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Review Article

TAXONOMY OF *PHYTOPHTHORA Spp.* CAUSING BLACK POD DISEASE OF COCOA (*THEOBROMA CACAO* L.)

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ABSTRACT

Phytophthora **pod rot, commonly known as black pod, is a major disease of cocoa in all cocoa growing countries. Information available on the taxonomic status of causal organism and criteria viz., morphological, physiological, biochemical, serological, molecular, pathological and non-morphological for classification of causal organism are reviewed.**

INTRODUCTION

Black pod disease of cocoa (*Theobroma cacao* L.) was first reported from Guyana and West Indies by Jenman and Harrison in 1897 and referred to the disease as black cocoa. Since then it has been reported from various cocoa growing countries. Even though the occurrence of *Phytophthora* was confirmed earlier on several hosts, black pod disease of cocoa was reported for the first time from India in 1965 (Ramakrishnan and Thankappan, 1965). At present, pod rot of cocoa caused by *Phytophthora* is prevalent in all cocoa growing countries (Lass, 1985). Black pod disease of cocoa has been referred by different names in different languages. In English, the common name 'black pod' has been widely used. The common name 'pourriture brune' in French and 'podridaopardo' in Portugese language are literally translated into English as 'brown rot'. In Spanish it is known as 'podredumbre negra' which is literally translated as 'black rot'. The disease is commonly known as 'black pod' for the last eighty years (Thorold, 1974). Therefore, in the present review, the term black pod has been adopted for the *Phytophthora* pod rot of cocoa. Pods of all ages are susceptible to black pod disease. The infection appears as one or more small chocolate brown to dark brown lesion on any part of the pod surface. Within four to seven days of infection, lesion enlarges assuming an elliptical shape and is partially covered by a whitish mycelial growth. After about 15 days, the entire pod and beans are invaded by the fungus and the pod turns black in colour, hence the name black pod disease (Thorold, 1974).

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TAXONOMIC STATUS OF CAUSAL ORGANISM

When Jenman and Harrison (1897) recorded the occurrence of black pod disease, they noticed the spore masses of the causal organism on the lesion surface and demonstrated the spread of the disease through natural contact between pods as well as through the spores brought into contact with healthy fruits. Carruthers (1898) was the first to record the fungus associated with pod and identified it as *Pernospora corda* in Sri Lanka. Masee (1899), after examining infected pods from Trinidad, identified the fungus on the lesions as *Phytophthora omnivora* de Bary. In Trinidad, Hart (1900) made the first successful isolation of the causal organism of black pod disease. Faber (1909), after a study of the fungus on pods in Cameroon, was dissatisfied with Masee's identification and pointed out the difference between *P.omnivora* and his isolate. Therefore, he retained only the generic name *Phytophthora* de Bary for his isolate. Based on Faber's work, Maublanc (1909) described the *Phytophthora* infecting cocoa as a new species and identified it as *Phytophthora faberi* Maubl., thereby distinguishing the fungus from *P.omnivora.* Rorer (1910) also reported that *Phytophthora* associated with the disease in Trinidad differed in several respects from the description of *P. omnivora.* Though Coleman (1910) described the fungus associated with black pod disease in Sri Lanka as *Phytophthora theobromae* Coleman, he withdrew it on recognition of the priority of Maublanc's (1909) nomenclature. Rosenbaum (1917) received a culture of *Phytophthora* from Rorer which he identified as *P. faberi.* Reinking (1919) reported that the fungus causing coconut bud rot was identical with *P. faberi*, the causal organism of pod rot and stem canker of cocoa, fruit rot and canker of rubber *(Hevea brasiliensis L.)* and papaya (*Carica papaya L.*) fruit rot. He also noted that *P. faberi* isolated from cocoa caused bud rot of coconut. Later, after a careful examination of Reinking's figures and descriptions of *P.faberi,* Butler (1925) confirmed that this fungus was morphologically identical with *P. palmivora* (Butl.)Butl. which was reported as the causal organism of bud rot of palmyrah palm *(Borassus flabellifer L)* (Butler, 1907 and 1910).

Ashby (1922), while accepting the morphological identity of *P. faberi* and *P. palmivora* to include cultural characters and recognised it as an omnivorous tropical species widely existing as number of morphologically, sexually, culturally and in some instances pathologically distinguishable strains. He described *P. palmivora* as having "sporangia which when mature, fall away from the sporangiophores by natural abscission with a short, often stout and more or less occluded pedicel, mostly 2-4 µm long and not exceeding 6 µm in length".

Tucker (1931) recognised two groups of *P. palmivora* isolates and referred them as 'typical' and 'atypical' forms which were distinguishable on the basis of sporangial morphology. The average length:breadth (L/B) ratio of sporangia of typical forms was 1.5 -2.1 and that of 'atypical' was 1.3-1.6 Based on the morphological distinction among isolates of *P. palmivora* infecting rubber and cocoa, Orellana (1959) suggested that *P. palmivora* isolates should have varietal status: *Phytophthora palmivora* (Butl.)Butl. var *theobroma* (Colm.) Orell. for the strain infecting cocoa and *P. palmivora var. heveae* (Thomps.) Orell for that on rubber. Other workers did not accept host specialization as a basis for the subdivision of the species of *Phytophthora.* Based on differences in morphology, pathogenicity and oospore formation, Turner (1960) classified West African isolates of *P. palmivora* on cocoa into three groups viz., 'G' type (Ghanian), 'N' type (Nigerian) and 'A' type (Angolan) in which he placed 'atypical' forms. In Nigeria, he found both 'N' and 'G' types on cocoa. On the other hand, studies on isolates of *P. palmivora* from many hosts have tended to have confusing results often leading to the assumption that *P. palmivora* was simply an extremely variable species exhibiting a wide range of cultural types (Zentmyer, 1974).

