Ultrastructural evidence for two types of proliferation in a single conidiogenous cell of *Septoria chrysanthemella*

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Conidiogenesis in *Septoria chrysanthemella* was studied *in vitro* with light and transmission electron microscopy (TEM). For the first time, proof is given with TEM that both percurrent and sympodial proliferation can occur in a single conidiogenous cell. Ontogeny of conidia is holoblastic. After delimitation by a transverse, uniperforate septum, each conidium is liberated schizolytically. Three kinds of conidiogenous cells occur in *S. chrysanthemella*: (i) annellides, proliferating only percurrently; (ii) sympodulae, proliferating only sympodially, and (iii) cells proliferating either way. No influence of illumination by diffuse daylight or nuv, or medium was observed on qualitative aspects of conidiogenesis. The annellations in *S. chrysanthemella* seen with TEM are not resolved by light microscopy (LM). It is concluded that LM is not sufficient to assess conidiogenesis in species of *Septoria* with equally minute conidiogenous cells.

Concepts of conidiogenesis initially were developed on the basis of light microscopic observations and first surveyed by Hughes (1953). These concepts were adjusted whenever new evidence emerged from electron microscopy (Cole & Samson, 1979; Kendrick, 1971; Minter, Kirk & Sutton, 1982, 1983). Ultrastructural findings proved indispensable for a correct interpretation of conidiogenesis in several groups of hyphomycetes (Cole & Samson, 1979; Wingfield, 1985, 1993; Wang, 1990; Wingfield, Kendrick & Schalk van Wyk, 1991). In modern systematic treatments of coelomycetes, conidiogenesis is used to refine generic concepts (Sutton, 1973, 1980; Nag Raj, 1993), but very few ultrastructural data have been collected for some critical cases (Sutton & Sandhu, 1969; Boerema & Bollen, 1975; Jones, 1976; Buchanan, 1987).

Septoria Sacc. s.l. is generally recognized as a heterogeneous genus (Sutton, 1980; Constantinescu, 1984; Sutton & Pascoe, 1987, 1989, van der Aa, Noordeloos & De Gruyter, 1990; Farr, 1991; Sutton & Hennebert, 1994). A segregation in more natural groups still is not within sight. Some species have been correlated with *Mycosphaerella* Johanson, but for most teleomorphs are unknown. Sutton (1980) redescribed the type species, *S. cytisi* Desm., and confined *Septoria* to pycnidial species with holoblastic sympodial conidiogenous cells. Since then, Constantinescu (1984), Sutton & Pascoe (1987, 1989), and Farr (1991, 1992) discovered different types of conidiogenesis within certain *Septoria* species. In particular, Farr (1991) pointed out that the occurrence of annellations diagnostic of percurrent proliferation could not always be ascertained reliably with LM, and that EM would be necessary in such cases.

More knowledge of the variability in conidiogenesis is necessary to assess its value to *Septoria* systematics. By studying selected species of *Septoria s.l.* also with TEM, the reliability of LM can be tested. *Septoria chrysanthemella* Sacc. causes black leaf spot in cultivated *Chrysanthemum* spp. (Waddel & Weber, 1963). The small size of the conidiogenous cells hampers determination of the type of conidiogenesis with LM.

MATERIALS AND METHODS

Septoria chrysanthemella CBS 354.73, isolated by G. F. Laundon from *Chrysanthemum morifolium*, Taranaki, New Zealand (LEV 6809) was used. The fungus was grown in Petri dishes with oatmeal agar (OA), cornmeal agar (CMA) or 2% malt extract agar (MEA) and incubated in the laboratory under diffuse daylight at room temperature or in an incubator under nuv (12:12 h light:darkness) at 18 °C.

