PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 8, Part 2, pp. 111–144 (1975)

CONIDIOGENESIS AND CONIDIAL SEPTATION AS DIFFERENTIATING CRITERIA BETWEEN PHOMA AND ASCOCHYTA

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(With 6 Text-figures, 1 Table and Plates 19–29)

New definitions of the form-genera *Phoma* Sacc. and *Ascochyta* Lib., based on developmental criteria, are presented.

Phoma species show phialidic ontogeny. The first conidium is produced within a papillate protrusion of the undifferentiated parent cell; after a conidium secedes the basal part of the papilla remains as a collarette on the conidiogenous cell which may show two or three layers corresponding with the layers of the original papilla. The conidia secede by a three-layered septum and are in principle one-celled, although secondary septation may occur, especially in vivo (0–95%). In vitro under normal laboratory conditions the majority of conidia however always remain one-celled. The pycnidia are usually glabrous but also may be hairy or setose.

Ascochyta species show annellidic ontogeny. The conidia arise as relatively thin-walled protrusions from undifferentiated parent cells. The secession of successive conidial primordia by a three-layered septum however may occur at approximately the same level, resulting in an increasing collar of periclinal annellations, which under the light microscope looks like the collarette of a phialide. After or incidentally also before secession the conidia become two- (or more-) celled by invaginations of a newly produced inner wall layer (distoseptation). In this genus therefore conidial septation is an essential part of the conidium completion, which explains that in vivo as well as in vitro the conidia are always mainly two- (or more-) celled. The pycnidia are glabrous or sometimes hairy.

These new generic concepts imply that most of the species usually placed in *Ascochyta* viz. those in *Ascochyta* sect. *Phyllostictoides* Zherbele, belong to *Phoma* as are many species at present placed in *Phyllosticta*, *Diplodina* and *Pyrenochaeta*. *Pyrenochaeta mali* Smith is shown to be identical with *Phoma herbarum*. For *Pyrenochaeta acicola* (Lév.) Sacc. the new name *Phoma leveillei* is introduced.

INTRODUCTION

In the Saccardoan system of classification of *Phoma*-like pycnidial fungi considerable emphasis was placed on the substratum and the presence or absence of septa in the conidia. In general *Phoma* was used for species with one-celled hyaline conidia

growing on stems and twigs of vascular plants, and *Phyllosticta* for similar fungi on leaves. Species with two-celled hyaline conidia occurring on stems and twigs were placed in *Diplodina*, whereas similar states growing on leaves were included in *Ascochyta*. Of course many *Phoma*-like fungi occur as well on leaves as on stems or twigs and besides in many cases both one-celled and two-celled conidia are found in one pycnidium. This chaotic situation has lead to various *Phoma*-like fungi having synonyms in all four genera (compare van der Aa & van Kesteren, 1971, and Boerema & Dorenbosch, 1973).

With the necessary revision of this unreal classification the first principle has to be that in accordance with the International Code of Botanical Nomenclature, genera are characterized by their type-species. Recent studies of the relevant type-species have opened startling new perspectives.

The lectotype-species of *Phyllosticta* Pers. ex Desm. (1847; nom. cons.), *P. cruenta* (Kunze ex Fr.) Kickx causes leafspots of *Polygonatum* spp. and has recently been studied in detail by van der Aa (1973) and Punithalingam (1974). It represents a separate group of pycnidial fungi formerly commonly known under the name *Phyllostictina* Sydow and characterized by relatively large conidia with an apical appendage. This means that species of *Phyllosticta* sensu stricto are quite different from *Phoma* species. Van der Aa (l.c.) distinguished 46 species of *Phyllosticta* of which 12 are now known to belong to the Ascomycetous genus *Guignardia* Viala & Ravaz. He showed that the ascigerous state of *Phyllosticta cruenta* is *Guignardia reticulata* (DC. ex Fr.) van der Aa. Most *Phyllosticta* species also produce a spermatial state which is classified in *Leptodothiorella* Höhnel.

According to Boerema (1970a) the type-species of *Diplodina* Westend. (1857), *D. salicis* Westend., represents the conidial state of *Cryptodiaporthe salicella* (Fr.) Petr., the causal fungus of branch canker of willow. This conidial state also formed the basis of *Discella* Berkeley & Broome (1850), which therefore has priority. Its type-species, *Discella salicis* (Westend.) Boerema is characterized by excipuliform (cup-shaped, dish-shaped) fruit-bodies with fusoid two-celled conidia. This means that *Diplodina* Westend., as a later synonym of *Discella* Berk. & Br., is not available for *Phoma*-like fungi. According to von Arx (1970) there are about 10 species of *Discella*, all representing conidial states of *Cryptodiaporthe* Petrak.

The characteristics of the lectotype-species of *Phoma* Sacc. (1880; nom. cons.), the ubiquitous saprophyte *P. herbarum* Westend., and the selected type species of *Ascochyta* Lib. (1830), the well-known pea-parasite *A. pisi* Lib., have been comparatively studied by Brewer & Boerema (1965) with the aid of the electron microscope. Both species have similar pycnidia but show substantial differences in conidiogenesis.

In mature pycnidia of *Phoma herbarum* and other typical *Phoma* species (cf. Boerema & Dorenbosch, 1973) conidia arise successively as 'buds' on somewhat cuspidate but otherwise undifferentiated cells lining the pycnidial cavity. The 'bud' of the first conidium originates from a papillate extension; successively produced basipetal conidia arise as 'buds' from the conidiogenous locus which is then surrounded by a distinct collarette. With this kind of conidiogenesis — characterized by Boerema

(1965) as a 'monopolar repetitive budding process' — the wall of the conidial primordia almost immediately attains its final thickness. The differentiation of the conidial wall is associated with abundant production of mucilaginous material. Independently of conidiogenesis in some conidia, septation may occur as an annular ingrowth from the lateral wall. In *P. herbarum* septate conidia are infrequent, but with other *Phoma* species in vivo a high percentage of the conidia may become two-celled.

In *Ascochyta pisi* the conidia are also formed in basipetal succession on undifferentiated inner cells of the pycnidial wall. In this case, however, the first conidium as well as successive conidia arise as long, extremely thin-walled protrusions at the apex of the parent cell. The secession of each successive conidium occurs usually at a somewhat higher level than the previous conidium, leaving the top of the parent cell with a series of scars (wall ridges) corresponding with the number of conidia that have seceded. After detachment, or incidentally also before secession, against the innerside of the initial conidial wall a new wall-layer arises, simultaneously dividing the conidia into two (or more) cells. This 'distoseptation'-process (term coined by Luttrell, 1963) is associated with the production of mucilaginous material.

These differences in conidiogenesis between *Phoma* and *Ascochyta*, however, are very difficult to observe with the light microscope. Especially since further study has shown that in *Ascochyta* spp. successive conidia may also secede at approximately the same level as the first conidium, which then results in an increasing collar of wall material, under optical microscopy similar to the collarette of *Phoma* spp. The differences in septation are also very difficult to observe with a light microscope. However the fact that septation in *Phoma* is a secondary process whereas in *Ascochyta* the septation of the conidia is an essential part of the 'finishing' (completion) of conidial-development offers a simple method for distinguishing species of both genera (Boerema, 1970b). It appeared that *Phoma* species, the pycnidia of which in vivo may contain a variable percentage of septate conidia ('pseudo-Ascochytas'), in agar-cultures under normal laboratory conditions always produce mainly one-celled conidia. To induce formation of conidia with more than one cell special 'experimental conditions' depending on the species involved, are needed. True *Ascochyta* species, however, always produce mainly two- (or more-) celled conidia in vitro. Cultural studies by Zherbele (1971),[1] have shown that the number of true Ascochytas is relatively small. They are true parasites and confined to few plant families.

In summary it can be said that recent studies of type-species showed that

i. only a small number of the more than 2000 described Phyllostictas are genuine species of *Phyllosticta* Pers. ex Desm.,
ii. a small number of the about 1300 described Ascochytas and Diplodinas concern true species of *Ascochyta* Lib.,

[1] Zherbele (l.c.) classes the species with mostly two-celled conidia in *Ascochyta* 'section *Stagonosporoides*'. The species producing in culture mainly one-celled conidia are placed in *Ascochyta* 'section *Phyllostictoides*'.

iii. most of the more than 5000 species described in *Phoma, Phyllosticta, Ascochyta* and *Diplodina* refer to species of *Phoma* Sacc.

Many *Phoma* species are plurivorous, which means that the number of species of this form-genus is much smaller than is suggested by the numerous descriptions in the literature. Only cultural studies in comparison with characteristics in vivo can solve the immense problem of synonymy and nomenclature of *Phoma* species.

The present study is a continuation and an elaboration of earlier work by Brewer & Boerema (1965). Their observations are checked and emended on the basis of electron micrographs of seven species of *Phoma* and three species of *Ascochyta*. Special study is made of the mechanism of conidial secession and septation in both genera and the development of the first conidium in *Phoma* spp. Observations on the germination of conidia are also included. In three schemes a survey is given of the processes of conidiogenesis, septation and germination in both genera.

In the discussion the characteristics of conidial development in both genera are further analysed, compared with electron microscopy observations in other fungi, and defined in accordance with modern terminology of conidial ontogeny as approved by the 'First international specialists workshop-conference on criteria and terminology in the classification of Fungi imperfecti' (Kendrick, 1971).

Finally redefinitions are given of the genera *Phoma* Sacc. and *Ascochyta* Lib.

MATERIALS AND METHODS

Specimens used.

The *Phoma*-type of conidial development was studied on the type-species of *Phoma* and six other form-species. Two represent conidial states of *Didymella* species and one concerns the conidial state of a *Leptosphaeria* species.

In cultures on agar media at 20–22 °C the mature pycnidia contain only or mainly one-celled conidia; however in other conditions and especially in vivo the mature pycnidia of some of these form-species may contain a variable percentage of two-celled, or even three-celled conidia.

1. *Phoma herbarum* Westend. At the 8th International Botanical Congress at Paris (1954) this species was selected as the type-species of the form-genus *Phoma* Sacc. (see Boerema 1964, 1970b). It is a ubiquitous saprophyte with usually only one-celled conidia. The pycnidia used in this study were taken from agar-cultures of an isolate made from bathroom paintwork (dried culture: L 972.109–054).

2. *Phoma chrysanthemi* Vogl., the conidial state of *Didymella chrysanthemi* (Tassi) Garibaldi & Gullino (1971). This fungus is known as the cause of (flower) ray blight of *Chrysanthemum morifolium* cultivars, though the stems and leaves of the plants may also be attacked. A large percentage of the conidia may become two-celled (synonym: *Ascochyta chrysanthemi* F. L. Stevens), depending among others on the temperature (Blakeman & Hadley, 1968). The pycnidia used were taken from agar-cultures of isolates obtained from stems of chrysanthemums (CBS 376.67 and CBS 729.74).

3. *Phoma complanata* (Tode ex Fr.) Desm., a very common species on Umbelliferae (see Grove, 1935) producing usually only one-celled conidia. It is the type-species of *Sclerophomella* von Höhnel (1918). The pycnidia studied were obtained from agar-cultures of the fungus obtained from stems of *Angelica archangelica* (CBS 633.68).

4. *Phoma exigua* Desm. (var. *exigua*), the most frequent *Phoma* species on herbaceous plants. As a weak or wound parasite it is often associated with distinct disease symptoms such as leaf spots, fruit spots, lesions on stems and roots, damping-off, and dieback, see Boerema & Höweler (1967) and Boerema & Dorenbosch (1973). In vivo a large percentage of the conidia may become two-celled (synonym e.g.: *Ascochyta phaseolorum* Sacc., cf. Boerema, 1972), but in vitro most conidia remain one-celled. Conidiogenesis was studied in pycnidia from a potato-stem (*Solanum tuberosum*) and an agar-culture obtained from fruit rot of tomato (*Lycopersicum esculentum*) respectively (L 972.109–045 and dried culture L 970.35–200).

5. **Phoma leveillei** nom. nov.,[2] well known as *Pyrenochaeta acicola* (Lév.) Sacc., a common saprophytic soil-fungus characterized by the occurrence of setae on the pycnidia, see Dorenbosch (1970). The conidia are usually one-celled and are produced on undifferentiated parent cells and not on elongated septate conidiophores as in true species of *Pyrenochaeta* De Not. (see the monographic study of this form-genus by Schneider, 1975). The pycnidia used were taken from agar-cultures of an isolate obtained from soil (CBS 536.66).

6. *Phoma lingam* (Tode ex Schw.) Desm. the conidial state of *Leptosphaeria maculans* Desm.) Ces. & De Not., a well-known seed-borne parasite of *Brassica* species, especially turnip, swede, broccoli and cabbage. The disease is known as dryrot and canker or black leg, but the fungus also causes leaf spots. The conidial state occurs in different phenotypes (Boerema & van Kesteren, 1964): pycnidia with common pseudo-parenchymatous wall structure (type I) and thick-walled pycnidia with a typical pseudo-sclerenchymatous structure (type II) similar to that of the pseudothecia of the perfect state. Pycnidial states with this character may be placed in a separate form-genus *Plenodomus* Preuss (1851), of which *P. lingam* represents the type-species (Boerema & van Kesteren, 1964). However, the fact that conidiogenesis in *P. lingam* without doubt is '*Phoma*-like', militates in favour of a classification under *Phoma* as already adopted by von Arx (1970): '*Phoma* section *Plenodomus*'. The conidia of *P. lingam* are usually one-celled. The pycnidia used in this study (type I) were taken from agar-cultures of an isolate made from stems of swede (CBS 532.66).

[2] Based on *Vermicularia acicola* Léveillé *in* Annls Sci. nat. (Bot.) III **9**: 259. 1848; neotype: dried culture of CBS 260.65 (Dorenbosch, 1970). A new combination with this basionym would result in a later homonym of *Phoma acicola* (Lév.) Sacc. ≡ *Sphaeropsis acicola* Lév., a different fungus.

Various otherwise typical *Phoma*-species occasionally produce pycnidia with 'setae', which may be sparse or numerous, stiff or rather hyphal-like and either short or relatively long. In our opinion this feature has to be considered only as a species-character. Smith (1963) has even described a strain of *Phoma herbarum*, obtained from spots on apples, producing setose pycnidia under the name *Pyrenochaeta mali* Smith (cf. the type-culture of *P. mali*, CBS 567.63; see also Schneider, 1975).

7. *Phoma lycopersici* Cooke, the conidial state of *Didymella lycopersici* Kleb., a well known parasite of tomato plants (*Lycopersicum esculentum*). The disease is known as stem rot (canker), although the fungus can also cause fruit rot and leaf infections. Besides one-celled conidia, two-celled ones can also occur in mature pycnidia, especially in vivo (synonym: *Ascochyta lycopersici* Brun.), see Boerema & Dorenbosch (1973). The pycnidia studied were obtained from tomato-stems and agar-cultures of the fungus (CBS 735.74).

The *Ascochyta*-type of spore development was studied on the type-species of *Ascochyta* and two other form-species of which one represents the conidial state of a *Mycosphaerella* sp.

1. *Ascochyta pisi* Lib., the lectotype-species of the form-genus *Ascochyta* Lib. (Diedicke, 1912, Clements & Shear, 1931; Sprague & Johnson, 1950; von Arx, 1970). A specialized seed-borne parasite of the pea (*Pisum sativum*). The disease is known as leaf and pod spot. It produces mainly two-, and occasionally three- or even four-celled conidia in vivo as well as in vitro, see Boerema & Dorenbosch (1973). The pycnidia studied were obtained from infected pea seeds (L 972.109–071).

2. *Ascochyta fabae* Speg., a seed-borne parasite of the broad bean (*Vicia faba*), which incidentally may occur on other Leguminosae. The disease is known as leaf spot, though the pods may also be attacked. It produces mainly two-, and occasionally three- or even four-celled conidia in vivo and in vitro, see Boerema & Dorenbosch (1973). The pycnidia studied were taken from agar-cultures of an isolate from infected pods of broad bean (CBS 649.71).

3. *Ascochyta pinodes* L. K. Jones, the conidial state of *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr. This seed-borne parasite is known as the cause of foot rot and leaf and pod spot of pea (*Pisum sativum*), but is also recorded from other plants. In vivo and in vitro the conidia are mainly two-celled (sometimes three- or even four-celled), see Punithalingam & Holliday (1972c). The pycnidia used in this study were taken from agar cultures made of an isolate from infected pea-pods (dried culture: L 974.306–481).

Methods.

Pycnidia were obtained from agar plates (cherry agar, pH 4.0, and oatmeal agar, pH 6.4) exposed to near ultra-violet light, and also from diseased plant material.