Waterhouse (1974a) regretted that studies on morphological differences among isolates of *P. palmivora* were neglected by preoccupation with compatibility types (Savage *et al.*, 1968; Brasier, 1972; Zentmyer *et al.*, 1973). She identified two morphological forms viz., MF1 and MF2 as 'typical' and 'atypical' forms respectively and also an additional 'pepper' form in the complex of *P. palmivora.* She further found

A1 and A2 compatibility types in each of the morphological forms. These groups were identified based on sporangial morphology. Sporangia of MF_1 were ellipsoidal or ovoid and having round base with short stalk (2-10 μm in length) and L/B ratio usually more than 1.4 whereas the sporangia of the MF_2 were shorter, obpyriform and ovoid with an average L/B ratio of 1.3-1.4 and oogonia tapering to a funnel shaped base. Black pepper form differed from the other two groups in forming only few sporangia and in the production of infrequent or no chlamydospores. Sporangia of this group were not shed or rather difficult to dislodge.

Studies on the taxonomy of *Phytophthora* were given a fresh dimension with demonstration of chromosome differences among isolates from cocoa in West Africa (Sansome *et al.*, 1975). Accordingly, one group termed 'S' type had 9-12 smaller chromosomes and the other termed 'L' type had 5-6 large chromosomes. The chromosome differences in other characters including colony morphology, growth rate and sporangial morphology. The A1 compatibility type was predominant in 'L' and A2 in 'S'. Based on the detailed studies, it has been concluded that 'S' and 'L' types were distinct species and that they were identical to 'cocoa' and 'rubber groups of *P. palmivora* described by Ashby (1929a), the 'typical' and 'atypical' groups of Tucker (1931) the 'G' and 'N' forms of Turner (1960) and morphological forms 1 and 2 of Waterhouse (1974a).

During the cocoa *Phytophthora* workshop at Rothamsted in 1976, at least three and possibly more distinct morphological forms within *P. palmivora* were identified. These forms were temporarily designated as MF_1 and MF_2 (Waterhouse, 1974a), MF3, MF_4, 'other types' and 'pepper forms' (Griffin, 1977). MF_1 corresponded to the 'S' type and MF_3 to the 'L' type of Sansome *et al.* (1975), whereas the 'other types' were identical to that of 'A' (Angolan) described by Turner (1960) and the 'black pepper' form to that reported by Tsao and Tummakate (1977). Differences in cultural morphology, sporangial morphology, pedicel length and chromosome number formed the basis for the grouping of the isolates into four different morphological forms (MF). The important characteristics of these four morphological forms (Griffin, 1977) are given below:

MF_1: Culture on carrot agar (CA) medium stellate/striate/smooth combed with sharply, well-defined edges; aerial mycelium usually sparse. Sporangia with round base, deciduous with a short, broad and occluded pedicel (<5 μm in length); L/B ratio 1.2 -1.8; small chromosomes, n=9-12. Isolates predominantly A2 compatibility type; A1 predominant only in Jamaica. An important cosmopolitan pathogen.

MF_2: Culture stellate or striate. Sporangia similar to MF1, round base and deciduous with a short occluded pedicel (25 μm). The economic importance of MF_2 as a pathogen of cocoa was uncertain and a geographical distribution was not known.

MF_3: Cultures on CA with plentiful cotton wool like mycelium over the entire colony, leading edge more diffuse and less regular than MF_1, no distinct colony pattern. Sporangia with round base, deciduous with thin stalk containing cytoplasm; stalk length 10-15 μm. L/B ratio of sporangia 1.2-1.6. Chromosomes large, n=5-6. Predominantly A1 compatibility type. Mainly restricted to Nigeria and Cameroon. An important cocoa pathogen.

MF_4: Cultures on CA mostly slightly petaloid with a moderate amount of aerial mycelium, rarely stellate/radiating pattern. Sporangia elongated, base usually tapered towards the stalk giving a 'sloping shoulder appearance, shed with thin long stalks (>15μm in length). L/B ratio of sporangia

1.9 some upto 2.3; A1 and A2 compatibility types were present. Chromosomes not determined. Predominant in South American countries.

'Other types' reported earlier from Brazil, Cameroon, Ecuador, Ivory Coast, Nigeria and Sierra Leone were found to be 'atypical' isolates. It was suggested in the cocoa *Phytophthora* workshop that MF_2 isolates could be included under MF_1. Dr. P.H. Tsao of California University, Riverside felt that black pepper isolates of *P. palmivora* should be described as a separate species on the basis of detailed studies made by him. There were obvious similarities between the black pepper isolates and the MF_4 cocoa isolates from Brazil.

Subsequently, Zentmyer *et al.* (1977) and Kaosiri *et al.* (1978) divided the 80 cocoa isolates of *P. palmivora* from Africa, America, the Pacific and South East Asia into three main groups based on the sporangial pedicel length. These three groups were identical to the morphological forms 1, 3 and 4 tentatively assigned to the isolates of *P. palmivora* at the cocoa *Phytophthora* workshop in 1976. They also identified a fourth group consisting of isolates with non-caducous sporangia (persistent) which probably corresponded to that of isolates assigned to the 'other types' at the Rothamsted cocoa *Phytophthora* workshop or to that of 'A' (Angolan) type described by Turner (1960). Kaosiri *et al.* (1978) also noted similarities between *P. palmivora* MF_4 isolates and *P. capsici*. Probably, the isolates of *Phytophthora* associated with cocoa in Trinidad, reported by Baker (1936) and Sreenivasan (1982) and the Brazilian isolates described by Medeiros (1976) might correspond to the MF4 type described in the cocoa *Phytophthora* workshop.

