ELECTRON MICROSCOPIC STUDY OF CONIDIUM ONTOGENY IN CONIOTHYRIUM CUPRESSACEARUM (COELOMYCETES)

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(With Plates 30–31)

Coniothyrium cupressacearum (Morelet) Morelet, exhibiting annellated conidiogenous cells in light microscopy, was examined by transmission electron microscopy. Since the conidial wall is formed de novo, i.e. not continuous with a pre-existent layer of the conidiogenous cell wall, the conidiogenesis is clearly phialidic. After production of a number of conidia, a percurrent proliferation of the phialide collar occurs; this explains the annellated appearance. The annellide is not considered to be a special kind of conidiogenous cell but a variation of either the phialide or the aleuriophore.

Among the Fungi Imperfecti electron microscopic studies of Coelomycetes are scarce. Ultrastructural data like that obtained for many Hyphomycetes would be very useful, particularly in species with small sporogenous structures. Many uncertainties still exist as to the phialidic or annellidic nature of the conidiophores of such taxa, among them Coniothyrium cupressacearum (Morelet) Morelet. In 1970 this fungus, together with other species, was isolated from twig cankers of Chamaecyparis lawsoniana (Murr.) Parl. and Cupressus sempervirens L. in France and was first described as Coniella cupressacearum (Morelet, 1971a). Later, when subsequent examinations with a light microscope had shown that the conidiophores are annellated (Morelet, 1971b), Coniella cupressacearum was transferred to the genus Coniothyrium Corda.

By the concept of Sutton (1971) the genus Coniothyrium Corda (nom. conserv.) sensu stricto, with C. palmarum as lectotype, is indeed characterized by annellidic conidiogenous cells. According to Sutton this distinguishes Coniothyrium from Microsphaeropsis Höhn. (type species: M. olivacea Höhn.), which is characterized by phialides and should include many phialidic species such as the well-known C. fuckelii Sacc. It has now been established (Oláh & Reisinger, 1973) however that the annellide may result from two distinctly different processes:

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– Holoblastic development (succession of vegetative proliferations after thallic conidiogenesis).
– Enteroblastic development (succession of conidiogenous apices or primordia). The present authors think that there is enteroblastic development when the primordium wall is not continuous with a pre-existent layer of the conidiogenous cell (Reisinger, 1972; Mangenot & Reisinger, 1976).

It is therefore of interest to investigate the annellides of *C. cupressacearum* by electron microscopy in order to establish the fundamental mechanism of its conidiogenesis. As far as we know, the only ultrastructural data on the *Coniothyrium*-group published so far deal with phialidic species such as *C. fuckelii* and *Microsphaeropsis olivacea* (Jones, 1976).

In accordance with previously established principles (Reisinger, 1972; Reisinger & Mangenot, 1973; Mangenot & Reisinger, 1976), in the study of conidiogenesis we have separated conidiophore development from conidial ontogeny. In conidial ontogeny we note three distinct steps: (i) primordium initiation, (ii) conidium maturation, and (iii) conidium liberation. These steps will be examined separately.

MATERIAL AND METHODS

The strain used for our study was the type culture of *C. cupressacearum* isolated in October 1970 from a twig canker of *Chamaecyparis lawsoniana* in the Southwest of France (Morelet, 1971a) and deposited in Baarn under the number *CBS 874.72*. The fungus was cultured in daylight on beer wort (18° Balling) with 2% agar at room temperature (c. 20° C.) for 24 days, by which time it had reached a diameter of 40 mm. Pycnidia of different ages were removed from the plates and fixed in 2% osmium tetroxide at pH 7.4 or in 2% unbuffered potassium permanganate for 2 h. at room temperature. After they had been dehydrated in an ethanol gradient and included in Epon, serial sections were made with a diamond knife. Sections were contrasted with lead citrate (Reynolds, 1963) or else with TCH (thiocarbohydrazide) by the method proposed by Thiéry (1967) for polysaccharide differentiation.

