The ontogeny of *Stagonospora nodorum* pycnidia in culture

M. N. Douaiher[1i], P. Halama[1ii] & M. C. Janex-Favre[2]

[1]Laboratoire de Biotechnologie des Micro-organismes, Unité de phytopathologie, Institut Supérieur d'Agriculture, Université Catholique de Lille, 41 rue du Port, 59046 Lille Cedex, France
[2]Laboratoire de Parasitologie Végétale, Université Pierre et Marie Curie, Boîte 155, 4 Place Jussieu, 75252 Paris Cedex 05-France

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The ontogeny of *Stagonospora nodorum* pycnidia was studied *in vitro*. After conidium germination and mycelium development, the pycnidial primordium arises through the interweaving of young hyphal branches to form a network that later becomes compact. This network develops into a thin wall and a fertile centre, made of radiating filaments. These originate the future conidiogenous cells that fill the pycnidium cavity, which is formed schizogenously. In the mature ovoid pycnidium, conidia are released through an ostiole opening at the top of the ostiolar cone. Results are discussed with previous pycnidium ontogenic data and taxonomic position.

Keywords: *Stagonospora nodorum*, ontogeny, pycnidium, conidiogenesis.

Several investigators have revised the taxonomy and nomenclature of the fungi responsible for the so-called "Septoria diseases of wheat". However, pathologists have been slow to adopt the revisions proposed by mycological taxonomists. Among these species, the necrotrophic pathogen *Stagonospora nodorum* (Berk.) Castell. & Germano (syn. *Septoria nodorum* Berk.) is the causal agent of glume blotch of wheat. Sprague (1950) stated that *Stagonospora nodorum* was closer to the description of the genus *Stagonospora* than *Septoria* only on the basis of the conidial shape. Yet, no change in the taxonomy was offered before the transfer to *Stagonospora* by Castellani & Germano (1977) on the basis of the conidial length-width ratio. This nomenclature was passed as a resolution by the participants of the 4th International *Septoria* Workshop in Poland (Cunfer, 1997).

The ontogenic and structural studies of pycnidia are fragmentary and not recent. In general, the development of Ascomycetes pycnidia was described and illustrated by Bauke (1876), De Bary

[i] mn.douaiher@isa-lille.fr, [ii] p.halama@isa-lille.fr

(1884) and Zopf (1890). These authors distinguished two pycnidial primordial developments: meristogenous and symphogenous types.

Reproductive organs (pseudothecia, pycnidia and micropycnidia) have been induced *in vitro* (Halama & Lacoste, 1992 a, b) and the pseudothecia ontogeny of the teleomorph of *S. nodorum* was studied (Halama & al., 1992). This present paper has two aims. The first is to complete this study by providing information regarding the pycnidia ontogeny. Secondly, it is hoped that this study would bring more discussion elements about the transfer *Septoria nodorum* to *Stagonospora nodorum*.

In this work, we first describe characteristics of mycelium in culture and of pycnidium ontogeny and structure. In the second part, with subsequent studies, we discuss the origin of the pycnidium, the cavity and the ostiole.

Material and methods

The origin of isolate 6T used in this study has been described previously (Rapilly & al., 1992). Based on previous studies (Halama & Lacoste, 1992 a), pycnidium development is obtained under optimal conditions (10° C, alternating 12h NUV light/12h dark cycle) on synthetic medium (MS). Two forms of culture were used. The first one, strips of culture medium ($76 \times 26 \times 2$ mm), supports the stages from conidia germination to formation of the pycnidial primordium. The MS medium was poured on slides of glass in Petri dishes and inoculated with a suspension of conidia. Strips were taken out daily, coloured with cotton blue and observed under the microscope. The second method consisted of culture on MS medium in Petri dishes. Developing pycnidia were taken out bi-daily with a small block of medium to facilitate the orientation of the samples. Afterwards, the samples were fixed in Westbrook liquid (1955) and embedded in paraffin. Cuttings of 5 µm are dyed in ferric hematoxylin and eosin (1%). Axial sections are selected for the drawings and the analysis of successive stages.