Based on detailed studies on morphological and physiological characteristics of 950 isolates of *Phytophthora* collected from all the major cocoa growing areas of the world including isolates studied by Gadd and Ashby in 1920's, Brasier and Griffin (1979) found that majority of the isolates of *P. palmivora* belonged to either one of the three main groups viz., MF1 ('S' type), MF_3 ('L' type) and MF_4 and they opined that the three forms should be designated as three separate species. Thus, MF_1 ('S' type) and MF_2 forms were redesignated as *P. palmivora* since detailed studies did not support MF_2 as a distinct form of *P. palmivora*. MF_3 ('L' type) could not be attributed to any known species and therefore it was described as a new species, *Phytophthora megakarya* Brasier and Griffin. They also noted that *Phytophthora* isolates from black pepper closely resembled with *P. palmivora* MF_4 in sporangial shape, sporangial pedicel type, oogonial morphology and their ability to respond to *Trichoderma* and therefore suggested both black pepper and MF_4 forms as one and the same species. According to Brasier and Griffin (1979), the suggestions of Sansome *et al.* (1975) that the 'S' and 'L' types corresponded to 'cocoa' and 'rubber' groups of Ashby (1929a), the 'typical' and 'atypical' of Tucker (1931), the 'G' and 'N' type of Turner (1960) and morphological forms 1 and 2 of Waterhouse (1974a) should be retracted. In their opinion, *P. palmivora* ('S' type) and *P. megakarya* ('L' type) corresponded to Turner's (1960) 'G' and 'N' forms respectively. But they did not correspond to the other forms listed.

Zentmyer *et al.* (1981) have examined isolates described as *P. palmivora* MF_4 and concluded that they should be renamed as *P. capsici* because of the many morphological similarities existing in the two *Phytophthora* groups. During the seminar on cocoa black pod at Rothamsted, England in 1980, it was decided that the widespread MF_1 and probably MF_2 were considered as *P. palmivora*, MF_3 as *P. megakarya* Brasier and

Griffin and MF_4 as a form of *P. capsici* Leonian (Waller, 1981).

Based on detailed comparative studies on morphology, reproductive physiology, electrophoretic protein patterns and isozymes of 25 typical isolates of *P. capsici* from 5 hosts and 29 isolates identifiable as *P. palmivora* MF_4 from 7 other hosts, Tsao and Alizadeh (1988) merged the two species into a single species. *Phytophthora capsici* Leonian amend A. Alizadeh and P.H. Tsao. Subsequently, the species concept of *P. capsici* Leonian emend A. Alizadeh and P.H. Tsao has come under additional scrutiny. Uchida and Aragaki (1989), using morphological characters such as chlamydospore production and sporangia size and shape, suggest that some isolates designated as *P.capsici* may be sufficiently distinct to warrant a separate species description. Cocoa *Phytophthora* isolates with non-caducous (persistent) sporangia described by Kaosiri *et al.* (1978) were found to be identical to *Phytophthora citrophthora* (Smith and Smith) Leonian (Campelo and Luz, 1981; Kellam and Zentmyer, 1981 and 1986a).

Now, it is recognised that at least four species of *Phytophthora* viz., *P. palmivora, P. capsici, P. megakarya and P. citrophthora* are the major species causing black pod disease of cocoa in various parts of the world. The distinguishing features of these major species are presented in Table 1. In addition to these four major species, several other species of *Phytophthora* have been described as minor pathogens of cocoa (Waterhouse 1974a and b; Zentmyer, 1988; Liyanage and Wheeler, 1989): *P. heveae* Thompson, *P. botryosa* Chee, *P.meadii* McRae, *P. drechsleri* Tucker, *P. nicotianae* (Breda de Haan) Waterhouse Var nicotianae, *P. nicotianae* Var parasitica (Dast.) Waterhouse, *P. megasperma* Drechsler and *P. katsurae* Ko and Chang. Most of these have been shown to be pathogenic only by wound inoculation of cocoa pods.

From the preliminary studies on *Phytophthora* associated with cocoa in Dakshina Kannada district of Karnataka, India, Sreenivasan and Chandra Mohanan (1984) reported that the *Phytophthora* inciting black pod disease in this locality was *P. palmivora.* Sastry and Hegde (1989) studied the characteristics of one isolate of cocoa *Phytophthora* collected from multistoried cropping system involving arecanut and cardamom as other component crops in Uttara Kannada district of Karnataka and identified this isolate as *Phytophthora meadii* McRae. Recently, detailed studies conducted on taxonomic complex of *Phytophthora* associated with black pod disease of cocoa in India revealed the occurrence of *P. capsici* and *P. citrophthora* in certain localities of Kerala State in addition to *P. palmivora* (Chowdappa and Chandra Mohanan, 1993; Chowdappa *et al.*, 1993; Chowdappa and Chandra Mohanan, 1996).

