Comparison between conidial development in *Sporendocladia bactrospora* and *Phialocephala virens*

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Conidium development was studied and compared in *Sporendocladia bactrospora* (thought to resemble *Chalara* spp.) and in *Phialocephala virens*. Techniques used in the study include bright field and fluorescence microscopy, as well as scanning and transmission electron microscopy. *Sporendocladia bactrospora* had cylindrical conidia produced in true chains from phialidic conidiogenous cells with long cylindrical collarettes. An area of wall building activity at the base of the conidiogenous cell was characterized by secretory vesicles indicating ring wall building development. In *Phialocephala virens*, conidia were formed by apical wall building and distinct periclinal thickening was evident. From this study it was possible to confirm the fact that *Phialocephala* s.l. can clearly be divided into two distinct groups on the basis of conidium development.

Key words: apical wall building, conidiogenesis, *Phialocephala*, ring wall building, *Sporendocladia*.

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Les auteurs ont étudié et comparé le développement conidien chez le *Sporendocladia bactrospora* (ressemblant aux *Chalara* spp.) et chez le *Phialocephala virens*. Les techniques utilisées à cette fin incluent la microscopie en fluorescence et à fond clair ainsi que la microscopie par balayage et par transmission. Le *Sporendocladia bactrospora* montre des conidies cylindriques produites en chaînes véritables à partir de cellules conidiogènes phialidiques, munies de longues colerettes cylindriques. Une région de déposition active de matériel pariétal à la base de la cellule conidiogène se caractérise par des vésicules secrétrices indiquant le développement d'anneaux pariétaux. Chez le *Phialocephala virens*, les conidies se forment par une déposition apicale du matériel pariétal et un épaississement périclinal distinctif et évident. Cette étude permet de conclure que les *Phialocephala* s.l. peuvent être divisés en deux groupes distincts sur la base du développement conidien.

Mots clés : déposition apicale du matériel pariétal, conidiogénèse, *Phialocephala*, déposition annulaire de matériel pariétal, *Sporendocladia*.

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Introduction

Initially, the *Leptographium* complex included the genera *Leptographium* Lagerberg & Melin, *Verticicladiella* Hughes, and *Phialocephala* Kendrick. These were separated on the basis of their annellidic, sympodial, and phialidic conidial development, respectively (Hughes 1953; Kendrick 1961, 1962, 1963). Subsequently, Wingfield (1985) showed that both annellidic and apparent sympodial conidial development occur in species of *Leptographium* and *Verticicladiella* and reduced these genera to synonymy.

All members of the *Leptographium* complex are characterized by the presence of dark mononematous conidiophores that are penicillately branched and produce hyaline ameroconidia accumulating in a slimy droplet at the apex of the sporogenous apparatus (Kendrick 1961, 1963; Wingfield et al. 1987). These fungi are commonly associated with small animals such as bark beetles and weevils (Goheen and Cobb 1978; Harrington 1988; Kendrick 1961; Upadhyay 1981; Wingfield 1983) and some are well-known tree pathogens (Alexander et al. 1988; Cobb 1988; Hansen et al. 1988; Morrison and Hunt 1988; Wingfield et al. 1988).

Leptographium spp. are best regarded as anamorphs of *Ophiostoma* H.&P. Sydow and related fungi such as *Ceratocystiopsis* Upadhyay & Kendrick (De Hoog and Scheffer 1984; Harrington 1987; Upadhyay 1981; Wingfield et al. 1987). In contrast, there are no known connections between *Phialocephala* and *Ophiostoma* (Harrington 1988). Only one species, *Ophiostoma francke-grosmanniae* Davidson, is purported to have a *Phialocephala* anamorph (Davidson 1971; Upadhyay 1981). It is, however, our opinion that *Leptographium* would be a more appropriate designation for this fungus (Mouton et al. 1992).

Leptographium spp. are characterized by percurrent proliferation of conidiogenous cells, sometimes with the illusion of sympodial conidial development (Van Wyk et al. 1988*a*; Wingfield 1985). Percurrent conidial development leads to an elongation of the conidiogenous cells and a series of annellations mark the sites of conidial secession (Cole and Samson 1979). In contrast, *Phialocephala* spp. have distinct phialidic conidiogenous cells (Jooste 1978; Kendrick 1961, 1963; Tsuneda and Hiratsuka 1984; Van Wyk et al. 1988*a*; Wingfield et al. 1987). No notable elongation of the conidiogenous cells occurs during the production of a succession of conidia (Cole and Samson 1979; Van Wyk et al. 1988*a*).

Wingfield et al. (1987) found that two distinct patterns of wall building occur in the genus *Phialocephala*. The one group is characterized by the presence of apical wall building and the accumulation of proliferation wall layers towards the inside of the conidiogenous cells (periclinal thickening) and occurs in species such as *P. fusca* Kendrick. In the second group, conidia appear to develop through ring wall building, similar to that in species of *Chalara* (Corda) Rabenh. Here, conidia are produced in chains (Minter et al. 1983; Wingfield et al. 1987). Based on these differences, Wingfield et al. (1987) transferred species of *Phialocephala* with apparent ring wall building development to *Sporendocladia* Arnaud: Nag Raj & Kendrick. After transferring certain species of *Phialocephala* to *Sporendocladia*, these authors noted that species

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remaining in *Phialocephala* still appeared to represent a heterogeneous group and that this genus should receive further consideration.