Results

Mycelium growth and first stages

Using the thin strip of medium, it was possible to observe developmental stages from mycelial colonization up to the formation of the pycnidial primordium. The mycelium is composed of hyphal cells that are 2–6 µm in diameter. Hyphal cells arise from swollen conidia well vacuolated and with walls strongly stained by cotton blue. Later, the hyphal cells begin to thicken and initiate the formation of a mycelium with ramifications for development, evolving into a mycelial network (Fig. 1a).

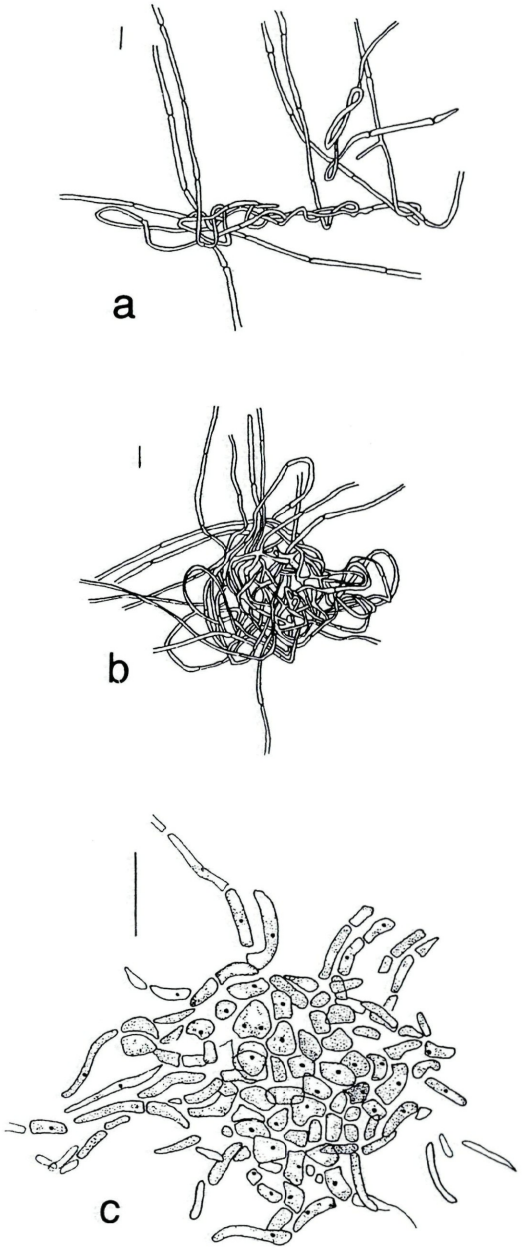

Fig. 1. Mycelial development. – a. Mycelial development and formation of a young knot. – b. Differentiation of a mycelial knot. – c. Pycnidial primordium. – Bars = 10 µm.

Three developmental stages of the pycnidium are recognized in culture :

Stage 1: The pycnidial primordium

Pycnidial primordium formation is initiated when several branches from different hyphae grow toward a common point and interweave to produce a mycelial knot or nodule (Fig. 1b). Cells with dense cytoplasmic content are differentiated in the central region (Fig. 1c). The pycnidial primordium grows via successive cell divisions in the peripheral layer. The structural organization of the pycnidial primordium becomes visible; a peripheral wall is apparent and a fertile centre differentiates (Fig. 2a), where the cells start to become conidiogenous cells (Fig. 2b). These oriented arrangements continue to establish throughout the fertile centre. The primordium increases in volume and develops into an almost piriform shape (Fig. 3a). Intense growth of the primordial pycnidium results in an ovoid mass. Tangentially oriented hyphae, with flattened cells, form the thin wall surrounding the primordium. At this stage, the upper part of the primordium consists of a cone, which is the future ostiole opening (Fig. 2b, 3a).

