MYCOTAXON

Volume XLVI, pp. 363-370 January-March 1993

CONIDIUM DEVELOPMENT IN THE KNOXDAVIESIA ANAMORPH OF CERATOCYSTIOPSIS PROTEAE

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Ceratocystiopsis proteae and its anamorph, *Knoxdaviesia proteae,* occur commonly in the infructescences of *Protea repens*. Conidia of *K. proteae* are produced in a gloeoid mass at the apices of phialidic conidiogenous cells. It would thus be expected that the conidia should be produced by apical wall building with enteroblastic proliferation and holoblastic ontogeny. TEM examination of conidium development in *K. proteae*, however, revealed the presence of a delimitation wall layer continuous with the new conidium inner wall. This might indicate some relationship with the ring wall building process. It is our opinion that the wall orientation in this case represents a hitherto unrecognized variation in phialidic conidial development.

INTRODUCTION

The genera *Ophiostoma* H. & P. Sydow, *Ceratocystis sensu stricto* and *Ceratocystiopsis* Upadhyay & Kendrick are included in *Ceratocystis* Ellis & Halst. *sensu lato*. (De Hoog & Scheffer, 1984; Upadhyay, 1981; Wingfield, Van Wyk & Marasas, 1988). *Ophiostoma* is characterized by having anamorphs with apical wall building conidial development (Minter, Kirk and Sutton, 1983a). These include representatives of genera such as *Graphium* Corda, *Sporothrix* Hekt. & Perkins, *Hyalorhinocladiella* Upadhyay & Kendrick and *Leptographium* Lagerberg & Melin (Upadhyay, 1981; Upadhyay & Kendrick, 1975). In contrast, *Ceratocystis sensu stricto* has anamorphs in *Chalara* (Corda) Rabenh. (De Hoog & Scheffer, 1984; Upadhyay, 1981) and possibly *Sporendocladia* Arnaud: Nag Raj & Kendrick, where conidia develop through a ring wall building process (De Hoog and Scheffer, 1984; Minter *et al.*, 1983; Wingfield, Van Wyk & Wingfield, 1987).

Ophiostoma and *Ceratocystis s.s.* can also be distinguished from each other by their cell wall components. *Ophiostoma* spp. have cellulose, chitin and rhamnose in their cell walls. In contrast, *Ceratocystis* spp. have cell walls more typical of Ascomycetes, containing chitin and no cellulose or rhamnose

(Harrington, 1987; Jewell, 1974; Rosinski and Campana, 1964; Smith, Patik & Rosinski, 1967; Weijman and De Hoog, 1974). *Ophiostoma* can also tolerate high concentrations of cycloheximide whereas *Ceratocystis s.s.* is sensitive to this antibiotic (Harrington, 1981).