Wingfield et al. (1987) noted that species of *Sporendocladia* have long collarettes and conidia produced in chains that indicate ring wall building development (Minter et al. 1983). The presence of this wall building ring has, however, never been confirmed using transmission electron microscopy. This would be interesting in view of the different morphology of *Sporendocladia* and *Chalara*, in which such conidium development is well defined.

Some species of *Phialocephala* have long, deep-set collarettes that can result in conidia arising in chains. These chains are usually considered as "false" chains in terms of Minter et al. (1982). Although *Sporendocladia* and *Phialocephala* can be differentiated by the presence of two points of attachment on conidia of the former genus and one point of attachment in the latter case, a detailed comparison of conidium development in the two genera, particularly including a *Phialocephala* sp. with long tubular collarettes, is needed. The aim of this study was, therefore, to produce a detailed view of conidium development in *Sporendocladia bactrospora* Kendrick, previously accommodated in *Phialocephala* and in which conidia are apparently formed through ring wall building. In addition, conidial development in a fungus recently described as *Phialocephala virens* Siegfried & Seifert (Siegfried et al. 1992) that has long collarettes was also studied. This fungus provided a basis for comparison of the two modes of conidial development associated with species of *Phialocephala* in the broad sense.

Materials and methods

The isolate of *Sporendocladia bactrospora* examined was obtained from the Institute for Fermentation, Osaka, Japan (as *Leptographium kitajimina*, isolate IFO 6908). The isolate of *Phialocephala virens* was obtained from Dr. K.A. Seifert, Forintek Canada Corporation, Eastern Laboratory, Ottawa, Ontario, Canada (isolate 164 C). Isolates were 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 sporulation.

Specimens for light microscopy were mounted in lactophenol on glass slides and examined using phase- and interference-contrast microscopy. Material for fluorescence microscopy was mounted on glass slides in a 0.05% w/v solution of calcafluor white M 2R optical brightener in 0.1 M phosphate buffer. The samples were examined with a Zeiss Axioskop fluorescence microscope, dark background and UV light.

Specimens for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were cut into blocks from the agar (approximately 5–8 mm^2), fixed in 3% glutaraldehyde, followed by 1% osmium tetroxide in a 0.1 M phosphate buffer (pH 7), and dehydrated in a graded acetone series (50, 70, 95, and 100%). The SEM material was then critical-point dried, mounted, coated with gold–palladium, and viewed with an ISI scanning electron microscope.

The TEM samples were fixed and dehydrated in a similar manner as for SEM and were embedded in epoxy resin (Spurr 1969). Thin sections (60 nm) were made using an ultramicrotome, mounted on copper grids, and stained with uranyl acetate (20–30 min) followed by lead citrate (10 min) (Reynolds 1963). Sections were viewed with a Phillips EM 300 transmission electron microscope.

Results

Both *P. virens* and *S. bactrospora* have conidiophores that were penicillately branched (Figs. 1 and 2). In both cases, the conidiogenous cells were lageniform, with distinct, deep-set phialides (Figs. 1 and 2). Small collarettes were visible in *P. virens* (Fig. 3), while *S. bactrospora* was characterized by having long cylindrical collarettes, and a slight narrowing could be detected just beneath the collarette (Fig. 4).

Conidia of *P. virens* were ovoid to ellipsoidal in shape, becoming narrower towards the very definite single attachment point (Fig. 5). In contrast, *S. bactrospora* was characterized by cylindrical conidia, and transmission electron micrographs as well as fluorescence microscopy indicated attachment points at both sides of these conidia (Figs. 6 and 13). From bright field microscopy and TEM, it was also clear that conidia of *S. bactrospora* are produced in true chains having a communal outer wall layer (Figs. 7 and 8).

Well-developed periclinal thickening could be seen in bright field and fluorescence micrographs of *P. virens* (Figs. 3 and 10). Transmission electron micrographs also confirmed the presence of this thickening, which was relatively deep set within the phialide and developed towards the inside of the conidiogenous cells (Figs. 9 and 10). No such periclinal thickening could be found in the conidiogenous cells of *S. bactrospora* (Fig. 11).

