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Conidial development in the anamorph of *Ophiostoma cucullatum*

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The anamorph of *O. cucullatum* is thought to belong to the genus *Phialographium* which is characterized by 'phialidic' conidial development. However, both distinct collarettes and annellations, indicative of phialidic and percurrent proliferation respectively, were observed at the apex of conidiogenous cells. Where collarettes were observed, TEM observations showed periclinal thickening indicating an accumulation of proliferation layers below the apex of the conidiogenous cell. Where proliferation had occurred above the apex of conidiogenous cells, these cells became longer and annellations were obvious. The presence of distinct 'phialides' as well as percurrent proliferation questions the value of using conidial development as the sole characteristic distinguishing genera in the *Graphium* complex.

Key words: *Ceratocystis*, Conidiogenesis, *Graphium*, *Ophiostoma*, *Phialographium*, Ultrastructure.

The *Graphium* complex includes the genera *Graphium* Corda, *Pesotum* Crane & Schokneckt and *Phialographium* Upadhyay & Kendrick, most species of which are anamorphs of *Ceratocystis sensu lato* or *Ophiostoma* H. & P. Sydow *sensu stricto* (Upadhyay, 1981). These fungi are characterized by dark, synnematous conidiophores with hyaline, single-celled conidia produced in gloeoid masses at the apices of synnemata. The mode of conidial development and associated features are the primary characters by which members of the *Graphium* complex are separated. *Graphium* has conidiogenous cells that proliferate annellidically whereas *Pesotum* and *Phialographium* have sympodial proliferation and 'phialidic' conidial development respectively (Crane & Schoknecht, 1973; Upadhyay & Kendrick, 1974).

Recent studies of conidial development in *Leptographium*, *Verticicladiella* and *Phialocephala* (Wingfield, 1985; Wingfield *et al.*, 1987; van Wyk *et al.*, 1988*a*), the mononematous analogues of *Graphium*, *Pesotum* and *Phialographium*, have shown that *Leptographium* and *Verticicladiella* cannot be distinguished on the basis of conidial development alone. As a result of these studies *Verticicladiella* was reduced to synonymy with *Leptographium* (Wingfield, 1985). Wingfield (1985) suggested that observations of conidial development in the *Leptographium* complex would probably also apply to synnematous homologues. If this were true, species with annellidic and sympodial proliferation development could not be separated and *Pesotum* would be reduced to synonymy with *Graphium*.

Unlike *Leptographium* which is best restricted to anamorphs of *Ophiostoma*, no connexions are known to exist between described species of *Phialocephala* and the teleomorph *Ophiostoma*. Rather, *Phialocephala* appears to represent an heterogeneous group of species, many of which are apparently unrelated (Wingfield, van Wyk & Wingfield, 1987). A single species, *C. franke-grosmanniae* is reported to have a *Phialocephala* state but this anamorph would be better accommodated in *Leptographium*. In contrast the single described species of *Phialographium*, *P. sagmatospora*, is an anamorph of *Ophiostoma* and various other *Ophiostoma* spp. are reported to have *Phialographium* anamorphs (Solheim, 1986; Upadhyay, 1981).

In preliminary studies of conidium development in the *Graphium* complex, the genus *Phialographium* was of special interest. It was suspected that, despite the purported 'phialidic' development, conidia would more likely develop in a manner similar to species of *Leptographium* and *Graphium*. Cultures of *P. sagmatospora*, the type species of *Phialographium*, are unobtainable. Therefore, the anamorph of *Ophiostoma cucullatum* Solheim, reportedly a *Phialographium* sp. (Solheim 1986), was chosen for detailed examination.

MATERIAL AND METHODS

An isolate of *O. cucullatum* (CBS 218.83) from *Ips typographus* L. was grown on 2% malt extract agar (20 g Merck malt extract: 20 g Merck agar; 1000 ml water) in Petri dishes.

