

Research Article

Developmental Morphology of Conidiomata in *Phyllosticta* Caryotae

Murugan M^{1*}, Arumugam P² and Arunkumar K³¹Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, India²Department of Zoology, School of Life Science, Bharathiar University, Coimbatore, Tamil Nadu, India³Department Of Plant Science, School of Biological science, Central University of Kerala, Kerala, India***Corresponding author:** Murugan M, Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, India**Received:** October 31, 2016; **Accepted:** December 09, 2016; **Published:** December 12, 2016**Abstract**

Coelomycetes are anamorphic Ascomycota that produce conidia within a flask-shaped ostiolate pycnidial conidiomata, acervular conidiomata or on a mass of vegetative hyphae (stroma). Coelomycetes fungi play an important role in terrestrial ecosystems. The objective of this work was to clarify the development of conidiomata in *Phyllosticta caryota* using light microscopical techniques. Light microscopic observations show symphogenous type of pycnidial formation. The conidia within young pycnidia were found with the sheath surrounding them. The mature conidium is multinucleate. The conidiogenous cells which are produced initially inside the cavity are referred to as "temporary conidiogenous cells". Subsequently, the cells lining the cavity elongate to form a palisade of permanent conidiogenous cells, which then start producing conidia. During dehiscence of the conidioma, these inner hyaline layers in the papilla dissolve to form the ostiole and the outer thick-walled cells form the beak-like structure of the conidioma. The papilla may also exert pressure to rupture the epidermis of the host for releasing the conidia outside.

Keywords: Appendages; Coelomycetes; Conidium ontogeny**Introduction**

Traditional classification of Coelomycetes was based on the morphology and was thus subjective, often resulting in artificial generic and species boundaries [1]. In view of the variability and diversity of the coelomycete conidiomata it is no easy task to accommodate them in a system of classification based on conidiomata satisfactorily. The fungi coelomycetes have conidia formed within a cavity lined by fungal or fungal/host tissue. The conidia-bearing structure (conidioma) is classified into five types according to exterior morphology: pycnidial, pycnothyrial, acervular, cupulate, and eustromatic [2] (Watanabe et al. 1998). Many researchers have described pycnidial-type conidiomatal development [3-8] (Mercer 1913). These facts suggest that the morphogenesis of conidioma has taxonomic value. In view of the variability and diversity in the form of the Coelomycete conidiomata it is no easy task to accommodate them in a system of classification based on conidiomata satisfactorily. Development is divided into three stages: primordia, cavity formation, and conidiogenesis, with each pycnidial fungus having a determinate mode in each stage.

The genus *Phyllosticta* (Teleomorph: *Guignardia* Viala & Ravaz was established by Persoon in 1818). Many species of *Phyllosticta* have been known as the causal fungi of leaf spot diseases of various plants [9]. The genus - *Phyllosticta* is also known as the endophytic fungus in a wide range of host plants, including *Ericaceae* [10-14]. Plant diseases caused by species of *Phyllosticta* are reported worldwide. Saccardo [15,16] revised the generic concept of *Phyllosticta*. The revision of Saccardo was based on perithecia which are subepidermal, lenticular, thin membranous, ostiolate, punctiform, areolate on leaf or rarely on stem, with small, ovoid to oblong, aseptate, hyaline to greenish, conidia. However, most subsequent investigators described the species based on the concept as "All leaf-inhabiting pycnidial fungi

with hyaline, one-celled conidia should be classified in *Phyllosticta*, whereas morphologically comparable fungi on stem in *Phoma*" [9]. The genus concept however was reconstructed by van der Aa and collaborators [17].

However, there is still controversy for the species concept. The species of teleomorph *Guignardia* and its anamorph are host species, with a host range confined to species of a single family, from the results of inoculation tests [18-21], whereas some are endophytic *Phyllosticta* cultures from 67 plant species in 54 genera of 38 families. All these cultures were identified as a single species, *Phyllosticta capitalensis* Henn, based on morphology and sequence analysis of ribosomal DNA internal transcribed spacer regions. Baayen et al. [10], revealed that *Guignardia citricarpa* Kiely (anamorph: *P. citricarpa* (McAlpine) Aa) was different from nonpathogenic species *Guignardia mangiferae*. Roy isolated from citrus, based on cultural characteristics, growth rate, and thickness of mucoid sheath surrounding the conidial wall. Also, they are different in nucleotide sequence data, although the size of their conidia was same. On the other hand, delimitation of the species of *Phyllosticta* relationship is not yet available. Hence, in this study, we applied Van der Aa and Vanev species concept [17,9] wherein the species epithet was given on the basis of fungus morphology on diseased leaf of host plant, cultural morphology on diseased leaf of host plant, cultural characteristics, and connection with teleomorph species.