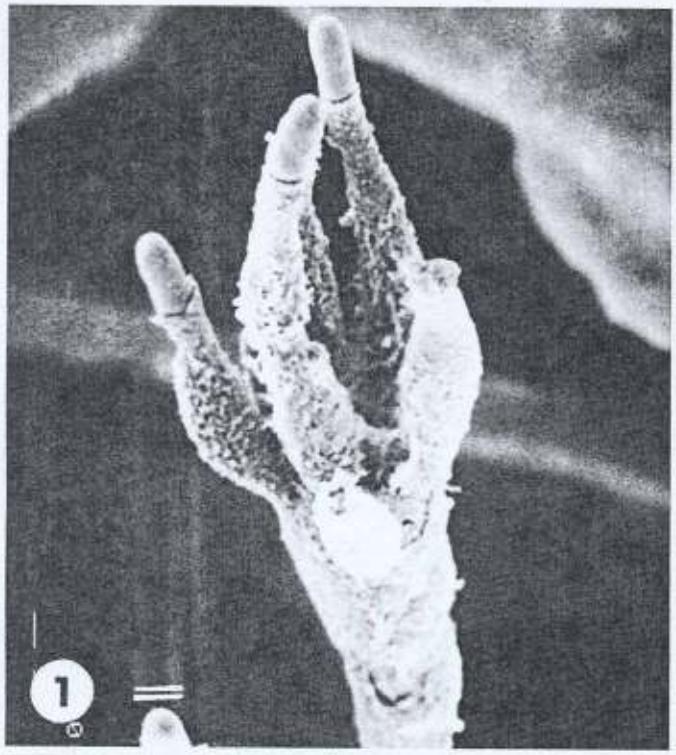
In *P. virens*, the secretory vesicles within the conidium were associated with wall building activity. In this fungus, conidial development was typical of apical wall building and vesicles formed a wall-building apex with walls produced in a downward direction (towards the base of the conidiogenous cell) (Fig. 12). Thus, each conidium was formed by a succession of enteroblastic proliferation, holoblastic ontogeny, conidial delimitation, and secession.

In *S. bactrospora*, fluorescence micrographs of conidiogenous cells verified the presence of a brightly fluorescing area just beneath the narrowing in the conidiogenous cells (Fig. 13). When ultrathin sections were made through these conidiogenous cells, the brightly fluorescing area was found to contain secretory vesicles within and along the sides of the conidiogenous cell (Fig. 14). These organelles represented the wall-building ring that gives rise to chains of conidia produced in an apical direction.

Discussion

The process of conidial development in *S. bactrospora* was fundamentally different from that observed in the *P. virens*. A brightly fluorescing area was observed beneath the narrowing of the conidiogenous cell in *S. bactrospora* and transmission electron micrographs revealed the presence of secretory organelles along the sides of the conidiogenous cell in this area. Minter et al. (1983) refer to this area as the wall-building ring,