After 2 wk, conidiomata were transferred onto a slide, gently pressed under a cover glass and studied with LM in tap water, using bright field and differential interference contrast (DIC) optics. For TEM, small blocks of agar containing conidiomata were fixed in 1% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2), and postfixed in 1% osmium tetroxide in cacodylate buffer. During dehydration samples were stained with 1% uranyl acetate in a graded series of ethanol. The material was embedded in Epon 3/7. Ultrathin sections were cut on a Reichert Jung Ultracut E ultratome. Contrasting of sections with uranyl acetate and lead citrate was insufficient to study wall structure in this material. Sections, therefore, were picked up on gold grids and treated with periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP, 'Patag'), modified from Thiéry (1967) as described by Verkley (1992). The contrast obtained, referred to as

'reactivity', is based on the presence of PAS-positive (periodic acid-Schiff) polysaccharides. Preparations were examined using a Jeol JM 1010 electron microscope at 60 kV.

RESULTS

Light microscopic observations

Conidiogenous cells are doliiform or lageniform (Sutton, 1980). Cells proliferating sympodially can be recognized when two or three conidia remain attached on separate loci. In addition, cells with scar-like irregularities at the apex, without any thickenings or longer projections from the body of the cell, are interpreted as 'sympodulae'. In other cells, the single locus is situated on a neck-like projection of variable length. Irregularities seen on these necks could be interpreted as scars resulting from sympodial proliferation. No periclinal thickenings are observed on loci (Fig. 1). No differences are observed between conidiogenous cells on the three media under diffuse daylight and nuv. On MEA confluent conidiomata and single pycnidia occur under diffuse daylight and nuv, while on CMA or OA only unilocular, simple pycnidia are observed.

