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CONIDIUM DEVELOPMENT IN THE SYNNEMATOUS ANAMORPHS OF OPHIOSTOMA.

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The anamorph genera *Pesotum* and *Phialographium* were recently reduced to synonymy with *Graphium* following studies of their mode of conidium development. Because there was limited ultrastructural evidence in the latter study, the present study was undertaken to examine additional species. All species examined showed percurrent proliferation of conidiogenous cells which is a distinguishing characteristic of *Graphium* species. Results of this study reaffirm that *Graphium* is the only acceptable name for synnematous anamorphs of *Ophiostoma.*

INTRODUCTION

The *Graphium* complex has included the genera *Graphium* Corda, *Pesotum* Crane & Schoknecht and *Phialographium* Upadhyay & Kendrick, which are well established anamorphs of *Ceratocystis* Ellis & Halst., *Ophiostoma* H. & P. Sydow and related fungi (Upadhyay, 1981). Species in the *Graphium* complex are characterized by darkly pigmented, synnematous conidiophores, bearing complex conidiogenous apparatus, capped by slimy heads of conidia (Upadhyay, 1981). This group of fungi, including other species of *Ceratocystis sensu lato,* are well known causative agents of blue-stain in logs and lumber (Bakshi, 1951; Upadhyay, 1981), and exist in close association with insects, such as ambrosia beetles (Bakshi, 1950; Wingfield & Gibbs, 1991).

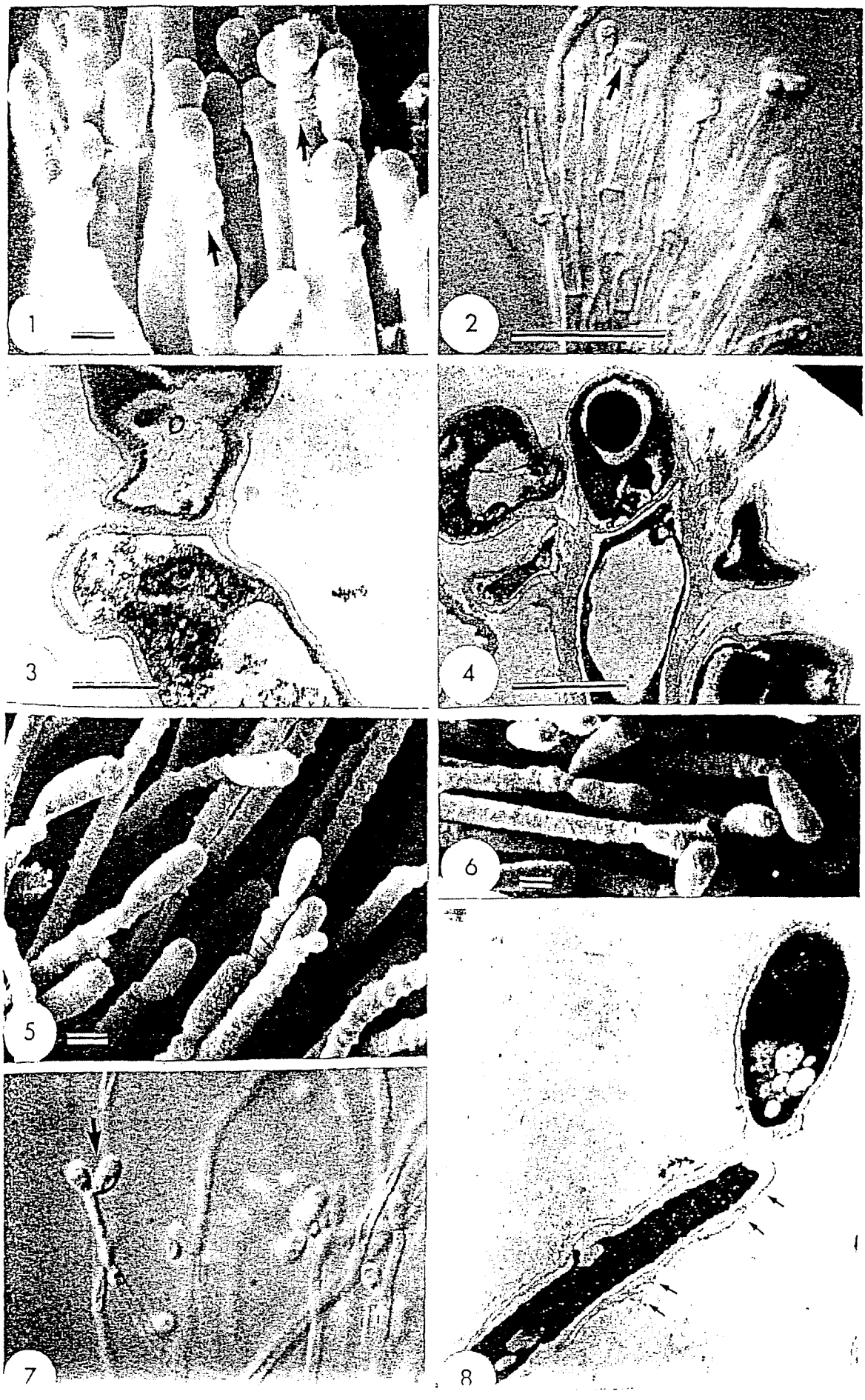
The genus *Graphium* was originally established for fungi with dark conidiophores that are penicillately branched with slimy heads of small aseptate, hyaline conidia (Corda, 1937). Corda's original description (1957) was amended by Saccardo (1886) and Goidànich (1935). However, after the establishment of conidium development as a taxonomic criterion by Hughes (1953), Crane and Schoknecht (1973) observed different modes of conidium development in *G. penicillioides* Corda, and the anamorphs of *O. ulmi* (Buisman) Nannf. and *O. piceae* (Münch) Syd. & P. Syd. The genus *Pesotum* was introduced for the anamorphs of the latter two species, to distinguish their sympodial conidial development from the annellated appearance of the conidiogenous cells of *G. penicillioides.* Upadhyay and Kendrick (1974) followed this trend when they established *Phialographium* for the *Graphium*-like anamorphs