To allow penetration of fixatives in most cases the pycnidia needed to be crushed on the substrate. Removal of conidia from the pycnidial fragments by the fixative was prevented by carefully covering the crushed pycnidia with a layer of water agar prior to fixation. The agar blocks (c. 2 mm^3) containing crushed pycnidia were fixed. Fixation was carried out by one of the following schedules:

1. Prefixation with 6% glutaraldehyde, buffered with Sörensen's solution (pH 7.0) for 2 hrs. at room temperature. The small pieces were then transferred to fresh buffer and rinsed, followed by postfixation in 1% OsO_4 buffered with Sörensen's solution (pH 7.0) or with veronal acetate (pH 7.4) for 4–5 hrs. at room temperature. This

schedule was used for specimens shown in the micrographs of Pls. 19A, B; 20B–D; 21A; 22D, F; 23C; 24C, D; 25C, D; 26A–E; 27A–E; 28C, D and 29A–C.

2. Prefixation with unbuffered 2% $KMnO_4$ for a period varying for the different specimens from 5 to 30 min. at room temperature. The fixed material was rinsed in fresh veronal acetate buffer (pH 7.4), followed by fixation with OsO_4 as indicated for (1). This schedule was used for specimens shown in the micrographs of Pls. 19C–E; 20A; 21D, E; 22A–C, E; 23A, B, D; 24A, B; 25A, B; 26D and 28A, B.

3. Fixation in unbuffered 2% $KMnO_4$ for 60 min. at 4 °C. The specimens were rinsed in several changes of distilled water, followed by staining in 0.5% uranyl acetate for 4 hrs. at room temperature. This schedule was used for specimens of *P. lycopersici* shown in the micrographs of Pls. 20E; 21B and C.

The application of different fixatives may explain why in the micrographs conidial walls and septa are sometimes electron-transparant and structureless and in other cases rather electron-dense with contrasting structure. For the same reason membranes and mucilaginous substances can clearly be discerned in some micrographs but not in others.

Following fixation, specimens were washed in buffer solutions, dehydrated in graded series of ethanol and embedded in 2:3 styrene butyl methacrylate at 60 °C, except for some specimens of *Phoma chrysanthemi* and *P. lycopersici* where epoxy resin was used.

Sections were cut with glass and diamond knives and examined in a Philips EM 100 and EM 300 electron microscope.

Germinated conidia were obtained by placing inverted plugs taken from agar plates with sporulating colonies onto the moist surface of water agar plates. After incubation for 16 hrs. at 18 °C germ tubes had been formed. Then the inverted plugs were removed and the plate was flooded with warm malt agar (c. 45 °C).

The difference in colour between malt agar and water agar facilitates the localization of the level at which germinated conidia were present. Small agar blocks (c. 2 mm^3) with germinated conidia were trimmed and fixed as indicated above.

ABBREVIATIONS USED IN FIGURES AND PLATES. — col, collarette. — cs, cloudy substance. — fr, basal frill. — gt, germ-tube. — il, inner layer of wall. — m, plasma membrane. — mcl, mucilage. — ml, middle layer of wall. — ol, outer layer of wall. — p, pore. — pl, plug. — pw, primary wall. — s, mucilaginous sheath. — sp, septal-plate. — srp, separation-plate. — sw, secondary wall. — ts, triangular space. — Wb, Woronin body. — wc^1, wc^2, etc., walls of successive conidia. — wr^1, wr^2, etc., annellations of successively seceded conidia.

RESULTS

The Phoma-type of conidial development.

In mature pycnidia conidia are produced by small parent cells which are usually indistinguishable from the inner cells of the pycnidial wall but for a single apical aperture. Each parent cell can produce a whole series of conidia (compare Pl. 19A);

under unfavourable conditions conidiogenesis may stop, but start again when conditions change. Finally the parent cells collapse and are replaced by new ones (compare Ciccarone & Russo, 1969).

The development of the first conidium by a parent cell is initiated by a papillate pronounced thickening of the wall at the top of the cell (Pls. 19B, C; 20A; Fig. 1A; see also Sutton & Sandhu, 1969 fig. 11, and Ciccarone & Russo, l.c. fig. 6). Within the papilla wall three layers can be distinguished, an outer almost electron transparent layer (ol) which is continuous with the wall of the parent cell, a thick, relatively electron dense middle layer (ml) which in cross section appears to be falcate, and an electron transparent inner layer (il). The plasma membrane adjoining the inner layer always shows a corrugation which is apparently associated with a concentration of elements of the endoplasmatic reticulum (compare Pl. 19D, E and Brewer & Boerema, 1965). Subsequently the papilla bulges outwards forming a bud-like protrusion (Pl. 19D, E; Fig. 1B). The outer layer (ol) sooner or later for the most part dissolves into mucilage (Pls. 19E; 20A; Fig. 1B). In some species, e.g. *Phoma exigua*, the middle layer (ml) becomes more prominent at the outset (Pl. 19E; Fig. 1B) and appears as if it will develop into the conidium wall (compare Brewer & Boerema, l.c.). The wall of the first conidium primordium (wc^1) however arises from the inner layer (il). The middle layer (ml) may have a function as an 'opener' of the conidiogenous locus of the parent cell. Like the outer layer it finally dissolves into mucilage. In some species it remains at first recognizable as a sheath surrounding the first conidium (Pl. 20A; Fig. 1C–a). In other species it seems to dissolve synchronously with the upper part of the outer layer or even earlier (Fig. 1C–b; compare Ciccarone & Russo, l.c. fig. 7). The process of wall differentiation — modification from homogenous wall substance into different outer, middle, and inner wall layers — is associated with increasing production of an electron transparent cloudy substance (cs) (Pl. 20A; Figs. 1, 2).

Conidium secession proceeds very rapidly; it looks often if the conidia are 'pinched off' (Pl. 20A, compare Brewer & Boerema, l.c.), especially because the cytoplasma of the parent cell may contract immediately after secession. However, from numerous micrographs (e.g. Pl. 20B–D) it is evident that secession is initiated by centripetal development of a very thin electron-transparent layer (Fig. 2E, F). Separation

Fig. 1. *Phoma* spp. Diagrammatic representation of our electron microscopy interpretation of the formation of the first enteroblastic phialidic conidium (A→D).

The wall of the first conidium (wc^1) arises within the inner layer (il) of the papillate thickening of the wall at the top of the parent cell (A) (m= plasma membrane). The differentiation of the conidial wall in the following bud-stage (B, C) is associated with the production of a mucilaginous cloudy substance (cs). The middle layer (ml) of the papilla seems to function as an 'opener' of the conidiogenous locus; later on it dissolves into mucilage and forms the outer layer of the mucilaginous sheath (s) around the first conidium (D). The upper part of the outer layer of the papilla (ol) sooner or later disintegrates completely (C-a, C-b). After secession of the first conidium (D) the basal part of the original papilla-wall remains as a collarette (col); sometimes the different layers of the papilla (ol, ml, il) are still recognizable in the collarette (wc^2= wall second conidial initial).

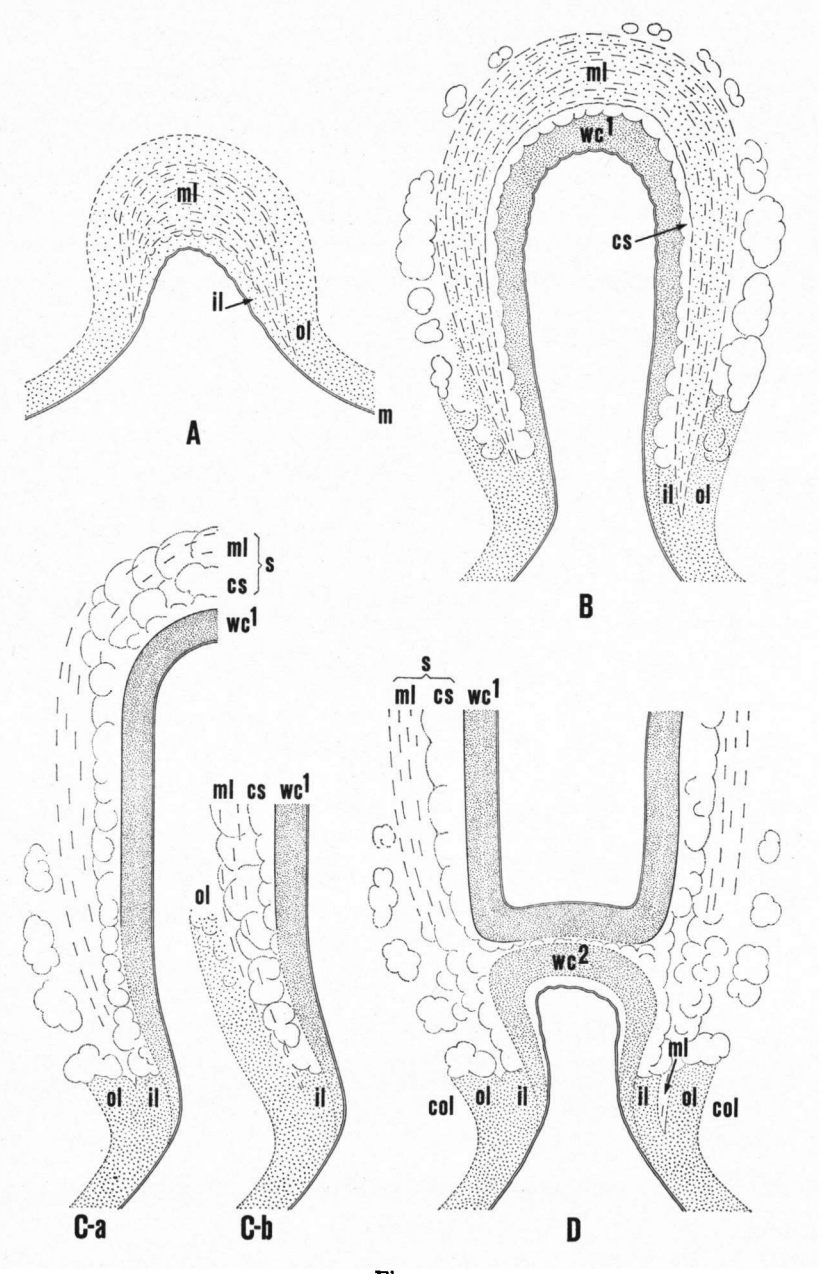
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Fig. 1

apparently occurs along this layer, indicated as separation-plate (srp). Almost simultaneously the basal wall of the conidium is produced, whereas the top of the parent cell also becomes closed by wall substance (Pls. 20E; 21A–C; Fig. 2F). The flattened wall at the base of the conidium always runs parallel to the thin separation-plate. This may also be the case with the wall material covering the parent cell (compare Pl. 21A, D; Fig. 1D), but — as already noted — often the cytoplasm of the parent cell contracts and the closing wall material is produced somewhat lower leaving sometimes plasma rests in the disorganized area between conidium and parent cell (Pls. 20E; 21B, C; Fig. 2F). At secession the separation-plate (srp) and the connecting periclinal wall areas disintegrate to mucilage (Pl. 21D; Figs. 1D, 2G), covering the next primordium (mcl).

The secession of the first conidium leaves the parent cell with a distinct collarette consisting of the undissolved basal parts of the papilla wall. Sometimes the different layers of the original papilla can also be recognized in the collarette (compare Pls. 20E; 21B, E and Figs. 1D, 2G). The collarette surrounds the fixed conidiogenous locus from which the second and subsequent conidia arise (Pl. 19A).

Apart from the papilla the development of subsequently produced conidia is the same as for the first conidium. They arise as outgrowths of the wall closing the parent cell after secession of the preceding conidium (Fig. 1D→Fig. 2E–G). The primordia are covered by some mucilaginous material (mcl, see above) whereas differentiation of the conidial wall is again associated with the production of a cloudy mucilaginous substance (cs).

It should be noted that the conidial wall in this conidiogenous process almost immediately attains its final thickness (see e.g. Pl. 20A) and that the abundant mucilaginous mass surrounding the mature conidia apparently originates partly from dissolved wall material (papilla wall or separation-plate with connecting peri-

Fig. 2. *Phoma* spp. Diagrammatic representation of our electron microscopy interpretation of (i) second and successive enteroblastic phialidic conidial development (E→G), (ii) frequently occurring secondary septation of the conidia (H→J), and (iii) conidial germination (K).

The second and successive conidia (wc^2, wc^3 etc.) arise as buds from the conidiogenous locus which is surrounded by a collarette (col), being the remnants of the papilla originally enclosing the first conidial primordium (ol, ml, il, see Fig. 1). Differentiation of the conidial wall is associated with production of a mucilaginous cloudy substance (cs). The conidia secede by separation of a three-layered septum. The initial separation-plate (srp) and the periclinal wall parts disintegrate into mucilage (mcl) which then covers the next primordium and will form the outer layer of the mucilaginous sheath (s) of the next conidium (m= plasma membrane).

Secondary septation of the conidia (H→J) occurs as an annular ingrowth from the lateral wall leaving a pore (p) in the centre. The septa consist of a middle lamella, the septal-plate (sp), at both sides covered with wall layers which for some distance are 'attached' to the lateral conidium wall.

Germination of the conidia (K) is initiated by a swelling. Then at the innerside a new layer can be distinguished which emerges through the ruptured wall of the conidium. Differentiation of the wall of the germ-tube (gt) is associated with the production of a mucilaginous cloudy substance (cs).

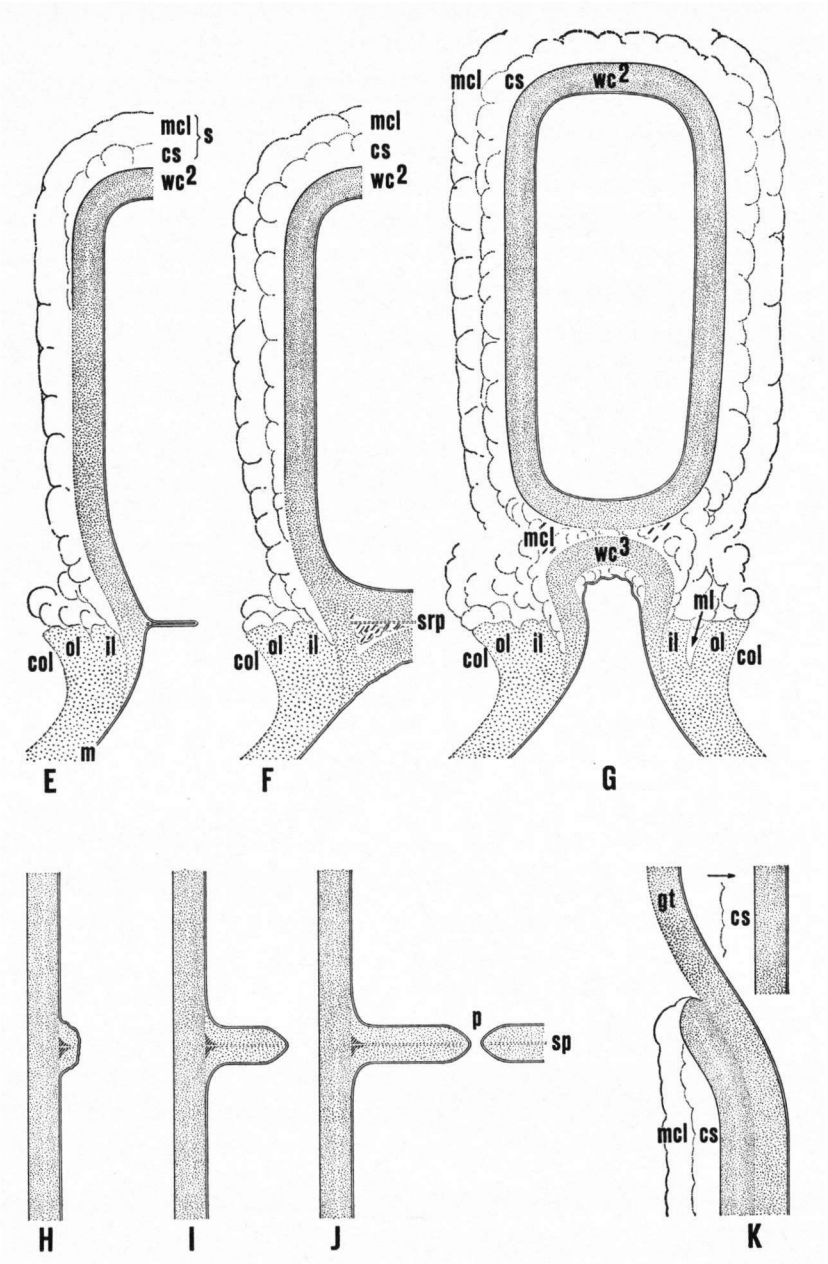


Fig. 2

clinal wall parts) and partly from a substance produced during conidial wall differentiation (cs).