Stage 2: The formation and the extension of the pycnidial cavity and conidiogenesis

Cavity formation begins (Fig. 3a) and excavates schizogenously in the fertile centre; the process is related to the important growth phase of the pycnidium. The wall retains the same structure as in the previous developmental stage. Conidiogenous cells can be distinguished and are first located in the lower part of the fertile centre (Fig. 3b), then extend throughout. A subparietal layer of cubic cells is outlined between the fertile centre and the wall (Fig. 4a). The apical part of the pycnidium consists of a subconical tip, which is fairly morphologically differentiated but has a compact structure (Fig. 3b). The apical cone consists of flat meristematic darker cells.

At the time of maturity, conidiogenesis takes place in continuation of the cavity extension. The conidiogenous cells are numerous. Uninucleate conidia are produced actively at their tips (Fig. 4a). They detach from conidiogenous cells, and fill the cavity while it is still closed. Enteroblastic phialidic conidiogenesis (Fig. 5b) progresses centrifugally as follows: at the extremity of the conidiogenous cell, a swell occurs. It grows into a long terminal cell that is often uninucleate. The separation of the conidium takes place after the formation of a septum within the swelling side (Figs. 4a, 5a). Their final shape is elongated and they grow oriented toward the ostiole (Fig. 4b).

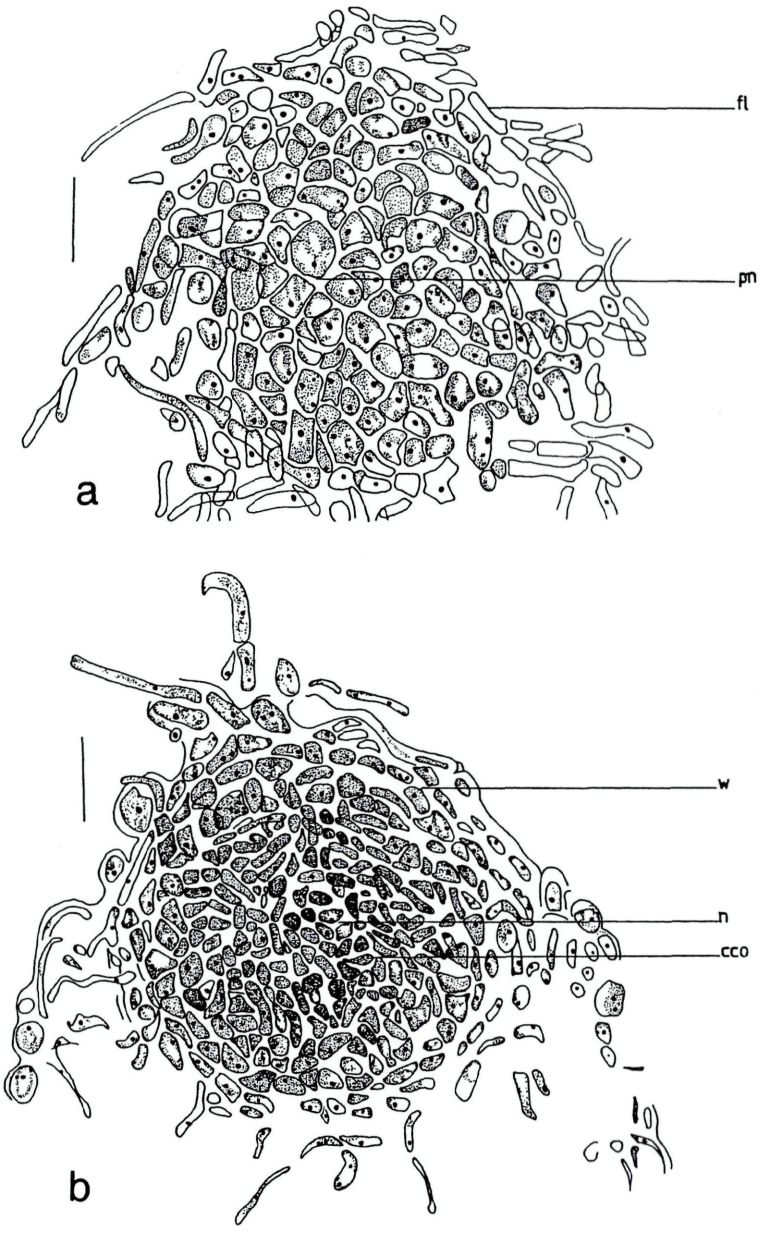

Fig. 2. – Pycnidial primordium. – a. Development of pycnidial primordium. – b. Initiation of a paraplectenchymatous nodule. – Bars = 10 µm. cco: conidiogenous cells, fl : future wall layer, n : nodule, pn : primordial nodule, w : wall.

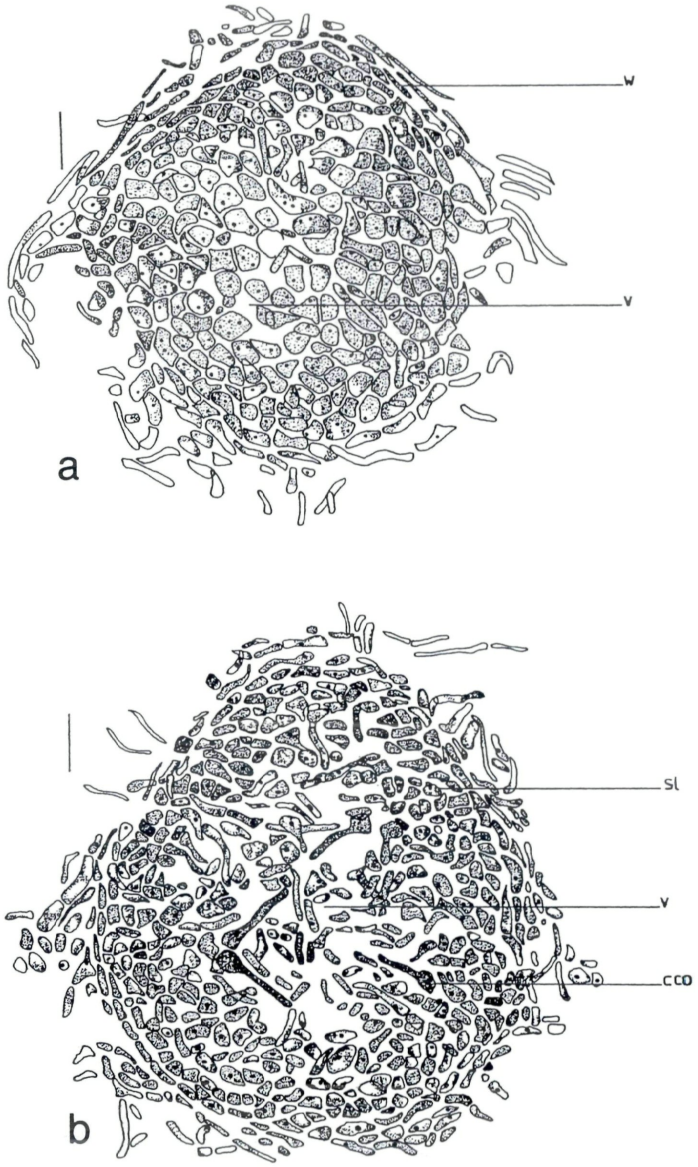

Fig. 3. – Development of the pycnidial primordium.. – a. Pycnidial primordium with the fertile centre, formation of the cavity and cells flattened at the ostiole side. – b. The formation and the extension of the pycnidial cavity and individualization of conidiogenous cells. – Bars = 10 µm. cco : conidiogenous cells, sl : subparietal layer, v : cavity, w: wall.