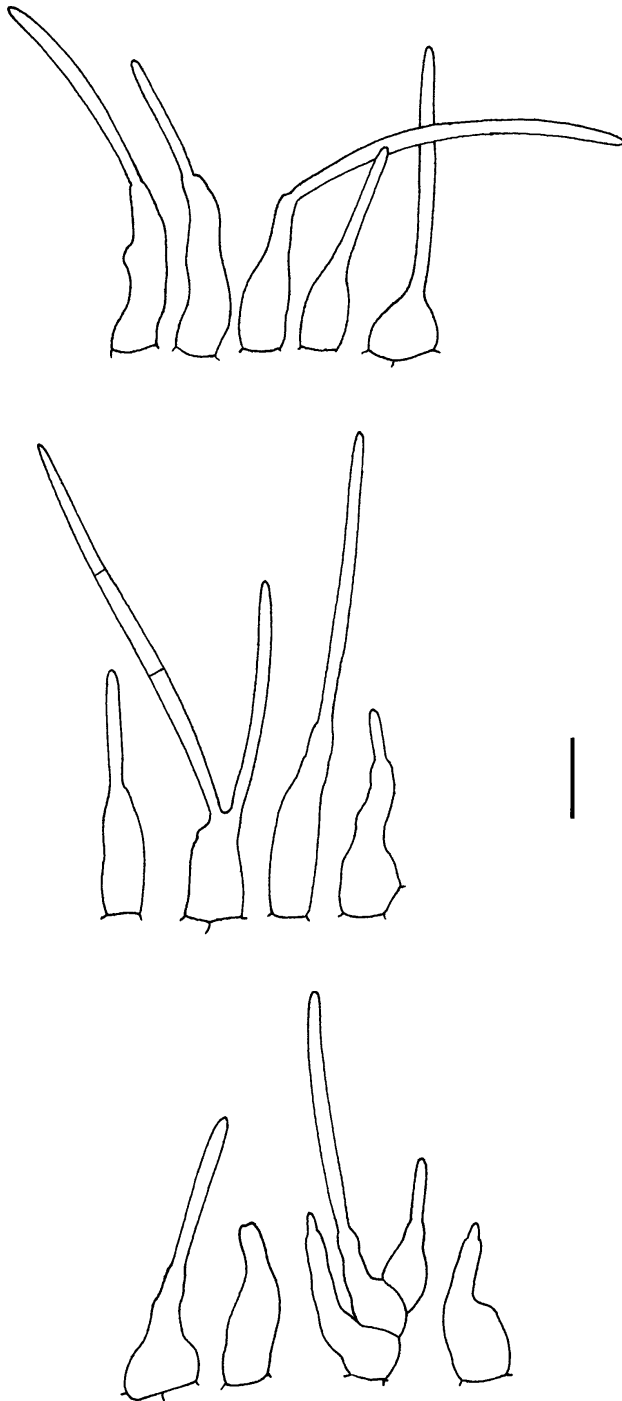


Fig. 1. Camera lucida drawings of conidiogenous cells of *Septoria chrysanthemella in vitro*. A. OA. B. 2% MEA. C. CMA. Bar = 5 μm.

Ultrastructural observations

During conidial initiation the transitional area of conidiogenous cell and conidium initial is constricted (arrows, Fig. 2), with walls in continuity. 'Conidial ontogeny' (Minter *et al.*, 1982) is therefore holoblastic. Initiation of the first conidium was not observed in TEM. However, scars of the first conidium and those formed later agree in structure, indicating that the first conidium also is formed holoblastically (Figs 2, 3, 5).

Conidia are delimitated by a transverse, uniperforate septum at the constriction. This delimiting septum consists of a septal plate, enclosed by walls of about equal thickness on either side. These walls are in continuity with the inner layers of the conidium and conidiogenous cell walls, respectively (Fig. 3). Conidial secession is schizolytical (Fig. 4). After a circumscissile rupture or dissolution of the outer layer, the walls separate along the septal plate.

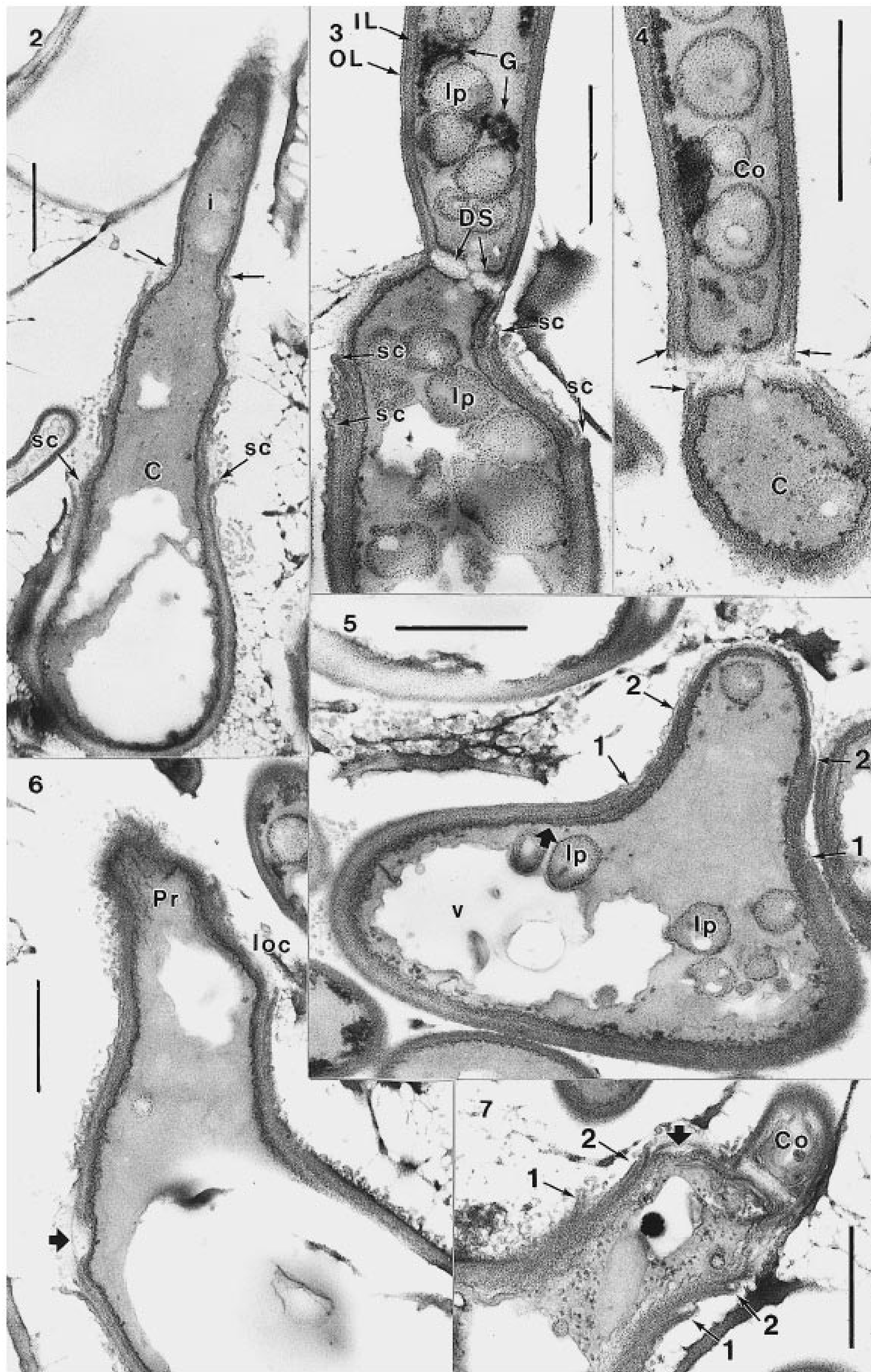
Three kinds of conidiogenous cells are observed: (i) annellides in the sense of Wang (1990), proliferating only percurrently, successive conidia seceding at progressive levels, leaving a series of close or more distant annellations (Figs 2, 5); (ii) sympodulae, proliferating only sympodially (Fig. 6); and (iii) cells proliferating either way (Fig. 7), first percurrently a number of times, then sympodially up to three times. Young 'annellides' could later also proliferate sympodially.

