Pycnidium development in the causal agent of ginger leaf spot and a taxonomic note on the fungus

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Various leaf diseases have been reported on ginger (*Zingiber officinale* Rescoe) from Taiwan [9]. Among them, leaf spot was the most common one in Taiwan (Fig. 1). The causal agent of leaf spot was originally identified as *Phyllosticta zingiberi* Hori [7]. In a floral survey funded by the Council of Agriculture, we collected several isolates of coelomycetous fungi from the leaves that appeared to have 'leaf spot' symptoms. Microscopic observations and pathogenecity tests confirmed that all isolates were the same pathogenic fungus. Colonies of the fungus on potato dextrose agar appeared lanate to densely felted, gray to dark olivaceous-gray to blackish-brown, frequently with a paler margin after a few days when grown at 24°C. Mycelium composed two types of hyphae: hyaline to subhyaline, thin-walled, smooth, sparsely branched, straight or slightly flexuous, 2-4 μm wide; and pale to mid brown, thicker-walled, smooth, ellipsoid to globose, frequently branched, straight, flexuous consorted,

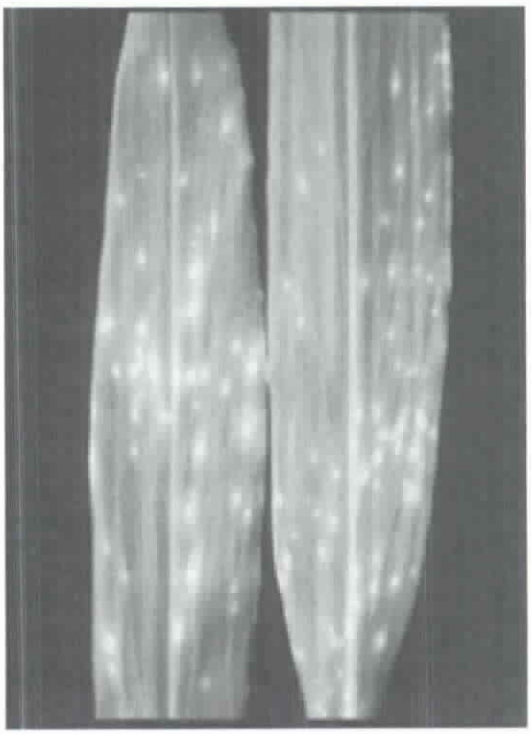

Fig. 1. The symptoms of ginger leaf spot.

