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Reviewed work(s):
Source: *Mycologia*, Vol. 90, No. 2 (Mar. - Apr., 1998), pp. 189-198
Published by: Mycological Society of America
Stable URL: http://www.jstor.org/stable/3761294
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Mycologia, 90(2), 1998, pp. 189–198.
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Ultrastructure of conidiogenesis and conidia in two species of *Septoria* sensu lato

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Abstract: Conidiogenesis and conidial morphology of *Septoria quercicola* and *S. aceris* was studied with light (LM) and transmission electron microscopy (TEM) in vivo and in vitro on three media. No fundamental differences in conidiogenesis were observed between these species. Ontogeny of conidia is holoblastic, and after delimitation by a transverse septum, conidia are liberated schizolytically. Proliferation of the conidiogenous cell can be percurrent or sympodial, and three categories of conidiogenous cells occur: (i) annellides, (ii) sympodulae, and (iii) cells proliferating both percurrently and sympodially. Conidiogenous cells of the last category were only observed in vivo, and most had a number of percurrent and one or two terminal sympodial proliferations. Annellides with close annellations had been interpreted as phialides with periclinal thickenings in LM. After secession, a majority of conidia of *S. quercicola* and *S. aceris* developed noncellular, mucoid appendages on either end, visible in LM and TEM; in *S. quercicola* appendages were formed in vivo and in vitro, in *S. aceris* only in vitro. No influence of either illumination (diffuse daylight or near-UV) or medium (OA, CMA, 2% MEA) was observed on qualitative aspects of conidiogenesis. The data are compared with those of *S. chrysanthemella* and their significance to *Septoria* systematics is discussed. The relation with *Septoriella* in respect of ontogeny of mucoid appendages is assessed.

Key Words: Conidial appendage, phialide, TEM, *Septoria quercicola, Septoria aceris, Phloeospora aceris*

INTRODUCTION

The anamorph genus *Septoria* Sacc. accommodates over 2000 species, and its heterogeneity is widely recognized (Sutton, 1980; Constantinescu, 1984; Sutton and Pascoe, 1987, 1989; Van der Aa et al., 1990; Farr, 1991; Sutton and Hennebert, 1994). Before more natural groups can be distinguished, many poorly described species must be reexamined for characters like conidial morphology and conidiogenesis. To date, a few selected groups of species occurring on certain host genera or families have been revised (Constantinescu, 1984; Sutton and Pascoe, 1987, 1989; Farr, 1991, 1992; McPartland, 1995; Wu et al., 1996). Characters of conidiogenesis are widely used to refine generic concepts of coelomycetes (Sutton, 1977, 1980; Nag Raj, 1993). Sutton (1980) redescribed the type species, *S. cytisi* Desm., and confined *Septoria* to pycnidial fungi with holoblastic sympodial conidiogenous cells, while subepidermal acervular forms with annellidic or sympodial conidiogenous cells were classified in *Phloeospora* Wallr.

Since then, Constantinescu (1984), Sutton and Pascoe (1987, 1989), and Farr (1991, 1992) discovered different types of conidiogenesis within certain *Septoria* species. Especially Farr (1991) noted, that the resolving power of the light microscope is insufficient to study species with minute conidiogenous cells, and that electron microscopy is necessary in such cases. Before conidiogenesis in *Septoria* can be exploited for systematic purposes, more information about its variability and ultrastructure is needed.

By comparing light and transmission electron microscopic observations of conidiogenesis in selected species of *Septoria* s.l., the reliability of light microscopic observations can be tested, and the stability of the characters after isolation in culture determined. In a first study of *S. chrysanthemella* Sacc., a species with very small conidiogenous cells, only sympodial proliferation was observed in LM. TEM revealed that the cells can also proliferate percurrently, but the scars diagnostic of this process are too small to be resolved in LM (Verkley, 1997).

This paper deals with two species causing leaf spots in *Quercus* and *Acer* in the Netherlands: *S. quercicola* Sacc. and *S. aceris* (Lib.) Berk. & Br. (*Phloeospora aceris* (Lib.) Sacc.). The first fungus produces conidiomata as described by Grove (1935) and Jørstad (1965), but the conidia bear conspicuous mucoid apical and basal appendages not reported in the literature. No teleomorph of this species is known, but its characters in culture are similar to those of species in *Mycosphaerella* Johansen. *Mycosphaerella latebrosa* (Cooke) Schroet. has been reported as the teleo-

Accepted for publication October 24, 1997
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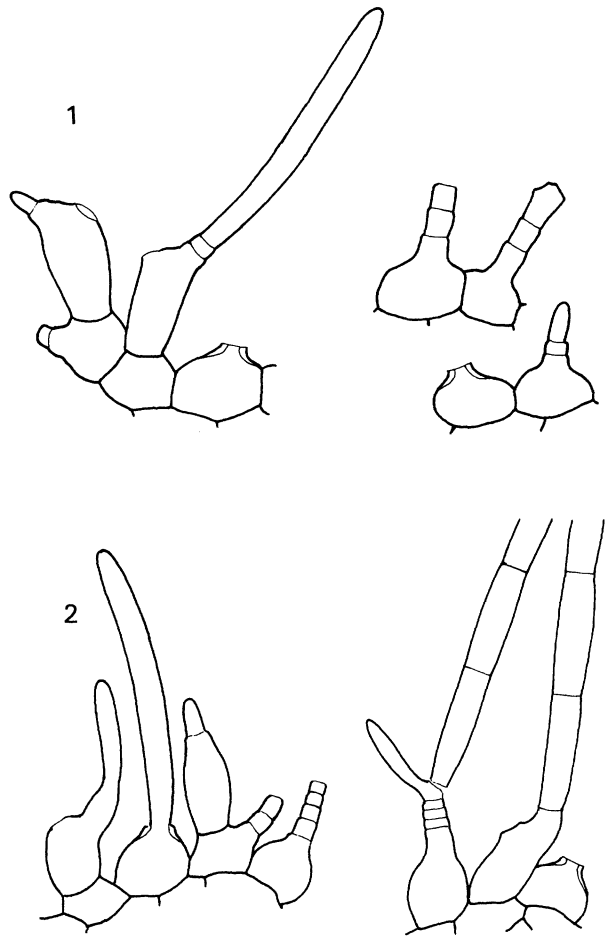

FIGS. 1, 2. Conidiogenous cells in vivo. Bar = 10 μm. 1. *Septoria quercicola.* 2. *Septoria aceris.*

morph of the second fungus, *Septoria aceris,* which produces more typically acervular conidiomata. The conidia lack appendages in vivo, but otherwise are similar to those in *S. quercicola.* The stability of conidiogenesis after isolation in culture is assessed and the ontogeny and structure of conidial appendages are studied. Conidiogenesis of the two species is compared to that of *S. chrysanthemella.* The significance of the results to *Septoria* systematics is discussed.

