Identity of Fungi inciting Charcoal Rot Disease

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Zusammenfassung: *Macrophomina phaseolina* (syn. *M. phaseoli*) ist die Ursache der Holzkohlenfäule verschiedener Getreidepflanzen. Die Pyknosporen von *M. phaseolina* sind hyalin und einzellig. Unsere Untersuchungen zeigten, daß Holzkohlenfäule aber auch durch andere Pilze verursacht werden kann, deren Sporen zuerst einzellig sind, später jedoch braun und zweizellig werden. Der Pilz mit braunen Sporen wurde früher als das Reifestadium von *M. phaseolina* betrachtet und in die Gattung *Botryodiplodia* gestellt. Er wird jetzt als neue Art, *B. solani-tuberosi*, beschrieben, da er von *M. phaseolina* verschieden ist.

Summary: *Macrophomina phaseolina* (syn. *M. phaseoli*) causes charcoal rot of several crop plants. The pycnidiospores of *M. phaseolina* are hyaline and one-celled. Our studies show that charcoal rot can also be caused by another fungus, which has spores at first hyaline and one-celled but later brown and two-celled. The brown-spored fungus was earlier regarded as the mature stage of *M. phaseolina* and was placed in the genus *Botryodiplodia*. This fungus is now described as a new species, *B. solani-tuberosi*, because it is distinct from *M. phaseolina*.

Macrophomina phaseolina (Tassi) G. Goidanich is an important pathogen on several crops in many parts of the world (Reichert & Hellinger, 1947). It is best known as the cause of a disease commonly called "charcoal rot". This fungus can also cause hollow stem rot, wilt, and pre- and post-emergence damping-off. However, the shredding of the infected tissues that bear black sclerotia the size of pinheads is the characteristic symptom.

M. phaseolina has distinctive vegetative and reproductive stages, and both stages have been described under several names. The fungus was first recognized as a cause of charcoal rot when Taubenhaus (1913) discovered the sclerotial stage, *Sclerotium bataticola* Taub., on *Ipomoea batatas* (L.) Lam., sweetpotato, in the United States. In India, Butler (1925) found a similar sclerotium-bearing fungus that, upon comparison with Taubenhaus' isolates, he named *Rhizoctonia bataticola* (Taub.) Butl.

Tassi (1901) first described the pycnidial stage of the fungus as *Macrophoma phaseolina*. In 1947 it was transferred to *Macrophomina*

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by GOIDANICH (1947). *M. phaseolina* (TASSI) G. GOIDANICH has nomenclatural precedence over *Macrophomina phaseoli* (MAUBL.) ASHBY (ASHBY, 1927), which MAUBLANC (1905) described as *Macrophoma phaseoli* in 1905 and which is apparently conspecific with the fungus named by TASSI. Names listed as synonyms of *Macrophomina phaseolina* in CMI Descriptions of Pathogenic Fungi and Bacteria No. 275, 1970, include *M. phaseoli* (MAUBL.) ASHBY and *M. philippinensis* PETR.; *Macrophoma phaseolina* TASSI, *M. phaseoli* MAUBL., *M. cajani* SYD. & BUTL., *M. corchori* SAWADA, and *M. sesami* SAWADA; *Sclerotium bataticola* TAUB.; *Rhizoctonia bataticola* (TAUB.) BUTL., *R. bataticola* (TAUB.) BRITON-JONES, and *R. lamellifera* SMALL; and *Dothiorella cajani* SYD. & BUTL.

The genus *Macrophomina* was established by PETRAK (1923) for *M. philippinensis*, parasitic on *Sesamum indicum* L., sesame. At present, *Macrophoma* and *Macrophomina* are differentiated chiefly by their mycelial stages, the hyphae of *Macrophomina* bearing numerous, irregular, minute, few- to many-celled sclerotia. Often, even in the absence of pycnidial structures, the charcoal-rot fungus is referred to as *Macrophomina phaseolina* on the basis of the sclerotium-bearing mycelial stage and the characteristic rot that it causes.

In studying charcoal rot of *Zea mays* L., maize, and *Solanum tuberosum* L., potato, on the Indian Plains, THIRUMALACHAR found that the hyaline spores of pycnidial material became two-celled and brown with longitudinal striations after overnight incubation in a moist chamber. He originally thought that the hyaline spores were *Macrophomina phaseoli* and, thus, that *M. phaseoli* was an immature phase of the brown-spored fungus, which he named *Botryodiplodia phaseoli* (MAUBL.) THIRUM. (THIRUMALACHAR, 1953). Since then we have studied numerous isolates of charcoal rot-inciting fungi from legumes in India, and from *Lycopersicon esculentum* MILL., tomato, and potato grown in the southern United States. We also have examined authentic material of MAUBLANC's fungus in the herbarium of the late Dr. Franz PETRAK, Vienna, Austria. Our studies indicate that two different fungi cause similar charcoal-rot symptoms and that they can be distinguished only when the pycnidial stages are produced. Apparently both fungi have worldwide distribution.

