated with gold/palladium and viewed with a JSM 6400 scanning electron microscope.

For transmission electron microscopy (TEM), the fixation and dehydration procedure was the same as that for SEM. Specimens were then embedded in epoxy resin (Spurr, 1969) and polymerized at 70° for 8 h. Sections (60 nm) were cut, mounted on copper grids, stained with uranyl acetate (20–30 min) and lead citrate (10 min) (Reynolds, 1963) and viewed with a Philips EM 300 transmission electron microscope.

RESULTS

Bright-field and fluorescence microscopy, as well as scanning and transmission electron microscopy, verified the presence of distinct phialidic conidiogenous cells in *P. dimorphospora* (Figs 1–4). Prominent, tubular collarettes were present (Figs 1, 3, 4) and conidia accumulated in slimy masses at the apices of the conidiogenous cells (Fig. 5).

Up to three conidia could be found within the collarettes (Figs 4, 6). Ameroconidia had distinct single attachment points (Figs 6–8), and were ovoid in the case of the primary conidia and spherical in all successive conidia (Fig. 7). Fluorescence microscopy revealed chains of conidia emerging from the collarettes although periclinal thickening was not obvious (Fig. 2).

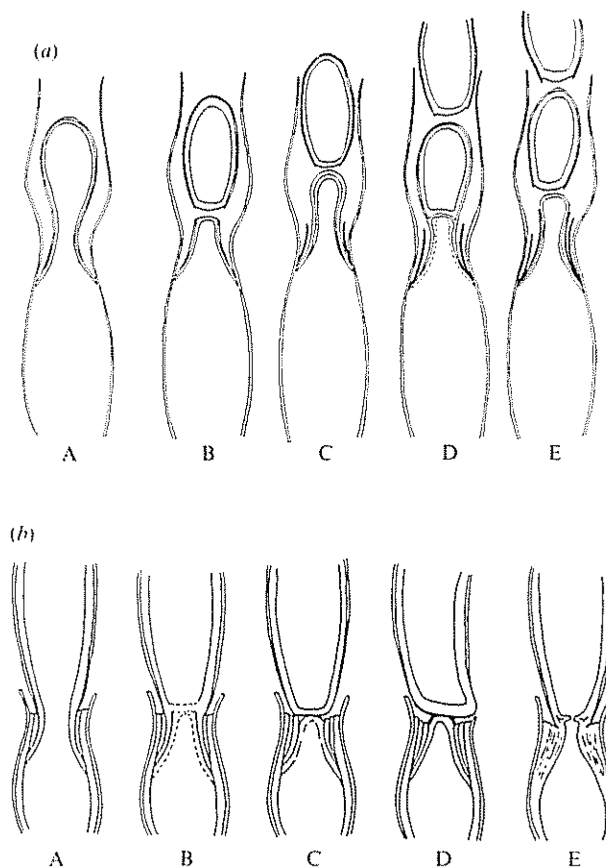


Fig. 13 (*a*, *b*). Schematic representation of conidiogenesis in *P. dimorphospora* and *F. crookwellense*. (*a*) A–E. Conidium development in *P. dimorphospora*. A. Enteroblastic proliferation and holoblastic ontogeny. B. After conidial delimitation and delayed secession, a 'spent' wall is left behind. C. Enteroblastic proliferation and holoblastic ontogeny. D. Conidial delimitation followed by delayed secession and early onset of proliferation. E. Conidial secession resulting in the deposition on a second 'spent' wall. (*b*) Schematic interpretation of macroconidium development in *F. crookwellense* showing the formation of proliferation wall thickening (redrawn from Van Wyk, Wingfield & Marasas, 1988*b*).

Figs 1–6. Conidia and conidiogenous cells of *P. dimorphospora*. **Fig. 1.** Bright-field micrograph revealing distinct phialidic conidiogenous cells (bar = 1 µm). **Fig. 2.** Phialidic conidiogenous cells with apparent chains of conidia evident as seen using fluorescence microscopy (bar = 1 µm). **Fig. 3.** SEM showing phialidic conidiogenous cells with tubular collarettes (bar = 1 µm). **Fig. 4.** TEM of a section through the conidiophore of *P. dimorphospora* showing phialidic conidiogenous cells with long tubular collarettes (bar = 1 µm). **Fig. 5.** Scanning electron micrograph showing an accumulation of conidia at the apex of the sporogenous apparatus (bar = 10 µm). **Fig. 6.** Section through a conidiogenous cell revealing up to three conidia within the collarette of the conidiogenous cell (bar = 1 µm).

Figs 7–12. Conidia and transmission electron micrographs of sections through conidiogenous cells in *P. dimorphospora*. **Fig. 7.** SEM of spherical secondary and successive conidia with single attachment points and an ovoid primary conidium (bar = 1 µm). **Fig. 8.** Section through the collarette of a conidiogenous cell showing the distinct single attachment point of the conidium (bar = 0·5 µm). **Fig. 9.** Apex of conidiogenous cell showing secretory vesicles (arrows) (bar = 1 µm). **Fig. 10.** Apex of conidiogenous cell showing the replacement of the wall building apex in the newly forming conidium (bar = 0·5 µm). **Fig. 11.** Apex of conidiogenous cell showing conidium outer wall layers remaining after delayed secession ('spent walls') of the newly formed conidium (arrows) (bar = 0.5 µm). **Fig. 12.** Accumulation of outer wall layers towards the inside on the conidiogenous cell at the base of the collarette (bar = 1 µm).