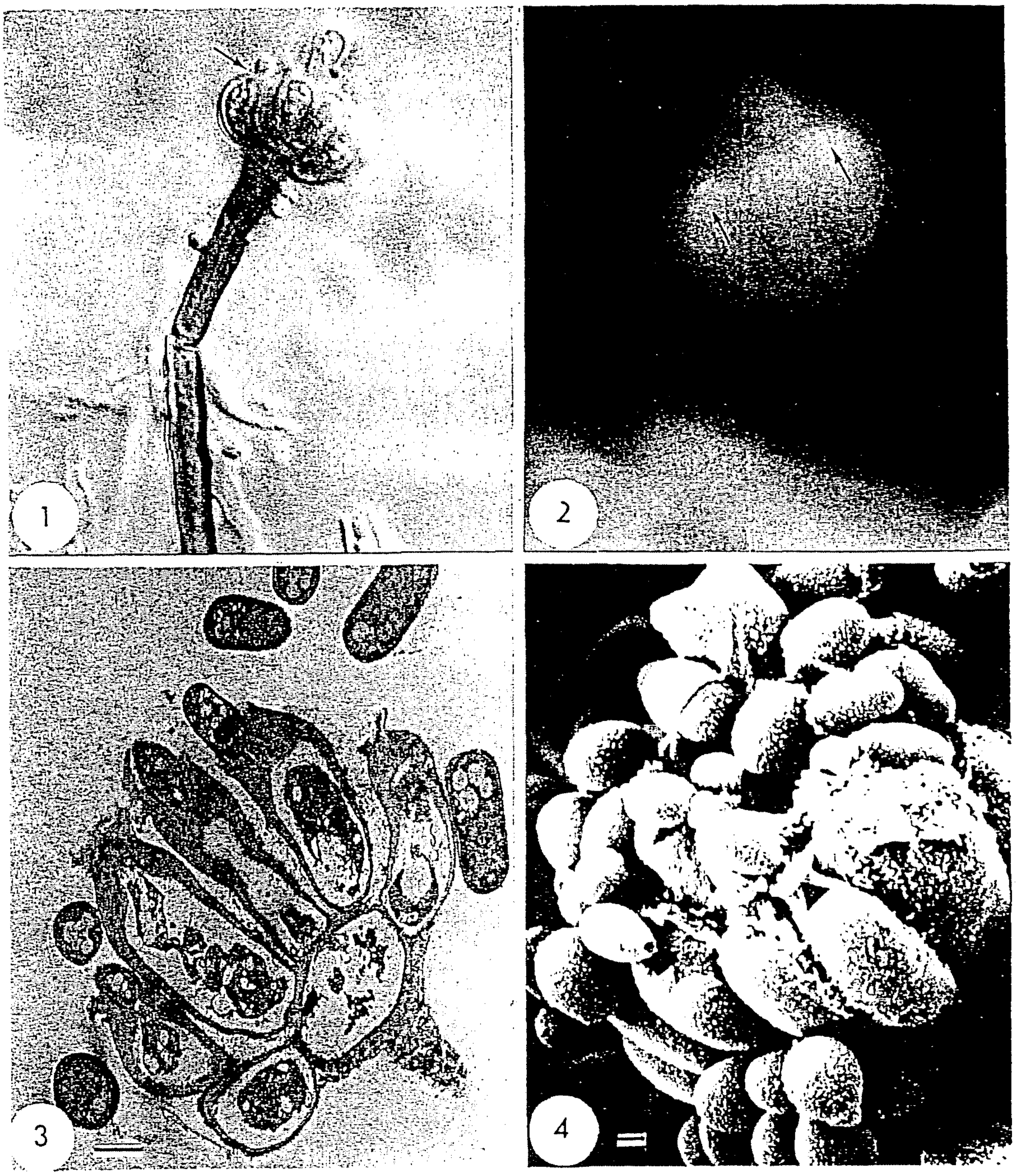
Ceratocystiopsis was established by Upadhyay and Kendrick (1975) to accommodate those species of *Ceratocystis s.l.* with falcate ascospores. Wingfield *et al.* (1988) described a new species of *Ceratocystiopsis*, *C. proteae*, that occurs commonly in the insect-infested infructescences of *Protea repens* (L.) L. *Ceratocystiopsis proteae* was found to share characteristics of *Ceratocystis s.s.* (cycloheximide sensitivity), *Ceratocystiopsis* (falcate ascospores) and *Ophiostoma* (relatively long perithecial necks, divergent ostiolar hyphae and an anamorph with holoblastic conidium development), but because its ascospores were apparently sheathed and falcate, it was thought to be best accommodated in *Ceratocystiopsis*.