No differences are observed in ultrastructure of conidiogenesis between material grown on OA, CMA or MEA 2% under diffuse daylight or nuv.

DISCUSSION

Percurrent proliferation (annellidic conidiogenesis) could be observed only with TEM, whilst the diagnostic scars (annellations) were not resolved with LM. Punithalingam (1967), who described the morphology and phytopathology of *S. chrysanthemella*, only referred to the conidiogenous system with 'blastospores from cylindrical to subulate conidiophores'. According to Sutton (1980), the species is characterized by 'holoblastic sympodial' conidiogenesis. Obviously, LM is insufficient to visualize all features of conidiogenesis in this species and others with equally minute conidiogenous cells.

The heterogeneity of *Septoria*, *Phloeospora* Wallr., *Cylindrosporium* Grev. *sensu auct.*, and other genera in respect of conidiogenous cell proliferation is well-known (Sutton, 1973). Sutton (1980) tentatively outlined three groups within *Septoria*



Figs 2–7. Transmission electron micrographs of conidiogenous cells in *S. chrysanthemella* (PA-TCH-SP). Bar = 1 μm. **Fig 2.** Conidial initiation. The transitional area of the conidium initial (i) and conidiogenous cell (C) is constricted (arrows). Note the scars of percurrent proliferation (sc) in this annellide. **Fig. 3.** The walls of the delimiting septum (DS) are barely reactive in PA-TCH-SP. Note the scars (sc) of earlier percurrent proliferations. The sporoplasm contains oil droplets (lp) and concentrations of PA-TCH-SP reactive glycogen particles (G). The conidium wall shows an outer (OL) and an inner layer (IL). **Fig. 4.** The walls of the conidium (Co) and conidiogenous cell (C) are completely separated. Note the remains of the outer wall layer (arrows). **Fig. 5.** Percurrent proliferation. Doliiform annellide, with two more distant scars (1, 2). Note the depth to which new wall material was deposited against the inner side of the old wall (bold arrow), after secession of the first conidium at scar 1; lp, lipid droplets; v, vacuole. **Fig. 6.** Sympodial proliferation. The first formed locus on this conidiogenous cell has been medianly cut (bold arrow). The cell has proliferated sympodially, and the next locus (loc) and following proliferation (Pr) are out of the plane of sectioning. **Fig. 7.** Conidiogenous cell showing two types of proliferation. Two percurrent proliferations (through scars 1 and 2) have preceded sympodial proliferations. Next to an obliquely cut, naked locus (bold arrow), secession of a conidium (Co) is in progress.