In many *Phoma* species some conidia may become two celled. This occurs especially in vivo. The septation process proceeds rapidly, so only a few micrographs of the first stages of this process have been obtained (Pl. 22A–D). It appeared that septation occurs as an annular ingrowth from the lateral wall, which apparently from the start attains the thickness of the final septum. The process seems to be initiated by the formation of a very thin highly electron-transparent layer — the septal-plate (sp) — arising more or less perpendicularly from the lateral wall (Pl. 22B–D, F; Fig. 2H, I). Coincidently at both sides of the septal-plate electron-dense wall layers occur. In vertical section they appear decurrent with the periclinal wall to which they are 'attached' with decreasing thickness for some distance (Pl. 22E). Between the decurrent edges of these layers, the periclinal wall and septal-plate triangular 'spaces' (ts) occur, which differ in electron-density (usually more electron-dense: Pl. 22F). In the centre of the septum a pore remains (p; in most sections of course missed) (Fig. 2J) and it is associated with Woronin bodies (Wb) and membrane bounded plugs (pl) (Pl. 23A, C). Incidentally also a microporus has been observed in the septum (Pl. 23B). It should be noted that apart from the area near the septum, the wall of a septate conidium is apparently not thicker than that of a non-septate conidium.

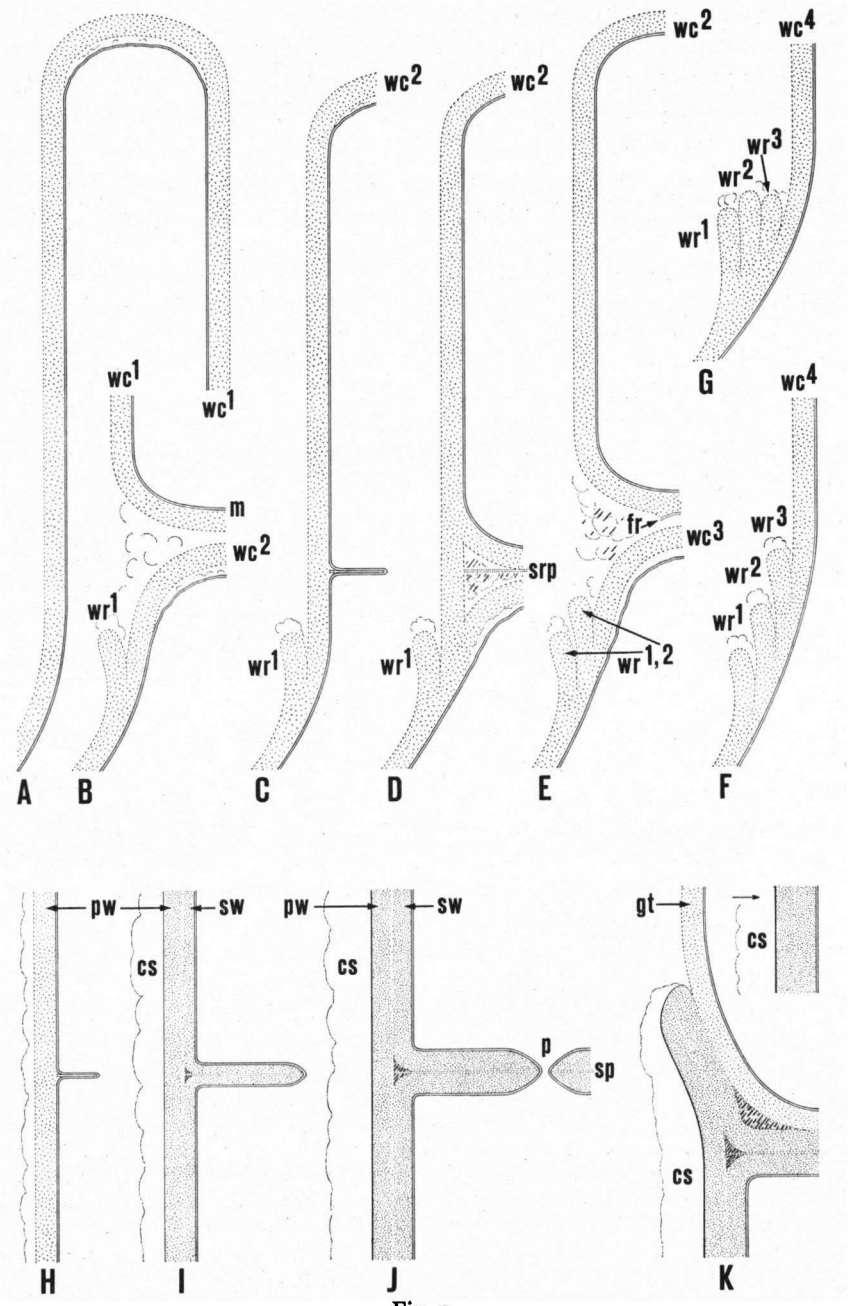
With germination (Pl. 23A, D; Fig. 2K) the conidia swell and a new electron-transparent layer can be distinguished inside the conidial wall and is continuous with the germ-tube which emerges through the ruptured outer wall of the conidium.

Fig. 3. *Ascochyta* spp. Diagrammatic representation of our electron microscopy interpretation of (*i*) annellidic conidial formation (A→G), (*ii*) distoseptation of the conidia (H→J), and (*iii*) conidial germination (K).

The first conidium arises as a long thin-walled protrusion (wc^1) at the top of the parent cell (A). When the first conidium secedes, the parent cell remains with a scar or annellation (wr^1) and the formation of the next conidium (wc^2) starts as a percurrent proliferation (B) (m= plasma membrane). The conidial secession (C→D: secession of second conidium) takes place by a three-layered septum and is initiated by the development of a thin separation-plate (srp). Transverse splitting along the separation-plate and circumscissile rupture (schizolytic) of the outer wall release the conidium. A part of the separation plate may be attached to the seceded conidium (E: fr = basal frill). The secession of each successive conidium may occur at some higher level (F), but also at approximately the same level (G) which then results in a gradually thickening collar, the annellated collar (wr^{1-3}=annellations of successively seceded conidia; wc^4=wall of fourth conidium).

The septation of the conidia (H→J) occurs by invagination of a secondary developing inner wall (sw; pw=primary wall): distoseptation. The invagination is initiated by the development of a septal-plate (sp). In the centre of the septum remains a pore (p). The process of distoseptation is associated with an abundant production of a mucilaginous cloudy substance (cs). (compare Fig. 4).

With germination (K) the conidia swell and a new layer can be distinguished inside the conidial wall which is continuous with the germ-tube (gt) which emerges through the ruptured wall of the conidium. The wall of the germ-tube thickens secondarily (probably distoseptation).


wc2
wc4
wr3
wr2
wr1
G
wc1
wc1
m
wc2
wr1
wr1
wc2
wc2
srp
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fr
wc3
wr1,2
wc4
wr3
wr2
wr1
A
B
C
D
E
F
pw
sw
cs
pw
sw
cs
p
sp
gt
cs
cs
H
I
J
K

Fig. 3

The Ascochyta-type of conidial development.

Conidia arise in succession on small undifferentiated parent cells of the meristematic tissue lining the pycnidial cavity. Under unfavourable conditions conidiogenesis may stop but start again when conditions are improved. New parent cells may replace old exhausted ones which then collapse.

The conidial initials are more or less oblong and have in comparison with mature septate conidia a thin wall (see e.g. Pls. 24A, B; 25A, B). They arise apparently simply as outgrowths of the wall at the apices of the parent cells (Pl. 24C, D; Fig. 3A, C). As in *Phoma* spp. the growing activity is associated with a corrugation of the plasma membrane and a concentration of endoplasmatic reticulum.

When the thin-walled protrusion has attained the (variable) dimension of a mature conidium the process of secession starts (Pl. 25A–D; Fig. 3C, D). This proceeds rapidly, which has made it very difficult to get pictures of the first stages. Nevertheless it was established that as in *Phoma*, three centripetally developing layers are involved: the basal conidial wall, the separation-plate (srp) and the wall closing the top of the parent cell. The initial separation-plate is very thin and highly electron-transparent just as the septal-plate (see below); it is only discernible in few micrographs (Pl. 26A, B). The basal conidial wall and the wall closing the parent cell often develop both close to and parallel with the separation-plate. Sometimes, however, the cytoplasma of the parent cell contracts and the wall closing the parent cell is partly or completely produced at some distance from the basal conidial wall; in the disorganized area between both walls then plasma rests may occur (Pl. 25C; Fig. 3D). Separation apparently takes place along the separation-plate (Pl. 25D); the periclinal walls rupture and the parent cell remains with a broken periclinal wall (wr) and a thin closing wall protruding from the inside of the periclinal wall (Fig. 3B, E). Incidentally the detached conidia show a basal frill (Pl. 25B: fr) which seems to be part of the separation-plate (compare Fig. 3E). The apical region of the closing wall of the parent cell will form the next primordium (Fig. 3E). The secession of each successive conidium may occur at a higher level than the previous conidium. This is usually the case with *Ascochyta pisi*. The top of the parent cell then becomes encircled with a series of wall ridges corresponding with the number of conidia that have seceded (Pls. 24A, B; 25A, B; Fig. 3F). Successive conidia, however, may also secede at approximately the same level as the first conidium. This is commonly the

Fig. 4. Line drawings of conidia and conidiogenous cells (phialides) of *Phoma* spp. as seen with the light microscope. — A. *Phoma herbarum* (after Sutton, 1964 figs. 4A, B, as *P. herbarum* var. *lactaria* Sutton, see Boerema, 1970b). — B. *Phoma exigua* (after Boerema & Höweler, 1967 figs. 3, 4). — C. *Phoma cucurbitacearum* (Fr.) Sacc. (after Punithalingam & Holliday, 1972a figs. C, D, as conidial state of *Didymella bryoniae* (Auersw.) Rehm, see Boerema & van Kesteren, 1972). — D. *Phoma tracheiphila* (Petri) Kantschaveli & Gikachvili (after Punithalingam & Holliday, 1973b fig. C, as *Deuterophoma tracheiphila* Petri, see Ciccarone, 1971). — E. *Phoma terrestris* Hansen (after Punithalingam & Holliday, 1973a fig. C, as *Pyrenochaeta terrestris* (Hansen) Gorenz & al., see Schneider, 1974). Note that the septate conidia in *Phoma* spp. (in vivo 0–95%) under optical microscopy cannot be distinguished from *Ascochyta*-conidia.

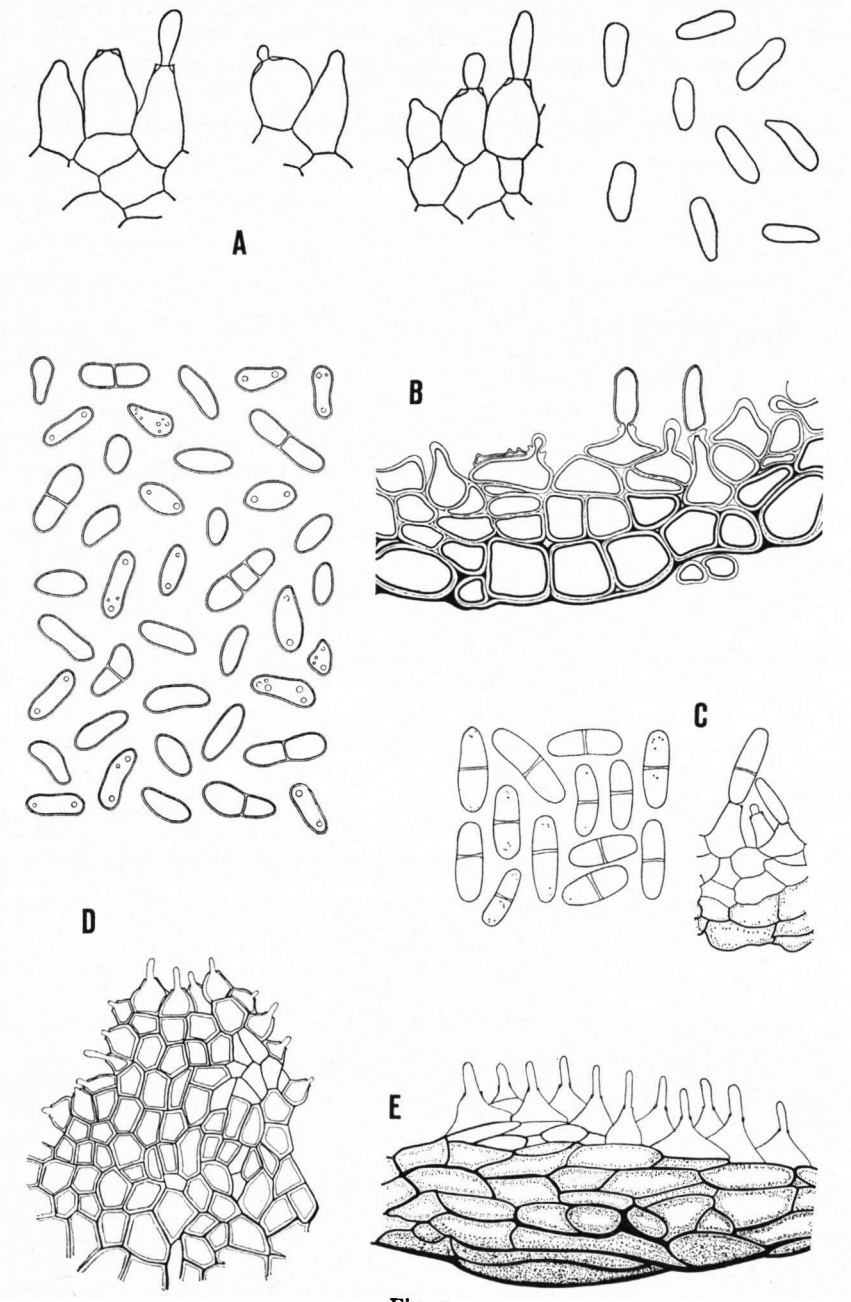

Fig. 4

case with *Ascochyta pinodes*. The top of the parent cell then shows a collar of periclinal wall ridges (Pl. 26C; Fig. 3G).

Immediately after detachment the conidia gradually become thick-walled and septate by the production of a new distinct inner wall (secondary wall: sw), see Fig. 3H–J. This process of wall-thickening and septation is associated with the production of a light cloudy substance (cs) representing the slime surrounding mature conidia (Pl. 26D, E). Most conidia form one septum but two or even three septa may be produced as well. Septation is initiated by centripetal development of a thin highly electron transparent layer, the septal-plate (sp). The development of this septal-plate occurs synchronously with the thickening of the new inner wall, which also covers the septal-plate (Pls. 27A–E; 28A, B; Fig. 3H–J). Between the outer wall, new inner wall and septal-plate, triangular 'spaces' (ts) occur which appear to be mostly electron-dense (e.g. Pl. 28C). In the centre of the septum there remains a pore (p) usually associated with Woronin bodies (Wb) and membrane-bounded electron-opaque plugs (pl) (Pls. 27E; 28D; Fig. 3J). Sometimes the septal-plate shows an undulation which then becomes levelled by the inner wall (Plate 26D; see also Brewer & Boerema, l.c. Pl. 4). Generally the production of the new inner wall and the septa starts immediately after secession; but occasionally this process occurs before secession of the conidium.

The plasma membrane of mature septate conidia sometimes shows typical invaginations (Pl. 29B, C).

Note that the mucilaginous mass surrounding the mature conidia is produced with the process of wall-thickening and septation and not during conidiogenesis.

Germination of the conidia (Pl. 29A; Fig. 3K) is initiated by a swelling of the conidial cells. Then at the innerside a new thin electron-transparent layer can be distinguished. The original wall apparently ruptures by protrusion of the new wall layer.

DISCUSSION

In addition to the electron microscopy observations by Brewer & Boerema (l.c.) this study has provided more information on conidiogenesis and secession, septation and germination of conidia in *Phoma* and *Ascochyta* species. The formation of the first conidium differs essentially in both genera, as does septation of the conidia. The

Fig. 5. Line drawings of conidia and conidiogenous cells (annellides) of *Ascochyta* spp. as seen with the light microscope. — A. Conidia of various graminicolous species (after Sprague & Johnson, 1950 fig. 1). — B. Conidiogenous cells in *Ascochyta pisi* (after Punithalingam & Holliday, 1972b figs. B, C). — C. Conidiogenous cells of *Ascochyta pinodes* (after Punithalingam & Holliday 1972c fig. E, as conidial state of *Mycosphaerella pinodes*). Note that under optical microscopy the annellides of *Ascochyta* show much resemblance with the phialides of *Phoma* (compare Fig. 4).