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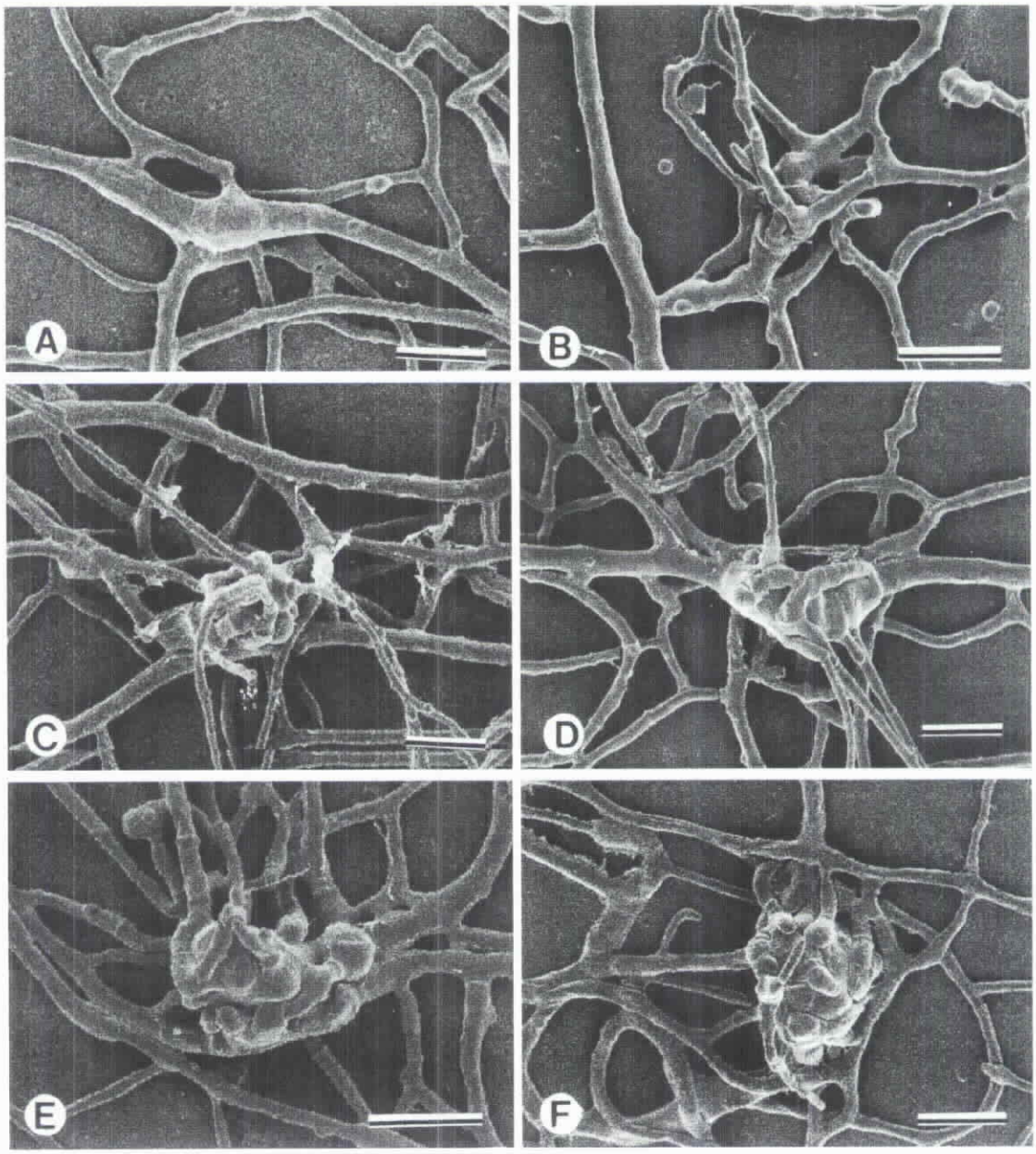

Plate 1. Development of conidiomata in *Phoma* species that causes ginger leaf spot. A. Different hyphae fuse to form a pycnidial initial. B. Hyphae lost orientation and multi-branched. C. More aerial hyphae develop from the cell complex and gradually become pycnidial primodium. D. Pycnidial primodium multiseptate and cell complex proliferated. E. Up-growth of pycnidial primodium. F. Pycnidial primodium develops through fusion of pseudoparenchyma cells. Bar=10 μm.

4-8 μm wide. The latter type frequently has individual cellular elements inflated up to 12 μm wide and clamydospore-like, constricted at the septa, aggregated and interwoven, especially in

the vicinity of pycnidial clusters to form loose, stroma-like masses. Pycnidia produced abundantly on PDA, seperated, more or less globose to subglobose, superficial, ostiolate, brown to black with age, 130-220 μm in diameter. Conidiogenous cells phialidic, hyaline, simple, smooth, ampuliform. Conidia enteroblastic, hyaline, smooth, oblong to ellipsoid or narrowly obvoid, straight, biguttulate, unicellular, 4.8-6.4 × 2.4-3.2 μm.

Since the dissemination of the disease mainly relies on the formation of the conidia, ontogeny of the pycnidium in this fungus is crucial. Unfortunately, ontogenesis of conidiomata in Coelomycetes has been studied only in a few taxonomic groups [2, 4, 10]. None has been done specifically on this fungus. In order to study the development of the fruiting structure, a scanning electron microscope (Jeol, FE6330, Tokyo, Japan) was applied for this purpose. The fixation, dehydration, critical point drying and metal coating followed the protocol reported elsewhere by Kuo [3].

The isolate used in this study was collected from leaf of ginger from Wufeng, Taichung in August, 1998 and maintained on potato dextrose agar in a 24°C incubator under continuous fluorescent light. For inducing pycnidial formation, a 3 mm diameter mycelium plug was inoculated on potato dextrose agar (Difco, Co., USA) overlaid with a layer of cellophane membrane. The earliest sign of pycnidial development shown on the medium was the fusion of surface slender hyphae and the enlargement of the fused cells (Plate 1-A).