Conidial development was examined using SEM, TEM and light microscopy. Material for SEM and TEM was prepared as described by Wingfield, van Wyk & Wingfield (1987).

RESULTS

Proliferation of conidiogenous cells could not be observed from light micrographs, but small collarettes could, in many cases be distinguished at the apices of these cells (Figs 1, 2). Scanning electron micrographs (Figs 3–5) were indicative of three different forms of conidial development. Small, yet distinct collarettes were obvious at the apex of the majority of the conidiogenous cells. However, closely packed annellations, indicative of percurrent proliferation were clearly present at the apex of some conidiogenous cells (Figs 4, 5). Signs of sympodial conidial development were also observed on conidiogenous cells (Fig. 5). In addition to proliferation of conidiogenous loci during conidial development, extended proliferation of conidiogenous cells, resulting in a new phase of conidial production at a higher level, was also common (Figs 4, 5).

Transmission electron micrographs showed that proliferation preceding conidium development, could result in distinct periclinal thickening (Fig. 6). In addition, conidia could be produced through percurrent proliferation of the conidiogenous locus to give distinct annellations at the apex of the cells (Figs 7–9). Conidiogenous cells also proliferated to form a new conidiogenous apex at a higher level and a septum was laid down at the base of this cell (Fig. 9).

DISCUSSION

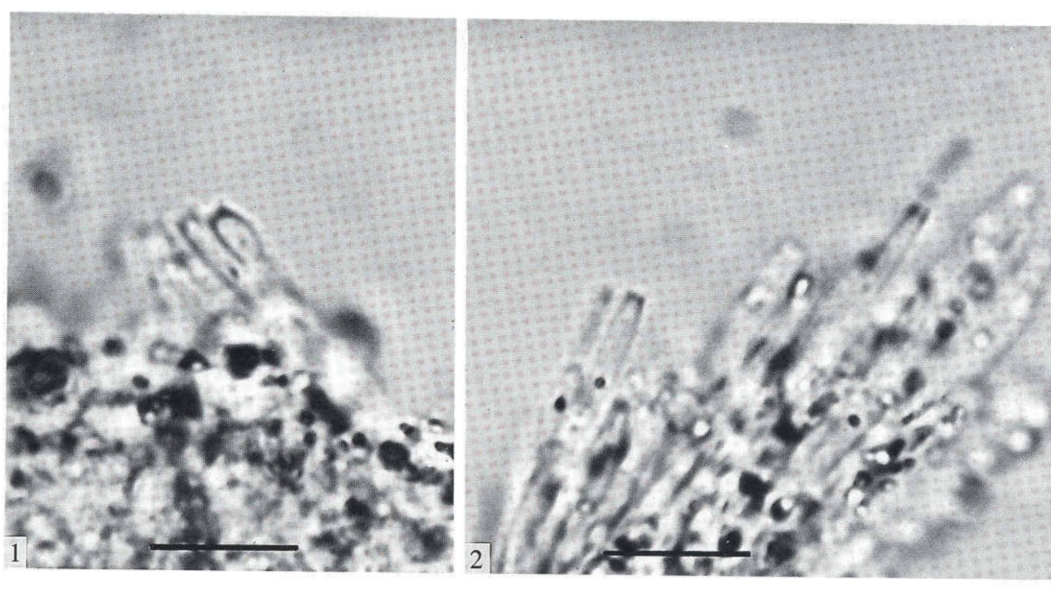
An interpretation of conidium development in the anamorph of *O. cucullatum* is illustrated in Fig. 10. The first conidium is produced holoblastically and secedes (Fig. 10 A). Two possible patterns of conidiogenous cell development can apparently then follow. In the first case (Fig. 10 B) proliferation layers preceding ontogeny, result in periclinal thickening as defined in 'phialidic' types of development (Subramanian, 1971). In the second pattern of development, proliferation is percurrent and extends above the apex of the collarette. This gives rise to an annellate conidiogenous cell apex (Fig. 10 C). As a result of delayed secession, development can also appear to be sympodial as previously explained for *Leptographium* spp. (van Wyk, Wingfield & Marasas, 1988*a*; Fig. 10 D).