Cryptosporiopsis radicola produces only excipular covering conidioma-like tissue with adhesive amorphous material and setae. Synnematos conidiomata with abundant macroconidia dominate the colony of *Cryptosporiopsis ericae*. *Cryptosporiopsis rhizophila* is different in its globose to subglobose conidiomata, consisting of loosely aggregated vegetative hyphae developing macroconidial conidiophores. *Cryptosporiopsis grisea*, being the only teleomorph-



Figure 1: Mature conidia. Conidia enveloped with a thick sheath (bar =12.5 μ m).

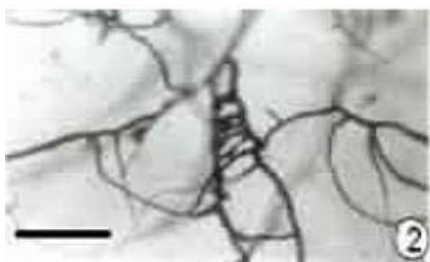


Figure 2: Compound meristogenous methods of primordial formation (bar =25 μ m).

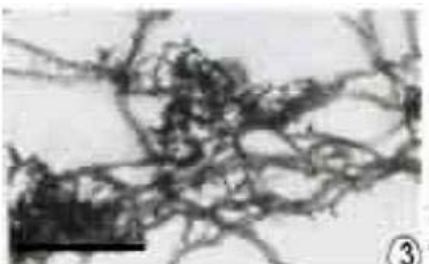


Figure 3: Symphogenous methods of primordial formation scale (bar =25 μ m).

connected species, differs from the others in its distinct columnar surface [22].

Therefore, as more and more data on this effect becomes available, the distinction between Hyphomycetes and Coelomycetes may be abandoned because of the presence of intermediary stages between hyphomycetes, acervular, stromatic and cupulate conidiomata. In this investigation developmental stages of the conidioma in the *Phyllosticta caryotae* state of the fungus were investigated. This forms part of a programme of work on developmental morphology and taxonomy of Coelomycetes which is in progress in this department.

Materials and Methods

Culture characters and identification

A culture of *Phyllosticta caryotae* Chen was isolated from leaf of *Citrus sinensis* from kodaikanal, India. It was grown on Oat Meal Agar (OMA) and Potato Dextrose Agar (PDA) in Petri dishes at room temperature (28°C). Cultures were incubated at 24°C in continuous light, and cultural morphology was examined after 7 days. Spore size was determined by measuring the length and width of 30 to 40 arbitrarily selected conidia from a conidial suspension. The isolates were identified initially by comparing morphological and cultural

characteristics (i.e., size of conidia, thickness and length of apical appendages).

Germination study

The initial stages of the development of conidiomatal primordia were studied by slide cultures [23]. For germination studies, conidia were collected aseptically from the teased out conidiomata in 1% glucose solution and allowed to germinate in cavity slides were kept at room temperature (28°C) and were examined every 5hrs for 36hrs to study germination.

Light microscope

To study the development of conidiomata, selected conidiomata with agar were trimmed into 2 mm square blocks and fixed in 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for 2hrs at room temperature (27°C) and 1h at 4°C and post fixed for 12hrs in 1% osmium tetroxide. Specimen were dehydrated through an ascending series of acetone (30-100%) at room temperature, each changes at 30min intervals, followed by 2-3 changes in fresh Spurr in the ratio of 3:1 (Acetone : Spurr) for 6hrs, followed by two changes with absolute Spurr mixture for 24hrs each lasting for 8hrs and polymerised in fresh spurr at 70°C for 8hrs. The samples infiltrated with the resin were transferred to a vacuum chamber for 1h for complete removal of air bubbles. Thin sections (0.5 μ m) were cut from these blocks and stained with 1% aqueous toluidine blue to study the development of conidiomata and conidiogenesis under the light microscope.

Results

Description of the fungus

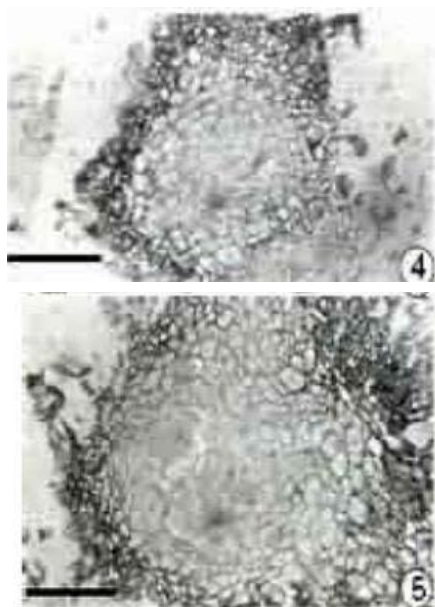
Conidiomata pycnidial, pycnidia black, spherical to subspherical, 75-120 μ m in diam. sometimes 200 μ m diam. with a small ostiole. In vertical section, the pycnidial wall 3-4 layered, the outer layer brown in colour, becoming pale brown to hyaline towards inside. Conidiogenous cells lining the pycnidial cavity, 1-2 celled, short, erect, 5-12 x 2.5 μ m, aseptate. Conidia formed singly at the apex of the conidiogenous cell, hyaline, aseptate, 7-13 x 5-10 μ m. The appendage arises from the apex of the conidium while the conidium is undergoing development and still attached to the conidiogenous cell (Figure 1).