During previous studies on the *Leptographium* Lagerberg & Melin -complex (the mononematous analogues of the *Graphium* complex), Wingfield (1985) reduced *Verticicladiella* Hughes to synonymy with *Leptographium*. This synonymy was based on studies showing that conidium development in *Verticicladiella* only had the appearance of being sympodial, when it was actually annellidic. Van Wyk, Wingfield and Marasas (1988) later showed that delayed secession of the newly formed conidia and the early onset of the successive proliferation stage sometimes results in the sympodial appearance of the conidiogenous cells in this group of fungi. This overlap in developmental stages eventually leads to conidia that are left hanging along the sides of the conidiogenous cell, creating an illusion of sympodial development. Wingfield, Kendrick and Van Wyk (1991) elaborated further on this view, and *Pesotum* and *Phialographium* were reduced to synonymy with *Graphium*. The latter study was, however, supported only by limited ultrastructural evidence from a few species. The aim of the present study was, therefore, to examine additional species in order to clarify this issue.

MATERIALS AND METHODS

Species examined included *Graphium ulmi* Schwarz, the type species of *Pesotum* obtained from the forest pathology culture collection of the Department of Plant Pathology, University of Minnesota; *Graphium piceae* (Crane & Schoknecht) Wingfield & Kendrick (= *Pesotum piceae*), from the culture collection of Dr. D.W. Davidson; the unnamed anamorph of *Ophiostoma olivaceum* Mathiesen, previously assigned to *Phialographium* by Upadhyay (1981) and from the collection of Dr. T. Hinds (C-382); the unnamed anamorph of *Ophiostoma davidsonii* (Olchowecki & Reid) Solheim (IMI 176524), formerly assigned to *Phialographium* by Upadhyay (1981); *Ophiostoma olivaceapinii* Davidson with an undescribed anamorph, which according to Upadhyay (1981) should be assigned to *Graphium*, from the forest pathology culture collection of the Department of Plant Pathology, University of Minnesota. All cultures 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 the onset of sporulation.