Conidial development progressed through an apical wall building process. Apical vesicles (secretory organelles) were observed at the apices of developing conidia (Fig. 9). After conidial delimitation, the wall building apex was lost by the mature conidium. This was then replaced with a new zone of wall building activity to form the apex of the newly developing conidium during early proliferation (Fig. 10).

Transmission electron micrographs indicated that wall remnants remained (Fig. 11) after the stages of delimitation and secession, to accumulate towards the inside of the conidiogenous cell just below the lower part of the collarette (Fig. 12). These wall remnants represent the outer wall layers of the previously formed conidia (Fig. 11) and could also be observed on fluorescence micrographs (Fig. 2).

The wall remnants in *P. dimorphospora* appeared to represent a previously unobserved pattern of periclinal thickening (Fig. 13*a*), morphologically distinct from that observed in ultrastructural studies of other phialidic fungi such as *Fusarium* Link (Fig. 13*b*). Here, wall remnants remained after delimitation and delayed secession of each conidium. In contrast, periclinal thickening in *Fusarium* spp. is the result of an accumulation of wall layers from the proliferation stage. Thus, in *P. dimorphospora*, the first conidium was produced holoblastically followed by delimitation and secession. Subsequent conidia were then formed by early enteroblastic proliferation, holoblastic ontogeny, conidial delimitation and delayed secession (Fig. 13*a*).

DISCUSSION

Species of *Phialocephala* have collarettes that vary from being quite inconspicuous to cup-shaped, loosely flaring or irregularly torn (Kendrick, 1961; Wingfield *et al.*, 1987). These are in contrast to those of *Sporendocladia* spp. that have distinct cylindrical collarettes (Wingfield *et al.*, 1987). In *P. dimorphospora*, collarettes are also long and cylindrical, but differ from those of *Sporendocladia* in being cup-shaped. These collarettes give rise to conidia that emerge in chains and represent what Minter, Kirk & Sutton (1982, 1983) referred to as 'false chains'. Conidia in these chains have single points of attachment and are in contrast to the true chains produced by ring wall building, where conidia are usually bacilliform with two attachment points (Kendrick, 1961; Wingfield *et al.*, 1987).

Apical secretory organelles were observed in the apices of all developing conidia of *P. dimorphospora*, which is also an indication of apical wall building (Minter *et al.*, 1983). A similar observation was made by Carroll & Carroll (1974) in their study on the ultrastructure of conidium development in this fungus. After delimitation, the wall building apex is lost by the mature conidium and replaced by new wall building vesicles in the apex of the newly developing conidium (Minter *et al.*, 1983).

Periclinal thickening at the bases of collarettes is a common feature of anamorphic fungi that produce conidia by apical wall building from phialides (Minter *et al.*, 1983; Van Wyk, Wingfield & Marasas, 1988*b*). This phenomenon has been observed in a variety of phialidic fungi such as *Trichoderma saturnisporum* Hammil (Hammil, 1974), in the development of microconidia in *Fusarium crookwellense* Burgess, Nelson & Toussoun and a wide range of other *Fusarium* spp. (Van Wyk *et al.*, 1988*b*), as well as *Stachybotrys atra* Corda (Campbell, 1972). It is generally thought that periclinal thickening results from an accumulation of wall layers, although some authors dispute this view (Tiedt, Jooste & Hamilton-Attwell, 1986). Distinct wall remnants were observed at the bases of the collarettes in conidiogenous cells of *P. dimorphospora* in this study. These structures were, however, not shown in the micrographs of Carroll & Carroll (1974) in their study of this fungus. This might be due to the different fixing methods used for the studies. For example, Carroll & Carroll (1974) used the glutaraldehyde fixative for 1 h, in contrast to our study in which this same procedure was done for approximately 24 h. Different buffers were also used during the fixing.

The wall remnants in *P. dimorphospora* were however, very different from those observed in previous ultrastructural studies of phialidic fungi. What made them unique was that they remained after delayed secession of the newly formed conidium and advanced onset of the subsequent proliferation stage (Fig. 13*a*). This is in contrast to a build-up of proliferation layers seen in other phialidic fungi such as *Fusarium crookwellense* (Van Wyk *et al.*, 1988*b*) (Fig. 13*b*).

The results of this study suggest that at least two distinct patterns of conidium development occur in anamorphic fungi that produce conidia through apical wall building in phialides. These groups can be distinguished by the morphological arrangement of expended wall layers associated with periclinal thickening. In the first case, wall layers arise during proliferation and adhere to proliferation wall layers from previously produced conidia, to give rise to typical periclinal thickening. This form of thickening has been found in all phialidic fungi that have been examined to date, and we refer to it as proliferation wall thickening. In the second case, exemplified by *P. dimorphospora*, wall remnants accumulate when proliferation of the newly forming conidium and the production of a new wall building apex proceed prior to secession of the preceding conidium. The delay in conidial secession results in wall remnants that are loosely aggregated at the base of the collarette. We refer to this form of thickening as 'spent wall thickening', because a wall layer remains (is 'spent') after conidial secession. It is thus the stage of conidial development during which the thickening is formed that results in the major difference between these two patterns of periclinal thickening.

The two different forms of conidial development and periclinal thickening ('proliferation' and 'spent' wall thickening) would be extremely difficult, if not impossible, to distinguish based on light microscopy or even scanning electron microscopy. At this stage, TEM studies are essential in order to recognize these developmental patterns. However, we hope that in future these differences will be more easily recognizable.

It is unlikely that the unusual form of conidial development observed in this study is restricted to *P. dimorphospora*. Further studies of conidial development in phialidic fungi that have similar morphology to *P. dimorphospora*, such as species of *Phialophora*, should be undertaken in order to clarify this situation.

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