Structure of conidia

The germination study showed that the germ tube is a stout structure observed at the basal region of conidium. Light microscopic studies by Punithalingam [24] showed that conidium is enveloped by a sheath which remains intact, while the outermost layer gradually gelatinizes with aging of the conidium. When observed with light microscope, the conidia within young pycnidia were found with the sheath surrounding them. The mature conidium is multinucleate (Figure 2).

Development of pycnidial primordia

The pycnidial primordium is initiated by the interweaving of hyphal branches to form a network, which is at first, loosely interwoven. This type of pycnidia initiation is termed as symphogenous. By repeated divisions, the primordial become pseudoparenchymatous (Figure 3). The earliest stage of pycnidial development seen in sectioned material consisting of small pseudoparenchymatous mass of tissue, the pycnidial primordium. The pycnidial primordium is



Figures 4,5: Sections of young primordium showing the formation of cavity (bar =25µm).

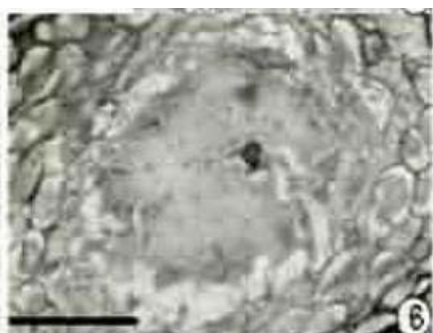


Figure 6: Sections of young primordium showing mucilaginous matrix (bar =50µm).

almost spherical consists of a compact cluster of thick walled cells. During further development, the primordium considerably increased in size by continued transverse and longitudinal divisions of the cells.

Formation of pycnidial cavity

As a prerequisite for the formation of pycnidial cavity, the cells at the periphery and centre of primordium were arranged as follows. The cells along the outer layer of the primordium became tangentially arranged which subsequently formed the conidiomatal wall. The inner cells of the primordium were thin walled and hyaline whereas the peripheral cells were thick walled and pigmented (Figures 4,5).

Formation of pycnidial cavity and sporogenous tissue

The central cells in the primordium showed signs of schizogenous activity to form the cavity. Further development stages showed the formation of the conidium simultaneously with the cavity formation. The cells lining the cavity commence conidial production by a budding-like process. Eventually, these conidiogenous cells along with conidia become detached from the wall of the conidiomata and remain floating inside the conidiomatal cavity (Figures 7-10). The

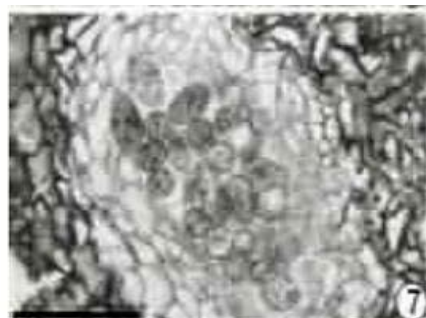
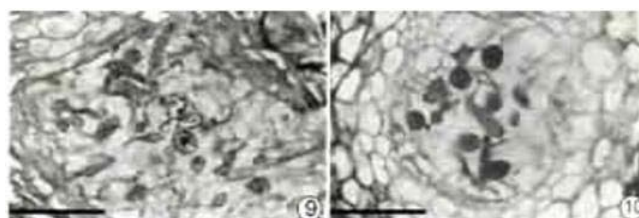


Figure 7: Vertical sections of conidiomata showing mature conidia (Note the well developed conidiomatal wall (bar =50 µm).



Figure 8: Section of young primordium showing conidial formation (bar =50 µm).