MATERIALS AND METHODS

The following material was used: *Septoria quercicola* Sacc., the Netherlands, Utrecht, Baarn, in leaf spots on *Quercus robur,* 11.VIII.1994, *G. Verkley 225* (Herb. CBS), isolate CBS 663.94; *S. aceris* (Lib.) Berk. & Br. (= *Phloeospora aceris* (Lib.) Sacc.), the Netherlands, Zuid-Holland, Wassenaar, in leaf spots on *Acer pseudoplatanus,* 8.VIII.1995, *G. Verkley 307* (Herb. CBS), isolate CBS 187.96 (as *Mycosphaerella latebrosa* (Cooke) Schroet.).

Shortly after collecting, hand sections were studied with LM in tap water, using bright field and differential interference contrast (DIC) optics. Drawings were made with a camera lucida. Mass-conidial isolates were grown on Petri dishes with oatmeal (OA, 20 g/L), cornmeal (CMA, 60 g/L) and 2% malt extract agar (MEA) and incubated in the laboratory under diffuse daylight at room temperature or in an incubator under near-UV (12 h) at 18 C. Fructifications in vitro were studied after 2–3 wk with LM as described above.

For TEM, shortly after collection, 5 mm squares of fresh leaf tissue containing carefully cut conidiomata

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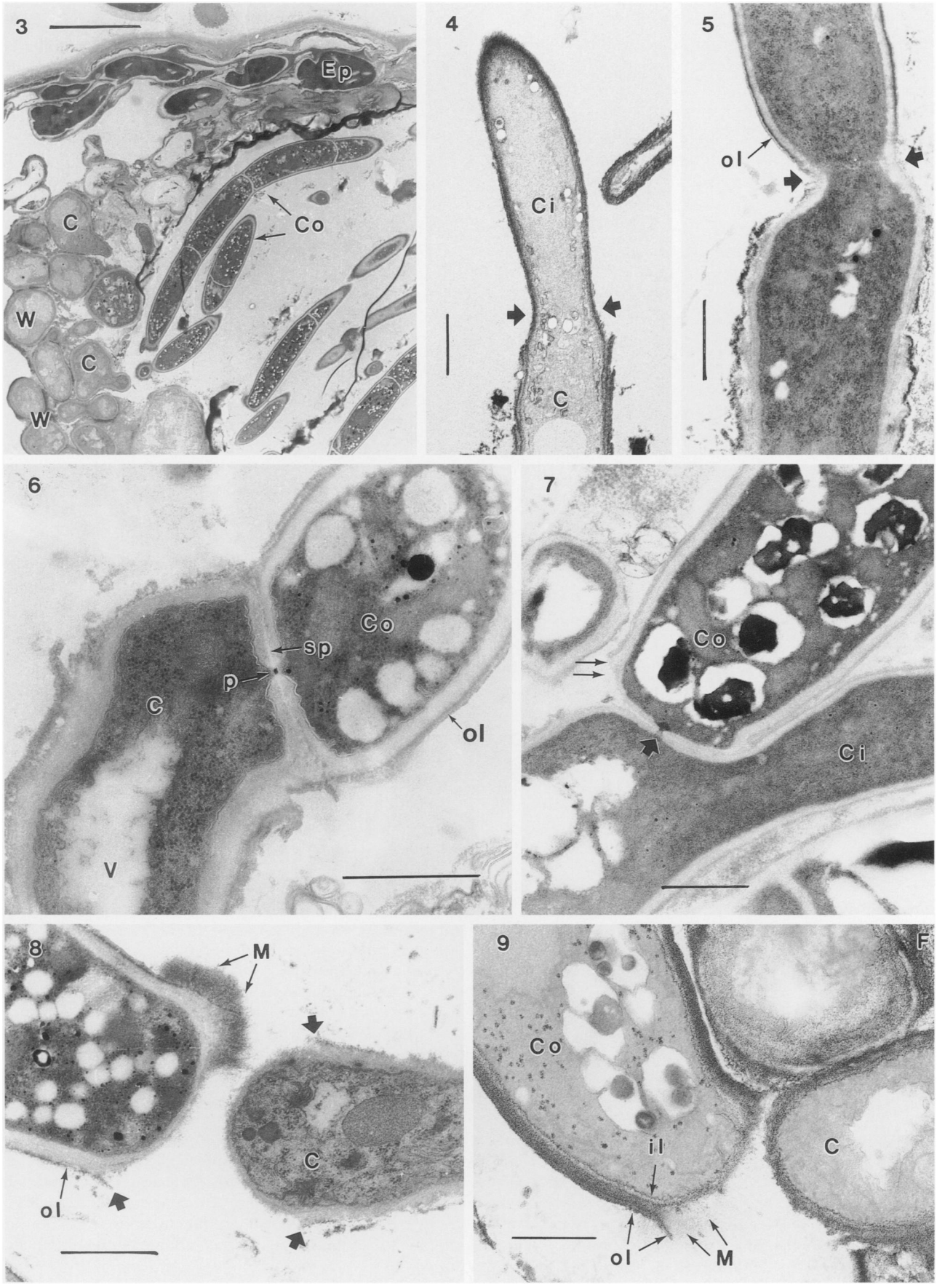
FIGS. 3–9. Transmission electron micrographs of *Septoria quercicola,* except FIG. 6, of *S. aceris,* fixed in glutaraldehyde (GA), unless stated otherwise. Bar = 1 μm. 3. Cross section of a conidioma in vivo, showing cells of the conidiomatal wall (W), conidiogenous cells (C) and conidia (Co), and epidermal cells (Ep) of the host, *Quercus robur.* Bar = 10 μm (Ur/Pb). 4, 5. Longitudinal sections of the transitional area between conidiogenous cell and conidium initial, before delimitation. 4. Young conidial initial (Ci) originating from a percurrent proliferation of the conidiogenous cell (C). The wall of the conidial initial and conidiogenous cell are continuous over the area of constriction (bold arrows), where the future delimiting septum will be laid down (PA-TCH-SP; on OA). 5. A well-differentiated outer layer (ol) is only visible above the constriction (bold arrows; Ur/Pb; in vivo). 6. Longitudinal median sections of the transitional area of conidium (Co) and conidiogenous cell after formation of the delimiting septum, showing a central pore (p), and an electron-transparent septal plate (sp). The outer layer (ol) is eroded near and below the delimiting septum (Ur/Pb; in vivo). 7–9. Conidial secession. 7. Early stage. The central pore in the delimiting septum (bold arrow) is plugged by an electrondense mass on the side of the conidium (Co). The basal conidial wall is already partly liberated at the delimiting septum (double arrows). Note the young conidial initial (Ci) formed by sympodial proliferation of the conidiogenous cell (GA + formaldehyde; Ur/Pb; on MEA). 8. Base of a conidium and apex of a conidiogenous cell shortly after secession. The outer layer (ol) is ruptured around the delimiting septum, protruding from the conidial base and demarcating the scar (bold arrows). Extramural material, well-contrasted by uranyl and lead salts (M), is associated with the base of the conidium (Co; Ur/Pb; in vivo). 9. Similar stage as in FIG. 8, but after treatment with PA-TCH-SP. Note the extramural, weakly to nonreactive, amorphous material emerging from one side of the conidial wall (M), while a matrix of strongly reactive fibrils (F) is found between various conidiogenous cells (C). The conidial wall consists of an outer layer (ol) and an inner layer (il; on OA).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were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), or 1% glutaraldehyde and 1% formaldehyde in the same buffer, and post-fixed in 1% osmium tetroxide in cacodylate buffer. After 2–3 wk of incubation, blocs of agar containing cut conidiomata were fixed in the same way. During dehydration, the material was 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. Sections were contrasted with uranyl acetate and Reynold's lead citrate (Ur/Pb). In addition, to facilitate study of wall structure, sections were picked up on gold grids and treated with periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) as described by Verkley (1992) and modified from Thiéry (1967). The contrast obtained, referred to as 'reactivity', is based on the presence of PAS-positive (periodic acid-Schiff) polysaccharides. Preparations were examined using a Philips CM10 transmission electron microscope at 60 kV.

