*Mycologia*, 87(3), 1995, pp. 298–303.


# Conidium development in the *Hyalorhinocladiella* anamorph of *Ophiostoma ips*

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***Abstract:*** The anamorph of *Ophiostoma ips* has been suggested to have both sympodial and phialidic conidium development. The phialidic form has consequently been referred to as *Acremonium*-like and the sympodial form as a species of *Hyalorhinocladiella*. The aim of this study was to determine the mode of conidium development and consequently the identity of the anamorph of *Ophiostoma ips*. Light microscopy clearly indicated that sympodial proliferation occurred during conidiogenesis. However, using scanning electron microscopy, conidiogenous cells were found to be annellidic. This was confirmed using transmission electron microscopy where distinct annellations could be distinguished on conidiogenous cells. We conclude that conidium development in the anamorph of *Ophiostoma ips* is annellidic and that an illusion of sympodial conidium development results from delayed secession of conidia. *Hyalorhinocladiella* could therefore be an acceptable genus for this fungus, although its circumscription would require modification.

***Key Words:*** conidiogenesis, *Hyalorhinocladiella*, *Ophiostoma*

## INTRODUCTION

*Ceratocystis sensu lato* Ell. & Halst. includes the genera *Ceratocystis sensu stricto*, *Ophiostoma* H. & P. Sydow and *Ceratocystiopsis* Upadh. & Kendr. (Weijman and De Hoog, 1975; Upadhyay, 1981; De Hoog and Scheffer, 1984). These genera have been separated by their ascospore morphology (Hunt, 1956; Griffin, 1968; Olchowecki and Reid, 1974), cell wall composition (Smith et al., 1967; Spencer and Gorin, 1971; Jewell, 1974) and cycloheximide tolerance (Harrington, 1981) and rDNA sequence data (Spatafora and Blackwell, 1994). They also have distinct forms of conidium development (Olchowecki and Reid, 1974; Weijman and De Hoog, 1975; Upadhyay, 1981; Minter et al., 1982).

*Ceratocystis s.s.* is characterized by *Chalara* (Corda) Rabenh. anamorphs (Weijman and De Hoog, 1975; De Hoog and Scheffer, 1984) with phialides (Nag Raj and Kendrick, 1975; Upadhyay, 1981; De Hoog and Scheffer, 1984). In these species, conidia are produced by ring wall building from the base of the deep cylindrical collarette (Minter et al., 1983). In contrast, *Ophiostoma* and *Ceratocystiopsis* have a variety of different anamorphs such as *Graphium* Corda, *Leptographium* Lagerberg & Melin, *Hyalorhinocladiella* Upadhyay & Kendrick, *Sporothrix* Hektoen & Perkins ex Nicot & Mariat, *Acremonium* Link: Fr. and *Hyalodendron* Diddens (Wright and Cain, 1961; Upadhyay and Kendrick, 1975; Upadhyay, 1981; Wingfield, 1985; Harrington, 1987). Conidium development in these anamorphs is primarily holoblastic, nonphialidic and occurs through apical wall building (Harrington, 1987; Minter et al., 1983).

Rumbold (1931) first isolated *Ophiostoma ips* (Rumb.) Nannfeldt from pine trees. A close association has been found between this fungus and Scolytid bark beetles (Rumbold, 1931, 1936; Ellis, 1939; Mathiesen-Käärik, 1953; Hunt, 1956; Mathre, 1964; Griffin, 1968; Wingfield and Marasas, 1980; Upadhyay, 1981; Perry, 1991). *Ophiostoma ips* is one of the most common causes of log and lumber stain in pine (Davidson, 1935; Verrall, 1939; Hunt, 1956). It has been recorded in many parts of the world including the United States (Rumbold, 1931, 1936; Hunt, 1956), Japan (Nisikado and Yamauti, 1933), Poland (Siemaszko, 1939), Sweden (Mathiesen-Käärik, 1960), and South Africa (Wingfield and Marasas, 1980).

Considerable confusion has existed regarding the nature of the anamorph of *O. ips*. Rumbold (1931) rather imprecisely described the anamorph as being composed of simple conidiophores that increase in number as the fungus ages, with small, hyaline, obovoid conidia formed in clusters. A similar description was given by Nisikado and Yamauti (1933) who described the anamorph of *O. ips* as being *Acremonium*-like with phialidic conidium development. Rumbold (1936) first illustrated (Fig. 1) the anamorph of *O. ips*, and her illustration suggested sympodial conidium development. Goidánich (1936) placed *O. ips* in the genus

Accepted for publication January 27, 1995.