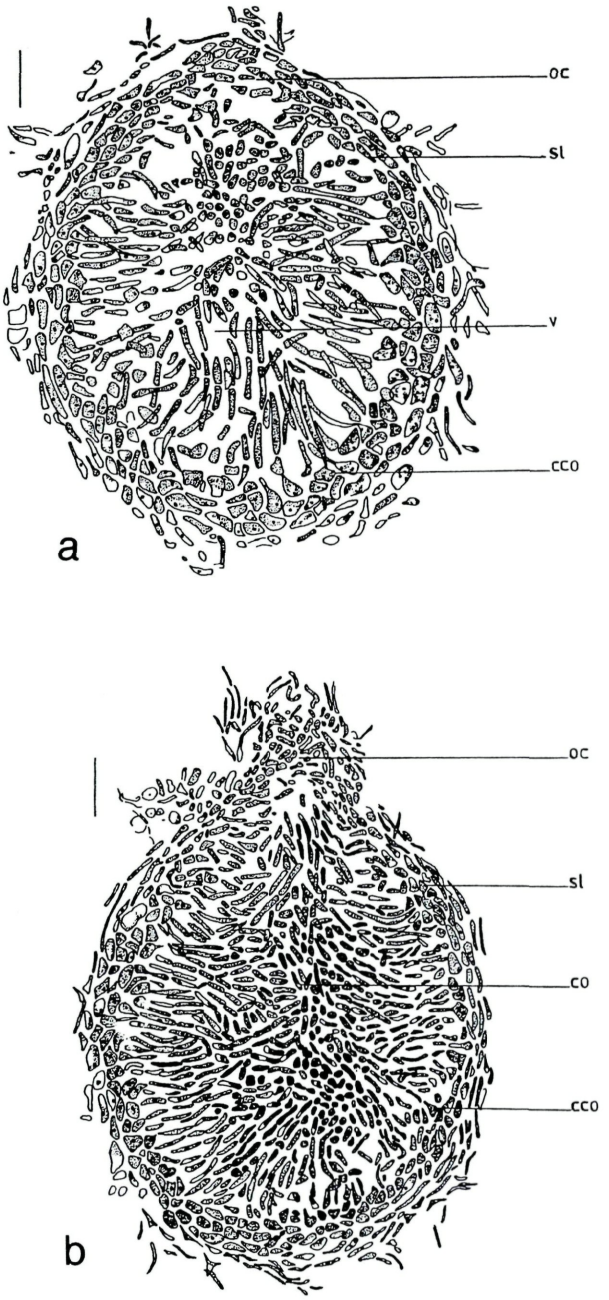

Fig. 4. – a. Young immature pycnidium.– b. Young pycnidium with conidiogenous cells which have produced conidia – Bars = 10 µm. cco : conidiogenous cells, co : conidia, oc : ostiolar cone, sl : subparietal layer, v : cavity.

Stage 3: The opening of the ostiole

At a later stage, as far as the constitutive parts are concerned, the organization of the pycnidium is unchanged. In the cavity, the conidia are increasingly numerous. The formation of the pycnidial ostiole does not coincide with the development of the fertile centre. The ostiole develops from the apical cone, composed of meristematic cells. At the time of opening, the internal cells of the apical cone break apart down under the pressure of the cavity contents. The cone empties from the base; it enlarges and a short cylindrical axial channel hollows it in continuation with the top of the cavity (Fig. 4b). Finally, the ostiole opening, which is the main characteristic of the mature pycnidium, permits the release of the conidia (Fig. 5c). A pink cirrhus is visible on each mature pycnidium. It consists of conidia lying in a mucilaginous exudate (Langeron & Vanbreuseghem, 1952). Most of the mature pycnidia have an ovoid to spherical form (Fig. 5c); some of them are pear-shaped (Fig. 4b). They contain a single cavity where the upper part narrows progressively up to the ostiole. At this stage, the pycnidia continue to produce pycnidiospores released through the ostiole. The conidiogenous cells remain active on the apical part of the pycnidium (Fig. 5c).