Collarettes observed using light microscopy are similar to those illustrated by Solheim (1986) and clearly give the impression of 'phialidic' development. In some species of *Leptographium*, similar collarettes have been observed and interpreted as remnants of outer walls produced during proliferation of conidiogenous cells (Wingfield, 1985; Wingfield, van Wyk & Wingfield, 1987). These collarettes were, however, seen in addition to obvious elongation of conidiogenous cells and were thought to arise after the production of unusually large conidia (Wingfield, 1985). In contrast, collarettes indicative of phialidic development in *O. cucullatum* were predominant and usually seen in the absence of any other form of proliferation.

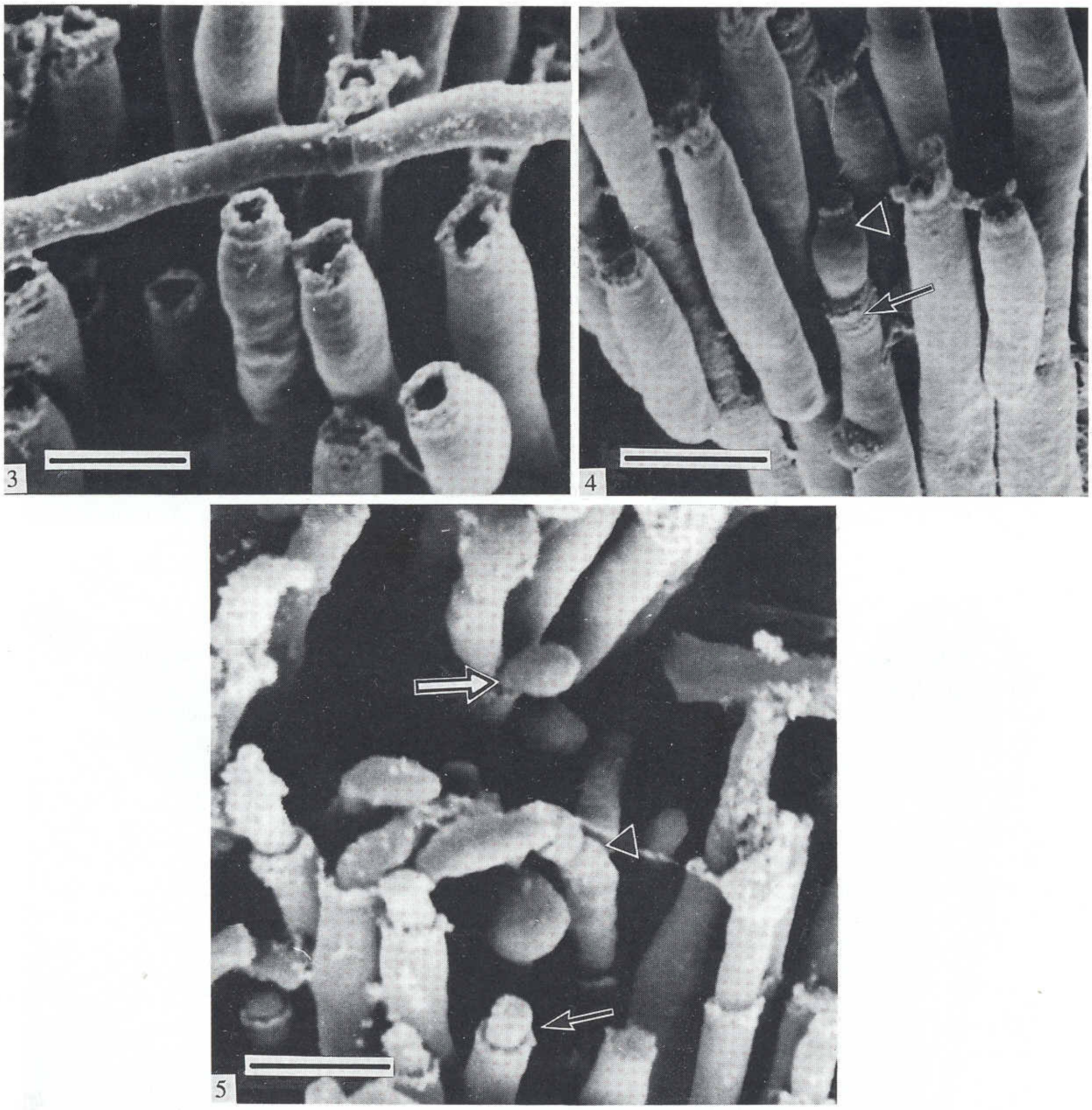
Transmission electron micrographs in this study clearly show that collarettes in *O. cucullatum* can represent the presence of conidiogenous cells with periclinal thickening such as those observed in species of *Fusarium* Link (van Wyk, *et al.*, 1987), *Trichoderma saturnisporum* Hammill (Hammill, 1974) and *Stilbella fimetaria* (Pers.) Lindau (Seifert, 1985) and which would loosely be termed phialides (Minter, Sutton & Brady, 1983). Here, the length of the conidiogenous cell does not change during the production of a series of conidia. These would, in terms of Minter, Kirk & Sutton (1982) be apical wall-building phialides with holoblastic conidial ontogeny following enteroblastic proliferation.