*Grosmannia* G. Goid. based on a *Leptographium*-like anamorph with annellidic conidium development, while Siemaszko (1939) described the anamorph as *Graphium*-like, having synnemmata with annellidic conidium development. Hunt (1956) and Mathiesen-Käärik (1960) described the anamorph of *O. ips* as a transitional form between *Leptographium* and *Graphium*. Griffin (1968) found that the *Leptographium* and *Graphium* anamorphs of *O. ips* were frequently absent from certain cultures. Wingfield and Marasas (1980) did not assign a name to the conidial state of *O. ips* because conidium development seemed to be sympodial, as well as phialidic. Upadhyay (1981) suggested that the anamorph was pleomorphic and could reside in any one of three genera: *Hyalorhinocladiella,* characterized by simple or branched conidiophores with conidiogenous cells that proliferate sympodially; *Graphilbum* Upadhyay & Kendrick, characterized by hyaline synnemata with conidiogenous cells that proliferate percurrently or, in a percurrent-phialidic manner; or *Acremonium* where conidia are phialidic and accumulate in a hyaline slimy head.

Since the introduction of conidium development (Hughes, 1953) as a taxonomic character for anamorphic fungi, it has been applied to distinguish many genera amongst the anamorphs of *Ophiostoma.* In recent years, many of these characters have been shown to overlap and genera have been synonymized (Mouton et al., 1994). The aim of this study was to consider the mode of conidial development in the anamorph of *O. ips.* In this way, it was hoped to clarify confusion regarding the identity of this anamorph.

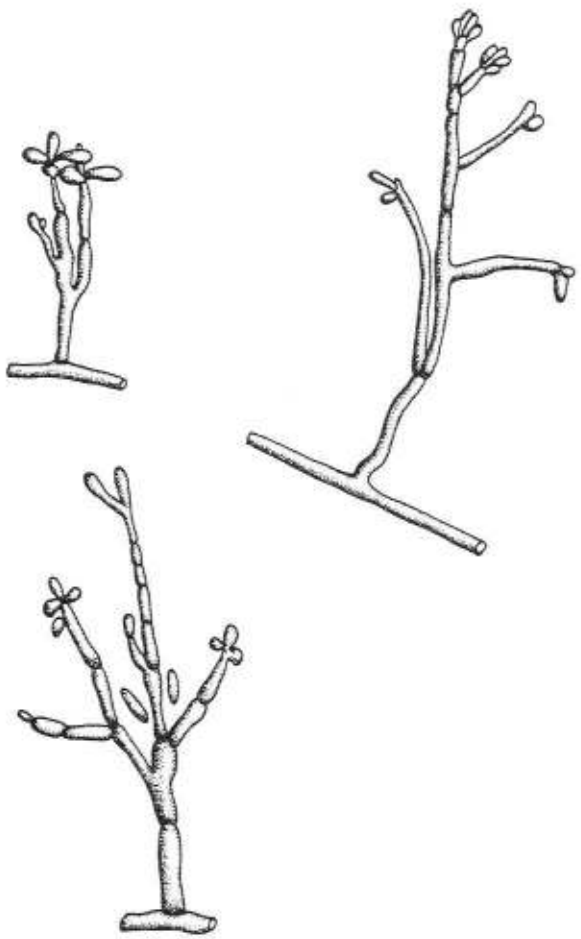
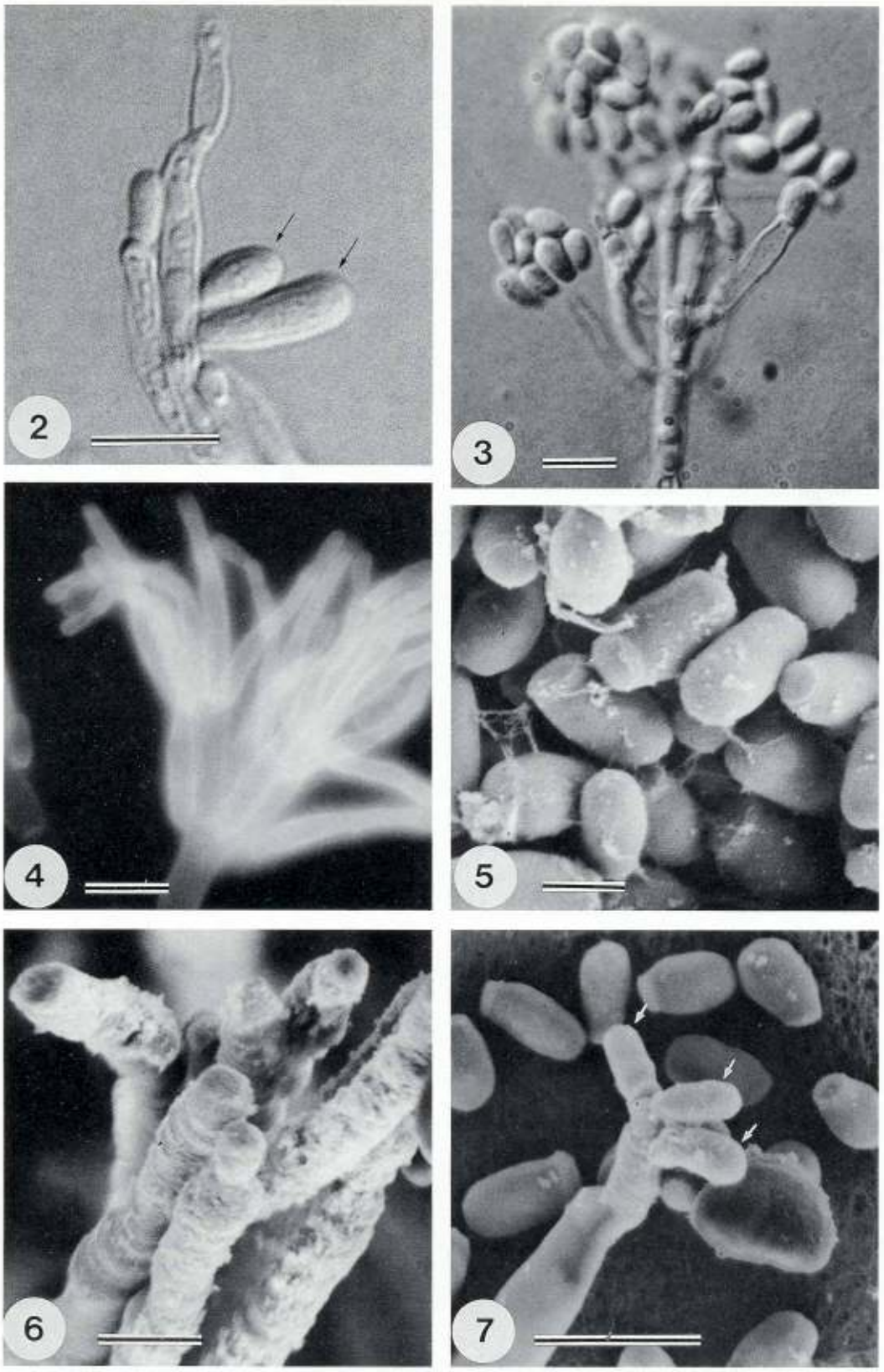