CRITERIA FOR CLASSIFICATION OF *PHYTOPHTHORA*

Studies on extent of biological variation within each species are necessary for the classification of species/subspecies. Most of the earlier classifications were based on morphological characteristics which tend to overlap species descriptions, creating confusion in identification and classification. The problem of taxonomy of *Phytophthora* is further compounded by the fact that species intercross readily, producing interspecific hybrids. Therefore, it is necessary to consider not only the morphological characteristics but also physiological and molecular variations to delineate the species and to determine the extent of variability within a species while studying the *Phytophthora* complex of cocoa.

Colony patterns: Colony morphology on carrot agar medium in darkness was found to be highly reliable character for separation of *Phytophthora* spp. of cocoa (Sansome *et al.*, 1975; Griffin 1977; Idosu and Zentmyer, 1978; Brasier and Griffin, 1979; Kellam and Zentmyer 1986a; Zentmyer, 1988). Colony patterns of four major species of *Phytophthora* on CA are presented in Table 1. Brasier and Griffin (1979) reported that differences between *P. palmivora*, *P. megakarya* and *P. capsici* were more pronounced in younger cultures than in old cultures. Though colony growth patterns on CA were more distinctive, this character alone could not be relied upon strictly for differentiating *P. palmivora, P. capsici* and *P. citrophthora* of cocoa due to the variations observed among isolates of same species of *Phytophthora* (Kellam and Zentmyer, 1986a).

Sporangial morphology: Size, shape and length to breadth ratio of sporangia were widely used by several workers in identifying *Phytophthora* spp of cocoa (Ashby, 1929a; Turner, 1960; Waterhouse 1974a; Griffin, 1977; Idosu and Zentmyer, 1978; Fagan, 1988; Zentmyer, 1988; Chowdappa, 1995) Sporangial characterstics of different *Phytophthora spp.* of cocoa on CA is presented in Table 1.

Sporangial caducity and pedicel length have been found to be valuable diagnostic characteristics in speciation of *Phytophthora* (Waterhouse, 1974b; Griffin, 1977; Tsao and Tummakate, 1977; Zentmyer *et al., 1977; Kaosiri et al.*, 1978; Idosu and Zentymyer, 1978; Newhook *et al.,* 1978; Al-Hedaithy and Tsao, 1979a and b., Ho 1981; Tsao *et al.*, 1981; Tsao, 1982; Stamps *et al.*, 1990). Cocoa *Phytophthora* isolates from Africa, America, Pacific and South East Asia, which were originally designated as *P. palmivora* were divided into four groups based on the pedicel length (Zentmyer *et al.*, 1977; Kaosiri *et al.*, 1978).

Group I - Short and thick sporangial stalk, average length >5µm
Group II - Thin sporangial stalk, intermediate in length (average 5-15 µm)
Group III - Unusual and characteristically long stalk with an average length of >15 µm
Group IV - Non-caducous sporangia

The first three groups corresponded to MF_1, MF_3 and MF_4 of cocoa isolates of *P. palmivora* (Griffin, 1977) which were subsequently identified as *P. palmivora, P. megakarya* and *P. capsici*, respectively (Brasier and Griffin 1979; Zentmyer, 1981). In contrast, *P. citrophthora* isolates had persistent sporangia.

Sporangium ontogeny was considered as taxonomic criterion in distinguishing *Phytophthora* species (Blackwell, 1949; Tsao and Tummakate, 1977; Alizadeh and Tsao, 1985a; Zentmyer, 1988). The formation of sporangia of *P.palmivora* and *P.megakarya* of cocoa has been reported as typically sympodial (Waterhouse, 1963 and 1974a; Idosu and Zentmyer, 1978; Brasier and Griffin 1979; Zentmyer, 1988), while sporangia of *P. capsici* were borne on umbels in addition to sympodial type (Idosu and Zentmyer 1978). Sporangia of *P. citrophthora* were produced in loose sympodium or in irregular patterns (Mchau and Coffey, 1994).

Chlamydospore morphology: Presence, position and size of clamydospore on vegetative hyphae have been listed in existing *Phytophthora* keys for species identification (Rosenbaum, 1917; Tucker, 1931; Frezzi, 1950; Waterhouse, 1963; Newhook *et al.*, 1978; Ho, 1981; Waterhouse *et al.*, 1983).

The four principal species on cocoa viz., *P. palmivora*, *P.capsici*, *P. megakarya* and *P. citrophthora* also differed in production of chlamydospores. *P. palmivora*, *P. megakarya* (Idosu and Zentmyer, 1978; Kaosiri *et al.*, 1978; Brasier and Griffin, 1979; Zentmyer, 1988) and certain isolates of *P. citrophthora* (Kellam and Zentmyer, 1986a; Mchau and Coffey, 1994) normally formed chlamydospores on carrot agar cultures, while some isolates of *P. capsici* (Kaosiri *et al.*, 1978; Idosu and Zentmyer, 1978; Brasier and Griffin, 1979; Zentmyer, 1988) did not form chlamydospores. However, no obvious differences in chlamydospore morphology between *P. palmivora* and *P. megakarya* were noted. The chlamydospore diameter of *P. palmivora* (mean 31.6 µm) and *P. megakarya* (mean 31.3 µm) did not vary (Brasier and Griffin, 1979). But the mean diameter of chalmydospore of *P. citrophthora* was reported as 26.5 µm (Kellam and Zentmyer, 1986a). The significance of chlamydospore morphology for classification of *Phytophthora* species was found to be restricted as these were formed by only certain isolates in some species of *Phytophthora* (Alizadeh and Tsao, 1985b).