Vegetative proliferation of conidiogenous cells also occurred in *O. cucullatum* to form a new conidiogenous cell capable of producing a succession of conidia at a higher level. This is not unusual in fungi with apical wall-building phialides

Figs 1–2. Light micrographs of conidiogenous cells of the anamorph of *O. cucullatum* showing collarettes at the apices of conidiogenous cells (Bar = 20 µm).



Figs 3–5. SEM of conidiogenous cells of *O. cucullatum* (Bar = 5 µm). **Fig. 3.** Collarettes at the apices of conidiogenous cells typical of 'phialidic' development. **Fig. 4.** Annellations (arrow) and percurrent proliferation (arrow head) amongst typical 'phialides'. **Fig. 5.** Conidiogenous cells illustrating 'phialidic' (arrow) 'annellidic' (arrow head) and 'sympodial' (white arrow) development.

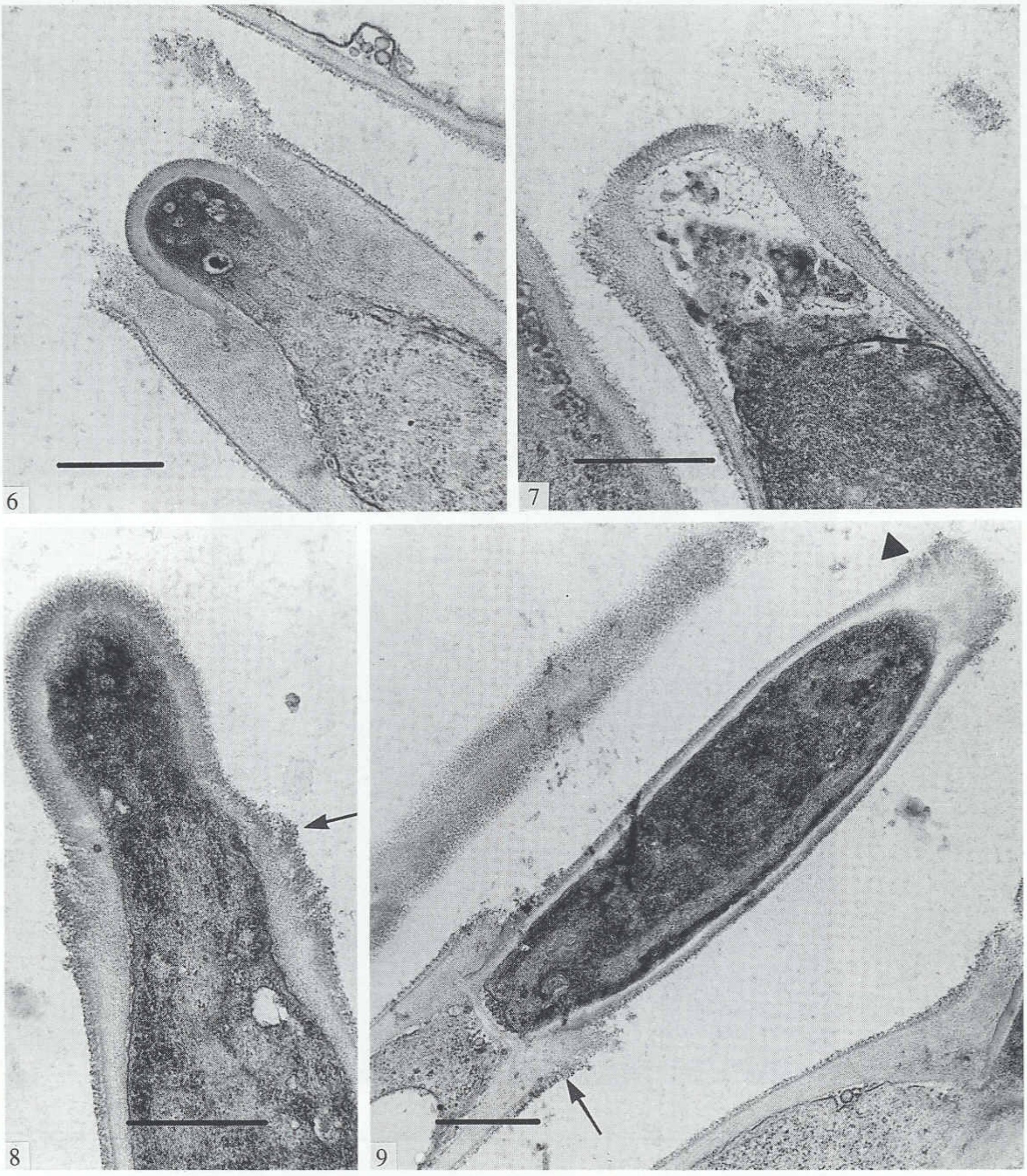


and in *Fusarium* it has been considered as a process of revitalization (van Wyk, Wingfield & Marasas, 1988*b*; van Wyk *et al.*, 1988). What is unusual, however, is the apparent change in the extent of proliferation. Thus a basipetal succession of conidia with resultant periclinal thickening appears to change to conidial development where percurrent proliferation extends above the collarette. This then gives rise to annellations and results in an increase in conidiogenous cell length.

Production of a phialide with a prominent collarette followed by proliferation extending above the collarette of the conidiogenous cell to produce subsequent conidia is seen in species of *Chloridium* Link (Cole & Samson, 1979). In *C. chlamydosporis* (Van Beyma) Hughes, secession of the first conidium leaves a remnant of the outer conidial wall and thus a collarette (Hammill, 1972; Cole & Samson, 1979). All subsequent conidia are apparently formed by holoblastic sympodial proliferation. Therefore, the 'phialide' of *Chloridium*, typified by a collarette, is unlike 'phialides' in *Fusarium* spp., *Trichoderma* spp. or the anamorph of *O. cucullatum* where a series of conidia are produced at a level below the base of the collarette. The result is an accumulation of outer conidiogenous cell walls and periclinal thickening which is apparently absent in *Chloridium* spp.