Discussion

In the genus *Septoria*, the origin of the pycnidial primordium is predominantly meristogenous. Indeed, the meristogenous mode was observed in *S. helianthi* Ell. & Kell. and *S. scrophulariae* Petrak by Kempton (1919) and in *S. chrysanthemella* Sacc., *S. socia* Pass. and *S. obesa* Syd. by Punithalingam (1966). In *Septoria polygonorum* Desm. the meristogenous mode is more often found, although a few primordia arise by the symphogenous method (Kempton, 1919). Both processes may occur in *Septoria leucanthemi* Sacc. & Speg. (Punithalingam, 1966). According to this typology, pycnidia of *S. nodorum* are related to the symphogenous type, the less frequent type of development among the few *Septoria* species previously studied.

For cavity formation in the pycnidia, two interpretations have also been proposed. Dodge (1923) and De Bary (1884) distinguished schizogenous and lysigenous processes. In *S. nodorum*, the central cavity is schizogenous. This one is formed as the central cells break down consequentially to the rapid growth of the pycnidial primordium. Among the Sphaeropsidales species, Archer (1926) claimed that in *Septoria lycopersici* Speg., the cavity was formed lysigenously by the lysis of the hyphae inside the pycnidium. Harris (1935) found that in *S. lycopersici* both processes interfered. A breaking of the cell layers first formed the pycnidial cavity but the presence of a

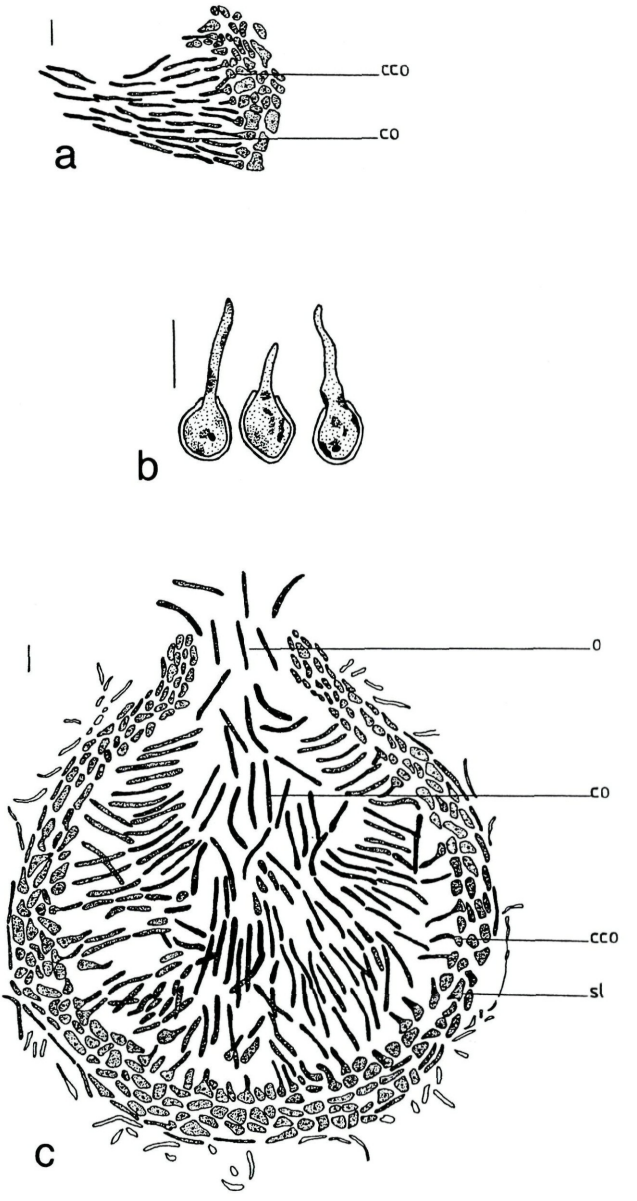

Fig. 5. – a. Details of the conidiogenesis. – b. Details of the conidiogenous cells. – c. Mature pycnidium, conidia released after the opening of the ostiole. – Bars = 10 µm. cco : conidiogenous cells, co : conidia, o : ostiole, sl : subparietal layer.

gelatinous substance in the pycnidium cavity suggested the dissolution of the hyphae prior to pycnidiospore formation. A similar mucilaginous substance is observed in the developing pycnidia of the *Septoria* species studied by Punithaligam (1966), and it seems that in these species the lysigenous process takes part in the cavity formation.