RESULTS

In vivo.—Camera-lucida drawings of conidiogenous cells are presented in FIGS. 1, 2. The conidiomata in *S. quercicola* are hypo- or epiphyllous, immersed, pycnidial with a wide, central ostiole (FIG. 3), while those in *S. aceris* are epiphyllous, subepidermal, typically acervular or more cup shaped. In both species, conidiogenous cells vary in shape from broadly barrel shaped or subglobose ('doliiform') to flask shaped with a straight or attenuated base ('lageniform'). Successive annellations or scars are observed at the apices of percurrently proliferating conidiogenous cells. Other cells show a sympodial proliferation, while previously formed conidia are still attached. Cells proliferating either way are also identified in both species. In addition, cells with distinct periclinal thickenings in the apical wall are observed, suggesting phialides (sensu Sutton, 1980). In *S. quercicola,* conidia are cylindrical, with a rounded apex and truncate base, mostly 3- to 5-septate, distinctly constricted at the septa in living state, mostly 38–50 × 4 μm (nonturgescent or rehydrated conidia 2–3 μm wide). Noncellular, apical and basal conidial appendages are observed using bright field, but are better resolved using DIC optics. Most free conidia show these appendages. The conidia in *S. aceris* agree in shape with those of *S. quercicola,* but are mostly 3-septate, 37–47 × 3–4 μm (nonturgescent 2–3 μm wide), and lack appendages.

In vitro.—Conidiomata of both species are pycnidioid or acervular, and conidiogenous cells agree with those on the natural substratum. However, conidiogenous cells proliferating both percurrently and sympodially are not observed in *S. quercicola* or *S. aceris.* The conidia also agree with those observed in vivo, but a minority of the free conidia in *S. aceris* develops basal and, less often, apical appendages in vitro, less conspicuous than those in *S. quercicola.* No influence of either illumination (diffuse daylight or n-UV) or medium (OA, CMA, 2% MEA) was observed on qualitative aspects of conidiogenesis.

Conidiogenesis.—Conidial ontogeny (conidial initiation) is holoblastic in both species: the newly formed wall in the apical part of the conidiogenous cell is continuous with the wall of the conidial initial (FIGS. 4-6). Sections treated with PA-TCH-SP show a strongly reactive outer and a weaker reactive inner layer in these walls, continuing over the constricted area,