Figs 1-8. Conidiogenous cells of the anamorph of *O. olivaceapinii* (Figs 1-4) and *Graphium ulmi* (Figs 5-8). **Fig. 1.** SEM of conidiogenous cells showing tightly packed annellations towards the apices (arrows) (Bar = 1 um). **Fig. 2.** Bright field micrograph showing the presence of incomplete dehiscence of newly formed conidia, giving a sympodial appearance to conidiogenous cells (arrow) (Bar = 10 um). **Fig. 3.** TEM of a section through a conidiogenous cell indicating an overlap in the stages of secession of the newly formed conidium and proliferation of the conidiogenous cell (Bar = 1 um). **Fig. 4.** TEM showing conidia left hanging along the sides of conidiogenous cells as a result of delayed secession (Bar = 0,5 um). **Fig. 5.** SEM showing annellations on the surface of the conidiogenous cells (arrows) as well as 'knobbly' structures that could be interpreted as sympodial development (arrowheads) (Bar = 1 um). **Fig. 6.** Annellated conidiogenous cells with newly formed conidia left hanging along the sides of the conidiogenous cells as a result of incomplete deshiscence (Bar = 1 um). **Fig. 7.** Bright field micrograph of a conidiogenous cell showing delayed secession of the newly formed conidium (arrow) (Bar = 1 um). **Fig. 8.** TEM of a section through a conidiogenous cell indicating annellations towards the apex (arrows) as well as delayed secession of the conidium (Bar = 1 um).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For bright field microscopy, fungal material was mounted on glass slides in lactophenol and photographed with Ilford FP4 film. Specimens for scanning electron microscopy (SEM) were cut from cultures in Petri dishes and fixed in 3 % glutaraldehyde, followed by 1 % osmium tetroxide for 2 h. 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.

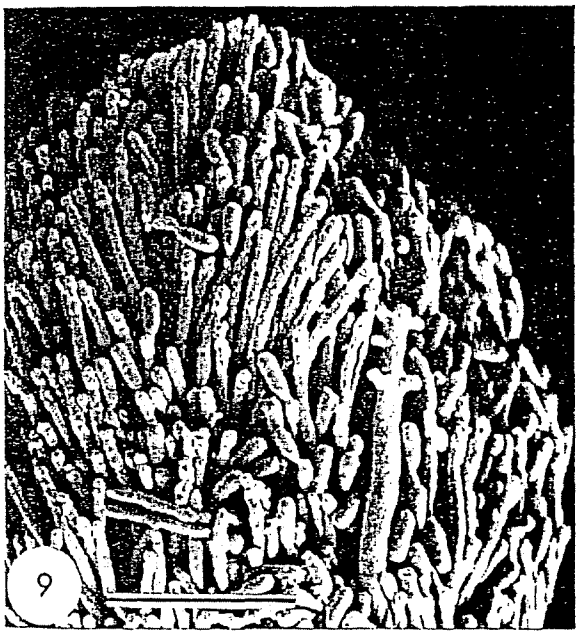
For transmission electron microscopy (TEM), the fixation and dehydration procedure was the same as that for SEM. Specimens were then embedded in epoxy resin (Spurr, 1969) and polymerized at 70 °C for 8 h. Sections (60 nm) were cut, mounted on copper grids, stained with uranyl acetate (20 - 30 min) and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

RESULTS AND DISCUSSION

The anamorph of *O. olivaceapinii* was characterized by dark synnematous conidiophores (Davidson, 1971), with tightly packed, yet distinct annellations at the apices of hyaline conidiogenous cells, indicating percurrent proliferation (Fig. 1). Bright field micrographs gave the impression of sympodial conidial development where two or more conidia could be observed at the apices of conidiogenous cells (Fig. 2). However, TEM studies clearly showed that the stage of secession of newly formed conidia was repeatedly not completed, leaving newly formed conidia hanging along the sides of the conidiogenous cells, creating the illusion of sympodial conidial development (Figs 3, 4). Conidium development in the anamorph of *O. olivaceapinii* is therefore similar to that in *Leptographium procerum* (Kendrick) Wingfield, *L. terebrantis* Barras & Perry, and *L. truncatum* (Wingfield & Marasas) Wingfield (Van Wyk *et al.*, 1988), where the stages of secession and proliferation overlap. The result is annellidic conidium development that can easily be mistaken for sympodial ontogeny using light microscopy.

According to Crane and Schoknecht (1973), conidia in both *Graphium ulmi* and *G. piceae* are produced sympodially on short nodules or denticles. Hiratsuka and Takai (1978) made the same comment for *G. ulmi* in their study of this fungus. In the present study we observed the same sequence of events in *G. ulmi* (Figs 5-8) and *G. piceae* (Figs 9-12), as those in the anamorph of *O. olivaceapinii*. Annellations were, however, not as tightly packed (Figs 5, 6, 9, 10), as in the latter species. No short denticles or nodules, as noted by Crane & Schoknecht (1973) and Hiratsuka & Takai (1978), were observed. We did, however note the presence of 'knobbly' scars on some of the conidiogenous cells in *G. ulmi* (Fig. 5) which could indicate sympodial development. The same structure could be observed in the transmission electron micrograph. However, annellations were distinctly visible in the same micrograph (Fig. 8).