In the genus *Septoria*, *S. cytisi* Desm. (species type), *S. adanensis* Petrak, *S. glycines* Hammi, *S. helianthi*, *S. lycopersici*, *S. obesa* and *S. socia* (Sutton, 1980), *S. chelidonii* (Lib.) Desm., *S. convovuli* Desm., *S. elaegni* (Chevall.) Desm., *S. gladioli* Pass., *S. hyperici* Desm., *S. lactucae* Pass., *S. lavandulae* Desm., *S. lepidii* Desm., *S. oenothera* Westend., *S. paeonia* Westend., *S. scutellariae* Thüm. (Andrianova & Minter, 1999) and *S. chrysanthemella* (Kempton, 1919; Punithalingam, 1966, Sutton, 1980) have holoblastic conidiogenesis. In *S. bromi* Sacc., *S. elymi* Ell. & Everh., *S. passerini* Sacc., *S. tritici* (Rob.) Desm. (Bissett, 1983) and *S. apiicola* Speg. (Sutton, 1980), on the other hand, the conidiogenesis is enteroblastic phialidic. In the genus *Stagonospora*, *S. paludosa* (Sacc. & Speg.) Sacc. (species type), *S. anglica* Cunnel, *S. caricinella* Brun., *S. caricis* (Oud.) Sacc., *S. cylindrica* Cunnel, *S. elegans* (Berk.) Sacc. & Trav., *S. gigaspora* (Niessl) Sacc., *S. macropycnidia* Cunnell and *S. vitensis* Unam. (Sutton, 1980) have holoblastic, occasionally enteroblastic annelidic conidiogenesis. In contrast, the conidiogenesis of *S. nodorum* is enteroblastic phialidic (Sutton & Waterston, 1966; Sunny & Zeyen, 1977). In the present study, the conidiogenesis in *S. nodorum* was also observed to be phialidic; therefore, the species should remain included in the phialidic subgroup of *Septoria* together with *S. apiicola S. bromi*, *S. elymi*, *S. passerini* and *S. tritici*.

According to Dodge (1923), the ostiolar channel of *Schizoparme straminea* Shear is formed as the result of the breaking down of the early stage wall cells. In the instance of *Septoria lycopersici* (Levin, 1916; Archer, 1926; Harris, 1935) and *Guignardia bidwellii* (Ellis) Viala & Ravaz (Janex-Favre & al., 1993), the ostiole opening is caused by the tension exerted by the conidia on the cells of the ostiolar cone. The pycnidial neck empties from the base, further enlarges and opens, thereby permitting the release of the conidia through the ostiole. The same process occurred in *S. nodorum* except that different observations have been made on the presence or absence of the pycnidial neck of *S. nodorum*. Halama & al. (1992) observed a short beak with periphyses. However, in this study we did not observe any pycnidial beak, an observation confirmed by Sutton & Waterston (1966). This might be the result of different humidity conditions in the incubation phase (Punithalingam, 1966).

In conclusion, this study shows that the ontogeny of pycnidium primordium would remove *S. nodorum* from *Septoria*. If the position

of the species in *Septoria* is not adequate for the conidial ontogeny, the same observation may be made in *Stagonospora* for its placement. Indeed, the conidiogenesis of *S. nodorum* is phialidic while the type species of *Stagonospora* is holoblastic, occasionally annelidic. With the exception of *Septoria apiicola*, the *Septoria* subgroup of species with phialidic conidiogenesis including *S. nodorum* is reported on *Gramineae*.

The genus *Septoria* contains more than 2000 described taxa and in the genus *Stagonospora* about 350 species are described (Sutton, 1980). The genus *Septoria* is extremely large and heterogenous, requiring considerable revision. Few studies have been made on the ontogeny of pycnidia so all possible techniques including molecular ones, pycnidial and conidial ontogeny are required to clarify phylogenetic relationships among *Stagonospora* and *Septoria* species.

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