FIGS. 1–6. Conidiophores, conidiogenous cells, and conidia of *P. virens* and *S. bactrospora*. Fig. 1. Scanning electron micrograph of the conidiophore of *P. virens* showing a penicillately branched conidiophore and phialidic conidiogenous cells. Scale bar = 1 μm. Fig. 2. SEM of the conidiophore of *S. bactrospora*, showing a penicillately branched conidiophore and phialidic conidiogenous cells with long cylindrical collarettes. Scale bar = 10 μm. Fig. 3. Light micrograph of the phialidic conidiogenous cells of *P. virens*. Scale bar = 1 μm. Fig. 4. SEM revealing a phialidic conidiogenous cell of *S. bactrospora* with an emerging cylindrical conidium. Scale bar = 1 μm. Fig. 5. Median section through the ovoid conidium of *P. virens* revealing a distinct single attachment point. Scale bar = 1 μm. Fig. 6. Transmission electron micrograph of a section through a conidium of *S. bactrospora* showing attachment points at either end. Scale bar = 1 μm.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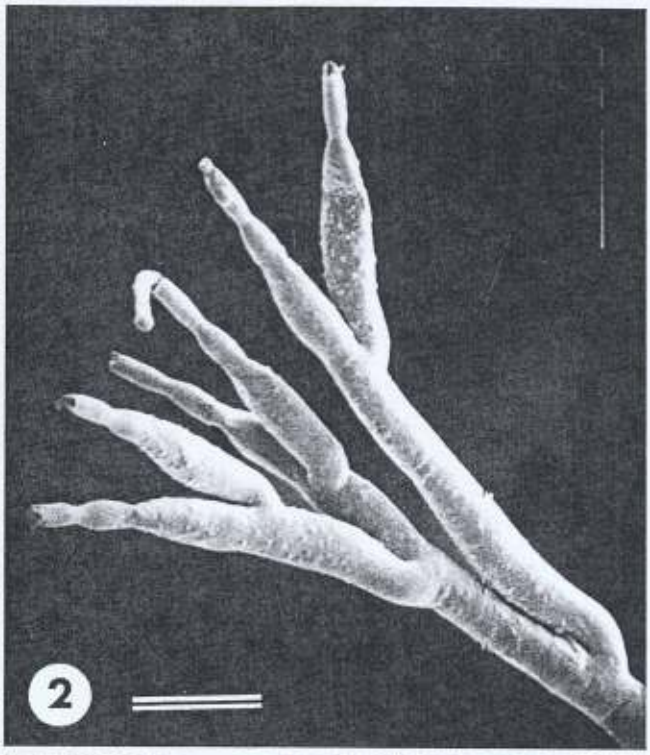
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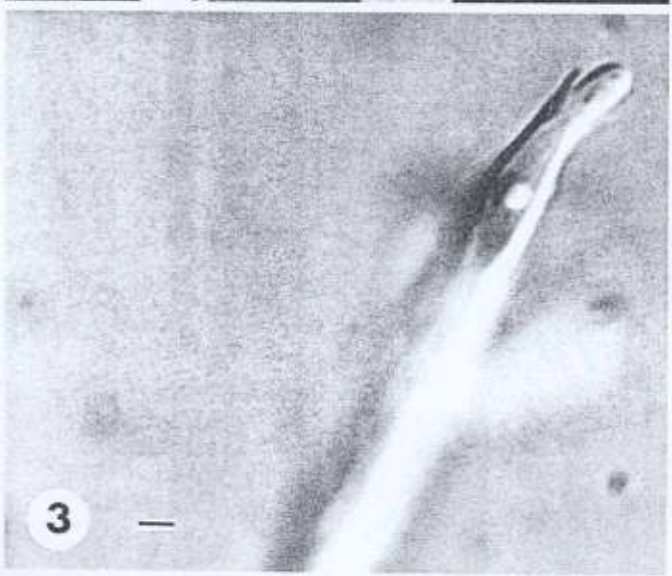
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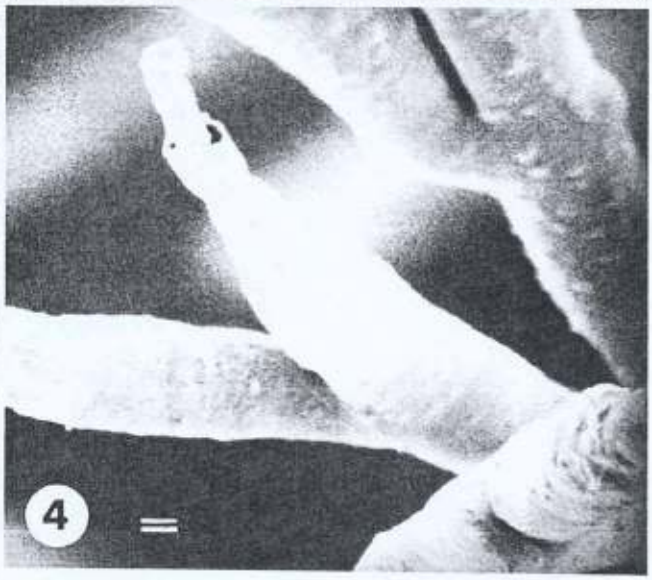
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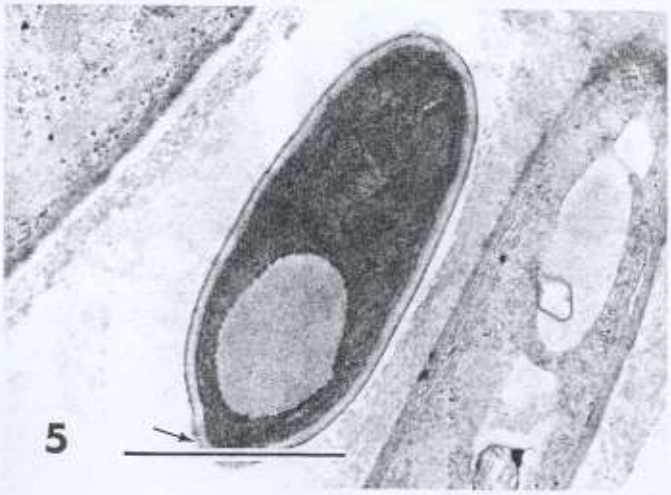