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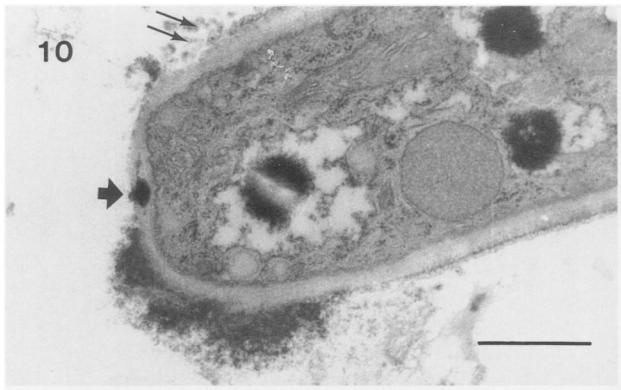
FIGS. 10–15. Transmission electron micrographs of *Septoria quercicola,* except FIGS. 12 and 15, of *S. aceris,* fixed in glutaraldehyde (GA), unless stated otherwise. Bars = 1 μm. 10. Base of a conidium shortly after liberation. The central pore is plugged with electrondense material (bold arrow). The sporoplasm contains vacuoles with osmiophilic material. Note the eroded outer layer near the base (double arrows; Ur/Pb; in vivo). 11. Longitudinal section of a percurrently proliferating conidiogenous cell, showing a series of scars (scl over 4) below the conidial initial (Ci). Note that the depth to which new wall material is deposited during the proliferation (scar 3), is below the level of scar 1, the arrows follow the thinning wall layer, till wdp 3 (PA-TCH-SP; on OA). 12. Percurrently proliferating conidiogenous cell with slight progression. The close, successive scars are covered by electrondense and reactive extramural material (M). The apical wall is thickened due to the deposition of new layers during successive percurrent proliferations (bold arrows). Note the glycogen particles in the apical cytoplasm (G; PA-TCH-SP; on CMA). 13. Conidiogenous cell showing scars (sc) of several percurrent proliferations, and a final sympodial one (Sy). The site where the latest conidium seceded (bold arrow), is only partly in the plain of sectioning (verified by comparison of serial sections; PA-TCH-SP; in vivo). 14. Conidiogenous cell forming a new conidial initial next of the previous locus (bold arrow) by a sympodial proliferation (Sy), here obliquely cut. Note the scar (sc) of an earlier percurrent proliferation, probably after a longer period of inactivity. Note the mitochondria (m) and nucleus (n) in the cytoplasm of the conidiogenous cell, rich in ribosomes (Ur/Pb; in vivo). 15. Sympodially proliferating conidiogenous cell with two obliquely cut loci (single arrows), and a near-medianly cut one (double arrows). Note a liberated conidium (Co; GA + formaldehyde, Ur/Pb; in vivo).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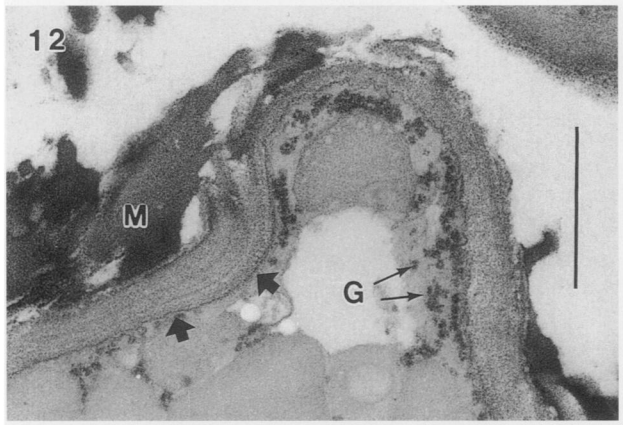
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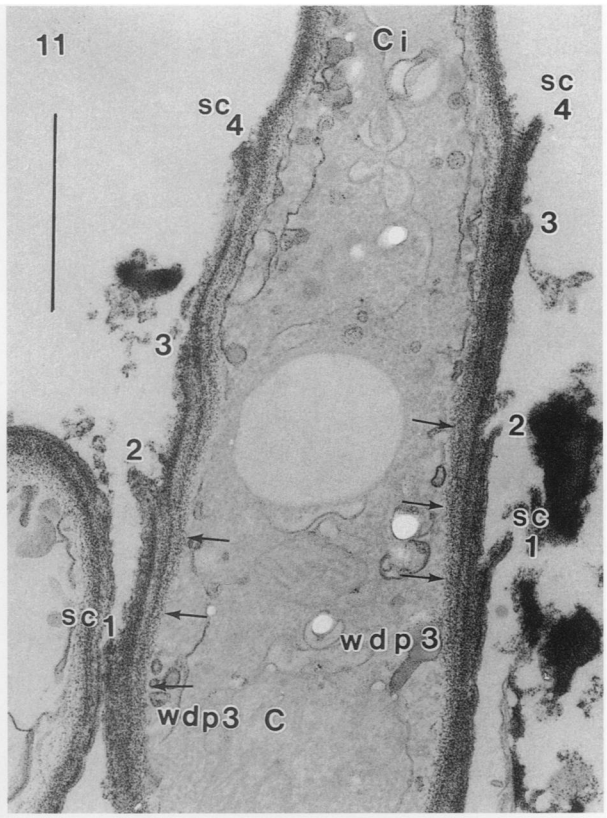
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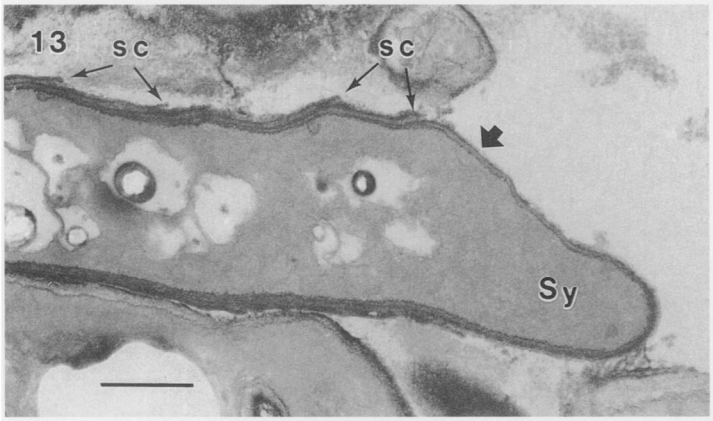
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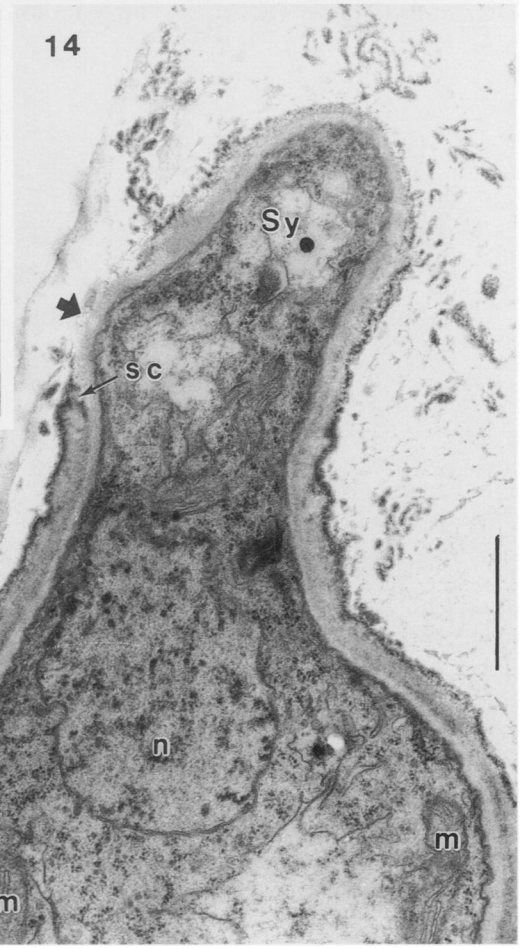
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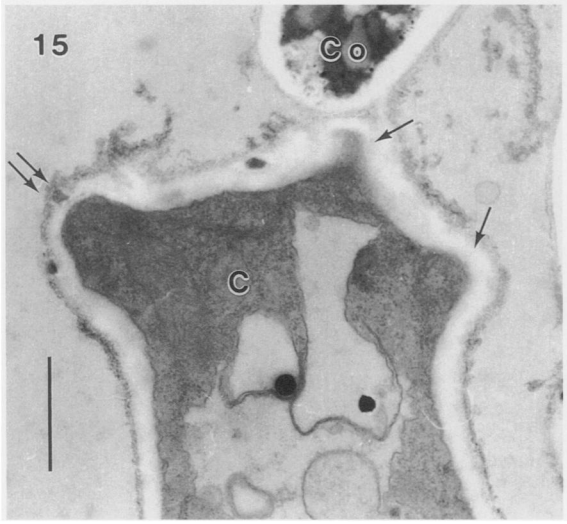
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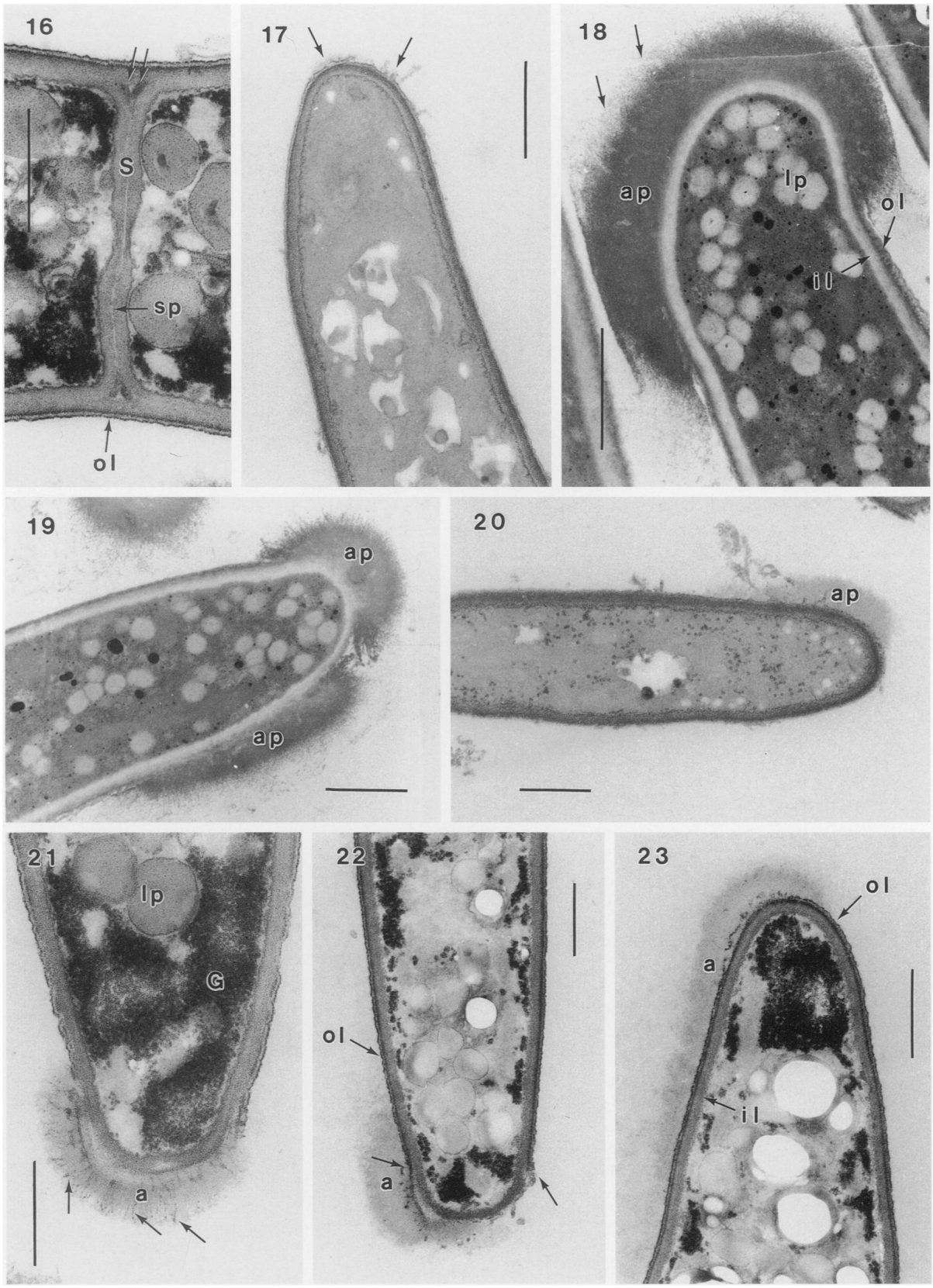