On some occasions, hyphae became disoriented and branched several times to form a multicell aggregation from which cell volume increased (plate1-B) and aerial hyphae developed (plate1-C). While the volume of the cells increased, enlarged cells also underwent a series of multi- septation and developed into a cluster of cell aggregate (plate 1-D). Notably, the aerial hyphae were only about 2-3 μm half to one-third the length of the surface slender hyphae. The up-growth of the cell aggregate gradually developed into a spherical body (plate1-E), and the cells of the aggregate rapidly proliferated, became repeatedly septate, and aggregate into a ball of pseudoparanchymatous cells (plate 1-F). Cells of the aggregate were bulbose and turned into spherical shapes while increasing in size. When the primodium reached about 30-40 μm in diameter, it started to develop conidiogenous cells internally. At this point, the outer layer of primodium autolysed and the surface thread-like hyphae integrated into the outer layer of the primodium (plate 2-A). The young pycnidium continued to grow and more surface hyphae integrated into the pycnidial outer wall (plate2-B). While developing, the pycnidium began to develop an out-growth which turned into an ostiole (plate 2-C). The hyphae surrounding the pycnidial ostiole which remained filamentous but compact. These hyphae are arranged in parallel (plate 2-D). Mature pycnidium was globose with a distinct ostiole, from which conidia extruded (plate 2-E). In conditions of high humidity, the ostiole was forced to open and large amount of conidia extruded (plate 2-F).

Seaver used to treat leaf-borne *Phoma*-like fungi as *Phyllosticta* [8]. This is now considered as inappropriate [1]. Van der Aa indicated that conidia of *Phyllosticta* bear appendage and have *Guignardia* teleomorph. Neither of these could be found in this fungus. Based on Sawada, the distribution of this fungus only was restricted in China and Taiwan[7].