FIG. 1. Conidia and conidiophores of *Ophiostoma ips* suggesting sympodial conidium development (redrawn from Rumbold, 1936).

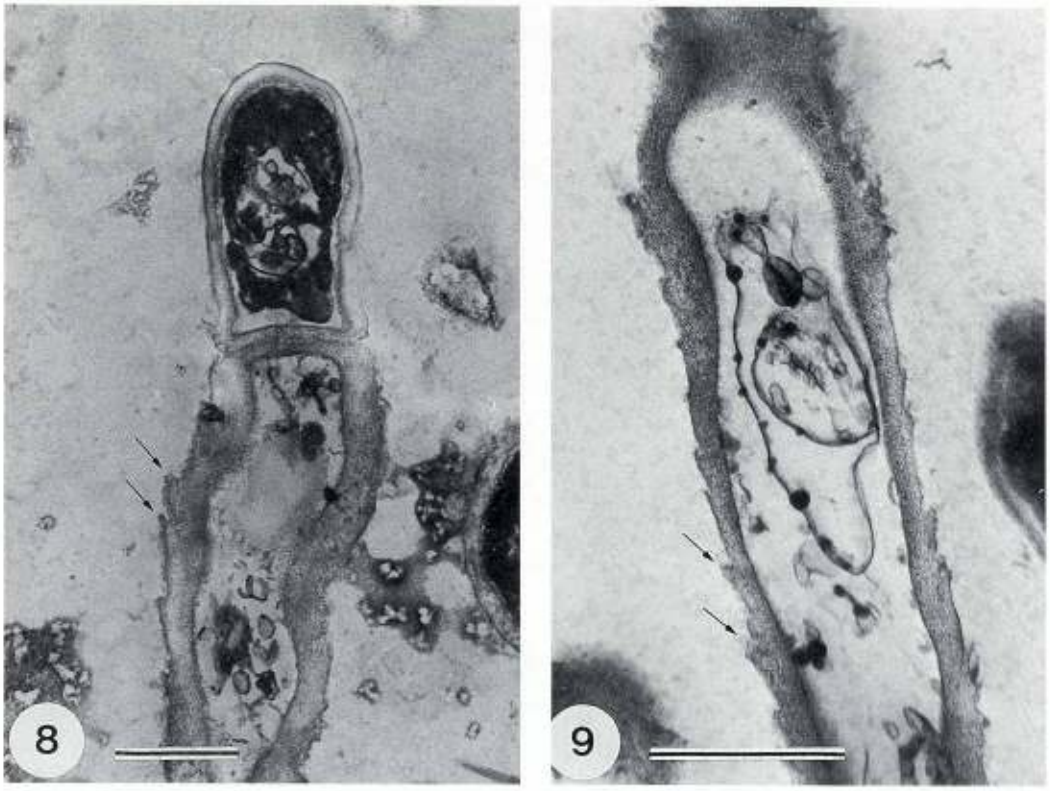
## MATERIALS AND METHODS

Three isolates of *O. ips* were used in this study. These isolates are all maintained in the culture collection of the second author (CMW) and have been deposited in the culture collection of the International Mycological Institute (IMI). They include CMW 311 (IMI 355935) from *Ips* De Geer (Coleoptera: Scolytidae) infested pine logs collected in the USA, CMW 1167 (IMI 355937) from pine logs infested with *Orthomicus erosus* (Woll.) and CMW 2259 (IMI 355938) isolated from *Ips* sp. from Israel in Taiwan. All isolates were examined using light and scanning electron microscopy (SEM). A single isolate (CMW 2259) that sporulated profusely was used for transmission electron microscopy (TEM). Cultures were grown on 2% malt extract agar plates (20 g Biolab malt extract; 20 g Biolab agar/1L water) and incubated at 25 C for approximately 2–3 wk until the onset of sporulation. Material for brightfield microscopy was mounted in lactophenol on glass slides and examined using phase and interference contrast microscopy. For fluorescence microscopy, material was mounted on glass slides in a 0.05% (w/v) solution of calcofluor white M 2R optical brightener in 0.1 M phosphate buffer. Material was examined with a Zeiss Axioskop fluorescence microscope, dark background and UV light.

Specimens for SEM and TEM were cut from agar and fixed sequentially in 3% glutaraldehyde and 1% osmium tetroxide in 0.1 M buffer (pH 7), and dehydrated in a graded acetone series. SEM specimens were then critical point dried, coated with gold/palladium and viewed with a JSM 6400 scanning electron microscope. Material for TEM was fixed in the same way and embedded according to Spurr (1969). The epoxy resin was polymerized at 70 C for 8 h. Ultrathin sections (60 nm) were made and mounted on copper grids. Sections were stained for 20 min in uranyl acetate followed by 10 min in lead citrate (Reynolds, 1963), and examined with a Phillips 301 transmission electron microscope.




FIGS. 2–7. Conidia and conidiogenous cells of the anamorph of *Ophiostoma ips* (IMI 355938). 2. Light micrograph of hyaline, one-celled, ovate to ellipsoidal conidia (arrows); bar = 2 μm. 3. Light micrograph of the conidiophore of *O. ips* showing ampulliform to lageniform conidiogenous cells; bar = 4 μm. 4. Fluorescence micrograph showing the conidiogenous




FIGS. 8, 9. Conidiogenous cells and conidia of the anamorph of *Ophiostoma ips* (IMI 355938). 8. Transmission electron micrograph of a section through a conidiogenous cell showing distinct outer wall remnants (arrows). This is indicative of percurrent development; bar = 2 μm. 9. TEM of a conidiogenous cell with distinct annellations (arrows); bar = 1 μm.

## RESULTS

Light microscopy showed hyaline, one-celled, ovoid to ellipsoidal conidia (FIG. 2) produced on *Leptographium*-like conidiophores (FIG. 3). Light and fluorescence microscopy suggested ampulliform to lageniform conidiogenous cells with small collarettes (FIGS. 3, 4). The mode of conidium development could not be determined with certainty at this level of magnification and the question as to whether conidiogenesis was sympodial, phialidic or annellidic (percurrent) could not be resolved.