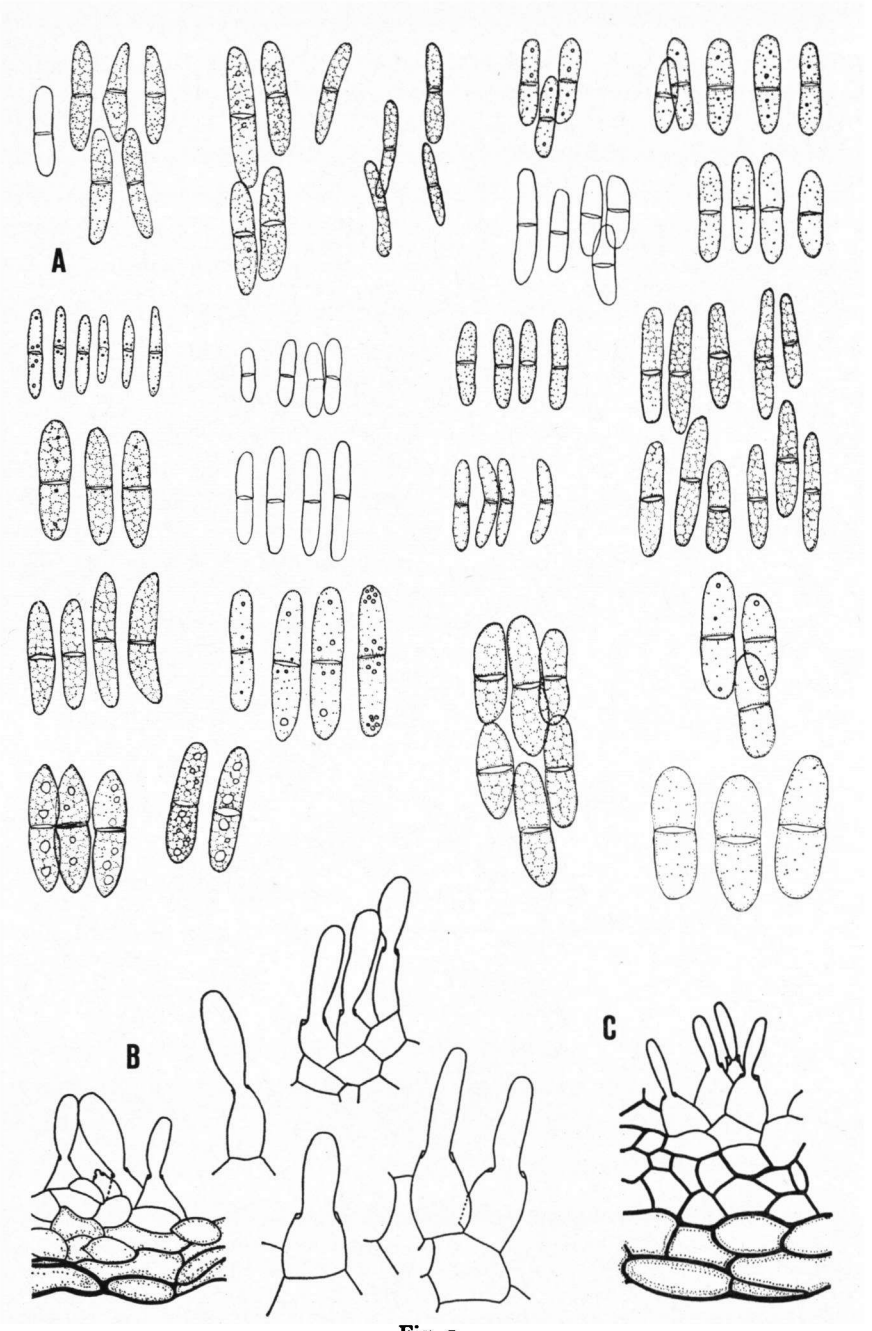

Fig. 5

processes of conidial secession and germination however show much resemblance. In Text-figures 1, 2 and 3 we have tried to reflect the differences and similarities.

First we emphasize that in both genera the conidia secede by septation. For *Ascochyta pisi* this was already established by Brewer & Boerema (l.c.) but in the case of *Phoma* spp. they referred to 'conidia which apparently become pinched off'. Conidial secession in species of both genera can now be characterized as separation by a t h r e e - l a y e r e d s e p t u m ('double septum', sensu Kendrick, 1971): two layers of wall material with a very thin abscission layer, s e p a r a t i o n - p l a t e (srp), between them (Pls. 20E; 21A-C; 26A, B). The abscission layer appears to break down (by autolysis?) and allows the conidium to separate from the parent cell (Pls. 21D; 25D). In *Ascochyta*, remnants of the separation-plate may remain attached to the detached conidium (basal frill, fr: Pl. 25B; Fig. 3E).[3] Conidial secession by a three-layered septum seems to be exclusive in Deuteromycotina and has been shown with the electron microscope for various types of conidial ontogeny (cf. Sutton, 1971; Hammill, 1974).

That the septa in conidia of *Phoma* and *Ascochyta* spp. also consist of two wall-layers with a very thin layer, the s e p t a l - p l a t e (sp), between them (Pls. 22F; 28C) is also in accordance with electron microscopy observations of septa in other fungi (see e.g. Bracker & Butler, 1963; Brenner & Carroll, 1968; Kreger-van Rij & Veenhuis, 1971; Littlefield & Bracker, 1971, and Reisinger, 1970). The septal pores of *Phoma* and *Ascochyta* spp. that are associated with Woronin bodies and membrane-bounded electron-opaque plugs (Pls. 23C; 28D), can be characterized as of the Ascomycete-type (see e.g. Bracker, 1967). The incidental observation of a kind of micropore in a conidial septum of *Phoma exigua* (Pl. 23B) is remarkable, because micropores or plasmodesmata are only known from a few fungi in septa that are lacking a central pore (cf. Bracker, l.c.).

Conidial development in both *Phoma* and *Ascochyta* can be characterized as forms of b l a s t i c c o n i d i a l o n t o g e n y (Kendrick, 1971): the conidia differentiate from a part of the parent cell — conidiogenous locus — and there is a marked

[3] From electron microscopy studies of other fungi it is known that the separation-plate does not always disintegrate. In *Doratomyces nanus* it remains a part of the truncate base of the detached conidium (Hammill, 1972a); in *Endomycopsis platypodis* it remains attached to the parent cell (Kreger-van Rij & Veenhuis, 1969).

Fig. 6. Line drawings of conidia and conidiogenous cells of some 'problematic species' (see text Addendum) as seen with the light microscope. — A. *Ascochyta nigripycnidiicola*. Large two- and more-celled conidia as occurring in pycnidia in vivo and in vitro, and one-celled microconidia as produced in other pycnidia also in vivo as well as in vitro. — B. *Phoma oleracea* var. *solidaginis*. 'Mixture' of large two-celled conidia and one-celled microconidia as occurring in pycnidia in vitro. — C. *Ascochyta bohemica*. Large two-celled conidia as produced en masse in vivo together with some one-celled microconidia. The mature two-celled conidia easily break into two parts. In vitro only microconidia are formed which then sometimes arise from elongated cells which look like undetached macroconidia. The parent cells producing microconidia have an obvious collarette in contrast with those producing macroconidia.

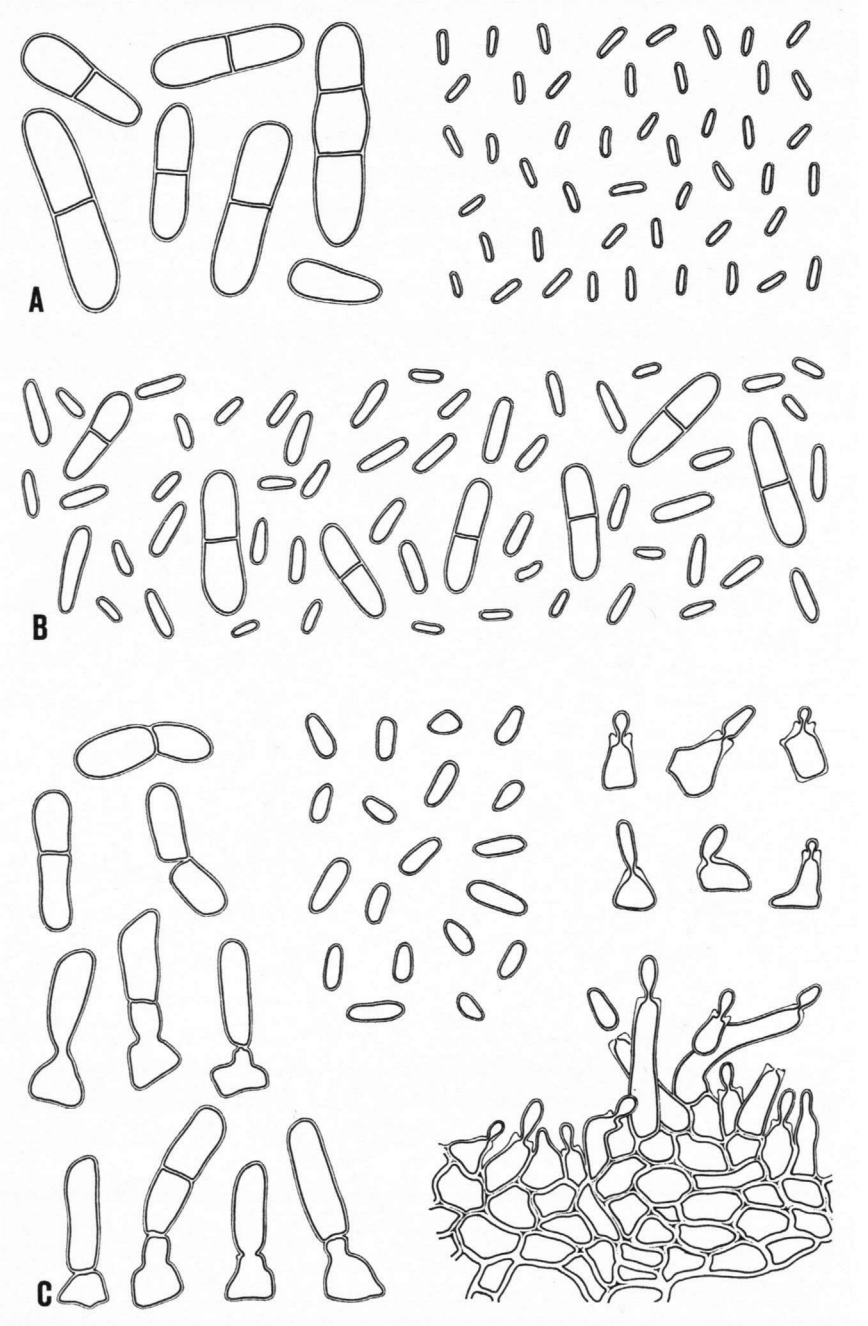

Fig. 6

enlargement of recognizable conidial initial before the initial is delimitated by a three-layered septum.

The conidia of *Phoma* spp. can further be regarded as enteroblastic (Kendrick, l.c.) because the outer layers of the wall at the apex of the parent cell are not involved in the formation of the conidial wall (Pls. 19A, E; 20A; 21E; Figs. 1, 2E–G; see also Sutton, 1971). Conidiogenous cells which produce enteroblastic conidia in basipetal succession (youngest at the base of the chain, the oldest at the tip) from a fixed conidiogenous locus are termed p h i a l i d e s (Kendrick, l.c.). A typical feature of phialides is the c o l l a r e t t e representing remnants of the wall layers originally enclosing the first conidium (compare Fig. 1A–D). On account of the conspicuous collarette the parent cells in *Phoma* spp. have already been interpreted as phialides (Sutton, 1964, Ciccarone & Russo, 1969). Boerema (1965) and Sutton & Sandhu (1969) however had raised doubts concerning this interpretation. They thought that the two or three periclinal layers that are sometimes visible with the electron microscope in the collarettes of *Phoma* spp. (compare Pl. 21B, E) represented wall ridges of detached conidia ('annellations'). The conidiogenous cells in *Phoma* then should not have a fixed meristem producing more than one conidium from it, but instead — as is the case with annellides (see below under *Ascochyta*) — various growing points each producing one conidium. The present study however has shown that the two or three layers or ridges sometimes occurring in *Phoma*-collarettes correspond with the different layers of the original papilla (ol, ml, il) enclosing the first conidium. The successively seceding conidia at the most add some mucilage to the collarette (Pl. 21D; Fig. 2G) but no real scars or annellations. Therefore conidiogenesis in *Phoma* spp. can be characterized as enteroblastic and phialidic, comparable with the enteroblastic phialidic ontogeny in some hyphomycetes as e.g. *Neuropora crassa* (Lowry & al., 1967; Subramanian, 1971: 'type 1'). A typical feature of enteroblastic phialidic ontogeny in *Phoma* spp. is that it starts with the development of a thick-walled papilla with a conspicuous usually more or less electron-dense middle layer (ml) which seems to function as 'opener' of the fixed conidiogenous locus. An additional characteristic is that the differentiation of the conidial wall to its final structure is associated with production of mucilage (cs) which together with dissolved wall material resulting from the liberation of the first conidium and secession of successive conidia, forms the abundant mucilaginous mass surrounding the conidia in *Phoma* spp. In many aspects conidiogenesis in *Phoma* spp. agrees with conidial development in the acervular fungus *Colletotrichum coccodes* (=*C. atramentarium*) as shown in the electron microscopy study by Griffiths & Campbell (1972).

For diagnostic purposes conidial development in *Phoma* spp. was described as 'monopolar repetitive budding' (Boerema, 1965), because under optical microscopy the conidia seemed to arise in succession as buds from the conidiogenous locus with often only a small cytoplasmic channel between bud and parent cell (comp. Pls. 19A; 21E). Originally 'budding' has been considered as secession without septal formation but recent electron microscopy studies have shown that buds in fungi secede by septation also (for references see Sutton, 1971 and Donk, 1973).

For *Ascochyta* spp. the formation of the first conidium by a parent cell can be described as holoblastic (Kendrick, 1971) because the whole (thin) wall at the apex of the parent cell is apparently involved in formation of the conidium wall (Pl. 25B; Fig. 3A). Holoblastic conidial development in basipetal sequence should be typical for annellides, i.e. (Kendrick, l.c.) conidiogenous cells which produce a single conidium from the apex (conidiogenous locus) of each of a succession of very short percurrent vegetative proliferations involving the basal part of the septum remaining after secession of the previous conidium (percurrent indicates a mode of vegetative proliferation in which each successive apex arises through the previous apex). From Pl. 24A–D and Pl. 25A, B, however, it cannot merely be concluded that in *Ascochyta* the conidia formed after the first one by a parent cell are truly holoblastic. In a critical discussion on the distinction between phialides and annellides Hammill (1974) also pointed out that for a number of other fungi in which conidiogenesis is considered to be typically annellidic, the conidia are 'clearly not holoblastic, at least not after the formation of the first conidium in the basipetal sequence'

Characteristic for annellides is the presence of narrow bands of wall material encircling the periclinal wall near the apex of the conidiogenous cell; these annellations are the vegetative portions of the proliferations left behind after each conidium has seceded. In contrast with the fixed meristem of phialides (see above under *Phoma*), annellides thus have various growing points at each of which one conidium is produced. With reference to the conidial ontogeny described and illustrated by Brewer & Boerema (l.c.) for *Ascochyta pisi* (compare Pls. 24A, B; 25A, B), Madelin (1966) has already interpreted the conidiogenous cells of *Ascochyta* as true annellides. As a result of our further observations we agree with this interpretation (Fig. 3). It must be noted however that the micrographs obtained from *A. fabae* and *A. pinodes* (Pls. 25C, D; 26A, C) have indicated that in *Ascochyta* spp. the conidia can originate and secede at approximately the same level as, or possibly even at a lower level than the previous conidium. The result is then not a series of annellations one above another as shown in the micrographs of *A. pisi* (Pls. 24A; 25A; cf. Fig. 3E, F), but an annellate collar composed of a number of periclinal layers corresponding with the number of conidia produced (Pl. 26C and Fig. 3G). Similar observations are made by Sutton & Sandhu (1969) on the conidiogenous cells in the acervuli of a *Cryptosporiopsis* sp.: 'annellides with successive conidium secession below, at, or above the level on which the first conidium seceded'. See also the electron microscopy study of the annellides in the acervular fungus *Steganosporium pyriforme* by Hammill (1972b). The annellate collars in *Ascochyta* spp. show much resemblance with the phialidic collarettes in *Phoma* spp., even with the electron microscope (compare Pl. 21B, C with Pl. 26A, B); under optical microscopy they cannot be distinguished at all.

Table I presents a comparison of characteristics of conidiogenous cells of *Phoma* and *Ascochyta* with those of phialides and annellides, as defined by Kendrick (1971).