Figs 9-14. Conidiophore and conidiogenous cells of *Graphium piceae* and *G. ulmi*. Fig. 9. SEM of the synnematous, sheathed conidiophore of *G. piceae* showing conidiogenous cells with apparent sympodial conidial development (arrows) (Bar = 10 um). Fig. 10. SEM of the conidiogenous cells of *G. piceae*, showing conidia left hanging along the sides of the conidiogenous cells resulting from delayed secession (arrow) (Bar = 10 um). Fig. 11, 12. TEM of sections through conidiogenous cells indicating annellations towards the apices (arrows), as well as delayed secession of the conidia (Bars = 1 um). Fig. 13. SEM of the *Sporothrix* synanamorphic state of *G. ulmi* (Bar = 1 um). Fig. 14. *Sporothrix* synanamorph of *G. piceae* (Bar = 10 um).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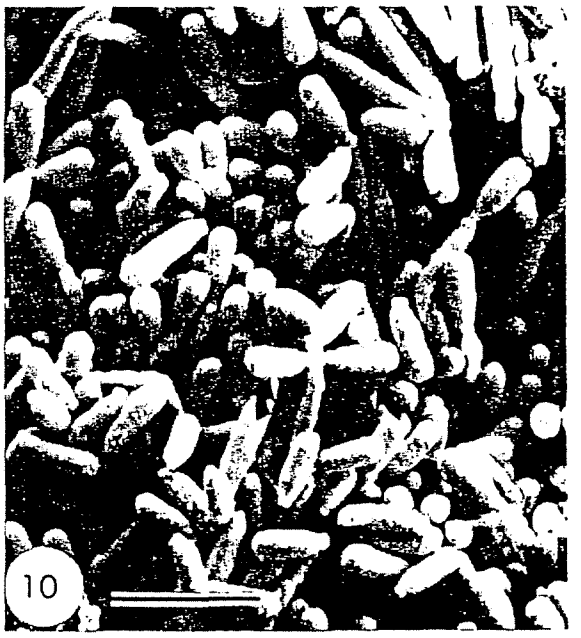
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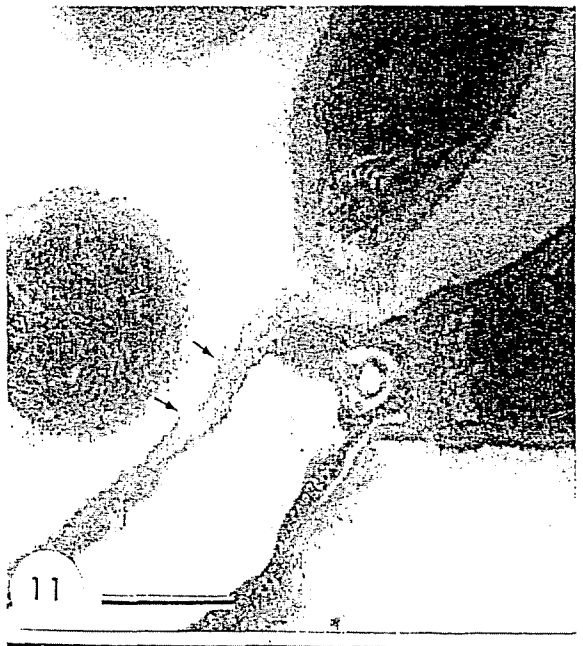
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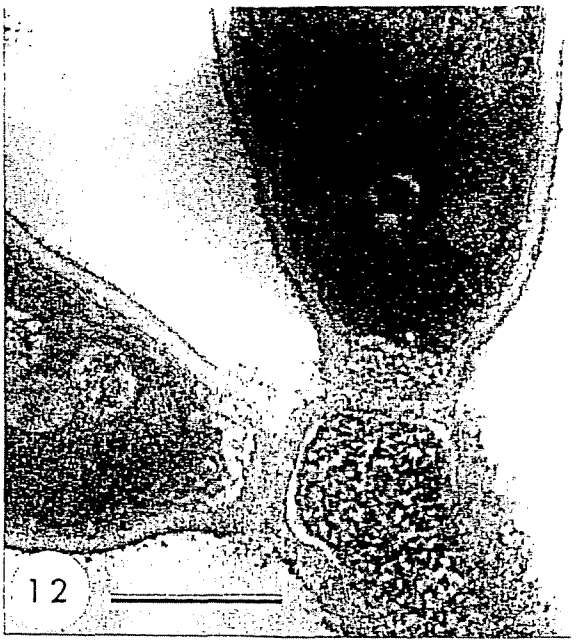
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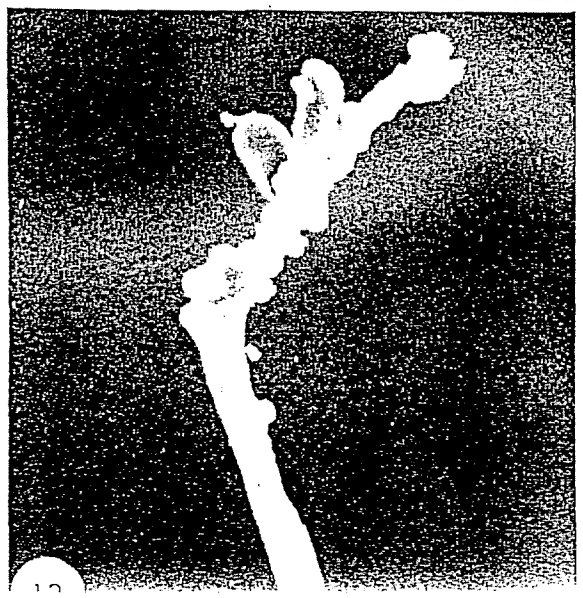