FIGS. 16–23. Transmission electron micrographs of longitudinal sections of free conidia, fixed in glutaraldehyde (GA), unless stated otherwise. Bar = 1 μm. 16. *Septoria aceris*. The near-medianly cut conidial septum (S) contains a thin, non-reactive septal plate (sp). Strongly reactive material encloses the periphery of this plate (double arrows). The moderately

where the future delimiting septum will be laid down (FIGS. 4, 11). In sections contrasted with Ur/Pb, a thin, electrondense outer layer is distinguishable only above the constriction in the future conidium of *S. quercicola* (FIG. 5) and *S. aceris* (FIG. 6). Ontogeny of a first conidium could not be observed, but scars of first and successive conidia are always identical in these species, and ontogeny is therefore regarded holoblastic throughout.

Conidia are delimited by the formation of a transverse septum at the constriction (FIGS. 6, 7). In both species, this delimiting septum consists of a middle, electron-transparent septal plate (FIG. 6), nonreactive with PA-TCH-SP. The plate is enclosed by a moderately reactive wall on either side, which is continuous with the innermost part of the inner layer of the lateral wall of conidium and conidiogenous cell (FIG. 9). A single pore perforates the center of the delimiting septum, which becomes plugged by electrondense material (FIGS. 6, 7).

Conidial secession is schizolytic in both species. The outer layer is ruptured around the delimiting septum (FIG. 8). The remnants are particularly conspicuous in sections treated with PA-TCH-SP (FIG. 9). The separation of the two septal walls proceeds centripetally (FIG. 7). Shortly after secession, the outer layer may erode further along the conidial wall (FIG. 10).

Both percurrent and sympodial proliferation of the conidiogenous cell are observed. The percurrently proliferating conidiogenous cell grows through the locus after liberation of a conidium. Successive conidia secede at progressive levels, leaving a series of scars or annellations on the wall surface of the conidiogenous cell (FIGS. 11, 12). Successive scars can be close together (FIG. 12) or more distant (FIGS. 11, 13). Once, in *S. quercicola* a percurrent proliferation was observed where the delimiting septum was formed below the level of the previous one, resulting in a retrogressive conidiogenesis. During percurrent proliferation, new wall material is deposited inside the wall of the conidiogenous cell to various depth, sometimes below the level of the previous scar or even the one before (FIG. 11). The sympodially proliferating conidiogenous cell forms a new growing point next to the previous locus before or during secession of the previous conidium (FIGS. 7, 13-15).