The diagnostic most typical characters of conidial formation in *Ascochyta* species is the septation of the conidia as an essential part of the completion of conidium

TABLE I.—A comparison of characteristics of conidiogenous cells of *Phoma* and *Ascochyta* with those of phialides and annellides as defined by Kendrick (1971).

	phialidic sensu Kendrick	annellidic sensu Kendrick	conidiogenesis in *Phoma*	conidiogenesis in *Ascochyta*
first conidium				
enteroblastic	+		+	
holoblastic		+		+
successive conidia				
enteroblastic	+		+	?
holoblastic		+		
conidial wall 'formed de novo'	+			
conidial wall involves distal layer of separation-septum		+	+	+
conidiogenous cell				
with collarette	+		+	
with annellations or annellate collar		+		+
conidiogenous locus				
fixed, more conidia are formed at the same locus	+		+	
each conidium is formed at a new locus		+		+

development (Pls. 27, 28; Fig. 3H–J). It usually takes place just after secession of the conidium but it may also occur before the conidium has seceded. This septation process in *Ascochyta* spp. agrees with the septation of conidia in some dematiaceous hyphomycetes; compare the detailed electron microscopy study by Campbell (1969) on *Alternaria brassicicola*. For this kind of septation Brewer & Boerema used the term distoseptation (=pseudoseptation sensu Ellis pro parte), employed by Luttrell (1963) in his taxonomic study of *Drechslera* (*'Helminthosporium'*). Luttrell's study was based on optical microscopy and emphasizes the double walled structure of distoseptate conidia as appeared from crushed conidia: 'extruding hyaline cells from ruptured (dark) epispore'. Campbell (1970) considered the essential criterion of distoseptation to be the ability of distoseptate conidia to break up in their constituent cells. He interpreted that the conidia of the albino-type of *Alternaria brassicicola* were distoseptate, but not the wild-types of this fungus, because: 'they maintain their integrity as multicelled spores, even though each cell is surrounded by its own wall'. The essential character of distoseptate conidia however is that septation results from invagination of the secondary developing inner wall: 'Invaginations of the inner wall divide the protoplast into a series of cells resembling peas in a pod' (Luttrell, l.c.). See also the study of the ultrastructure of conidiogenesis in *Drechslera sorokiniana* by Cole (1973, fig. 27d, p. 634): 'a new wall layer ... developed by apposition adjacent to the thin plasma membrane ... thickens and is continuous with

septa traversing the conidia'. The invagination of the inner wall is apparently initiated by the development of the septal-plate arising perpendicularly from the outer wall (Fig. 3C). How far in mature distoseptate conidia the outer wall can be separated by pressure from the inner wall depends on differences in structure of both wall layers and is in fact not essential for the distoseptation process. In *Ascochyta pisi*, Brewer & Boerema (l.c.) incidentally could separate both layers by pressure.

The division of the conidia of *Ascochyta* spp. into two (or more) cells is on account of the distoseptation for this genus as typical as the multicellular characters of the conidia in hyphomycete genera such as *Drechslera* and *Alternaria*. The distoseptation process fully explains that true *Ascochyta* species always in vivo as well as in vitro produce mainly septate conidia (Zherbele, 1971: '*Ascochyta* sect. *Stagonosporoides*'). Just like the number of septa in conidia of *Drechslera* and *Alternaria* species show a certain variability, in *Ascochyta* spp. not all mature conidia are two-celled; one-celled and three- (or even four-) celled conidia occasionally occur.

The septation in conidia of *Phoma* spp. Pls. 22, 23A–C; Fig. 2H–J) is a secondary process which only occurs under special conditions, among others in conditions promoting germination (Pl. 23A). It thus occurs independently of conidiogenesis. Brewer & Boerema (l.c.) called this kind of septation 'euseptation', a term introduced by Luttrell (l.c.) for conidial-septation in dematiaceous hyphomycetes such as *Sporidesmium* and *Nakataea* (=*Vakrabeeja*) spp. Septal formation in the conidia of these hyphomycetes however has not been studied by electron microscopy, so we cannot judge at present if it agrees with out observations on conidial septation in *Phoma* spp. It is conspicuous that the secondary developing septa in *Phoma*-conidia apparently almost from the start attain their final thickness. In *Phoma chrysanthemi* septal formation is associated with only a partial thickening of the lateral wall at both sides of the septum (zone of attachment; Pl. 22E). This agrees with the general impression that septate conidia in *Phoma* spp. do not have thicker walls than one-celled conidia. Nevertheless it may be possible that in certain species the thickening of the lateral wall is not restricted to the 'zone of attachment' but includes the whole inside of the conidial wall. Schmid & Liese (1970) in their electron microscopy study of hyphae of the basidiomycete *Armillaria mellea* have observed two types of septa. Firstly, septa of which the wall layers at both sides of the septal-plate are continuous with the inner layers of the lateral wall and secondly, septa which, as in *Phoma chrysanthemi*, show only a restricted zone of attachment to the lateral wall. They called the latter type pseudosepta! Therefore, it seems to us inopportune to describe secondary septation of *Phoma*-conidia as euseptation. In this area many more comparative ultrastructural studies are urgently needed.

Under conditions favouring germination in the swollen conidia of *Phoma* as well as of *Ascochyta* species the initial wall of the germ-tube becomes discernable as a new layer at the innerside of the conidial wall (Pls. 23D; 29A; Figs. 2K, 3K). In *Phoma* the wall of the germ-tube seems to attain at once 'hyphal-thickness'. Optical microscopy has shown that septation of the germ-hypha occurs soon after emergence but without visible wall-thickening. At later stages the wall appears to be defined

more sharply and to be surrounded by a diffuse mucilaginous sheath (comparable with the slime surrounding conidia?; see Fig. 2K and the study of the ultrastructure of germinating conidia of *Botrytis cinerea* by Hawker & Hendy, 1963). In *Ascochyta* species the wall of the germ-tube is relatively thin. Light microscopy observations suggest that the wall of the germ-hypha thickens during septation that occurs quickly, which process can then be characterized as distoseptation.

EMENDED DEFINITIONS OF PHOMA AND ASCOCHYTA

The differences in conidial ontogeny and septation of typical *Phoma* and *Ascochyta* species and the results of cultural studies of both kind of pycnidial fungi (e.g. Zherbele, 1971 and Boerema & Dorenbosch, 1973) make it now possible to redefine both genera.

PHOMA

Phoma Sacc. *in* Michelia **2**(1): 4. 1880 (as *Phoma* 'Fr. em.'; nomen genericum conservandum, 8th Int. Bot. Congr., Paris 1954). — Lectotype-species (8th Int. Bot. Congr., Paris 1954): *Phoma herbarum* Westend. *in* Bull. Acad. r. Belg. Cl. Sci. **19**(3): 118. 1852; lectotype (Boerema, 1964) in herbarium Westendorp & Wallays (BR): exs. Herb. crypt. Belg., Ed. Beyaert-Feys, Fasc. **20**, No. 965, 1854, on stems of *Onobrychis viciifolia*.

Ascochyta sect. *Phyllostictoides* Zherbele *in* Trudy vses. Inst. Zashch. Rast. **29**: 20. 1971. For other synonyms, see von Arx (1970).

Pycnidia mostly glabrous but sometimes hairy or setose especially towards the ostiole, usually globose-subglobose or globose-ampulliform to obpyriform but also more irregular in shape, separated or in small groups, usually sub-epidermal then erumpent with mostly one, but sometimes more, distinct, impressed but more often papillate openings (ostiole or porus); wall pseudoparenchymatous or prosenchymatous, sometimes pseudosclerenchymatous, the outer cells mostly dark and thick-walled, the inner cells hyaline and more or less isodiametric, giving rise to conidiogenous cells.

Conidiogenous cells usually indistinguishable from the inner cells of the pycnidial wall but for a single aperture.

Under light microscopy the conidiogenesis may be characterized as monopolar repetitive 'budding'. The 'bud' of the first conidium arises from a papillate extension; subsequently conidia arise as 'buds' in basipetal succession from the apex of the conidiogenous cell surrounded by a distinct collarette.

Under electron microscopy the conidiogenous cells appear to be phialides producing, from a fixed conidiogenous locus, enteroblastic conidia which secede by a three-layered septum. The first conidial initial is produced within the inner layer of the papillate thickening of the wall at the apex of the conidiogenous cell (Fig. 1A, B). The upper part of the papilla wall sooner or later dissolves, but its basal part remains as a conspicuous collarette (Fig. 1C, D). The walls of successively produced conidia arise from the fixed meristem as outgrowths of the basal layer of the three-layered septum remaining after secession of the previous conidium (Fig. 2E–G). Differentiation of the conidial wall is associated with abundant production of mucilage.

Conidia hyaline or sometimes slightly coloured (yellow to pale brown), globose, obovoidal, ellipsoidal or clavate, mostly once or twice as long as wide, generally measuring between (2)2.5–10(12) × (0.5)1–3.5(5) μm. Conidia one-celled, but secondary septation may occur resulting in two- (or even more-) celled conidia (Fig. 2H–J); the percentage of septate conidia depends on the environmental conditions and may vary between 0–95% (in vivo).

Many species of *Phoma* are morphologically similar and besides, many of these species are exceedingly variable as regards size, form and structure of pycnidia and conidia. The existing Saccardoan classification of these fungi is mainly based on substrate-criteria and ignores the existence of numerous unspecialized species with wide host ranges, parasites as well as weak parasites and saprophytes (compare Boerema, 1969). The only way to reach a practical and useful classification of *Phoma* species has proved to be the study of the characteristics in vitro (see e.g. van der Aa & van Kesteren, 1971; Boerema, 1964, 1965, 1967a, 1967b, 1969, 1970b, 1972; Boerema & Dorenbosch, 1968, 1970, 1973; Boerema, Dorenbosch & van Kesteren 1965, 1968, 1971, 1973; Boerema, Dorenbosch & Leffring 1965; Boerema & Höweler, 1967; Boerema & de Jong, 1968; Dorenbosch, 1970; Dorenbosch & Boerema, 1973; Dorenbosch & Höweler, 1968; van Kesteren, 1972; Maas, 1965; Zherbele, 1971). A real and practical differentiation then appears to be possible when the morphological characteristics are combined with growth characteristics in culture including the general growth habit, rate of growth, pigment production, crystal formation, etc., in addition to the structure of the mycelium and of any chlamydospores. Classification based on study in vitro also solves the problem of differentiating *Phoma* species producing septate conidia in vivo (pseudo-Ascochytas) from true *Ascochyta* species. Under normal laboratory technique of culturing fungi on standardized agar media in petri dishes and tubes, conidial septation of *Phoma* species is always restricted (0–10%), whereas true *Ascochyta* species produce mainly septate (distoseptate) conidia (compare Boerema & Dorenbosch, 1973; Zherbele, 1971). *Phoma* species with hairy or setose pycnidia are readily distinguished from *Pyrenochaeta* by the shape of the conidiogenous cells. True species of *Pyrenochaeta* De Not. (Schneider, 1975) have elongated, septate conidiophores with lateral phialidic apertures below the septum delimiting each cell (acropleurogenous conidiophores). The pycnidia of the type-species of *Phoma*, *P. herbarum*, have a predetermined opening or ostiole; that means that structural provisions for the production of the opening are apparently already present in the pycnidial primordia (Boerema, 1964). This is the case with most typical species of *Phoma*. In certain species, however, pycnidia remain closed for an extended period; the opening then appears almost at the end of the growing process: porus instead of an ostiole. This occurs in e.g. *Phoma lingam* (Boerema & van Kesteren, 1964) and other *Phoma*-states of *Leptosphaeria* spp. (*Phoma* section *Plenodomus*, compare von Arx, 1970).

Different types of *Phoma*-parent cells and conidial shape are shown in Fig. 4 taken from Boerema & Höweler (1967), Punithalingam & Holliday (1972a, 1973a, b) and Sutton (1964).

ASCOCHYTA

Ascochyta Lib. *in* Pl. crypt. Ard., Fasc. 1: 8. 1830; *in* Mém. Soc. Sci. Agric. Lille 1829–1830: 175. 1831 (as '*Ascoxyta*'; see discussion by Sprague & Johnson, 1950). Lectotype-species (cf. Diedicke, 1912: 139; Clements & Shear, 1931: 363; Sprague & Johnson, 1950: 529; von Arx, 1970: 135): *Ascochyta pisi* Lib. *in* Pl. crypt. Ard., Fasc. 1, No. 59. 1830 (as '*Ascoxyta Pisi*'); holotype in herbarium Libert (BR): on pods of *Pisum sativum* (as '*Ascospora Pisi* N').

Ascochyta sect. *Stagonosporoides* Zherbele *in* Trudy vses. Inst. Zashch. Rast. **29**: 20. 1971.

Pycnidia usually glabrous but sometimes hairy, mostly globose-subglobose or ampulliform to mammiform, sometimes more irregular in shape, separated or in small groups, usually subepidermal then erumpent with usually one, but sometimes more openings (pores) which may be simple, impressed, slightly protruding or papillate; wall mostly relatively thin, usually pseudoparenchymatic, the outer cells darker and more thick-walled than the hyaline inner cells.

Conidiogenous cells not distinctly differentiated from the inner cells, but recognizable by the cuspidate or somewhat elongated apex.

Under optical microscopy the conidia arise in basipetal succession as thin-walled protrusions at the apices of the conidiogenous cells. A kind of collar may be present or not evident at all.

Under electron microscopy the conidiogenous cells appear to be annellides producing from successively developing conidiogenous loci, thin-walled conidia seceding by a three-layered septum. The wall of the first conidium arises as an outgrowth of the thin wall at the apex of the conidiogenous cell (Fig. 3A). The walls of successive conidia arise from the apical part of very short percurrent proliferations of the conidiogenous cell involving the basal layer of the three-layered septum remaining after secession of the previous conidium (Fig. 3B–E). Percurrent growth however not only occurs at increasingly higher levels resulting in a series of annellations one above another encircling the apex of the conidiogenous cell (Fig. 3F), but also at approximately the same level and then appears like a collar of periclinal annellations, the annellate collar (Fig. 3G). Conidiogenesis proceeds without evident production of mucilage.

Conidia, hyaline or sometimes slightly coloured (yellow to pale brown), usually cylindrical to ellipsoidal or cymbiform, mostly twice or three times as long as wide, generally measuring between (5)8–25(29) × (2)2.5–6(8) μm. Conidia after secession often one-celled but then soon becoming two- or occasionally three- or even four-celled.

Under electron microscopy the crosswall formation is characterized as distoseptation: production of a new inner conidial wall layer which concurrently by invagination divides the conidia in two or more cells; the invagination is initiated by the development of a septal-plate (Fig. 3H–J). This distoseptation process is associated with abundant production of mucilage.

Most species of *Ascochyta*, if not all, are typical parasites with restricted host ranges. According to Zherbele (1971) they occur especially on the Campanulaceae, Chenopodiaceae, Gramineae, Leguminosae, Solanaceae and Umbelliferae. Some of them have one specific host but then incidentally also may occur on related species of the same genus. They are in form, structure and size of pycnidia and conidia relatively stable and therefore usually easy to differentiate. Study in vitro is nevertheless also essential for identification of *Ascochyta* species, because the cultural characteristics (e.g. pigment production, occurrence of chlamydospores etc.) are often even more

specific than the purely morphological characters. Study in vitro is further necessary to distinguish *Ascochyta* species with annellate collars from 'pseudo-Ascochytas' i.e. *Phoma* species producing in vivo many conidia with septa (see above). On account of the distoseptation process, the mature conidia of true *Ascochyta* species are always, in vivo as well as in vitro, two- or more-celled, whereas *Phoma* species in culture produce mainly one-celled conidia. The openings in the pycnidia of *Ascochyta pisi* and other typical species of *Ascochyta* apparently occur towards the end of the growing process and are interpreted as a porus instead of an ostole. However it does not appear opportune to include this character as a generic criterion (see above under *Phoma*; compare also von Arx, 1973).

For the various types of conidial shape in *Ascochyta* spp., and the conidiogenous cells as seen under optical microscopy see Fig. 5 taken from the study of graminicolous species by Sprague & Johnson (1950) and the descriptions of *Ascochyta pisi* and *Mycosphaerella pinodes* by Punithalingam & Holliday (1972b, c).

ADDENDUM

Phoma- and Ascochyta-like fungi the position of which needs further study.

Most pycnidial fungi with one- or/and two-celled hyaline conidia can by study in culture easily be classified into the form-genera *Phoma* or *Ascochyta* as defined above. The observation that true Ascochytas have usually larger conidia than pseudoforms of *Phoma*, facilitates prognostication of the genus for the species producing many two-celled conidia in vivo. However as already noted by Zherbele (1971) a small number of species remain difficult to classify because they show 'mixed' or intermediate characteristics. This may be illustrated with three examples:

1. In eastern Europe *Ascochyta nigripycnidiicola* Ondřej (1968) causes spots on leaves and stems of vetches, *Vicia* spp. In the spots pycnidia are produced with extremely large two- or more-celled conidia, 20–45 × 7–12 μm. On the leaves sometimes pycnidia also occur with one-celled microconidia, 5–8 × 1.5–2 μm. In culture the fungus produces at first pycnidia with two-celled conidia, 35–62 × 10–15 μm, thus even larger than those in vivo. Later on, however, only pycnidia with microconidia, 5–12(15) × 1.5–3(3.5) μm, are formed in vitro (Ondřej, 1970). See Fig. 6A. Although the fungus probably also has a perfect state (Ondřej, 1968) the microconidia are not spermatia, but true conidia as appeared from inoculation experiments. Similar observations were made by Ondřej (1970) on *Ascochyta viciae* Lib., a parasite of *Vicia sepium*.

Does this species form pycnidia with phialides like *Phoma* and pycnidia with annellides like *Ascochyta*?