Knoxdaviesia Wingfield, Van Wyk & Marasas, a new genus of dematiaceous Hyphomycetes, was established to accommodate the anamorph of *C. proteae*. This genus superficially resembles the Hyphomycete genera *Stachybotrys* Corda and *Phialocephala* Kendrick (Wingfield *et al.*, 1988). *Knoxdaviesia*, like the genera *Phialographium* Upadhyay & Kendrick and *Phialocephala* appears to have distinctly phialidic conidium development. However, only one species of *Ophiostoma*, *O. francke-grosmanniae*, has a *Phialocephala* anamorph (Davidson, 1971; Upadhyay, 1981). Moreover, the latter fungus is thought to be better placed in *Leptographium* (Harrington, 1988). Similarly, studies of conidium ontogeny in species of *Phialographium* suggest that both percurrent and apparent false sympodial proliferation can be observed in single species assigned to the genus (Wingfield, Van Wyk & Van Wyk, 1989). Light microscope studies showed that conidium development in *Knoxdaviesia* is unlike that of the anamorphs of *Ophiostoma* and is possibly more like that of anamorphs of the Hypocreales. In this study, a detailed examination of conidium development in *Knoxdaviesia proteae* was undertaken in the hope that this might improve our understanding of the relationships between this fungus and other anamorphs of *Ceratocystis sensu lato*.

MATERIALS AND METHODS

Knoxdaviesia proteae was isolated from ascomata of *C. proteae* in a *Protea repens* infructescence at Stellenbosch in the Cape Province of South Africa. The culture was grown on 2 % malt extract agar (20 g Difco malt extract; 20 g Difco Bacto agar/ 1 L water) in Petri dishes and incubated at 20 °C for approximately two weeks until onset of sporulation.
Conidial development was examined using scanning electron microscopy (SEM), transmission electron microscopy (TEM), light and fluorescence microscopy. Specimens for SEM and TEM were cut from agar and fixed in 3 % glutaraldehyde and 1 % osmium tetroxide in 0.1 M buffer (pH 7.0), and dehydrated in a graded acetone series. Material for SEM was critical point dried (Cohen, 1970), coated with gold/palladium and viewed with an ISI scanning electron microscope.

Material for TEM was fixed in a similar way, embedded according to Spurr (1969) and the epoxy resin was polymerized at 70 °C for 8 hours. Ultrathin sections (60 nm) were cut with glass knives, mounted on copper grids and stained for 20 minutes in uranyl acetate followed by 10 minutes in lead citrate (Reynolds, 1963). Sections were examined with a Phillips EM300 transmission electron microscope.



Figs 1-4. Conidiophores and conidiogenous cells of *K. proteae*. **Fig. 1.** Light micrograph of the conidiophore of *K. proteae* showing a collarette at the apex of a conidiogenous cell (arrow). **Fig. 2.** Fluorescence micrograph of the conidiogenous cells of *K. proteae*, revealing the presence of periclinal thickening (arrows), on the inside of the apices of the conidiogenous cells. **Fig. 3.** TEM of a section through the conidiogenous cells of *K. proteae*, showing distinct phialidic conidiogenous cells with well developed periclinal thickening (Bar = 1 um). **Fig. 4.** SEM of conidiogenous cells of *K. proteae*. Collarettes at the apices of the conidiogenous cells (arrowhead) and conidia with single attachment points (arrow) are typical of phialidic conidial development (Bar = 1 um).

Conidia and conidiophores to be examined using fluorescence microscopy, were mounted on glass slides in a 0.05 % w/v solution of cellifluor white M 2R optical brightener in 0.1 M phosphate buffer. Samples were examined with a Zeiss Axioskop fluorescence microscope, dark background and ultraviolet light (365 nm) and photographed using Ilford FP4 film.

RESULTS

Light and fluorescence microscopy, as well as TEM and SEM examination of conidium development in *K. proteae*, verified the presence of distinctly phialidic conidiogenous cells (Figs 1-4). Small, yet distinct collarettes were obvious at the apex of the majority of conidiogenous cells (Fig. 4). Conidia had single attachment scar (Fig. 5) and were produced in a gloeoid mass at the apex of the phialidic conidiogenous cells.