Heterothallism: The formation of oospores in paired cultures of *P. palmivora* (earlier identified as *P. faberi*) was first reported by Ashby (1922). Gadd (1924) classified the isolates of *P. palmivora* into '+' or 'cocoa' group (A2 mating type) and - or 'rubber' group (A1 mating type) based on the production of sexual stages in paired cultures. Subsequently, several workers confirmed the heterothallic nature of *P. palmivora* (Ashby, 1929b; Leonian, 1931; Venkatarayan, 1932; Thomas *et al.*, 1948; Turner, 1960, 1961a and b; Spence, 1961; Savage *et al.*, 1968; Brasier and Griffin, 1979; Kaosiri *et al.*, 1980; Fagan, 1988; Zentmyer, 1988). In addition to production of oospores in intraspecific crosses, there have been number of reports of oospores production from interspecific pairings involving *P. palmivora* as one parent (Barret, 1948; Cohen, 1950; Haasis and Nelson, 1963; Savage *et al.*, 1968; Zentmyer and Erwin, 1970).

P. megakarya of cocoa was also reported as essentially heterothallic species (Brasier and Griffin, 1979; Zentmyer, 1988). The sexuality of *P. citrophthora* is not well defined. Some workers characterized it as sterile (Leonian 1925; Tucker 1931; Stamps *et al.*, 1990) while others have reported it as heterothallic (Haasis and Nelson, 1963; Savage *et al.*,1963; Riberio *et al.*, 1975., Kellam and Zentmyer, 1986b). Recently, Mchau and Coffey (1994) recorded both sterility and heterothaleism among isolates of *P.citrophthora* on cocoa. They recorded only A2 mating type in *P. citrophthora*.

Both A1 and A2 compatibility types have been reported as existing in the population of *P. capsici* on cocoa (Brasier and Griffin, 1979; Kaosiri *et al.*, 1980; Kellam and Zentmyer, 1986a; Fagan, 1988; Zentmyer, 1988, Chowdappa, 1995). Eventhough both A1 and A2 compatibility types of cocoa isolates of *P. palmivora, P. megakarya* and *P. capsici* were reported to exist in nature, in general one type seemed to attain dominance over the other in distribution. Thus, in *P.palmivora* population A2 compatibility type was found to be predominant world wide (Zentmyer, 1974 and 1988; Brasier and Griffin, 1979; Brasier, 1992, Chowdappa, 1995) except in Jamaica where A1 mating type was reported to be prevalent (Griffin, 1977; Fagan, 1988). In *P.megakarya*, the A1 compatibility type was reported to be wide spread in distribution (Brasier and Griffin, 1979; Zentmyer, 1988). Both A1 and A2 types were found to occur commonly in *P. capsici* population (Brasier and Griffin, 1979; Fagan, 1988; Zentmyer, 1988; Brasier, 1992).

Morphology of sexorgans: Gadd (1924 considered morphology of sex organs as the most reliable characteristic in identification of *P. palmivora*. Oospores recorded by other workers (Ashby, 1929b; Turner, 1961a) indicated high variability. Morphology of sex organs has been used as one of the criteria in the taxonomy of *P. palmivora* (Waterhouse, 1963). Further, Waterhouse (1974a) reported that the size of oospores of MF_1 and MF_2 of *P.palmivora* of cocoa were 21-25 μm and 24-30μ m respectively. Kaosiri *et al.* (1980) reported that size of oospores of MF_1 *(P. palmivora)*, MF_3 (*P. megakarya*) and MF_4 (*P.capsici*) from cocoa on CA averaged 21-26, 21-27 and 22-31μm respectively, indicating that these morphological forms could not be differentiated on the basis of oospore size. Based on the detailed studies on *P. palmivora* complex of cocoa, Brasier and Griffin (1979) reported that size of oogonia had little diagnostic value in separating MF_1 (*P.palmivora*), MF_3 (*P. megakarya*) and MF_4 (*P.capsici*). They further stated that the range of oogonia size within a single morphological form was very large.

Temperature/growth response: Cardinal temperatures have often been used as one of the aids in *Phytophthora* taxonomy (Tucker, 1931; Waterhouse, 1963; Newhook *et al.*, 1978; Ho, 1981; Waterhouse *et al.*, 1983). Waterhouse (1974a) reported that the minimum, optimum and the maximum temperatures for growth of *P. palmivora* of cocoa were 11°C, 27.5-30° and 35°C respectively. Idosu and Zentmyer (1978) studied the temperature growth response of 12 'typical' (*P. palmivora*) and four 'atypical' (*P.capsici*) isolates of *Phytophthora* complex of cocoa from 10 countries. Their results indicated that the minimum temperatures for growth of 'typical' and 'atypical' isolates were 12 and 9°C respectively which could be considered as an additional characteristic in distinguishing 'typical' and 'atypical' isolates. None of the isolates studied grew at 36°C. The temperature requirements for growth of five MF_1 isolates consisting of one A1 from Jamaica and four A2 (from Ghana, Costa Rica, Nigeria and Cameroon), five MF_3 isolates consisting of four A1 (Nigeria and Cameroon) and one A2 type (Cameroon) and three MF_4 isolates from Brazil consisting of two A2 and one A1 on CA in darkness were examined by Brasier and Griffin (1979) using 1° steps on growth/no-growth basis to determine the upper limit and lower limits and comparative growth rate studies at 2° steps over 22 and 32°C to determine optima. The results of this study revealed striking differences between the three types. The upper limit of temperature requirement for growth of MF_1, MF_3 and MF_4 of *P.palmivora* varied markedly. It was 34, 29-30 and 33°C for MF_1, MF_3 and MF_4 respectively. The lower limits for growth were 10-11°, for MF_1 and MF_3 and 8° for MF_4. The optimum temperature for the MF_3 type (24-26°C) was considerably lower than that for MF_1 and MF_4 types (28-30°C). Based on the studies on temperature requirements for the growth of one isolate each of *P. palmivora* (Costa Rica), and *P. capsici* (Brazil) and 10 isolates of *P.citrophthora* (Brazil) of cocoa, Kellam and Zentmyer (1986a) found that minimum, optimum and maximum temperature for growth were 9, 24 and 36°C for *P. capsici* and *P.citophthora* and 9, 24 and 33° for *P.palmivora*. The isolates of *P.citrophthora* and *P.capsici* exhibited variations in growth rates at different temperatures though minimum and the maximum temperatures for the growth were similar. Since the composition of the medium was found to influence the growth rates, emphasis should be given to the culture medium while considering growth response to temperature as one of the taxonomic criteria in species classification (Shepherd and Pratt, 1974; Zentmyer *et al.*, 1976).