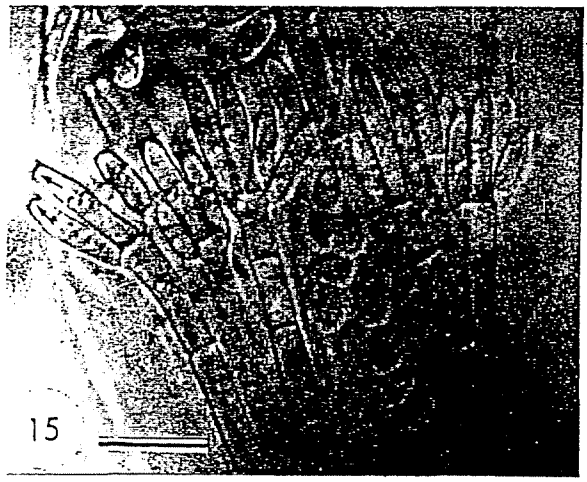
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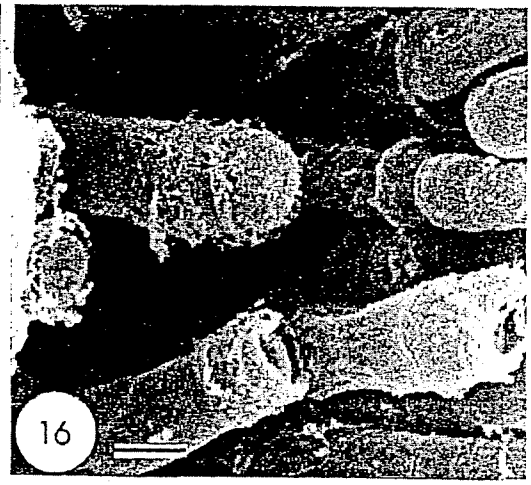
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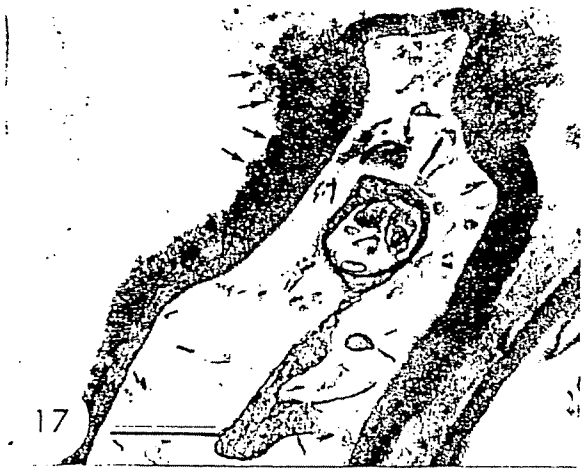
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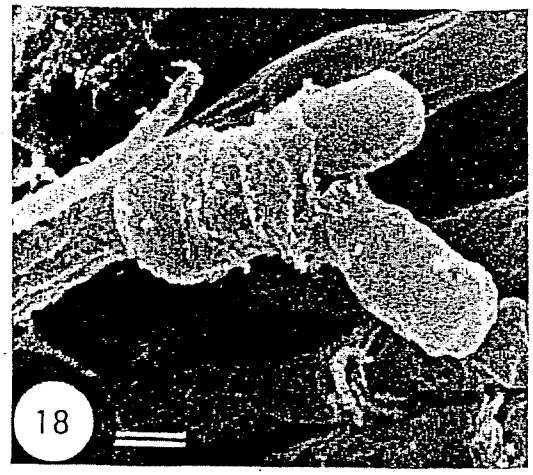
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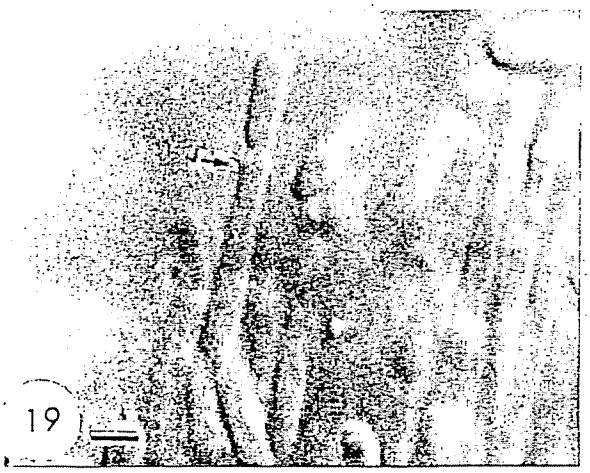
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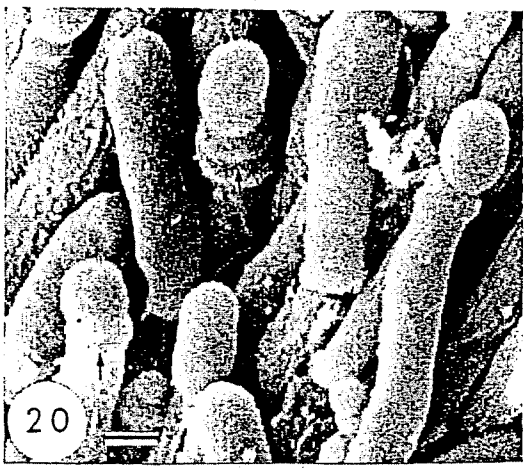