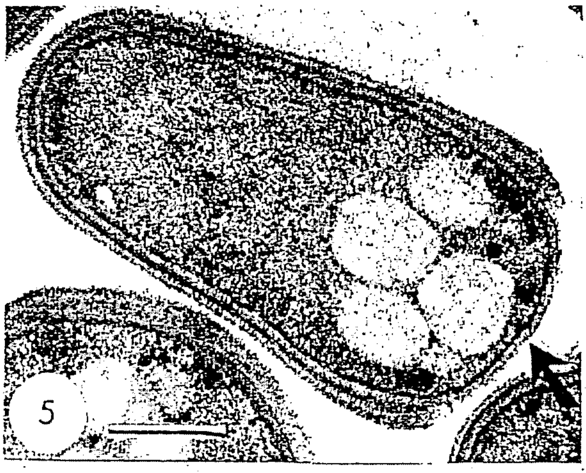
In *K. proteae* the first conidium was produced holoblastically (conidium ontogeny) by apical wall building. At the base of the newly developing conidium, a delimiting septum was formed (Fig. 6). Distinct wall material was observed at the base of newly developed conidia (Fig. 7). It therefore appeared that conidial secession was delayed. A new wall building apex was formed, just below the septum delimiting the preceding conidium. The secretory organelles (apical vesicles), which are responsible for wall synthesis, were also visible below the delimiting septum (Fig. 8). A new wall building apex was therefore simultaneously being produced in the region where the new conidium would develop (Fig. 8). The preceding conidium then seceded (conidial secession) and the second conidium was thus formed by enteroblastic proliferation followed by holoblastic ontogeny.

In young conidiogenous cells, very little periclinal thickening was observed (Fig. 9) and, as subsequent conidia were produced, there was an accumulation of proliferation wall layers adjacent to the conidiogenous cells inner walls and, just beneath the collarettes (Figs 6-8). The proliferation wall layer is continuous with the outer wall layer of the new conidium (Figs 7, 8). These wall layers eventually led to the narrowing of the apices of the conidiogenous cells.

DISCUSSION

Conidia of *K. proteae* were formed by an apical wall building process. This is also a common feature in the anamorphs of *Ophiostoma* and *Ceratocystiopsis* in contrast to the anamorphs of *Ceratocystis s.s.* where conidia are produced by ring wall building (Minter *et al.*, 1983a).

Figs 5-9. TEM of the anamorph of *C. proteae*. **Fig. 5.** A conidium having only one, basal attachment scar (arrow, ←) (Bar = 0.5 um). **Fig. 6.** Delimitation of the conidium, preceded by enteroblastic proliferation and holoblastic ontogeny (Bar = 0.5 um). **Fig. 7.** Conidial secession appears to be delayed and new wall layers are formed at the base of the newly developed conidium (arrows) (Bar = 0.5 um). **Fig. 8.** The wall building apex is replaced below the delimiting septum and enteroblastic proliferation occurs in this area (Bar = 0.5 um). **Fig. 9.** A young conidiogenous cell in which very little periclinal thickening is visible, indicating that periclinal thickening results from an accumulation of proliferation wall layers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Conidia were oval to cylindrical with a single attachment scar (Fig. 10 A), also indicative of apical wall building. Fungi with ring wall building tend to have cylindrical conidia with two attachment scars (Minter *et al.*, 1982) (Fig. 10 B).