Two distinct patterns of conidiogenous cell proliferation occur in the anamorph of *O. cucullatum*. In one case proliferation layers do not extend above the apex of the collarette and periclinal thickening results. This is apparently

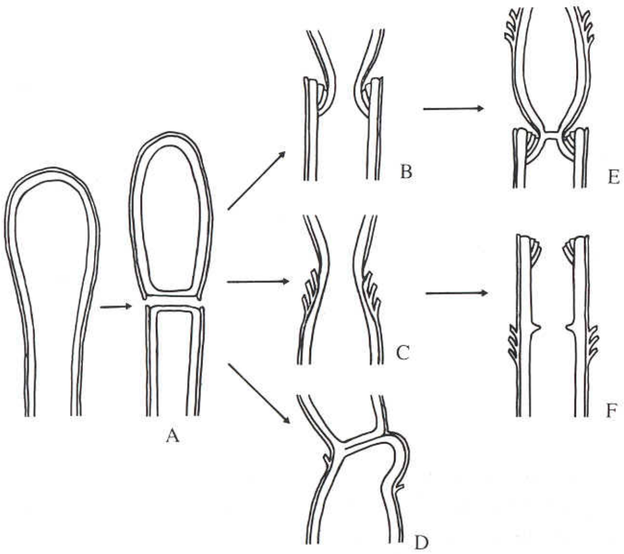
Figs 6–9. TEM of conidiogenous cells of the anamorph of *O. cucullatum* (Bar = 0·5 µm). **Fig. 6**. Conidiogenous cell with periclinal thickening. **Fig. 7**. Annellate conidiogenous cell. **Fig. 8**. Closely-packed annellations at the apex of a conidiogenous cell (arrow). **Fig. 9**. Percurrent proliferation of the conidiogenous cell. Note periclinal thickening at the apex of older conidiogenous cell (arrow) and annellations at the apex of the new conidiogenous cell (arrow head).



very different from conidiogenous cells where proliferation extends well above the base of the collarette resulting in an increase in conidiogenous cell length and distinct annellations. Despite the fact that these two forms of development appear distinct, the processes involved in conidial development are fundamentally the same. In both cases conidia are produced by a succession of holoblastic conidial ontogeny, secession, delimitation and enteroblastic proliferation (Minter, Kirk & Sutton, 1982). The only difference in the two forms of conidial development is in the extent of proliferation. We know of no precedent for these two forms of conidial development occurring in a single fungus.

Observations in this study confirm the sentiments of Minter, Sutton & Brady (1983) that the term phialide can only be used loosely and in specific cases needs careful definition. Clearly, a collarette is a most misleading structure and need not imply that a succession of conidia has been produced below the level of that collarette. In contrast, such proliferation might have occurred despite the presence of obvious percurrent or sympodial proliferation on some conidiogenous

Fig. 10. Schematic representation of conidial development in the anamorph of *O. cucullatum* A, Holoblastic development of the first conidium; B, conidial development resulting in periclinal thickening. Proliferation preceding ontogeny never extends above the collarette; C, annellate conidiogenous cell apex where proliferation extends above the previously formed collarette; D, delayed secession giving the impression of sympodial development. E, F, percurrent proliferation of the conidiogenous cell; E, proliferation below the apex of the collarette and periclinal thickening changing to typical percurrent proliferation and annellations above the collarette on a single conidiophore. The reverse of this process (F) can also occur.



cells such as we have shown here in *O. cucullatum*. Thus, while Wingfield, van Wyk & Wingfield (1987) may have been correct in interpreting the origin and structure of collarettes in some *Leptographium* spp., they may have not fully recognized the function of these structures. Further studies using TEM are required to interpret the processes of conidial development in *Leptographium* spp. where collarettes are present.

Genera in the *Graphium* complex can clearly not be separated on the basis of conidium development alone and urgently require revision. However, prior to such a revision, a thorough study of conidium development in these fungi will be essential.

These interpretations would not have been possible without the clarification that Drs D. W. Minter and co-workers have contributed to our understanding of the processes involved in conidial development.

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