Three categories of conidiogenous cells are observed in *S. quercicola* and *S. aceris*: (i) cells proliferating only percurrently, annellides (FIGS. 6, 11, 12); (ii) cells proliferating only sympodially, sympodulae (FIGS. 7, 15); (iii) cells proliferating either way (FIGS. 13, 14), only observed in vivo. In *S. quercicola,* cells of the last category may start proliferating sympodially, to continue percurrently on one, occasionally two projections, but most cells proliferate first percurrently one or more times, and then continue sympodially (FIGS. 13, 14). In *S. aceris,* cells proliferate percurrently and sympodially in varying sequences. TEM revealed no phialides as seen with LM in both species. Most likely, the scars in some annellides are to close to be distinguished, and therefore appear in LM as periclinal thickenings characteristic of phialides, as depicted for *S. aceris* in FIG. 12.

Conidial wall structure.—The lateral wall of the conidium consists of two layers. The outer layer is moderately electrondense (FIGS. 6, 7), with an irregular surface strongly reactive in PA-TCH-SP (FIGS. 17, 22, 23). The conidial septa agree in ultrastructure with the delimiting septa (FIGS. 6, 16). The outer layer becomes thinner over the apex of the conidium, and its constituents often loosen from the wall surface in an early stage (FIG. 17).

Conidial appendages.—In *S. quercicola,* noncellular appendages appear after liberation at the conidial apices, where the outer layer has disappeared. These appendages consist of fibrillar to amorphous mate-

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reactive septum wall is continuous with the inner part of the inner layer in the lateral conidial wall. The outer layer (ol) is rough and strongly reactive. 17–20. *Septoria quercicola.* 17. Conidial apex shortly after liberation. The outer layer is partly loosened from the wall over the tip (arrows; PA-TCH-SP; in vivo). 18–20. Conidial apices, later stage (compare FIG. 17), bearing apical, noncellular appendages. 18. Moderately electrondense material originates from a continuous area over the conidial tip, where the outer layer is now absent. At the periphery of the appendage (ap) the constituents appear to be fibrillar and radiating (arrows). The mature sporoplasm contains numerous oil droplets (lp) and very electrondense osmiophilic bodies (Ur/Pb; in vivo). 19. As previous FIGURE, but material of the appendage (ap) originating from a discontinuous area (Ur/Pb; in vivo). 20. Oblique section of conidial apex with a weakly reactive appendage (ap; PA-TCH-SP; on CMA). 21–23. *S. aceris* (PA-TCH-SP, on OA). 21. Basal appendage (a), showing radiating fibrils of reactive material (arrows) in a largely nonreactive matrix. Oil droplets (lp) and large concentrations of glycogen particles (G) fill the sporoplasm. 22. Base of a conidium of similar age. The outer layer (ol) is partly loosened around the truncate base and appears to be disintegrating (arrows). A basal appendage (a) of similar material partly covers basal and lower lateral wall of the conidium. 23. Apex of an older conidium. The apical wall is partly covered by a noncellular apical appendage (a) of material contrasted by uranyl, but nonreactive.

rial, well-contrasted by Ur/Pb (FIGS. 18, 19), but weakly to nonreactive in PA-TCH-SP (FIG. 20). Shortly after secession, noncellular appendages also emerge near the conidial base, first usually on one side (FIGS. 8–10), later often from the whole basal area. Basal appendages in *S. quercicola* consist of material with a similar electrondensity as in the apical ones, but no reactivity in PA-TCH-SP. In *S. aceris,* a minority of the conidia develops noncellular basal, and, less frequently, also apical appendages (FIGS. 21–23), but only in vitro. Some material can already appear near the basal wall before, but most of it appears after complete liberation. Apical and basal appendages consist of material stainable with uranyl acetate, and partly also of fibrillar material reactive in PA-TCH-SP (FIGS. 21, 23).

DISCUSSION

Septoria quercicola and *S. aceris* generally agree with *S. chrysanthemella* in ultrastructure of all important stages of conidiogenesis, viz. holoblastic conidial ontogeny (conidial initiation), structure of the delimiting septum, schizolytic secession, and conidiogenous cell proliferation, which can be both sympodial and percurrent. Single cells proliferating both ways were observed frequently on the host, but never on artificial media, despite elaborate light microscopical examinations. Possibly the constant conditions of temperature, humidity and light maintained in vitro are not conducive to such behavior. Apart from this difference, features of conidiogenesis are stable after isolation. Cells proliferating either way occur in cultures of *S. chrysanthemella,* but this species was not studied in vivo (Verkley, 1997).

As in *S. chrysanthemella,* percurrent proliferation is enteroblastic. After conidial secession, annellides deposit new wall material in the upper part which becomes intermixed with the remnants of the septum wall. The new wall formed is either partly continuous with the inner wall layer of the scar, or merely lines its inner surface. Sympodial proliferation is holoblastic, with complete continuity between new parts of the wall and the old, that are reshaped in the process. As Madelin (1979) already envisaged, modes of blastic (conidial) ontogeny are related to the age and the degree of plasticity of the involved wall parts in the conidiogenous cell.

Conidiogenesis is widely used to refine generic concepts in coelomycete systematics. Most genera have been described as either annellidic or phialidic (Sutton, 1973, 1980; Nag Raj, 1993). The scarce ultrastructural data available indicate that among the 'phialidic' taxa different types exist (Sutton and Sandhu, 1969; Boerema and Bollen, 1975; Buchanan, 1987), and study of more taxa is required to assess the value to systematics. It has been suggested that the annellide and phialide are the extremes of a continuum of developmental types (Cole and Samson, 1979; Minter et al., 1982). However, this is not the case for all 'phialides'. From that perspective, the suggestion by Hawksworth et al. (1995) to regard the term 'phialidic' obsolete cannot be accepted.