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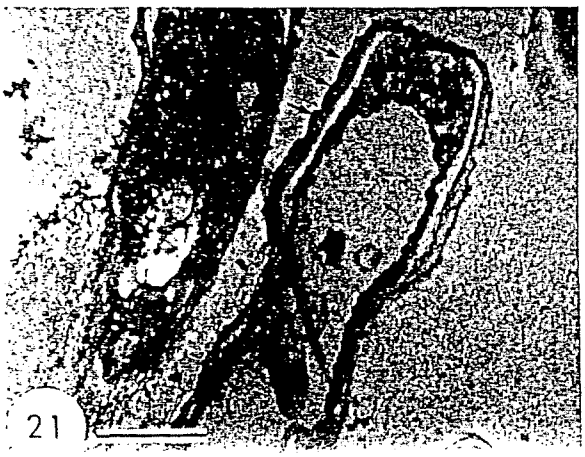
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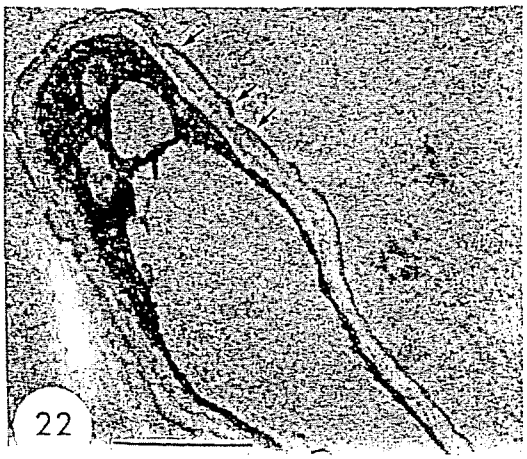
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We, therefore, confirm the reassignment of these two species to *Graphium* (Wingfield *et al.*, 1991). *G. ulmi* and *G. piceae* were also characterized by the presence of sympodial *Sporothrix* Hekt. & Perkins synanamorphic states (Figs 13, 14), a common feature among *Graphium* anamorphs of *Ophiostoma* (De Hoog, 1974).