RESULTS

Conidial ontogeny.

PRIMORDIUM INITIATION.—The conidiogenous structures inside pycnidia are closely entangled with other fertile or sterile cells. They are either long and phialide-like or short obconical (Pl. 31 fig. 7). In mature pycnidia the apex of the conidiogenous cells is usually provided with an opening surrounded by a typical thickening, well known in subendogenous phialides (Pl. 30 figs. 1–5, Pl. 31 figs. 6, 7).

Successive conidia are formed by extrusion through the collar opening. Each

primordium is separated from the preceding conidium by a perforated wall (Pl. 30 fig. 1) structurally analogous to ascomycetous walls but without Woronin bodies. The wall is made up of two layers originating in the lateral wall and separated by a cleavage zone; the lower layer later becomes the fundamental layer of the next primordium (Pl. 30 figs. 1, 3, 4). The primordium wall originates in the zone below the annular thickening of the collar; since it is not continuous with a pre-existing layer (Reisinger, 1972) it is formed *de novo*. The detection of polysaccharides by Thiéry's TCH technique is well suited for the study of this zone in which neither lead citrate (Pl. 30 fig. 1) nor permanganate (Reisinger, unpublished data) gives enough contrast. The same was observed earlier in phialides of *Phialophora richardsiae* (Oláh & Reisinger, 1974). Provided the section is favourably oriented TCH coloration will reveal a stratification in the annular thickening of the collar. These layers seem to result from deposits left in the course of successive conidium production. Lead citrate fails to give enough contrast to the young wall of the primordium (Pl. 30 fig. 1) but TCH provides good differentiation between the collar thickening and the newly formed wall of the new primordium (Pl. 30 fig. 2). The plasmalemma in the primordium and the upper part of the phialide is deeply corrugated, thus revealing strong physiological activity in these zones.

CONIDIUM MATURATION.—The wall of the young primordium thickens with age but exhibits no differentiation between the layers. In young pycnidia, conidia are hyaline even after secession and their outer surface is covered with a loose granular structure. In accordance with our terminology (Reisinger, 1972; Mangenot & Reisinger, 1976) we can distinguish an A-layer and a B-layer (Pl. 31 fig. 11). During maturation dark pigments are deposited in the wall by a process described by Reisinger & al. (1977) and in this way delimit the two sublayers B_1 and B_2 (Pl. 31 fig. 8). Such a development in the conidial walls is consistent with that of haplothecate conidia (Mangenot & Reisinger, 1976). Some pictures suggest that the primary wall participates in the formation of the mucilage surrounding the conidia (Pl. 30 figs. 2, 4, 5).

CONIDIUM LIBERATION.—The successive steps in conidium liberation can be reconstructed from Figs. 1, 3, 4, 5, 2 and 6 on Plates 30 and 31. The wall delimiting the young conidium from the next primordium is progressively lysed in the cleavage zone (Pl. 30 figs. 4, 5). Conidia remain tied together by residual products of lysis remaining between the layers of the wall (Pl. 30 fig. 2, Pl. 31 fig. 6).

Development of the conidiogenous cell.

In old pycnidia long annellide-like conidiogenous elements were seen, which confirms previous observations with light microscopy. A thorough ultrastructural examination of these structures led us to interpret them as the result of a series of successive proliferations. The whole structure is made up of encased phialide collars. We assume that each stage functions for some time as a phialide producing a number of conidia; this might account for the thickness of the collar deposit (Pl. 31 fig. 9).

Mucus.

Different contrasting techniques reveal slight variations in the appearance of the mucus in the pycnidial cavity. With lead citrate its granules seem to be arranged in lines, giving them a fibrillar aspect. TCH shows a more random disposition of the strongly reactive granules, except in zones surrounding primordia (Pl. 30 figs. 2, 4, 5). This led us to the hypothesis that the mucus might originate from the primary wall.