Repetitive DNA polymorphism: Taylor (1986) reviewed value of studying mitochondrial DNA (mt DNA) restriction fragment length polymorphisms (RFLP's) and other mt DNA characteristics in fungal systematics. Recently, mt DNA RFLP's was found to be a useful tool in *Phytophthora* taxonomy (Forster *et al.*, 1987, 1988, 1989 and 1990; Mills *et al.*, 1991; Forster and Coffey, 1991).

This technique involves digesting a DNA preparation with a restriction enzyme and separating the fragments according to their size by gel electrophoresis. After transfer on to a membrane, it is possible to localize an unique or newly repeated sequence by hybridization with an appropriate radio isotope labelled probe. Applying this method, some researchers (Panabieres *et al.*, 1989; Chowdappa, 1995) demonstrated that, after digestion and electrophoresis, the total DNA of *P. palmivora, P. megakarya, P. capsici* and *P.citrophthora* of cocoa had distinct restriction digestion patterns, whereas within any single species, all isolates showed identical profiles regardless of the enzymes used. *Most of this repetitive DNA was of* nuclear origin. Based on this finding, they suggested that this method has practical advantage over the RFLP's that it is possible to prepare DNA, digest it with restriction enzyme, and to obtain its electrophoretic patterns in a single day and therefore, there is no need to develop radio isotope-labelled probes. A defect of this method is that it does not provide information at the sub species level.

Electrophoretic protein profiles: Several characteristics, including protein profiles, have been proposed (Brasier, 1983; Gallegly, 1983) to supplement morphological characters that were being used as sole determinants for identification and classification of *Phytophthora* species. The validity of protein banding patterns as a major determinant for distinguishing species and subgroups within a species of various *Phytophthora* species was highlighted by various workers (Kaosiri and Zentmyer, 1980; Erselius and de Vallavieille, 1984; de Vallavielle and Erselius, 1984; Hansen *et al.*, 1986; Bielenin *et al.*, 1988). Comparative studies on *Phytophthora* isolates of cocoa revealed that *P. palmivora*, *P.capsici*, *P. megakarya* and *P.citrophthora* could be resolved into distinct groups based on protein patterns and this variation has been correlated with differences in sporangial stalk length and other morphological characters (Kaosiri and Zentmyer, 1980; Chowdappa and Chandra Mohanan, 1995). Further, two electrophoretic sub groups were distinguished among isolates of *P.citrophthora* (Chowdappa and Chandra Mohanan, 1995). Thus electrophoresis of proteins has been found to be useful for distinguishing species and sub groups within a species associated with cocoa.

Isozyme pattern: Variations in isozyme pattern have been successfully applied in *Phytophthora* taxonomy. (Oudemans and Coffey, 1991; Mills *et al.*, 1991; Mchau and Coffey, 1994). Isozyme profiles provided valuable information about genetic relationships and variability within and between isolates of *P.palmivora, P.capsici, P.megakarya* and *P. citrophthora* of cocoa. These species demonstrated much higher levels of variation in isozyme patterns and subgroups were readily identified within each species. Isolates of *P.palmivora* were very uniform. *P.capsici* isolates were separated into three subgroups (CAP1, CAP2 and CAP3). Two subgroups (CTR1 and CTR2) were distinguished among isolates of *P.citrophthora, P. megakarya* isolates were divided into two groups (MGK1 and MGK2).

Serology: The usefulness of serology in *Phytophthora* taxonomy was first demonstrated by Burrel *et al* (1966). Later on, serological techniques have been used successfully for diagnostic and taxonomic evaluation of many *Phytophthora* species (Amos and Burrell, 1967; Merz *et al.*, 1969; Cristinsio, 1982; Nachmius *et al.*, 1979). Recently, Chowdappa (1995) reported that isolates of *P.palmivora, P.capsici* and *P.citrophthora* of cocoa from India exhibited distinct serological variation in their reaction with antisera to either *P.capsici* or *P.citrophthora* in the agar double diffusion

Table 1. ***Summary of important characteristics of P. palmivora, P. megakarya, P. capsici and P. citrophthora on carrot agar (CA), the major Phytophthora species associated with black pod disease of cocoa***