The unnamed anamorph of *O. davidsonii* has been described by Olchowecki and Reid (1974) as being synnematous with phialidic conidiogenous cells. Upadhyay (1981), therefore, stated that it was a species of *Phialographium*. Bright field micrographs showed that the conidiogenous cells of the anamorph of *O. davidsonii* appeared to be phialidic with small collarettes and periclinal thickening towards the inside of the cell (Fig. 15). However, SEM and TEM studies clearly showed distinct annellations towards the apices of conidiogenous cells (Figs 16, 17). Scanning electron micrographs also indicated the presence of incomplete deshiscence of newly formed conidia, giving a sympodial appearance to such conidiogenous cells (Fig. 18), whereas TEM studies verified the occurrence of sympodial or angled percurrent proliferation in terms of Wingfield *et al.* (1991) (Fig. 17). This study reaffirms that the placement of the anamorph of *O. davidsonii* in *Phialographium* was incorrect and that it should be assigned to *Graphium* as suggested by Wingfield *et al.* (1991).

Another anamorph accepted as a species of *Phialographium* is that of *O. olivaceum* (Upadhyay, 1981). Olchowecki and Reid (1974) also noted that the morphology of the 'phialides' and the arrangement of the individual stalks composing the synnema of this fungus, were very similar to that of the anamorph of *O. davidsonii*. Neither Mathiesen (1951), nor Hunt (1956), discussed the mode of conidiogenesis in their descriptions of the fungus, although it appears to be phialidic in Mathiesen's (1951) schematic representations. It was difficult to interpret with certainty the nature of the conidiogenous cells from bright field or SEM studies because the apices of these cells were characterized by a prominent widening (Figs 19, 20). From these micrographs the conidiogenous cells appeared to be phialidic. TEM studies, however, showed the presence of annellations on the surface of the widened apices of the conidiogenous cells. This anamorph should, therefore, also be assigned to *Graphium*.

This study has reaffirmed that anamorphs of *Ophiostoma* assigned to the *Graphium* complex cannot be further segregated on the basis of mode of conidium development. *Graphium* can, therefore, be the only acceptable name for synnematous anamorphs of *Ophiostoma*.

Figs 15-22. Conidiogenous cells of the unnamed anamorph of *O. davidsonii* (figs 15-18) and *O. olivaceum* (Figs 19-22). **Fig. 15.** Bright field micrograph showing apparent phialidic conidiogenous cells with periclinal thickening towards the apices (arrows) (Bar = 10 um). **Fig. 16.** SEM revealing the presence of distinct annellations towards the apices of the conidiogenous cells (arrows) (Bar = 1 um). **Fig. 17.** TEM of a section through a conidiogenous cell showing distinct annellations at the apex (arrows) (Bar = 0,5 um). **Fig. 18.** Example of incomplete secession of a newly formed conidium resulting in an illusion of sympodial conidial development (Bar = 1 um). **Fig. 19.** Light microscopy showing a widening towards the apex of the conidiogenous cell (arrow) (Bar = 1 um). **Fig. 20.** SEM of conidiogenous cells with a prominent widening at the apex (arrows) (Bar = 1 um). **Fig. 21, 22.** TEM of sections through the conidiogenous cell showing the presence of annellations on the surfaces of the widenings at the apices (arrows) (Bars = 0,5 um).

Other synnematous anamorphs such as *Graphilbum* Upadhyay & Kendrick, *Hyalopesotum* Upadhyay & Kendrick and *Graphiocladiella* Upadhyay are separated from *Graphium* species only on the basis of pigmentation and spore septation. They will also have to be examined to clarify whether it is justifiable to separate them from *Graphium.*

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