In old pycnidia the cells in the pycnidial cavity degenerate. The autolysis causing the degeneration affects even the cell wall. The cell wall ruptures and the altered cell contents leak out (Pl. 31 fig. 10). A similar process was seen in lysis of *Coprinus* carpophores (Sokolski & al., 1976).

CONCLUSIONS

Ultramicroscopic examination of the steps of conidium ontogeny in *C. cupressacearum* reveals that its spores are phialoconidia with all the production mechanisms judged to be typical of this kind of propagulum (Reisinger, 1972; Reisinger & Mangenot, 1973; Mangenot & Reisinger, 1976; Kiffer & al., 1971). The nature of its conidiogenesis places this species in the group of phialidic fungi with conidial heads as recently defined by Roquebert (1976). It should be emphasized that the first part of the primordial conidial wall is formed by the lower layer of the septum that separates the previous conidium from the newly developing conidium.

Initially this septum is perforated and similar to the hyphal septa but it lacks the Woronin bodies associated with vegetative septa. The photographs obtained to date do not reveal the mechanism that eventually closes the septal perforation.

It is our conclusion that the functioning of the phialide of *C. cupressacearum* is identical in all respects to that of *Phialophora richardsiae* (Oláh & Reisinger, 1974). Except in minor details, it is also similar to the conidiogenesis in *Doratomyces purpureofuscus* (Kiffer & al., 1971), which possesses annellides of the first type, viz. the type with enteroblastic development.

The other type of annellide, that with holoblastic development, results from a quite different process, the best known example of which is *Monotosporella* (=*Acrogenospora*) *sphaerocephala* (Reisinger, 1972). In this fungus the annellated appearance of the conidiophore results from the emission of proliferations after the formation of each of the blown-out conidia (Hammill, 1972a; Oláh & Reisinger, 1973; Mangenot & Reisinger, 1976; Reisinger & al., 1977).

Separate analysis of conidiophore development and conidial ontogeny reveals the annellide-like structures of *C. cupressacearum* as merely vegetative proliferations whose apices resume conidiogenous activity for varying periods of time.

Such a mode of evolution of conidiophores by successive proliferations is not rare among fungi, especially phialidic species (Hughes, 1971). In Coelomycetes it was described by Morgan-Jones & al. (1972).

We consider the conidia of *C. cupressacearum* to be typical phialoconidia. They are characterized by a primordium which is surrounded by a newly formed wall that is not continuous with any of the pre-existing layers of the phialide wall. They are different from thalloconidia (sensu Reisinger & Mangenot, 1973) whose primordium originates from the usual mechanisms of hyphal growth and whose walls are continuous with a pre-existing layer of the conidiogenous cell, either internally or externally.

Because of its multilayered aspect the thickening around the phialide neck seems to be produced by successive deposits of wall material during conidiogenesis. This material may exist in phialides however before conidiogenesis (Jones, 1976). Its existence seems to be very widespread among phialidic fungi, including species with monoconidial phialides (Gams, 1973), the few Coelomycetes so far investigated (Brewer & Boerema, 1965; Sutton & Sandhu, 1969; Boerema & Bollen, 1975; Jones, 1976), and many Hyphomycetes. The thickening has even been compared to the denticle plug of 'Radulasporae' (Gams, 1973) and to the apical swelling of the ascus (Delespine & Chadefaud, 1960; Chadefaud, 1974). Therefore even if any deposition of wall material occurs it cannot entirely explain the swelling of the phialide necks.

As already stated, electron microscopic studies of Coelomycetes are scarce (Brewer & Boerema, 1965; Sutton & Sandhu, 1969; Hammill, 1972b; Boerema & Bollen, 1975; Jones, 1976).