Characters	*P. palmivora* (Butl.) (Butl.)	*P. megakarya* Brasier & Griffin	*P.capsici* Leonian emend Alizadeh and Tsao	*P. citrophthora* (Smith & Smith) Leonian
1) Colony morphology	Stellate/striate pattern; Sharply defined edge; Aerial mycelium sparse except in centre.	Faint lobed/floral pattern; diffuse edge; fairly uniform deep cotton-wool like aerial mycelium	Petaloid pattern with diffuse edge; uniform dense aerial mycelium over entire colony.	Rosette with diffuse edge; uniform moderate aerial mycelium over entire colony.
2) **Growth rate (mm/day) at 24°C in dark**	7.5	6.7=0-24h 9.6=24-72h	14.3	8.7 to 14.4
3) **Sporangial characteristics**				
a) Shape	Near spherical to ovate, elongate, rounded base	Near spherical to ovate, elongate, rounded base	Spherical, ovoid, obvoid ellipsoidal, fusiform, pyriform, rounded base/tapered base	Irregularly shaped, large
b) Papilla	Prominent; occasionally two or three	Prominent; occasionally two or three	Prominent; occasionally two or three	Prominent; occasionally two or three
c) Sporangial size (µm) (Length x Breadth)	44x24(range 32-60x19-30)	36x26 (range 20-60x13-41)	47x27(range 40-52x20-31)	64x35 (20-95x17-32)
d) L/B ratio	1.2-1.8	1.2-1.6	1.6-2.0	1.6-2.2
e) Caducity	Caducous	Caducous	Caducous	Non-caducous(persistent)
f) Pedicel type	Broad, short and occluded	Narrow, medium length and not occluded	Narrow, long and not occluded	
g) Pedicel length (µm)	Mostly 2-5	Mostly 10-30	20-150, sometimes upto 250	
h) Ontogeny	Sympodium	Sympodium	Umbellate/irregular	Sympodium/irregular
4) **Chlamydospores**				
a) Morphology	Promptly produced; abundant, terminal/intercalary, spherical	Abundant, mostly terminal, spherical	Most isolates do not form chlamydospores	Abundant, spherical
b) Size (µm)	31 (Range 23-40)	31 (Range 20-40)		27 (19-40)
5) **Compatibility Types**	Predominantly A2 except Jamaica where A1 is common	Predominantly A1 (A2 rare)	A1 and A2	Sterile; very few isolates reported as A1 and A2

6) **Sex Organs**				
a) Oogonial morphology	Spherical, meeting stalk fairly abruptly; stalk narrow, tubular or broadening towards oogonium.	Pyriform, tapering downwards to a funnel shaped base.	Spherical to slightly oval meeting stalk fairly abruptly; stalk narrow, tubular or broadering towards oogonium.	-
b) Oogonial size (μm)	22-34	26-31	21-27	-
c) Antheridial morphology	Amphigynous	Amphigynous	Amphigynous	-
d) Oospore morphology	Spherical, plerotic	Spherical, plerotic	Spherical, plerotic	-
e) Mean oospore size (μm)	24-29 (Range 19-43)	23-28 (Range 18-34)	20-21 (Range 15-30)	-
f) Staining of oogonial wall with aceto-orecin	Nil	Stains darkly	Stains darkly	-
g) Response to *Tricho-*	Negative	Negative	Positive	-
7) **Cardinal Temperature (min, opt, and max)**	10-11°, 28-30°, 34°C	10-11°,24-36°, 29-30°C	8°,28-30°,33°C	9°,24°,36°C
8) **Electrophoretic Profiles of Native Proteins (no. of bands)**	10-13	12	9-11	10-16
9) **Distribution**	Predominant world wide	Confined to several countries of West Africa. Togo, Nigeria, Cameroon, Equatorial Guinea, Gabon, Ghana, Fernando po. It is the principal species associated with cocoa in Nigeria.	Confined to South ad Central America and West Indies. Mexico, Gautemala, El Salvador, Honduras, Caribbean Islands, Trinidad Venezuela, Cameroon, Jamaica, Cost Rica, Tobago and Chipas, India but rarely in Africa. It is the principal species on cocoa in Brazil.	Brazil, Mexico, Gautemala, Costa Rica, Tobago and Chipas, India.

Source: (1) Kaosiri *et al.* (1978)
(2) Brasier and Griffin (1979)
(3) Kellam and Zentmyer (1986)
(4) Zentmyer (1988)
(5) Tsao and Alizadelh (1988)

test. Two distinct sub groups were distinguished among the isolates of *P. capsici*. Their results showed that serological technique can be successfully used as additional criterion in distinguishing the three species of *Phytophthora* in support of morphological criteria.

Response to antibiotics and fungicides: Antibiotics: The species of *Phytophthora* and *Pythium* are generally considered to be highly sensitive to several 'antibacterial antibiotics' compared to other fungi by virtue of unusual permeability characteristics which allow access to antibiotics and thus represent distinct specialised group of organisms. The possible mechanisms of fungicidal action of these antibiotics have been reported to inhibit protein synthesis and RNA metabolisms (Tsao, 1970). Chowdappa (1995) studied the response of *P. palmivora, P. capsici* and *P.citrophthora* of cocoa to five anbiotics and found considerable variation among and within a species of *Phytophthora* in their reaction to these antibiotics at lower concentration. ED_{50} values also demonstrated diversity in response to every single antibiotic. *P.palmivora* was generally more sensitive to all these antibiotics than isolates belonging to *P. capsici* and *P. citrophthora*. There were two distinct subgroups among isolates of *P.capsici* and *P.citrophthora* that could be distinguished by their reactions to tetracycline hydrochloride and chloramphenicol. Growth responses to antibiotics could be used as an additional non-morphological criterion in distinguishing different *Phytophthora* spp. and even sub-specific groups within a single species.