Annellations and axial elongation are diagnostic of the annellide (Kendrick, 1971), but both can be difficult to observe in LM (Verkley, 1997). The phialide is widely understood as a nonproliferating conidiogenous cell, with holoblastic ontogeny of the first conidium, and enteroblastic ontogeny of all consecutive conidia (Kendrick, 1971; Cole and Samson, 1979). A collarette and periclinal thickening are often present. Annellides in *S. quercicola* and *S. aceris* can be recognized in LM, except when progression is minimal during proliferations, then they are interpreted as phialides. In that case several wall layers become overlaid around the apex of the conidiogenous cell, visible as periclinal thickenings.

Under the light microscope, the conidiogenous cells of *Phoma* Sacc. and *Ascochyta* appear as typical phialides with collarettes and periclinal thickenings (Punithalingam, 1979; Sutton, 1980). On the basis of TEM, Boerema and Bollen (1975) concluded that *Phoma* has true phialides with fixed loci and *Ascochyta* percurrently proliferating cells with 'annellate collars', showing little progression (elongation). Buchanan (1987) also studied the conidiogenous cells in *Ascochyta pisi* Lib., and showed truly fixed loci in his electron micrographs. He therefore interpreted the cells as phialides instead, with a short collarette formed by rupture of the phialide apex, surrounding a periclinal thickening of up to six wall layers. In *Phoma,* accumulation of such layers was not observed (Boerema and Bollen, 1975). Buchanan (1987) did not show delimiting septa in *A. pisi,* but they appear to be formed on the same level for successive conidia and leave no trace on the conidial base. In contrast, conidia in *Septoria* species as studied here always show a basal scar, and the lateral wall is already differentiated at the time of secession. Moreover, the level of delimiting septa is not fixed.

In a *Cryptosporiopsis* sp. studied by Sutton and Sandhu (1969), the points at which conidia secede are at about (so not exactly) the same level, or, retrogressive. This is also the case in some annellides of *S. quercicola* and *S. aceris.* Sutton and Sandhu (1969) interpreted these conidiogenous cells as 'annellophores', annellides in current sense, not 'annellophore' sensu Wang (1990). In *Cryptosporiopsis* more distant percurrent proliferations also occur, resulting

in up to three distinct scars in LM (Sutton, 1980; Verkley, unpubl. results). Cole and Samson (1979) regarded conidiogenesis in *Cryptosporiopsis* as a special case of phialidic development, but they rather overemphasized the significance of the 'collarette', which arises during secession from a circumscissile rupture of the outer wall of the first conidium above the level of the delimiting septum, not occurring in *Septoria* species studied here. Conidia of *Septoria* and *Cryptosporiopsis* differ from conidia of *A. pisi* and *Phoma* spp. by having persistent basal scars.

Based on differences in conidiogenesis, Sutton (1980) outlined three groups within *Septoria* s.l.: (i) species with 'mainly holoblastic sympodial conidiogenesis', congeneric with *S. cytisi,* including *S. chrysanthemella, S. obesa* Syd., and *S. passerinii* Sacc.; (ii) species with 'phialidic' conidiogenesis, *S. apiicola* Speg. and *S. tritici* Rob., and (iii) species with 'simple holoblastic' conidiogenesis (without proliferation), e.g. *S. adanensis* Petr., and several others. Later it was found that certain species, including the type species *S. cytisi* (Constantinescu, 1984; Farr, 1992) and *S. chrysanthemella* (Verkley, 1997; TEM) of Sutton's first group, are capable of both percurrent and sympodial proliferation. This combination of features characterizes a core group of both pycnidial species, e.g. *S. cytisi, S. chrysanthemella,* and *S. quercicola,* and more typically acervular ones, e.g. *S. aceris.* Species reported to have only 'phialides', e.g. *S. aureocorona* B. Sutton and Pascoe (Sutton and Pascoe, 1987), require further study. If conidiogenesis is not cryptically percurrent (annellidic) but truly phialidic with enteroblastic conidial ontogeny, a segregation of such species from the core group as suggested by Sutton (1980), is feasible.

Some authors also mention the phenomenon of single conidiogenous cells proliferating either way in species of *Septoria* (Constantinescu, 1984; Sutton and Pascoe, 1987, referring to unpublished work by Yip and Punithalingam), but it is unclear whether this occurs in *S. cytisi.* Wingfield (1985) reported that single conidiogenous cells of *Leptographium* Lagerb. & Melin and *Verticicladiella* S. Hughes proliferate both sympodially and percurrently, but later SEM led to the assumption that incomplete secession of annellidic conidia caused an 'optical sympodial illusion' (Wingfield et al., 1991; Wingfield, 1993). The work on *S. chrysanthemella* (Verkley, 1997) and that presented here are the first to illustrate the joint occurrence of both modes by TEM.

Cellular and noncellular appendages are widely used to define genera in coelomycetes (Nag Raj, 1993). Expression of mucoid appendages is inconstant in *S. quercicola* and *S. aceris,* and in the latter none were observed on the natural substratum. According to Nag Raj (1993), some *Septoria* species may in fact belong to *Septoriella* Oudemans, which has been characterized by pycnidial, pycnidioid to indeterminate conidiomata, and conidia with apical and basal mucoid appendages. However, the presence of such appendages should not be given undue significance. *Septoria quercicola* and *S. aceris* do not belong to *Septoriella,* and the ontogeny of appendages is clearly different. According to Nag Raj (1993), appendages of *Septoriella* originate from a small region of the conidial wall delimited at each end of the conidium by a transverse wall layer formed in an early stage (type H, Nag Raj, 1993). The apical end zone undergoes gelatinization and becomes the apical appendage. The basal conidial appendage in *Septoriella* arises differently, a collar of mucus appearing at the base of the conidium (Nag Raj, 1993, p. 41, 42). In *S. quercicola* and *S. aceris* appendages arise from gelatinization of the outer layer of the conidial wall, mostly after liberation. More material is probably excreted through the wall, especially in *S. aceris.* A delimitation of apical or basal conidial walls, as in *Septoriella,* does not occur. At this point, the appendages of these *Septoria* species cannot be assigned to any of the other of Nag Raj's types. This may be due to differences in methods applied. In *Linochorella striiformis* Syd. & P. Syd., a *Septoriella*-like species for which a separate genus was retained by Nag Raj (1993), the mucoid apical appendages arise by eversion of a mucoid sheath (type C), much like in *Tiarosporella graminis* var. *karoo* B. Sutton & Marasas (Roux et al., 1990; TEM). In the latter, a layer of electron translucent material covers the entire conidial body, and is everted upon conidial release to form the apical appendage. Mucoid appendages may play a role in the adhesion of the fungus to a host plant.