s.l.: (i) species with 'mainly holoblastic sympodial conidiogenesis', congeneric with the type species *S. cytisi*, including *S. chrysanthemella*, (ii) species with 'phialidic conidiogenesis', *S. apiicola* Speg. and *S. tritici* Roberge, and (iii) species with 'simple holoblastic conidiogenesis', so without proliferation, e.g. *S. adanensis* Petr., and several others. However, since then different types of conidiogenesis have been reported within some species of *Septoria*, e.g. in three occurring on Betulaceae (Constantinescu, 1984). Interestingly, Farr (1992) has observed percurrent and sympodial proliferation in the type species *S. cytisi*, but whether both types of proliferation occur within single conidiogenous cells of this species remains to be clarified.

The occurrence of more types of conidiogenous cell proliferation within a single anamorph genus now is accepted widely. Anamorphs currently classified in *Cladobotryum* Nees are known to proliferate percurrently or sympodially, while others are retrogressive, yet form a homogeneous group in respect of their *Hypomyces* teleomorphs and apparently are monophyletic (Rogerson & Samuels, 1993). It is known that in certain *Septoria* species more types of proliferation may be found in a single conidiogenous cell (Constantinescu, 1984; Sutton & Pascoe, 1987), but proof with TEM has never been given before, neither in *Septoria* nor in any other filamentous fungus.

Conidium ontogeny in *S. chrysanthemella* is holoblastic, namely, the entire (apical) wall of the conidiogenous cell 'contributes' to the conidium wall (Minter *et al.*, 1982; Hawksworth *et al.*, 1995). Percurrent proliferation is enteroblastic, as newly formed walls are partly continuous with the inner layer of the old wall in the scar, or merely line its inner surface. Madelin (1979) envisaged that the modes of blastic conidial ontogeny are related to the juvenility or maturity of the wall at the conidiogenous loci. He also considered enteroblastic and holoblastic conidial ontogeny as being extremes of a continuum. This equally applies to the walls involved in conidiogenous cell proliferation. Sympodial proliferation is holoblastic, as complete continuity is observed between the new, undifferentiated and the older wall of the conidiogenous cell. The cell is able to re-shape this apparently still fairly plastic wall.

Cercospora beticola Sacc. is the nearest related anamorph of which conidiogenesis has been studied with TEM (Pons, Sutton & Gay, 1985). Conidiogenous cell proliferation is obligately sympodial in that species, but interpreted as 'enteroblastic' by Pons *et al.* (1985), because the outer layer of the conidiogenous cell wall ruptures and is not continuous with the newly formed wall of the proliferating apex. The conidiogenous cells of *C. beticola* are pigmented, and show an outer layer which probably is less plastic (Madelin, 1979).

A broad concept of *Septoria*, allowing percurrently or sympodially proliferating as well as non-proliferating forms, has become generally adopted in the last decade or so (Constantinescu, 1984; Sutton & Pascoe, 1987, 1989; Farr, 1991, 1992). However, this concept is merely an *interim* stage. Many species that apparently lack a teleomorph would need genotypical characterization, to trace their affinities with pleomorphic fungi and to create a stable system of smaller, monophyletic groups. For many more species full phenotypic characterizations are also needed, which should appreciate conidiogenesis, conidial morphology, and ecology. Too little is known of the diversity in conidiogenesis in particular, and more species must be examined with TEM before a proper assessment of the value of these characters to *Septoria* systematics can be made.

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