2. On last-year stems of goldenrod, *Solidago* spp., a species described by Saccardo (1884) as *Phoma oleracea* var. *solidaginis* (type in PAD) can be found. On the stems the pycnidia contain only one-celled conidia, (4)5–6(7) × 1.5–2 μm. In culture, however, apart from one-celled conidia, (2.5)3.5–7.5(8.5) × 1.5–2.5(3.5) μm, often also some

large two-celled conidia are produced in the same pycnidium, measuring (14.5) 15.5–22(24) × 4–6(7) μm. See fig. 6B.

Does this mean that phialides and annellides can be formed in one pycnidium?

3. A well-known leafspot-disease of *Campanula* spp. is caused by a fungus described as *Ascochyta bohemica* Kab. & Bubák (cf. Sauthoff, 1962). On the spots, which may also occur on stems and flowers, the pycnidia at maturity contain mainly large, two-celled conidia, (11)13–23 × (3.5)4–6 μm. Together with these conidia also some one-celled microconidia, 4–6 × 1–2 μm can be found. In culture the pycnidia usually contain only microconidia, (2.5)3.5–6(8.5) × 1.5–2(2.5) μm. Sometimes however in the same pycnidium at maturity some large, two-celled conidia are produced with dimensions similar to those in vivo. In contrast with the parent cells of the two-celled macroconidia, the parent cells producing the microconidia always show an obvious collarette. In vitro the microconidia are sometimes produced on elongated cells which look like macroconidia that have not seceded. The mature two-celled macroconidia easily break into two parts (Brewer & Boerema, 1965), which is never observed for distoseptate *Ascochyta*-conidia. See Fig. 6C.

Can parent cells of this fungus produce phialidic conidia as well as annellidic conidia? Moreover are the annellidic conidia not distoseptate?

The occurrence of aberrant forms is a common phenomenon in nature. It often reveals a stumbling-block for taxonomists. Even with the most 'natural systems' at one's disposal problematic species may remain. Using the new definitions of *Phoma* and *Ascochyta* the number of problematic species has been shown to be few (Zherbele, 1971). Electron microscopy studies of conidial ontogeny and septation in the deviating species is not only necessary for classification of these species but it may also lead to a deeper understanding of the process of conidial formation in this kind of fungi (relation between phialides and annellides) and its relevance for taxonomy.

ACKNOWLEDGEMENTS

We are indebted to Miss Christien B. de Jong for competent assistance and for helpful suggestions concerning the modification of conidial germination technique. We are also grateful to Prof. Dr. A. J. P. Oort without whose initial stimulation this work would not have commenced. A contribution towards the cost of the plates provided by the 'Landbouwhogeschool Fonds' is gratefully acknowledged. Electron microscopy was done at the Technical and Physical Engineering Research Service at Wageningen. Dr. B. C. Sutton, C. M. I., Kew, very kindly improved the English text.

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EXPLANATION OF PLATES 19–29

PLATE 19

Fig. A. Conidial development in *Phoma leveillei*; the conidia produced in basipetal succession arise as buds from the conidiogenous locus surrounded by a collarette (col) at the top of the parent cells.

Figs. B-E. Parent cells of *Phoma* spp. producing their first conidium. — B. *P. herbarum.* — C. *P. lingam.* — D, E. *P. exigua.* Papillate pronounced thickening of the wall at the top of the parent cells, gradually developing into a bud-like protrusion. The wall of the first conidium (wc^1) arises within the electron-transparent inner layer (il) of the thickened wall. The more or less electron-dense middle layer (ml) seems to function as 'opener' of the conidiogenous locus. The outer electron-transparent layer (ol) in fig. E is already partly dissolved.

PLATE 20

Fig. A. Micrograph showing three parent cells of *Phoma exigua* in different developmental stages of the first conidium (the 'free' conidia originate from parent cells not visible in this section). The dark middle layer (ml) of the papillate stage is in the process of conidial secession still visible as the outer layer of the mucilaginous sheath around the conidia. The electron-transparent outer layer (ol) of the papilla is in the process of conidial secession, for the most part dissolved to a series of mucilaginous 'drops'. The differentiation of the conidial wall (developed within the original electron-transparent inner layer of the papilla, see Pl. 19 fig. E) is associated with the production of an electron-transparent cloudy substance (cs). This is also present around the free conidia. So far the free conidia are not first conidia, the outer layer of the mucilaginous sheath of these conidia represents dissolved wall material resulting from the process of secession of the previous conidium (compare Text-figs. 1D and 2E-G). The precise way in which the conidia become detached from the parent cells cannot be seen in this micrograph.

Figs. B-E. Micrographs of *Phoma* spp. showing the development of the initial electron-transparent separation-plate (srp) immediately followed by the formation of the basal conidial wall and a wall closing the parent cell (compare Text-fig. 2E-G; col=collarette being the basal part of the original three-layered papilla wall: ol, ml, il). — B. *P. leveillei.* — C. *P. complanata.* — D, E. *P. lycopersici.*

PLATE 21

Figs. A–C. Stages just before conidial secession in *Phoma* spp. — A. *P. herbarum.* — B, C. *P. lycopersici.* In secession three layers are involved: the basal conidial wall, the separation-plate (srp) and the wall closing the parent cell. In the collarette (col) the layers of the original papilla (ol, ml, il) sometimes can still be distinguished (e.g. in fig. B).

Fig. D. Final stage of conidial secession in *Phoma exigua*, showing the disintegration of the separation-plate and the original periclinal wall parts into mucilage.

Fig. E. *Phoma lingam*; characteristic picture of conidiogenesis in *Phoma* spp. Conidial initial arising as a bud from the conidiogenous locus which is surrounded by a distinct collarette (col), being the basal part of the original three-layered papilla-wall (ol, ml, il), compare Pl. 19 fig. C.

PLATE 22

Figs. A–F. Development of septa in conidia of *Phoma* spp. From the start three layers can be distinguished: a thin electron transparent septal-plate (sp) at both sides covered with a thicker wall layer making round edges with the lateral wall to which they are 'attached'

with decreasing thickness for some distance. Near the lateral wall at both sides of the septal-plate triangular 'spaces' (ts) occur (mostly electron-dense). Note that the developing septa apparently almost immediately attain their final thickness. — A. *P. exigua.* — B, D, E. *P. chrysanthemi.* — C, F. *P. lycopersici.*

PLATE 23

Fig. A. Conidia of *Phoma chrysanthemi* obtained from a suspension in water which encourages septation and germination. Note the closed pori in the septa with associated electron-dense membrane-bounded plugs.

Fig. B. Septum in a conidium of *Phoma exigua*, apparently perforated by a microporus (plasmodesmium).

Fig. C. Septal pore in meristematic pycnidial tissue of *Phoma exigua* showing the clumped or stocked membrane profiles (pl) similar to those usually associated with pores in septa of *Phoma*-conidia. Note also the Woronin-bodies (Wb) near the porus.

Fig. D. Germinating conidium of *Phoma lycopersici*: at the innerside of the original conidial wall a new layer can be distinguished which is continuous with the germ-tube. Note the ruptured conidial wall at both sides of the germ-tube.

PLATE 24

Figs. A–D. Different stages of conidial development in *Ascochyta* spp. — A, B. *A. pisi.* — C, D. *A. pinodes.* The conidial initials are, in comparison with the mature (=septate) conidia, extremely thin-walled. All parent cells show at the base of the conidial initials wall-ridges (wr) meaning that each cell has already produced conidia before (wr^1=wall-ridge first conidium etc.; wc^2=wall second conidium; the first conidium arises apparently simply as an outgrowth of the thin-walled apex of the parent cell, see Pl. 25 fig. B). Note that the cloudy electron transparent substance (cs; = slime) surrounding the mature (septate) conidia in figs. A, B does not occur around the initials.

PLATE 25

Figs. A-D. Micrographs showing various stages of conidial secession in *Ascochyta* spp. — A, B. *A. pisi.* — C, D. *A. pinodes.* With secession in fact three wall-layers are involved; the basal conidial wall, the wall closing the parent cell and a very thin electron transparent layer, the separation-plate (srp in fig. C; compare Pl. 26 figs. A, B) along which separation takes place. Note the different wall ridges at the base of the conidial initials (wr^1=wall ridge first conidium, etc.; wc^1=wall first conidium, etc.). The large detached conidium in fig. B shows a basal frill (fr), probably remnants of the separation-plate.

PLATE 26

Figs. A, B. Stages just before conidial secession in *Ascochyta fabae* showing three layers: basal conidial wall, the very thin electron-transparent separation-plate (srp) and the wall closing the parent cell. Note the similarity between separation-plate and septal-plate (sp) as shown in figs. D, E.

Fig. C. Collar of parent cell in *A. pinodes*: different layers (wr) corresponding with the number of conidia produced (14?); wc, wall of probably the 15th conidium formed by the parent cell.

Figs. D, E. Mature conidia of *Ascochyta* spp. showing that the septum consists of three layers: a thin electron-transparent septal-plate (sp) at both sides with a wall-layer making round edges with the lateral wall. Note that the septal-plate may be undulated. — D. *A. pisi.* — E. *A. fabae.*

PLATE 27

Figs. A–E. Various stages of septation in conidia of *Ascochyta pisi*. Centripetal development of a thin electron-transparent septal-plate (sp) concurrently with the production of a new inner conidial wall-layer which gradually increases in thickness (compare Text. fig. 3 H–J). In the centre of the septum a pore remains, usually associated with Woronin bodies (Wb) and membrane-bounded electron-dense plugs (pl).

PLATE 28

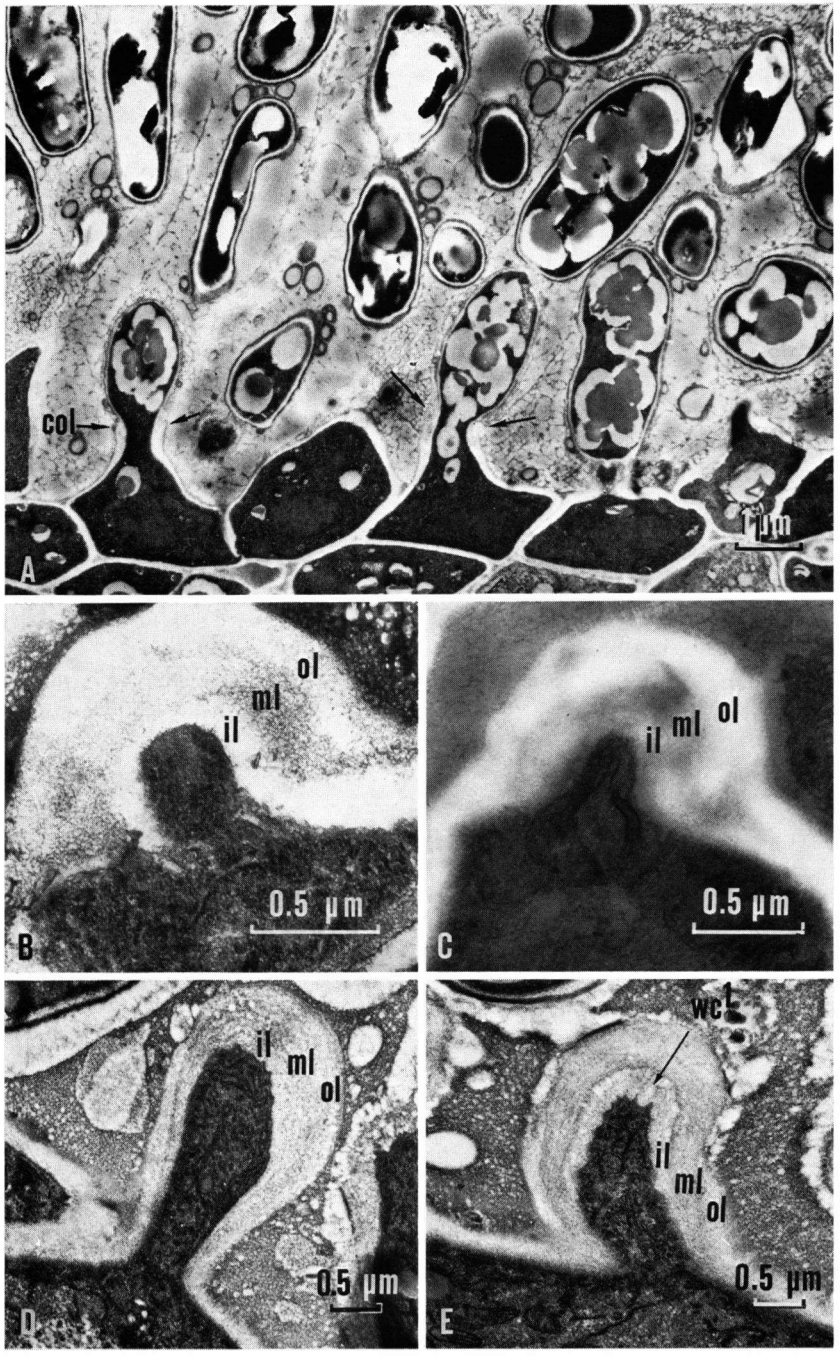
Figs. A, B. Septal formation in *Ascochyta fabae* (compare Plate 27).

Figs. C, D. Septa in mature conidia of *Ascochyta pinodes*. Three layers: the thin electron-transparent septal-plate (sp) at both sides covered with a wall-layer continuous with the inner part of the lateral wall. At the points of attachment electron-dense triangular spaces (ts) occur. Fig. D shows a pore with electron-opaque plug (pl) and two Woronin bodies (Wb).

PLATE 29

Fig. A. Micrograph showing a germinating septate conidium of *Ascochyta fabae*. The wall of one cell is just ruptured by protrusion of the wall of the germ-tube (compare Text-fig. 3 K; the septal-plate is not visible in this micrograph).