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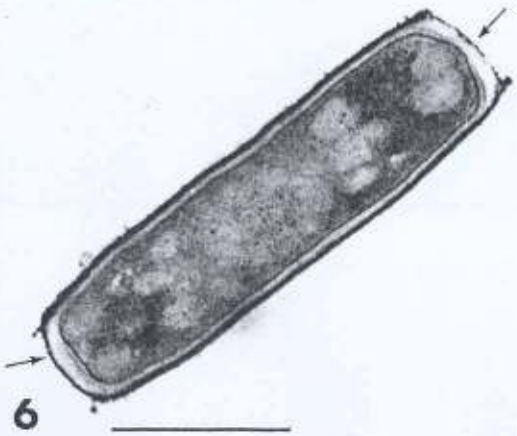
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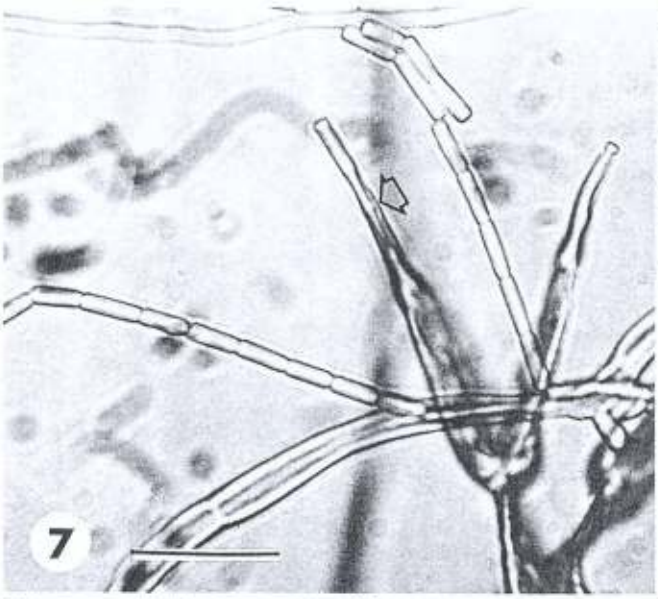
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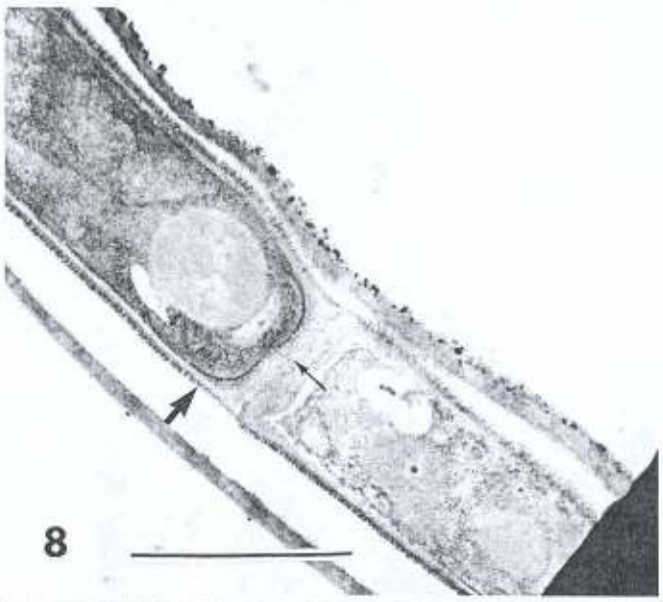
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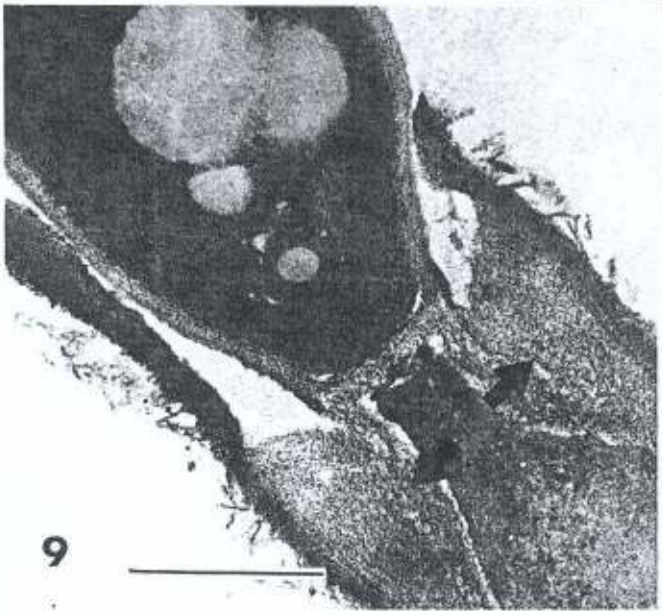
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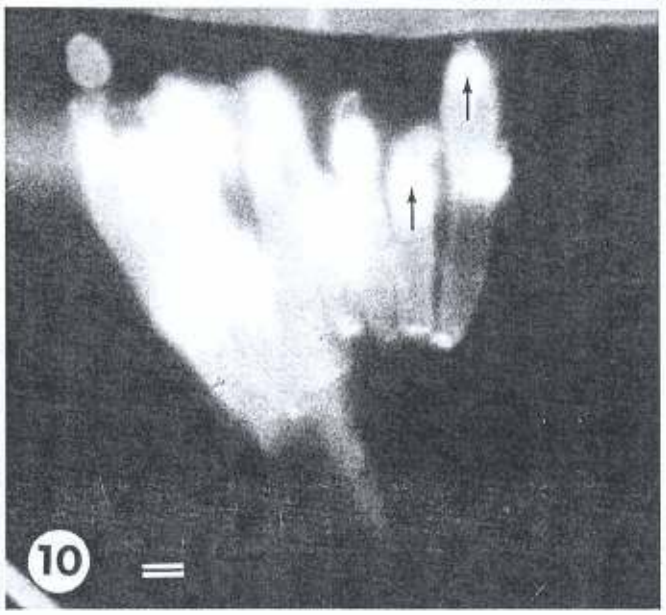
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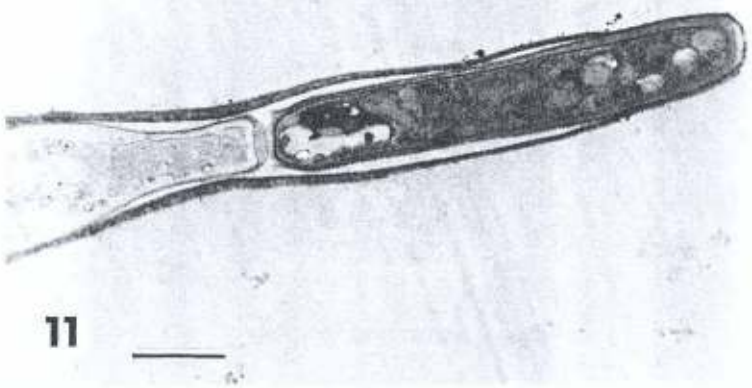
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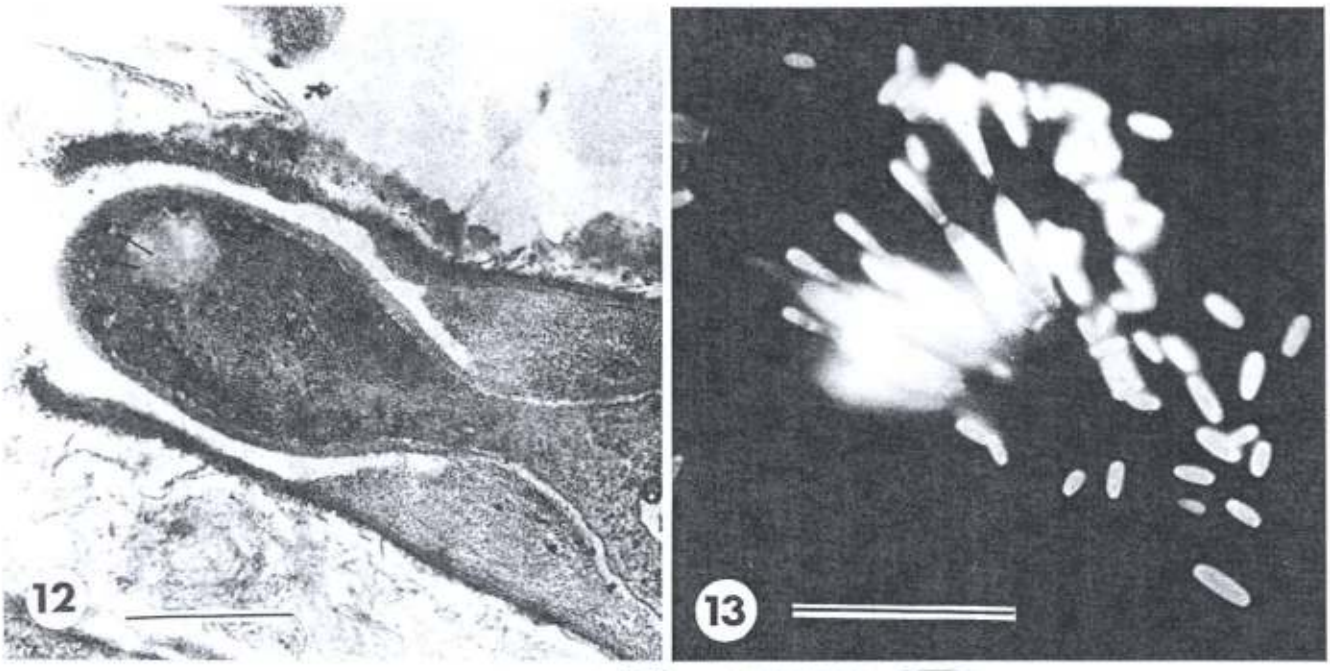