TEM studies revealed the presence of periclinal thickening toward the inside of the walls of the conidiogenous cells just beneath the collarettes. This phenomenon is common in fungi producing conidia from phialides by apical wall building and occurs in species of *Trichoderma* (Cole & Samson, 1979; Hammill, 1974), *Fusarium* (Marchant, 1975; Van Wyk *et al.*, 1987) and *Cryptosporiopsis* (Sutton & Sandhu, 1968). According to Minter *et al.* (1983a) the presence of periclinal thickening indicates a succession of replacement wall building apices and its absence shows the presence of a wall building ring. However, it has also been suggested in *Fusarium* that periclinal thickening has no connection with production of successive conidia (Tiedt, Jooste & Hamilton-Attwell, 1986; Tiedt & Jooste, 1988). We however contest this view and believe that this accumulation of wall layers within the phialide, if it went on long enough, would eventually lead to the blockage of the conidiogenous cell. Our sections through young conidiogenous cells, showing only slight periclinal thickening, support our suspicion. It is surprising however, that a certain degree of periclinal thickening also occurs in the conidiogenous cells of fungi with "true" conidial chains (Minter *et al.*, 1983a). It may be possible that some degree of periclinal thickening is always present and may have a function in protecting the conidiogenous cell or supporting the conidial mass. In this case, we would not expect to see a gradual increase in this thickening, nor would striations indicative of individual walls be present.

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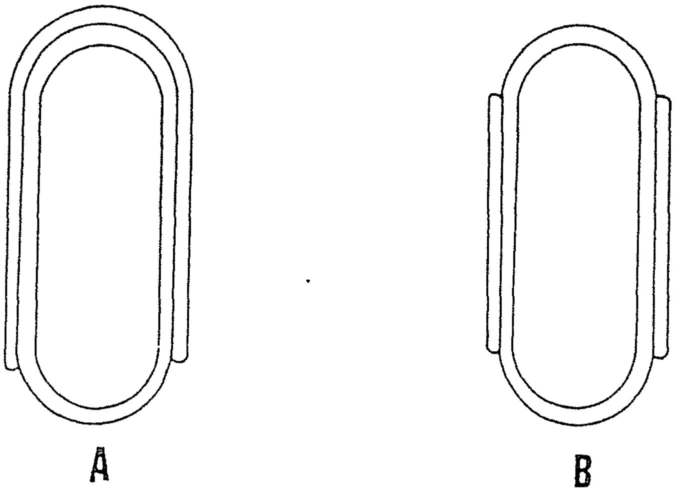

Fig. 10 A, B. Schematic representation of conidia illustrating different arrangements of dehiscence scars. A. Conidium with a singlle, basal dehiscence scar, indicative of apical wall building. B. Conidia produced by ring wall building are characterized by apical and basal dehiscence scars.

From sections through conidiogenous cells, distinct wall material was observed to form during early stages of proliferation, below the delimiting septum, and it therefore appeared that conidial secession was delayed. However, these cell walls apparently represent a form of apical wall building with a centripetal (lateral) onset of the proliferation stage and might indicate some relationship with the ring wall building process. This is in contrast to *Fusarium* spp. in which the very early stages of proliferation is only apical (centrifugal). The consequence of this phenomenon is still uncertain.

Observations in this study confirm the sentiments of Minter, Sutton and Brady (1983b) that the term phialide can only be used relatively and in specific cases needs careful definition. Certain supposedly diagnostic structures, such as collarettes and periclinal thickening can be misleading.

From our study it was clear that conidia in *K. proteae* develop by an apical wall building process and that no percurrent proliferation occurs. This is an uncommon phenomenon amongst anamorphs of *Ophiostoma*, where percurrent proliferation is usually present (Wingfield, 1985; Wingfield *et al.*, 1987). Conidium development in *K. proteae* was therefore more similar to that of anamorphs of the Hypocreales with apical wall building, phialidic conidiogenous cells and periclinal thickening (Minter *et al.*, 1983a). This adds to the very unusual characteristics of *Ceratocystiopsis protea*. Other anamorphs of *Ceratocystis s.l.*, and particularly species of *Ophiostoma*, should be examined to characterize their patterns of conidium development and to evaluate their taxonomic significance in this group of fungi.

ACKNOWLEDGMENTS

We are grateful to Dr. P.W.J. van Wyk for assistance and advice and appreciate financial support of the Foundation for Research Development. We also thank Dr. W.B. Kendrick for constructive review of this manuscript.

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