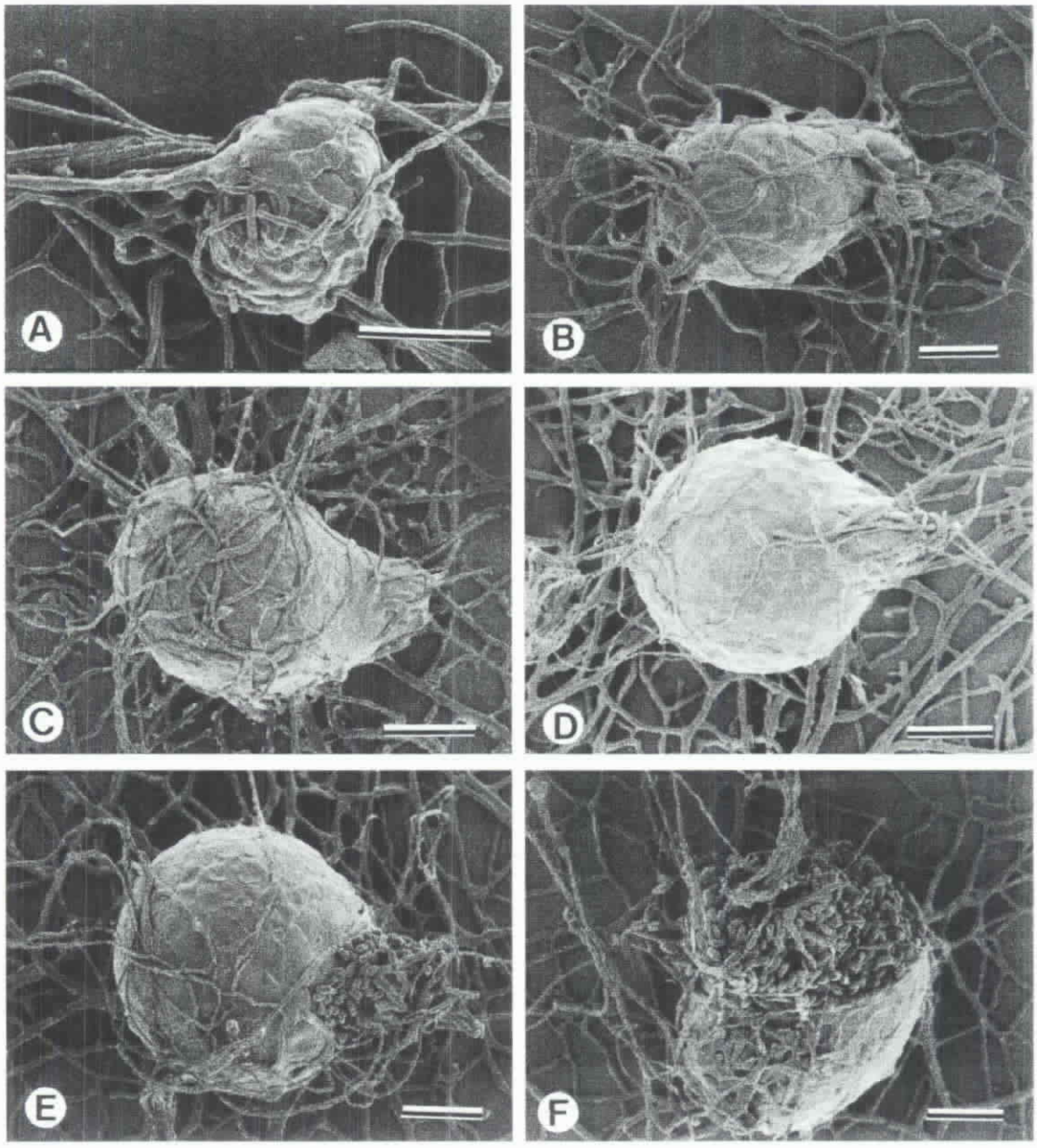

Plate 2. Development of conidiomata in *Phoma* species that causes ginger leaf spot. A. The outermost hyphae disintegrate into the pycnidial primodium. Some of the aerial hyphae collapsed and fused together to form this outermost layer. B. early stage of ostiolar development. The right hand side of hyphae start to form an ostiole. Another part of aerial hyphae devlops into pycnidial walls. Notice the morphological change that occurs when aerial hyphae touch the pycnidium. C. Surface hyphae of pycnidium continue to distintegrate into its outer layer, and the pycnidium increases in size due to the development of conidia inside. D. Surface hyphae integrate into cell wall of pycnidium and reveal pseudoparenchymatous cells. The ostiole continues to develop and forms a strand of hyphae that surround the ostiole outer walls. E. A mature pycnidium. Outer cells irregularly polygonal and conidia extruded from the ostiole. F. The ostiole is forced to open and large amounts of conidia are extruded when humidity is high. Bar=10 μm .

However, a similar fungus, *Phyllosticta zingiberis* F. Stevens & Ryan, was reported on ginger to cause leaf spot in Hawaii [5]. An Indian report indicated that the causal agent of ginger leaf spot was *Phoma exigua* var. *exigua* [6]. It is necessary to locate all of these holotypes and examine whether they are the same fungus. However, before a firm conclusion can be made, it is clear that the causal agent of ginger leaf spot is nothing close to *Phyllosticta* and therefore the earlier identification done by Sawada (1959) was incorrect. The generic name of the causal agent of ginger leaf spot should be transferred to *Phoma*.

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摘 要

郭克忠[1]*、李祈益[2]、鄭婉如[2] 2000 薑白星病原柄子殼發育及其分類 植保會刊 42：195－200.（1.台北市 行政院農委會動植物防疫檢疫局；2.臺中縣霧峰鄉行政院農委會農業藥物毒物試驗所）

薑白星病的病原最早訂名為 *Phyllosticta zingiberi* Hori. 根據澤田兼吉的記載，本菌最初只發生於中國及臺灣。七十年來關於本菌在培養皿中的性狀甚少有人加以描述。本試驗利用馬鈴薯葡萄糖培養基上覆賽絡芬膜的方法誘導柄子果的發育，發現柄子果芽體最早係由一般匍伏菌絲進行細胞融合，隨後融合處細胞膨大，並向上發育氣生菌絲，並進行一連串的菌絲分叉及多重分隔，最後形成一球狀芽體，芽體的一部份並逐漸分化，由緊密的絲狀菌絲組形成一口狀構造成為柄子果口，其餘的部份則由擬柔膜狀組織自行融解發育成柄子殼壁。柄子果內則形成大量柄孢子。柄孢子釋放受培養皿濕度影響，濕度大時大量柄孢子自孔口擠出。柄孢子大小 4.8-6.4 × 2.4-3.2 μm，內具兩油球、無色、不分隔、不具附絲。由柄子果發育過程及菌落特性可知其正確屬名應為 *Phoma* 並非屬 *Phyllosticta*。

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