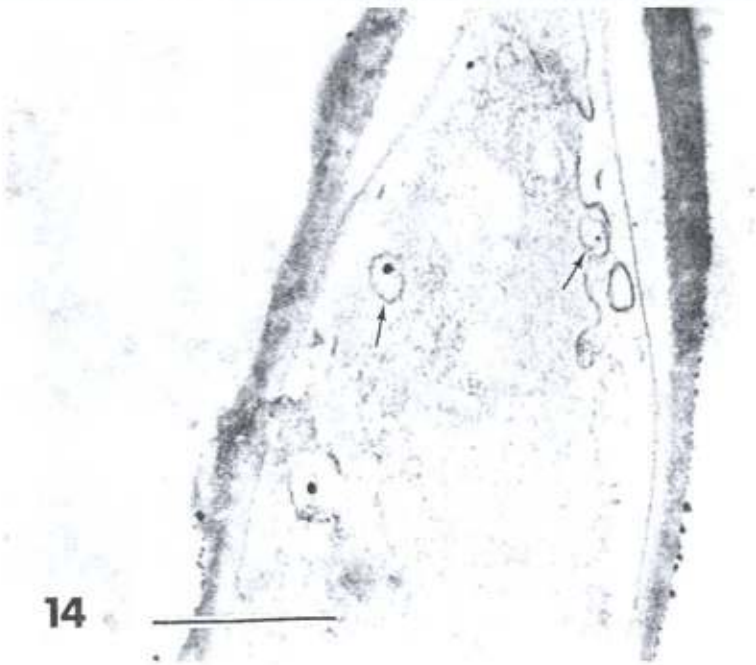

FIGS. 12–14. Conidiogenous cells of *P. virens* and *S. bactrospora*. Fig. 12. Section through the conidiogenous cell of *P. virens*, revealing secretory vesicles in the apex of the developing conidium (arrows). Scale bar = 0.5 μm. Fig. 13. Fluorescence micrograph of the conidiophore of *S. bactrospora* revealing a brightly fluorescing area below the narrowing in the conidiogenous cell as well as the attachment points on both ends of conidia. Scale bar = 10 μm. Fig. 14. Section through the conidiogenous cell of *S. bactrospora* indicating the presence of secretory organelles along the sides and beneath the narrowing in the conidiogenous cell (arrows). Scale bar = 0.5 μm.

FIGS. 7–11. Conidiophores and conidiogenous cells of *P. virens* and *S. bactrospora*. Fig. 7. Light micrograph of the conidiophore of *S. bactrospora* showing the production of chains of conidia from the phialidic conidiogenous cells (arrow). Fig. 8. Section through the conidiogenous cell of *S. bactrospora* showing the attachment point between two successive conidia (small arrow), with a communal outer wall layer (large arrow). Scale bar = 1 μm. Fig. 9. TEM of a section through the conidiogenous cell of *P. virens* revealing the relatively deep set conidiogenous locus and well-developed periclinal thickening (fluorescence) towards the inside of the cell (arrow). Scale bar = 0.5 μm. Fig. 10. Fluorescence micrograph of the conidiophore of *P. virens* indicating well-developed periclinal thickening in the apices of the conidiogenous cells (arrows). Fig. 11. TEM of a section through a conidiogenous cell showing a mature conidium and a newly developing one within the long cylindrical collarette of *S. bactrospora*. No periclinal thickening is present in this case. Scale bar = 1 μm.

as first described in species of *Chalara*. Conidium development in *S. bactrospora* is thus interpreted as typical ring wall building as opposed to apical wall building in *P. virens*. This conclusion is also supported by the presence of cylindrical phialoconidia produced in true chains with a communal outer wall layer surrounding the chain of conidia (Minter et al. 1983).

Phialocephala virens has deep-set phialidic conidiogenous loci, although these are not as distinct as those of *S. bactrospora*. In this case, the secretory vesicles are situated at the apex of the developing conidium. Minter et al. (1983) described the latter area as the wall building apex, and this process of conidial development is commonly referred to as apical wall building. This process is also characterized by the presence of periclinal thickening, which was well developed in *P. virens*.

Periclinal thickening provides evidence of a succession of wall-building apices and its absence indicates ring wall building (Minter et al. 1983). We believe that periclinal thickening in *P. virens* is the result of an accumulation of wall remnants produced during conidial proliferation and that it might lead to blockage of the conidiogenous cell if the latter is not able to regenerate prior to total blockage. Periclinal thickening has been observed in ultrastructural studies of a number of fungi, including species of *Fusarium* Link (Van Wyk et al. 1987, 1988*b*), *Trichoderma saturnisporum* Hammil (Hammil 1974), and *Stachybotrys atra* Corda (Campbell 1972). In contrast with the general belief that periclinal thickening results from an accumulation of proliferation wall layers during the production of successive conidia, some authors (Tiedt et al. 1986) contest this view. Mouton et al. (1993) have recently shown that an additional type of wall thickening occurs in *Phialocephala dimorphospora* Kendrick. In this fungus, delayed conidial secession results in wall remnants that loosely aggregate at the base of the conidiogenous cell's collarette. This was described as spent wall thickening and is distinctly different from the proliferation wall thickening found in *P. virens*. This provides additional evidence for the suggested heterogeneous nature of *Phialocephala* (Wingfield et al. 1987).

The results of this study support the decision of Wingfield et al. (1987) to divide *Phialocephala* s.l. into two groups with either ring or apical wall building conidial development. Circumstantial evidence that conidia in *Sporendocladia* would develop from a wall building ring (Wingfield et al. 1987) is also supported with more definitive evidence of this process. Despite the accentuated collarettes in *P. virens*, conidium development was typical of apical wall building.

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Alexander, S.A., Horner, W.E., and Lewis, K.J. 1988. *Leptographium procerum* as a pathogens of pines. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 97–112.

Campbell, R. 1972. Ultrastructure of conidium ontogeny in the Deuteromycete fungus *Stachybotrys atra* Corda. New Phytol. **71**: 1143–1149.

Cobb, F.W., Jr. 1988. *Leptographium wagneri*, cause of black-stain root disease: a review of its discovery, occurrence and biology with emphasis on pinyon and ponderosa pine. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 41–62.

Cole, G.T., and Samson, R.A. 1979. Patterns of development in conidial fungi. Pitman Publishing Ltd., London. pp. 54–83.

Davidson, R.W. 1971. New species of *Ceratocystis*. Mycologia, **63**: 5–15.

De Hoog, G.S., and Scheffer, R.J. 1984. *Ceratocystis* versus *Ophiostoma*: a reappraisal. Mycologia, **76**: 292–299.