Isolates of the two fungi grown on several media resemble each other closely in temperature requirements, morphology of the mycelium, and development of sclerotia. Both cause wilt of cotton seedlings when soil is inoculated by the method of MONIZ, PATEL & THIRUMALACHAR (1956). Both readily produce pycnidia on agar media seeded with filter paper discs saturated with ether extract of peanut meal (KNOX-DAVIES, 1965 & 1966). GOTH & OSTAZESKI (1965), using natural plant material, showed that light was essential for sporulation of *M. phaseoli*.

We produced pycnidia of both species on potato stems, on filter-

paper discs by the Knox-Davies method, and on agar by a method that we have recently developed for the production of fruiting bodies in diverse fungi. In this latter technique, which will be described in another paper, a few sterilized, dehusked sesame seeds are spread on solidified sterile one percent water agar in Petri plates to serve as the growth substrate.

A comparison of pycnidia and spores confirms that the two fungi studied are distinct species. *M. phaseolina* has hyaline, one-celled, ovate-ellipsoidal pycnidiospores measuring 13—25 $\mu \times 6.5$ μ. Such pycnidiospores have been observed on leguminous plants in India and in an isolate from tomato grown in Texas (USA). This fungus has been isolated from potato, blackened maize kernels, *Ricinus communis* L. (castor bean), *Brassica rapa* L. (turnip), and *Raphanus sativus* L. (radish) in India and the United States. It should be referred to as *Macrophomina phaseolina* (Tassi) G. Goidanich. The second fungus has black, ostiolate pycnidia. These pycnidia initially produce hyaline, glassy spores, 18—35 $\mu \times 8$—12 μ, that later become dark brown, two-celled, somewhat rounded at both ends, and longitudinally striated. This is the fungus that previously had been designated *B. phaseoli* (Maubl.) Thirum. by Thirumalachar (1953) on the assumption that the hyaline spores of *Macrophomina phaseoli* (Maubl.) Ashby eventually darken and become two-celled. However, they do not, and the two fungi are distinct. The one with the brown, two-celled spores is an undescribed species, for which we propose the name *Botryodiplodia solani-tuberosi* Thirum. & O'Brien. Because *B. phaseoli* (Maubl.) Thirum. is nomenclaturally attached to *Macrophoma phaseoli*, it becomes synonymous with *Macrophomina phaseolina* (Tassi) G. Goidanich, of which *Macrophoma phaseoli* Maubl. is a facultative synonym.

Botryodiplodia solani-tuberosi Thirum. & O'Brien, spec. nov.

Syn.: *Botryodiplodia phaseoli* sensu Thirum., Phytopathology **43**: 610. 1953; non *Macrophoma phaseoli* Maubl., Bull. Soc. Myc. France **21**: 90. 1905.

Mycelium fuscum, sclerotia numerosa minuta gerens; sclerotia pauca usque ad pluricellulata, irregularia, saepe 100 μ diam.; pycnidia atrofusca, caespitosa vel rare dispersa, immersa deinde erumpentia, 100—250 μ diam., ostiolata; paries pycnidicus multicellularis, extrinsecus valde pigmentosus et crassitunicatus; conidia matura fusca, ellipsoidea, glabra, uniseptata, 18—35 $\mu \times$ 8—12 μ longistudinaliter striata exappendiculata.

Hab. e tuberibus *Solani tuberosi* morbo "charcoal rot" dicto affectis, India June 1968 (typus HACC-250); et e plantis *Zeae maydis* e morbo eodem affectis isolata, India et USA. Cultura typica in American Type Culture Collection, Rockville, Maryland; Commonwealth Mycological Institute, Kew, England; Centraal Bureau voor Schimmelcultures, Baarn, Netherlands; Indian Agricultural Research Institute, New Delhi, India.

Mycelium dark, bearing numerous minute sclerotia; sclerotia few- to several-celled, irregular, often 100 μ in diameter. Pycnidia dark

brown, aggregated in clusters, rarely scattered, immersed, becoming erumpent, 100 μ to 250 μ in diameter, ostiolate. Pycnidial wall multicellular, heavily pigmented and thick-walled on the outer side. Mature conidia dark brown, ellipsoidal, smooth, one-septate, 18—35 μ× 8—12 μ, longitudinally striated, appendages lacking.

Hab. Isolated from charcoal-rot-affected potato tubers in India, June 1968 (HACC-250 type); from charcoal rot on maize (India and USA) and jute (India). Type culture deposited in American Type Culture Collection, Rockville, Maryland; Commonwealth Mycological Institute, Kew, England; Centraal Bureau voor Schimmelcultures, Baarn, Netherlands; and the Indian Agricultural Research Institute, New Delhi, India.

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