Figs. B, C. Invagination of the plasma membrane in septate conidia of *Ascochyta* spp. — B. *A. pisi*. — C. *A. pinodes*.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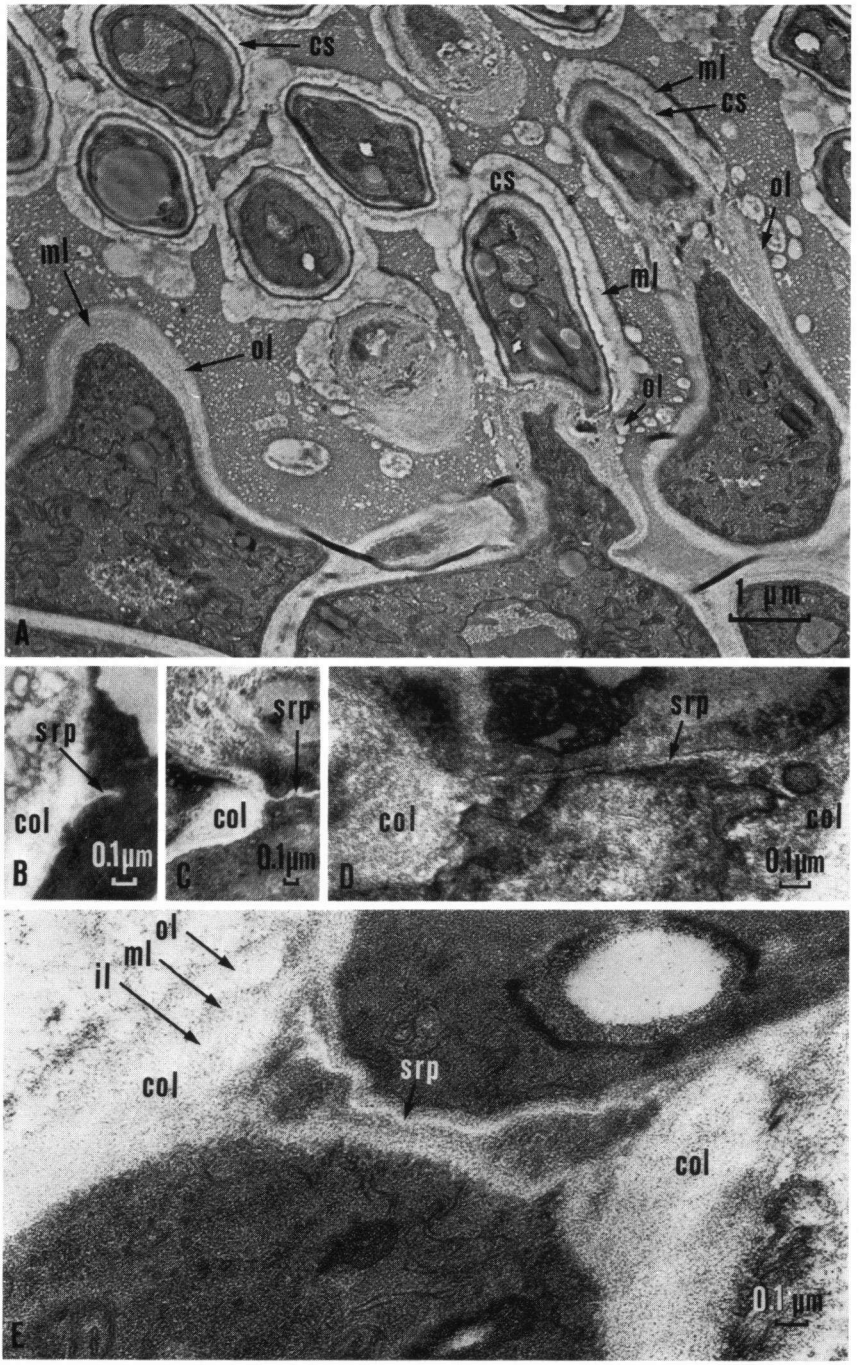
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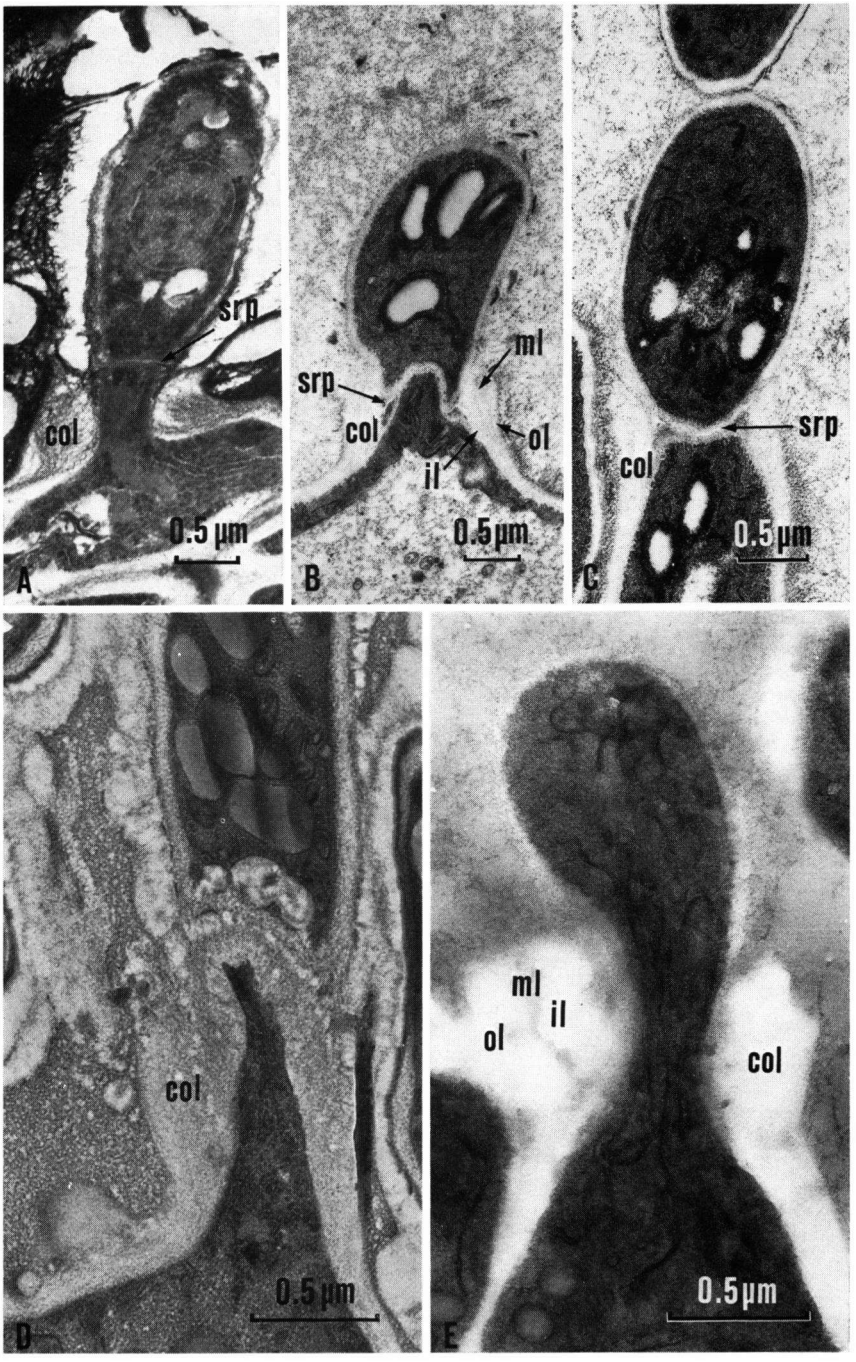
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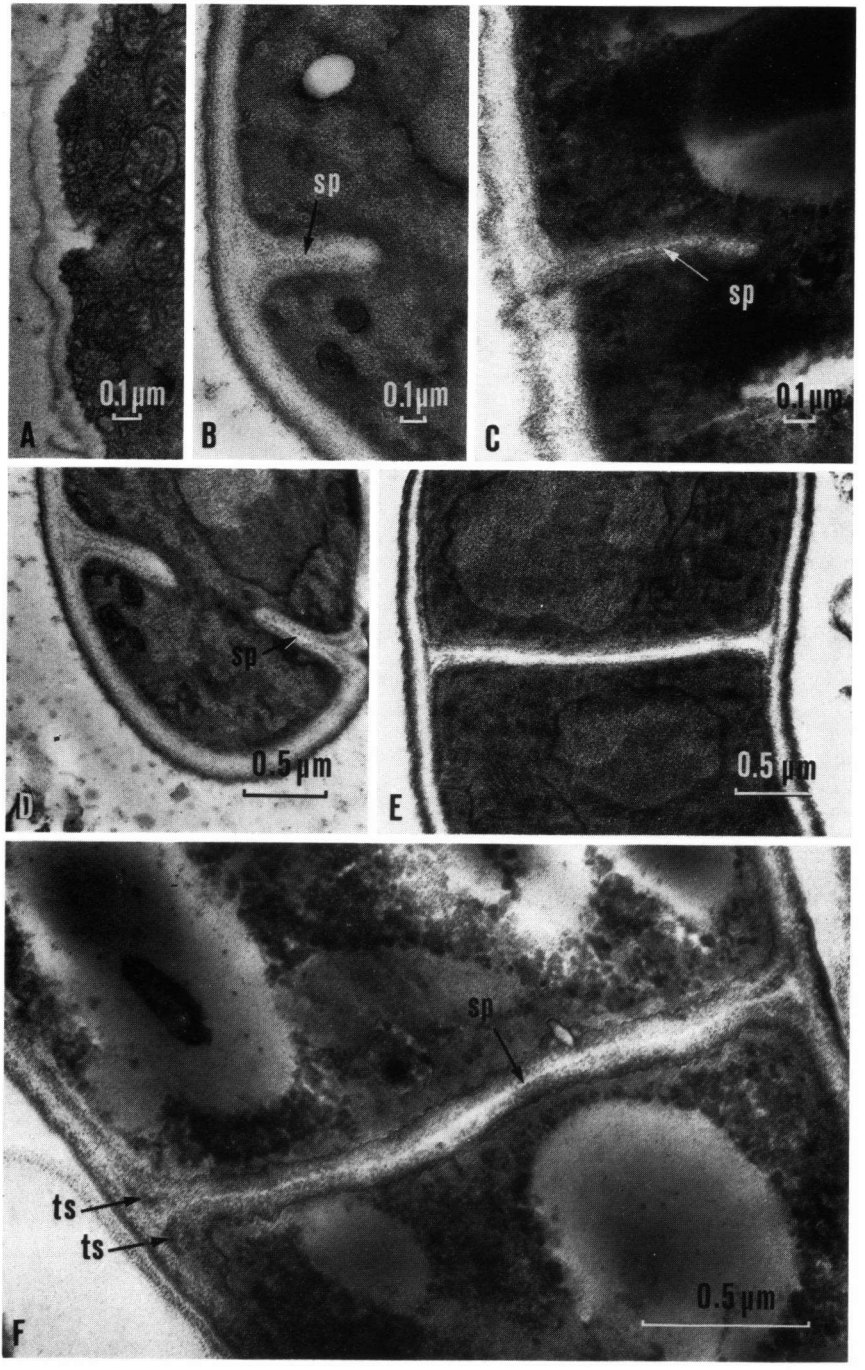
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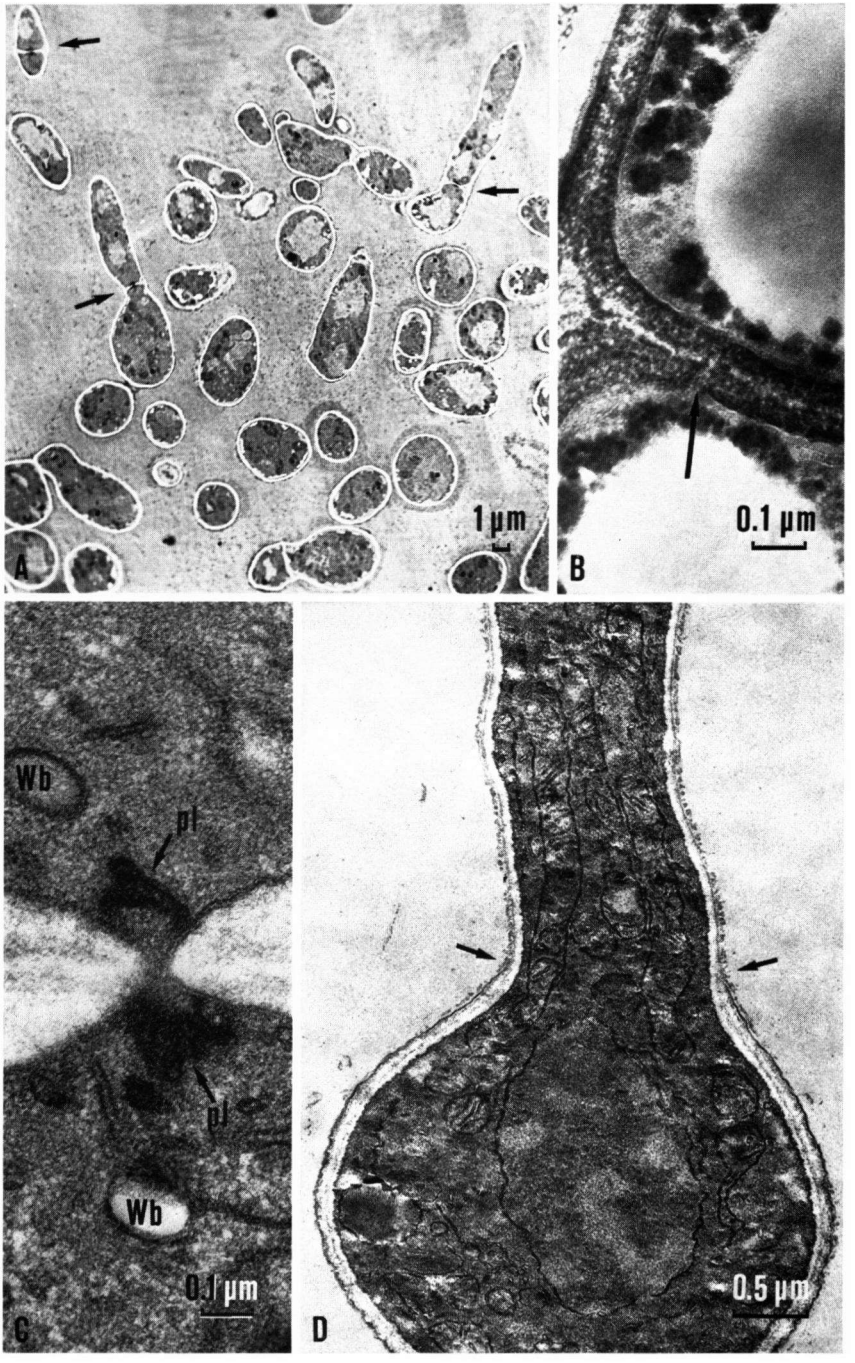
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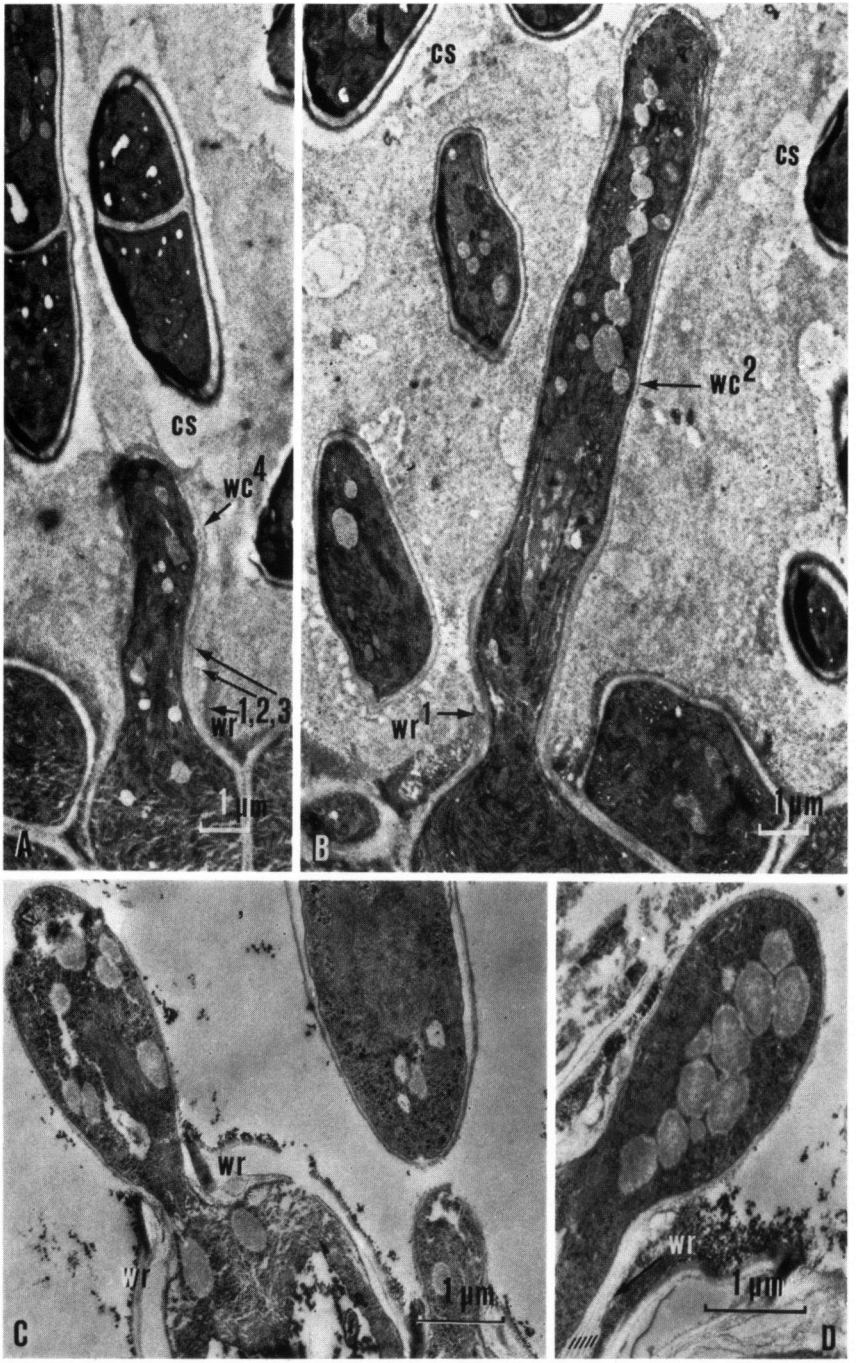