Scanning electron micrographs showed that the ovoid to ellipsoid conidia were attached only at the base (FIG. 5). Tightly packed yet prominent annellations were visible at the apices of the conidiogenous cells (FIG. 6). Scanning electron micrographs also showed an apparent sympodial arrangement of the conidia on the conidiogenous cells (FIG. 7).

In some cases, outer wall layers of conidiogenous cells were flared, and it is likely that these would be perceived as collarettes of phialides under the light microscope. Transmission electron micrographs clearly showed the remains of outer wall layers along the periclinal walls of conidiogenous cells. These annellations are evidence of holoblastic ontogeny and percurrent enteroblastic proliferation of the conidiogenous cells (FIGS. 8, 9).

## DISCUSSION

SEM and TEM studies revealed prominent tightly packed annellations at the apices of conidiogenous cells in the anamorph of *O. ips*. This implies that the development of conidia occurs through percurrent proliferation. Conidia are thus formed by a succession of percurrent enteroblastic proliferation, holoblastic ontogeny, delimitation and secession (FIG. 10). Scanning electron micrographs of the anamorph of *O. ips*

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cells; bar = 4 μm. 5. SEM showing conidia with single attachment points; bar = 2 μm. 6. SEM revealing prominent, tightly packed annellations at the apices of conidiogenous cells; bar = 4 μm. 7. SEM, revealing conidia sympodially arranged, along the sides of the conidiogenous cell (arrows); bar = 4 μm.

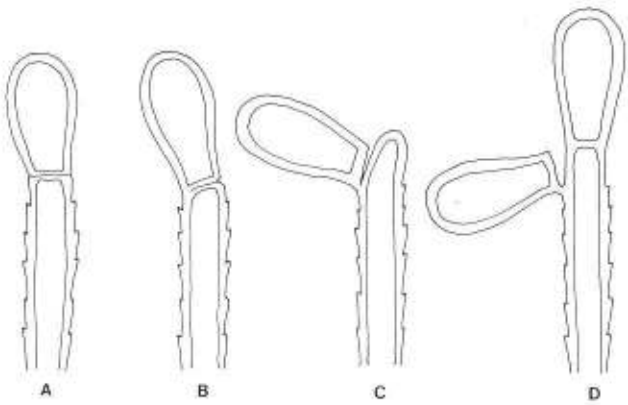


FIG. 10. Illustrative interpretation of conidial development in the amamorph of *Ophiostoma ips*. A. Delimitation of the conidium. B. Secession is delayed and the onset of early proliferation. C. Advanced proliferation and delayed secession. D. Delimitation of the succeeding condidium while the previous conidium is left hanging along the side of the conidiogenous cell creating an illusion of sympodial conidium development.

also, in some cases, indicated apparent sympodial conidium development, similar to that reported in *Leptographium* (Wingfield, 1985; Van Wyk and Wingfield, 1987; Van Wyk et al., 1988) and *Graphium* (Wingfield et al., 1991). In the anamorph of *O. ips*, the secession stage is delayed and overlaps the onset of proliferation. As a result, conidia are left hanging along the sides of the conidiogenous cell. This gives an illusion of sympodial development, although conidium development is actually annellidic (percurrent).

In this study, it became clear that light microscopy can be misleading in two ways: 1) delayed secession of percurrently produced conidia gives a false impression of sympodial conidiation, and 2) when tightly packed inconspicuous annellations and flaring wall layers give a false impression of phialidic conidiation. Both situations can be resolved conclusively only by SEM and TEM studies. This apparently misled previous authors (Wingfield and Marasas, 1980; Upadhyay, 1981) to refer to phialidic development and *Acremonium* anamorphs.

Upadhyay and Kendrick (1975) established the genus *Hyalorhinocladiella* for the anamorphs of *Ceratocystis s. l.* that were *Rhinocladiella*-like but without pigmentation. This genus is characterized by simple reduced conidiophores with hyaline, ovate, flat-based conidia hanging along the sides of the conidiogenous cells in an apparently sympodial fashion (Upadhyay, 1981). This is in contrast to *Sporothrix* that can apparently be distinguished from *Hyalorhinocladiella* by the distinct peg-like denticles associated with conidium development. Conidium development in the anamorph of *O. ips* is annellidic with delayed secession giving rise to an illusion of sympodial development. We believe that the anamorph of *O. ips* would best be considered as species of *Hyalorhinocladiella*. This would, however, necessitate a modification of the generic circumscription of *Hyalorhinocladiella* to include percurrent conidial development.

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