Fungicides: Metalaxyl has been found to be highly inhibitory to mycelial growth of *P. palmivora* (Kerkenaar and Kaarsiji pesteijen, 1981; Tey and Wood, 1983; Campelo *et al.*, 1984; Fuller and Gisi, 1985; Ramachandran *et. al.*, 1988, Chowdappa, 1995) of cocoa. Isolates of *P.palmivora*, *P.capsici* and *P. citrophthora* from cocoa exhibited differential responses to metalaxyl (Coffey and Bower, 1984; Campelo *et al.*, 1984). According to Coffey and Bower (1984), *P.palmivora* isolates were generally more sensitive to metalaxyl than *P.citrophthora* and *P. capsici* at lower concentrations particularly at 0.1 µg/ml. On the contrary, Campelo *et al* (1984) reported that *P.capsici* and *P.citrophthora* were sensitive to metalaxyl than *P.palmivora*. These three *Phytopthora* species also exhibited similar differential responses to phosphorous acid and Fosetyl-Al *in vitro* (Chowdappa, 1995). *P. palmivora* was most sensitive followed by *P.citrophthora* and *P.capsici*. There were no distinct subgroups among isolates of *P.palmivora* and *P.capsici* isolates but there was considerable heterogeneity in their response to metalaxyl, phosphorous acid and fosetyl-Al (Chowdappa 1995). However, two sub groups were distinguished among the isolates of *P.citrophthora* based on their reactions to metalaxyl, phosphorous acid. Thus, sensitivity to fungicide could also be used as additional tool in finding out the variability between and within the species of *Phytophthora*.

Pathogenic variability: Spence (1961) reported that several isolates of *P.palmivora* varied in the rate of lesion development on wounded cocoa pods when artificially inoculated. Based on the difference in virulence between two isolates of *P.palmivora*, Leather (1966) considered the isolates as two different strains of *P. palmivora;* one being more virulent and the other a less virulent strain. This observation formed the basis of *P. palmivora* causing black pod disease of cocoa in Jamaica. Tarjot (1967) and Rocha and Vello (1971) have demonstrated differences in virulence of ecologically distinct strains of *P. palmivora*. Ram and Ram (1973) found significant differences in the virulence of 22 isolates of

Phytophthora collected from different cocoa growing areas of Brazil. These studies were conducted before a clear understanding of the classification of *Phytophthora* of black pod disease into distinct species. Therefore, it may be considered that existence of more than one species of Phytophthora might be one of the reasons for pathogenic variability.

Turner (1960) was the first to study the pod lesion characteristics of 'G' and 'N' forms of *P.palmivora*' corresponding to MF_1 (*P.palmivora*) and MF_3 (*P. megakarya*) respectively and found significant differences between them. Sansome *et al*(1975) also found differences between lesions produced by 'S' type (MF_1) and 'L' type (MF_3). The 'S' type produced fast developing surface lesion with sharp edge while 'L' type formed lesion with diffuse edge. Later on Brasier and Griffin (1979) studied the growth characteristics of Nigerian MF_1 ('S' type) and MF_3 ('L' type) isolates of *P. palmivora* corresponding to *P. palmivora* and *P. megakarya* respectively on healthy green cocoa pods collected from open pollinated trees of three varieties. The lesions caused MF_1 isolates were significantly larger than those caused by MF_3 isolates on all the three varieties. MF_1 isolates usually produced pod lesions with a sharp, well-defined edge and sparse fungal growth on the lesion surface. The peripheral areas of the lesion were usually devoid of any fungal structures. In contrast, MF_3 isolates produced lesions with a more diffuse leading edge and considerably more fungal growth on the surface, often extending to within a few mm of the lesion edge. The lesions produced by MF_1 isolates were more dark brown than that produced by MF_3 isolates.

P. palmivora, P. capsici and *P. citrophthora* of cocoa from different countries differed in their pathogenicity. Lawrence *et al.* (1982) reported that *P. palmivora* was consistently more virulent than *P. capsici* on pods, germinating seeds and seedling stems of a number of cocoa types in Brazil. According to them, same cultivars reacted differently to the two species; 'Catongo' being moderately resistant to *P. capsici* but susceptible to *P. palmivora.* Further studies conducted in Brazil revealed *P. citrophthora* as the most virulent, *P. palmivora* moderately and *P. capsici* the least virulent on detached cocoa pods inoculated with mycelial disks (Campelo *et al.*, 1982). Comparative studies on the pathogenic variations of the three *Phytophthora* species conducted in Brazil again revealed that the diameter of lesion produced on cocoa pods due to *P. capsici* was 1/2 and 1/3 of those due to *P. palmivora* and *P. citrophora* respectively, (Luz and Yamada, 1984). Based on detailed pathogenicity studies, *P. palmivora* and *P. capsici* isolates were significantly less virulent than *P. palmivora.* Similarly, *P. capsici* isolates caused the smallest average lesion area followed by *P.palmivora* and *P.citrophthora* on seedlings in Brazil (Pinto *et al.*, 1989). From pathogenicity studies with *P.palmivora.*, *P. capsici* and *P.citrophthora* on 20 cocoa accessions in India, Chowdappa (1995) reported that *P. palmivora* is highly virulent than either *P.capsici* or *P.citrophthora* regardless of pathogenic parameter viz., lesion area, mycelial index, sporulation potential.

A knowledge of the range of variability in *Phytophthora* is the basic requirement for breeding disease resistant/tolerant varieties to develop new strategies in the better control/management of black pod disease of cocoa.

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