Sutton & Sandhu examined four species, among them a *Cryptosporiopsis*, and concluded that these fungi possess annellides. Since then a closer examination of this type of conidiogenesis has led to a distinction between two types of annellides. The first is linked to phialidic conidiogenesis, the second produces aleurioconidia with percurrent proliferations (Reisinger, 1972; Hammill, 1972a, 1974, 1977). Of the four species studied by Sutton & Sandhu *Cryptosporiopsis* species and *Phoma fumosa* belong to the first type (see Jones, 1976) and the two *Melanconium* species to the second type. According to data by Hammill (1972b) *Stegonosporium pyriforme* produces its conidia by the phialidic process. All the species studied by Jones (1976) are phialidic. Boerema & Bollen (1975) examined a number of species of *Phoma* and *Ascochyta*, and concluded that *Phoma* is phialidic while *Ascochyta* is annellidic. In *Phoma* all the conidia, including the first one, are enteroblastic; the collar thickening is a remnant of the initial thick papilla of the phialide and is not increased in width by the deposition of new material during conidiogenesis. In *Ascochyta*, in addition to the presence of the annellated collar typical of annellides, the first conidium is holoblastic but subsequent conidia could not be shown to be truly holoblastic. Boerema & Bollen used these and other characteristics to establish a clear-cut distinction between the two genera.

However, if the grouping of *Ascochyta*-type annellides and phialides together is accepted then the limited data on the ultrastructure of conidiogenesis in Coelomycetes gathered to date, reveal only two types of conidia: phialospores (common) and aleuria (rarer). The existence of percurrent proliferations, in some instances in both groups, explains the annellidic aspect seen in light microscopy.

Contrary to the opinion of Jones (1976) we think that phialo- and annelloconidio-genesis (first type) are truly similar and that a distinction between the two kinds of conidiogenous cells is only practical in value. Gams (personal communication) also thinks that the above distinction between phialidic *Phoma* and annellidic *Ascochyta* is 'very useful, but less fundamental than Boerema & Bollen suggest'. Hammill (1974, 1977) seems to share this opinion, stating that the distinction between phialides and annellides is tenuous; both types produce enteroblastic conidia (except in that the first conidium of annellides could be holoblastic, i.e. surrounded by the wall of the annellide apex). In both cases the lower part of the separating septum becomes part of the wall of the next conidium so that in the end a continuum rather than a sharp distinction might exist between phialide and annellide.

The purpose of our work was to closely examine the conidiogenous cells of *C. cupressacearum*; when seen with the light microscope these appear to be annellides like in the type species of *Coniothyrium*. Electron microscopy shows that these cells are true phialides with percurrent proliferations. A study of *C. palmarum*, type species of *Coniothyrium*, is needed to permit conclusions on the systematic position of this genus and related genera.

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RÉSUMÉ

Coniothyrium cupressacearum (Morelet) Morelet montre en microscopie photonique des cellules conidiogènes annelées. Il a été examiné en microscopie électronique à transmission. La conidiogenèse est clairement phialidique, la paroi conidienne est néoformée, c'est-à-dire qu'elle n'est pas en continuité avec une couche préexistante de la paroi de la cellule sporogène. Après production d'un certain nombre de conidies, le col de la phialide repousse de façon percurrente, ce qui explique l'aspect annelé vu au microscope photonique. L'annélide n'est pas considérée comme une cellule sporogène spéciale mais comme une variante, soit de la phialide, soit de l'aleuriophore.

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EXPLANATION OF PLATES 30–31

PLATE 30

Figs. 1–5. *Coniothyrium cupressacearum*, various steps in conidium formation, maturation, and secession. Mucus seems to originate in part from the outer layers of the young conidial walls (Figs. 4, 5). (Fig. 1, stained with lead citrate; Figs. 2–5, stained with TCH).

PLATE 31

Figs. 6–11. *Coniothyrium cupressacearum*. — 6. Conidium secession (TCH). — 7. Short obconical sporogenous cell (TCH). — 8. Mature conidium, A and B($=B_1+B_2$) layers (lead citrate). — 9. Successive encased collars of a phialide (TCH). — 10. Lysis of an aged cell, probably contributing to the production of mucus (TCH). — 11. Maturing conidia (TCH).