Figures 9,10: Vertical section of young primordium showing the dissolving central cells (bar =50 µm).

conidiogenous cells which are produced initially inside the cavity are referred to as “temporary conidiogenous cells” by Punithalingam [25]. These temporary conidiogenous cells occasionally bear an annellation and were normally blastic. As the temporary conidiogenous cells and conidium are detached inside the cavity, some of the conidiogenous cells might disorganize or dissolve to form a mucilaginous matrix which was clearly visible inside the cavity (Figure 6). Several sections of the conidiomata showed the presence of the mucilaginous matrix along with the detached conidia. Subsequently, the cells lining the cavity elongate to form a palisade of permanent conidiogenous cells, which then start producing conidia. Consequently, the conidioma becomes considerably larger in size by proliferation of outer pseudoparenchyma while the inner cells become arranged tangentially into several layers (Figures 11,12).

Formation of ostiole

When the conidioma becomes fully mature, a papilla-like projection appears at the apex. In vertical section, the papillate region showed thick walled, dark cells towards the outside and thin walled,

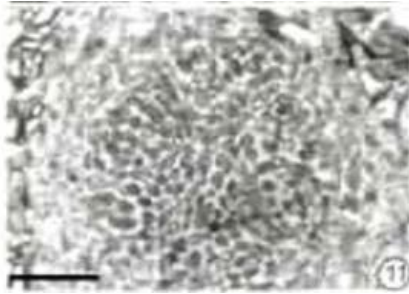


Figure 11: Vertical sections showing young conidiomata with the initially formed conidia (bar =25 µm).

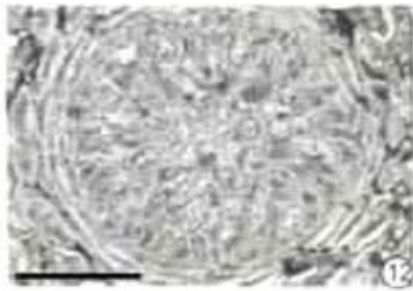


Figure 12: Vertical section of mature conidiomata showing the permanent conidiogenous cells and conidia (Note the developed wall layers) (bar =50 µm).

hyaline, elongated cells towards the inner side. During dehiscence of the conidioma, these inner hyaline layers in the papilla dissolve to form the ostiole and the outer thick walled cells from the beak like structure of the conidioma. The papilla may also exert pressure to rupture the epidermis of the host for releasing the conidia outside.

Discussion

The origin and structure of the apical conidial appendage of *Phyllosticta* spp. had already been fully described by Punithalingam and Woodhams [26]. The cultural characters and developmental studies of the *Phyllosticta* state of *Guignardia mangiferae* Roy along with the connection between the ascogenous state has also been thoroughly investigated [27,28]. Conidiomata developmental morphology of *Pestalotiopsis disseminate* is known to produce only acervular conidiomata on its host whereas in artificial culture media, pycnidial conidiomata were formed [29]. The morphology of conidia, appressoria and cultural characters of the ex-neo type culture was provided [30]. This work deals with different stages of development of the conidiomata within the natural host and also in artificial culture media. It was observed that the *Phyllosticta caryotae* investigated here produces temporary and permanent conidiogenous cells similar to those of *Ascochyta* spp [25] and *Macrophomina phaseolina* [24]. Normally the temporary conidiogenous cells produce a single conidium after which they get sloughed off from the wall layers. In the present fungus, the temporary conidiogenous cells occasionally show one annellation indicating that it is capable of producing more than one conidium. Probably after producing one or two conidia, the temporary conidiogenous cells may become detached from the wall layers. Another interesting finding is the presence of the thick sheath around the conidium. This sheath was also observed covering

the conidiogenous cell and the conidium initial. The presence of the sheath around the conidium confirms observation made by light microscope. Both transverse and longitudinal sections of the old conidia failed to reveal a sheath surrounding the conidia suggesting that the sheath gradually gelatinizes and disappears as the conidium becomes old.

The dual conidiation process was found as a general feature of *Ascochyta* species in culture [25] and also in *Macrophomina phaseolina* [24]. Punithalingam [25] reported that some of the observations made with *Ascochyta* in culture were in conformity with the interpretations of Archer [3] during his investigation of cavity formation and ostiole development in some members of *Sphaeropsidales*. Other coelomycete species studied by the authors, namely, *Botryodiplodia theobromae* Pat., *Ciliochorella mangiferae* Syd., *Coleophoma cylindrospora* (Desm.) Hohn., *Pestalotiopsis palmarum* (Cooke) Stey. And *Urohendersonia pongamia* Nag Raj & Ponnappa show this type of dual conidiation process in culture. In the present investigation *Phyllosticta caryotae* also was found to produce temporary and permanent conidiogenous cells. But *Robillarda depazeoides* (Welw. et Curr.) Sacc. was found to produce only one type of conidiogenous cells during conidiation in culture. Therefore the present study reveals that necessary data collected based on the developmental morphology of conidiomata for many more coelomycetes will be useful for segregating genera into different groups [31-33].

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