Ultrastructural findings have greatly influenced concepts of conidiogenesis, and often proved indispensable to correct interpretations of conidiogenesis in groups of hyphomycetes (Cole and Samson, 1979; Wang, 1990; Wingfield 1985, 1993; Wingfield et al., 1991). The problem of classifying the septorioid fungi is only one of many in coelomycete systematics. Ultrastructural data as presented here can contribute significantly to a balanced generic concept that complies with both phenotypic and genotypic characters.

ACKNOWLEDGEMENTS

Prof. A. J. Verkleij, Dept. of Molecular Cell Biology, EMSA, Utrecht University is kindly thanked for offering access to electron microscopy facilities, and Dr W. H. Müller for practical help. Drs H. A. van der Aa and W. Gams are gratefully acknowledged for critical reading of the manuscript.

LITERATURE CITED

Boerema, G. H., and G. J. Bollen. 1975. Conidiogenesis and conidial septation as differentiating criteria between *Phoma* and *Ascochyta*. *Persoonia* 8: 111–144.

Buchanan, P. K. 1987. A reappraisal of *Ascochytula* and *Ascochytella* (Coelomycetes). *Mycol. Pap.* 156: 1–83.

Cole, G. T., and R. A. Samson. 1979. *Patterns of development in conidial fungi.* Pitman, London. 190 pp.

Constantinescu, O. 1984. Taxonomic revision of *Septoria*–like fungi parasitic on Betulaceae. *Trans. Brit. Mycol. Soc.* 83: 383–398.

Farr, D. F. 1991. *Septoria* species on *Cornus*. *Mycologia* 83: 611–623.

———. 1992. Species of *Septoria* on the Fabaceae, subfamily Faboideae, tribe Genisteae. *Sydowia* 44: 13–31.

Grove, W. B. 1935. *British stem- and leaf-fungi (Coelomycetes).* Vol. 1. *Sphaeropsidales.* Cambridge University Press, London. 488 pp.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, and D. N. Pegler. 1995. *Ainsworth and Bisby's dictionary of the fungi.* 8th ed. CABI, Wallingford. 616 pp.

Jørstad, I. 1965. *Septoria* and septorioid fungi on dicotyledones in Norway. *Skr. Norske. Vidensk.-Akad. Oslo Mat.-Naturvidensk. Kl.* 22: 1–110.

Kendrick, W. B. (Ed.) 1971. *Taxonomy of fungi imperfecti.* University of Toronto Press, Toronto. 309 pp.

Madelin, M. F. 1979. An appraisal of the taxonomic significance of some different modes of producing blastic conidia. Pp. 63–80. *In: The whole fungus.* Ed., B. Kendrick. National Museums of Canada, Ottawa.

McPartland, J. M. 1995. *Cannabis* pathogens XI: *Septoria* spp. on *Cannabis sativa, sensu stricto.* Sydowia 47: 44–53.

Minter, D. W., P. M. Kirk, and B. C. Sutton. 1982. Holoblastic phialides. *Trans. Brit. Mycol. Soc.* 79: 75–93.

Nag Raj, T. R. 1993. *Coelomycetous anamorphs with appendage-bearing conidia.* Mycologue Publications, Waterloo, Ontario. 1101 pp.

Punithalingam, E. 1979. Graminicolous *Ascochyta* species. *Mycol. Pap.* 142: 1–214.

Roux, C., J. Coetzee, and K. T. van Warmelo. 1990. Ontogeny of conidiomata and conidia in *Tiarosporella graminis* var. *karoo*. *Mycol. Res.* 94: 254–262.

Sutton, B. C. 1973. Coelomycetes. Pp. 513–582. *In: The fungi.* Vol. IVa. Eds., G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman. Academic Press, New York.

———. 1977. Coelomycetes VI. Nomenclature of generic names proposed for coelomycetes. *Mycol. Pap.* 141: 1–253.

———. 1980. *The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata.* Commonwealth Mycological Institute, Kew. 696 pp.

———, and G. L. Hennebert. 1994. Interconnections amongst anamorphs and their possible contribution to ascomycete systematics. Pp. 77–100. *In: Ascomycete systematics. Problems and perspectives in the nineties.* Ed. D. L. Hawksworth. NATO ASI series 269. Plenum Press, New York.

———, and Pascoe, I. G. 1987. *Septoria* species on *Acacia*. *Trans. Brit. Mycol. Soc.* 89, 521–532.

———, and ———. 1989. Some *Septoria* species on native Australian plants. *Stud. Mycol.* 31: 177–186.

———, and Sandhu, D. 1969. Electron microscopy of conidial development and secession in *Cryptosporiopsis* sp., *Phoma fumosa, Melanconium bicolor,* and *M. apiocarpum*. *Canad. J. Bot.* 47: 745–749.

Thiéry, J. P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microscopie* 6: 986–1018.

van der Aa, H. A., M. E. Noordeloos, and J. de Gruyter. 1990. Species concepts in some larger genera of the Coelomycetes. *Stud. Mycol.* 32: 3–19.

Verkley, G. J. M. 1992. Ultrastructure of the ascus apical apparatus in *Ombrophila violacea, Neobulgaria pura,* and *Bulgaria inquinans* (Leotiales). *Persoonia* 15: 3–22.

———. 1997. Ultrastructural evidence for two types of proliferation in conidiogenous cells of *Septoria chrysanthemella*. *Mycol. Res.*, in press.

Wang, C. J. K. 1990. Ultrastructure of percurrently proliferating conidiogenous cells and classification. *Stud. Mycol.* 32: 49–64.

Wingfield, M. J. 1985. Reclassification of *Verticicladiella* based on conidium development. *Trans. Brit. Mycol. Soc.* 85: 81–93.

———. 1993. *Leptographium* species as anamorphs of *Ophiostoma*: progress in establishing acceptable generic and species concepts. Pp. 43–51. *In:* Ceratocystis *and* Ophiostoma. Eds., M. J. Wingfield, K. A. Seifert and J. F. Webber. APS Press, St. Paul, Minnesota.

———, B. Kendrick, and P. Schalk van Wyk. 1991. Analysis of conidium ontogeny in anamorphs of *Ophiostoma*: *Pesotum* and *Phialographium* are synonyms of *Graphium*. *Mycol. Res.* 95: 1328–1333.

Wu, W., B. C. Sutton, and A. C. Gange. 1996. Revision of *Septoria* species on *Hebe* and *Veronica* and description of *Kirramyces hebes* sp. nov. *Mycol. Res.* 100: 1207–1217.