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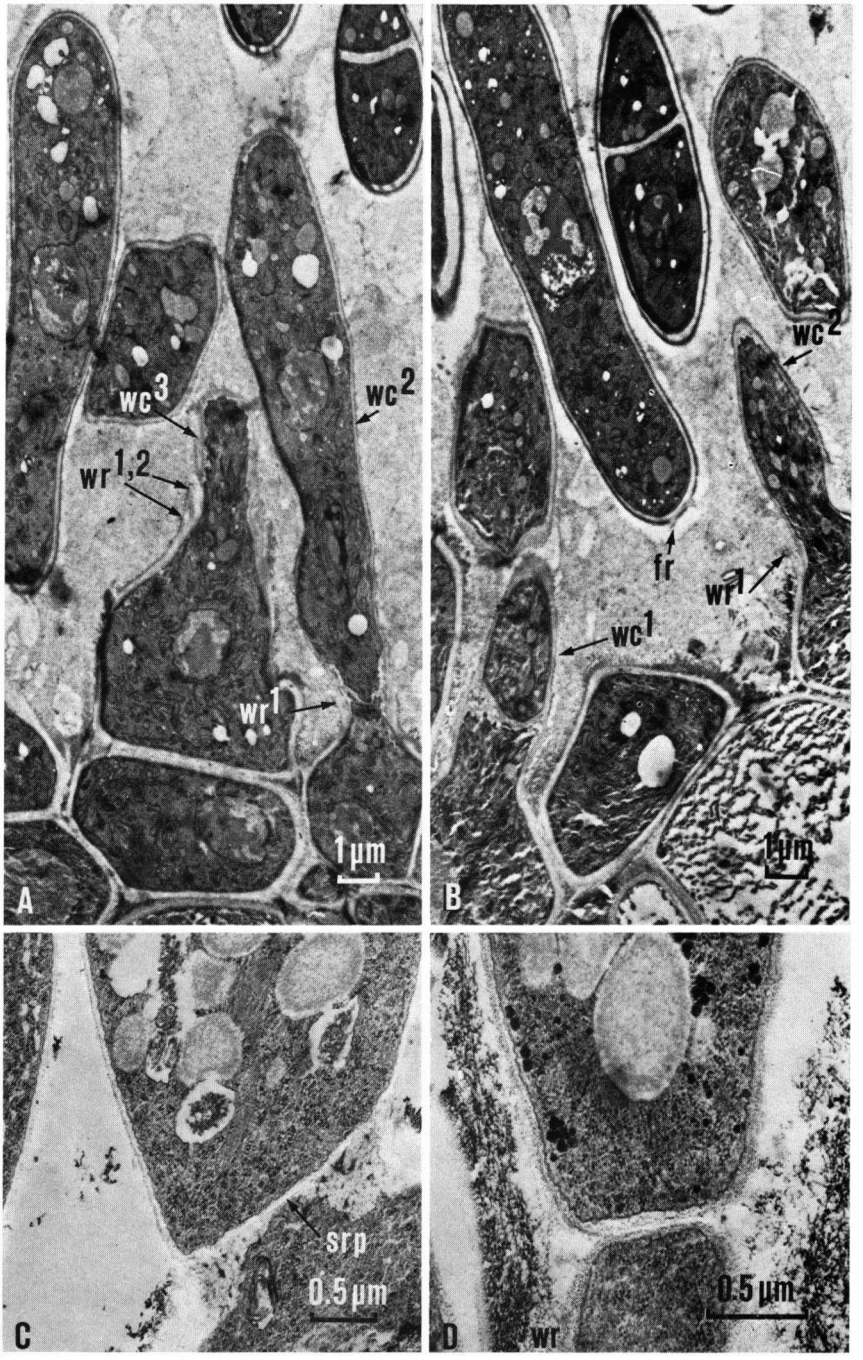
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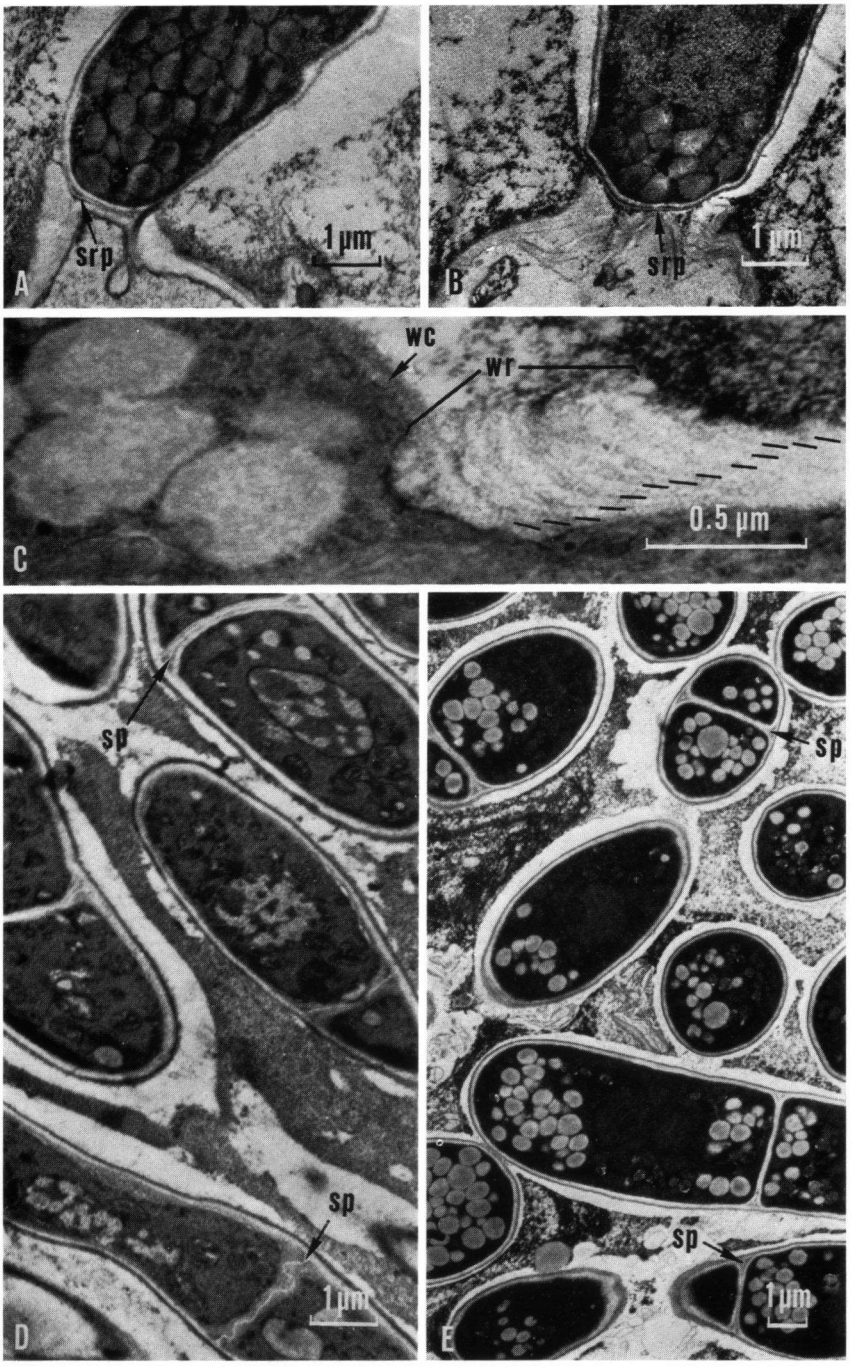
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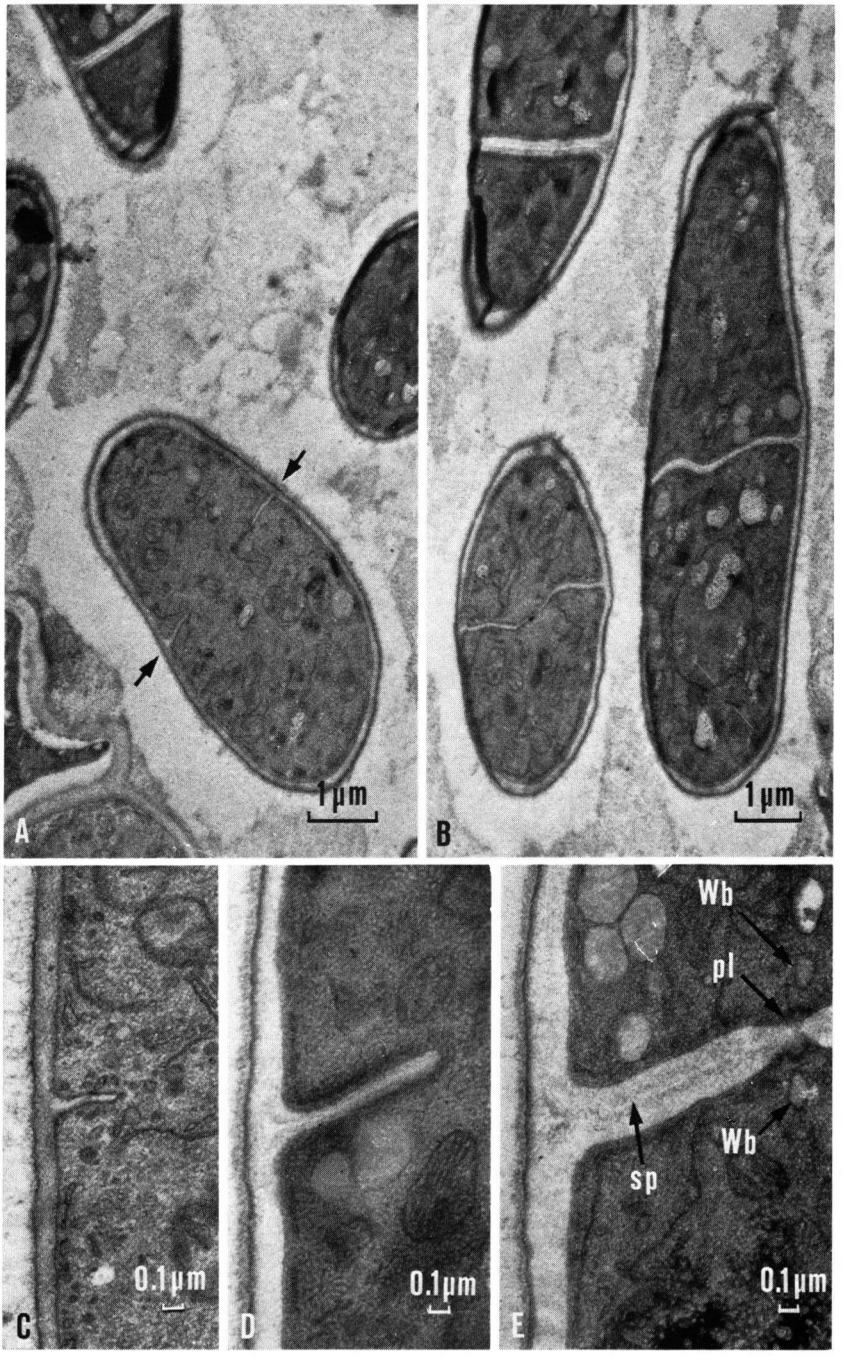
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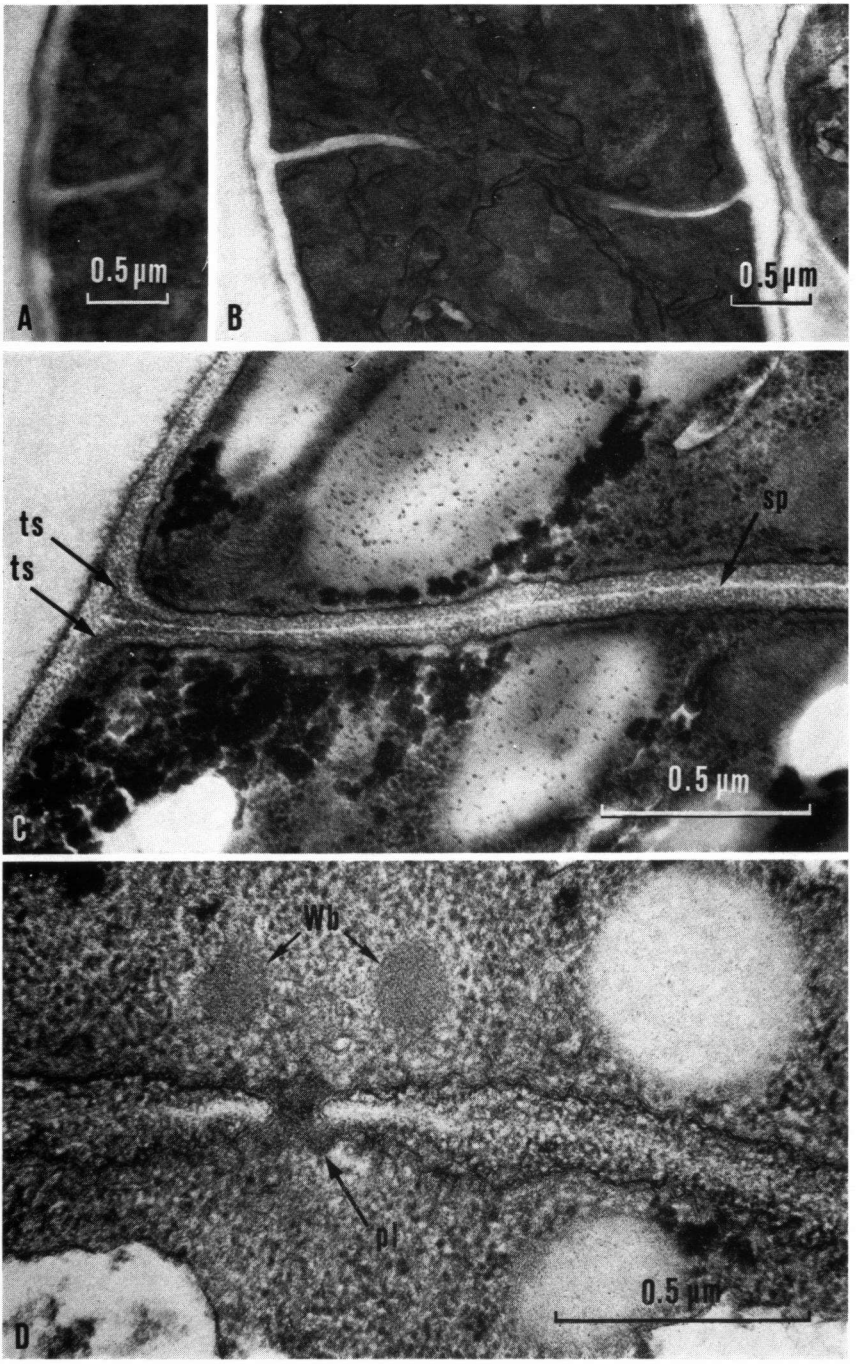
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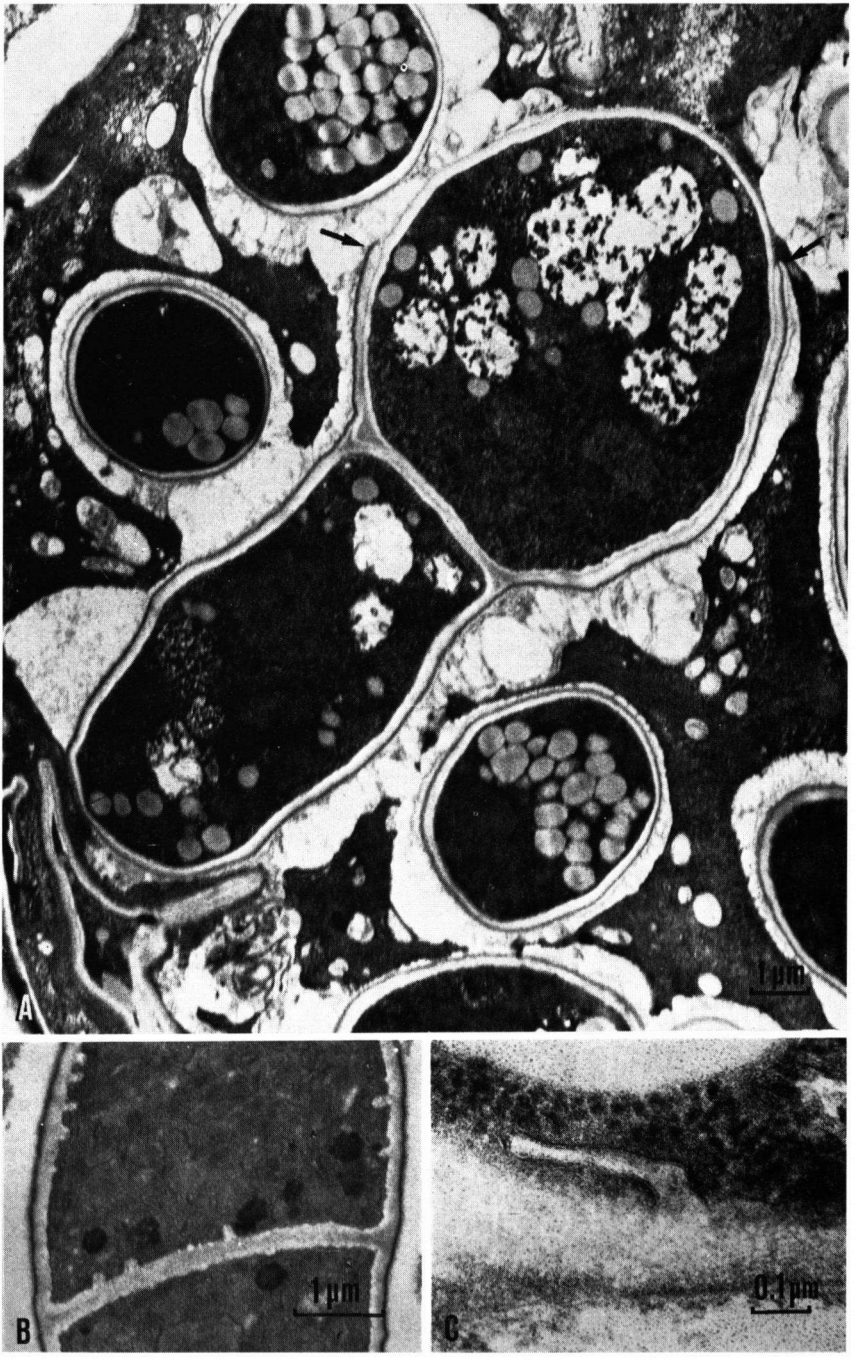
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