Goheen, D.J., and Cobb, F.W., Jr. 1978. Occurrence of *Verticicladiella wagnerii* and its perfect state, *Ceratocystis wagnerii* sp. nov. in insect galleries. Phytopathology, **57**: 1192–1195.

Hammil, T.M. 1974. Electron microscopy of phialides and conidiogenesis in *Trichoderma saturnisporum*. Am. J. Bot. **61**: 15–24.

Hansen, E.M., Goheen, D.J., Hessburg, P.F., *et al.* 1988. Biology and management of black-stain root disease in Douglas-fir. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 63–80.

Harrington, T.C. 1987. New combinations of *Ophiostoma* and *Ceratocystis* species with *Leptographium* anamorphs. Mycotaxon, **28**: 39–43.

Harrington, T.C. 1988. *Leptographium* species, their distribution, hosts and insect vectors. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 1–39.

Hughes, S.J. 1953. Conidiophores, conidia, and classification. Can. J. Bot. **31**: 577–659.

Jooste, W.J. 1978. *Leptographium reconditum* sp. nov. and observations on conidiogenesis in *Verticicladiella*. Trans. Br. Mycol. Soc. **70**: 152–155.

Kendrick, W.B. 1961. The *Leptographium* complex. *Phialocephala* gen.nov. Can. J. Bot. **39**: 1079–1085.

Kendrick, W.B. 1962. The *Leptographium* complex. *Penicillium repens* C. & E. Can. J. Bot. **41**: 573–577.

Kendrick, W.B. 1963. The *Leptographium* complex. Two new species of *Phialocephala*. Can. J. Bot. **41**: 1015–1023.

Minter, D.W., Kirk, P.M., and Sutton, B.C. 1982. Holoblastic phialides. Trans. Br. Mycol. Soc. **79**: 75–93.

Minter, D.W., Kirk, P.M., and Sutton, B.C. 1983. Thallic phialides. Trans. Br. Mycol. Soc. **80**: 39–66.

Morrison, D.J., and Hunt, R.S. 1988. *Leptographium* species associated with root disease of conifers in British Columbia. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 81–95.

Mouton, M., Wingfield, M.J., and Van Wyk, P.S. 1992. The anamorph of *Ophiostoma francke-grosmanniae* is a *Leptographium*. Mycologia, **84**: 857–862.

Mouton, M., Wingfield, M.J., and Van Wyk, P.S. 1993. Conidium development in *Phialocephala dimorphospora* and a new pattern of wall thickening. Mycol. Res. **97**: 99–104.

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

Siegfried, A.L., Seifert, K.A., and Bilmer, B.C. 1992. A new species of *Phialocephala* (Hyphomycetes). Can. J. Bot. **70**: 2484–2489.

Spurr, A.R. 1969. A low viscosity embedding medium for electron microscopy. J. Ultrastruct. Res. **26**: 31–43.

Tiedt, L.R., Jooste, W.J., and Hamilton-Attwell, V.L. 1986. Ultrastructural aspects of conidiogenesis of *Fusarium sacchari* f.sp. *subglutinans*. Trans. Br. Mycol. Soc. **87**: 237–241.

Tsuneda, A., and Hiratsuka, Y. 1984. Sympodial and annellidic conidiation in *Ceratocystis clavigera*. Can. J. Bot. **62**: 2618–2624.

Upadhyay, H.P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. The University of Georgia Press, Athens, Ga.

Van Wyk, P.S., Venter, E., Wingfield, M.J., and Marasas, W.F.O. 1987. Enteroblastic first macroconidia in *Fusarium crookwellense*. Can. J. Bot. **66**: 1364–1366.

Van Wyk, P.S., Wingfield, M.J., and Marasas, W.F.O. 1988*a*. Differences in synchronization of stages of conidial development in *Leptographium* species. Trans. Br. Mycol. Soc. **90**: 451–456.

Van Wyk, P.S., Wingfield, M.J., and Marasas, W.F.O. 1988*b*. Delimitation of *Fusarium crookwellense* macroconidia. Trans. Br. Mycol. Soc. **91**: 611–617.

Wingfield, M.J. 1983. Some insects associated with *Verticicladiella procera* and *Leptographium terebrantis* in the Lake States. Can. J. For. Res. **13**: 1238–1245.

Wingfield, M.J. 1985. Reclassification of *Verticicladiella* based on conidial development. Trans. Br. Mycol. Soc. **85**: 81–93.

Wingfield, M.J., Van Wyk, P.S., and Wingfield, B.D. 1987. Reclassification of *Phialocephala* based on conidial development. Trans. Br. Mycol. Soc. **89**: 509–520.

Wingfield, M.J., Capretti, P., and MacKenzie, M. 1988. *Leptographium* spp. as root pathogens of conifers. An international perspective. *In Leptographium* root diseases on conifers. *Edited by* T.C. Harrington and F.W. Cobb, Jr. The American Phytopathological Society. APS Press, St. Paul, Minn. pp. 113–128.