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PHYTOPHTHORA MEGAKARYA: A REVIEW ON ITS STATUS AS A PATHOGEN ON CACAO IN WEST AFRICA

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ABSTRACT

Phytophthora megakarya Brasier & Griffin, is one of the Oomycete pathogens reported on *Theobroma cacao*, and it is the most virulent of the *Phytophthora* species that causes black pod disease. *Phytophthora megakarya* was first reported as the causal agent of black pod disease in 1979 after the reclassification of *Phytophthora* species recovered from infected cacao tissues. The pathogen causes pod rot, also referred to as black pod disease of *T. cacao,* and is only endemic to West and Central Africa. *Phytophthora megakarya* has spread westwards from Cameroon through Nigeria and Togo, to the major cocoa producing countries of Ghana and Côte d'Ivoire, and southwards to Gabon and Equatorial Guinea. *Phytophthora megakarya* has become the main yield-limiting factor in cocoa production in the sub region, rapidly surpassing *P. palmivora.* The menace of *P. megakarya* on cacao is of great concern to cocoa farmers and scientists, but the processes underlying the emergence of *P. megakarya* on cacao are unknown. There is, thus, increased need for fundamental knowledge on the diversity and epidemiology of *P. megakarya* in order to develop effective and sustainable methods for its control. This paper reviews the current state of knowledge on the origin, distribution and biology of *P. megakarya,* in West Africa and evaluates the efficacy of current control methods. We highlight quarantine as a means of limiting the introduction of *P. megakarya* into other cocoa growing regions, and also discuss cultural and biological control and use of resistant/tolerant varieties as major components of an integrated disease management strategy for the disease. The need for research into integrated management of the disease with emphasis on biocontrol and use of resistant varieties, and applying genomic information and tools from *T. cacao* and from other Oomycetes for managing *P. megakarya* are also discussed.

Key Words: Black pod disease, CODAPEC, indigenous, *Phytophthora palmivora*

RÉSUMÉ

Phytophthora megakarya Brasier & Griffin, est l'un des agents pathogènes de la classe des Oomycètes identifié sur *Theobroma cacao*, c'est le plus virulent de l'espèce *Phytophthora*, qui est responsable de la maladie de la cosse noire. *Phytophthora megakarya* avait été identifié pour la première fois comme agent causal de la maladie de la cosse noire en 1979 après la reclassification de l'espèce *Phytophthora* récupérée de tissus infectés de cacaoyers. Le pathogène cause la pourriture de la cosse, qui est aussi appelée la maladie de la cosse noire de *T. cacao,* cette maladie est seulement endémique en Afrique de l'Ouest et en Afrique centrale. *Phytophthora megakarya* s'est développé dans le sens Ouest partant du Cameroun en passant par le Nigeria et le Togo, pour aller dans les grands pays producteurs du cacao comme le Ghana et la Côte d'Ivoire, il s'est aussi développé vers le Sud au Gabon et en Guinée Equatoriale. *Phytophthora megakarya* est devenu le facteur le plus important limitant le rendement en production du cacao dans la sous-région, dépassant rapidement *P. palmivora.* La menace exercée par *P. megakarya* sur le cacao est un problème pour les producteurs de cacao et les scientifiques, mais les mécanismes de survenue de *P. megakarya* sur le cacao demeurent inconnus. Il y a donc un besoin accru de connaissance sur la diversité et l'épidémiologie de *P. megakarya* dans le but de développer des méthodes de lutte efficace et durable contre cet agent pathogène. Cet article passe en revue l'état actuel des connaissances sur

l'origine, la distribution et la biologie de *P. megakarya,* en Afrique de l'Ouest et d'évaluer l'efficacité des méthodes actuelles de lutte contre ce pathogène. Nous mettons l'accent sur la mise en quarantaine comme un moyen d'empêcher l'introduction de *P. megakarya* dans d'autres régions productrices de cacao, et nous discutons aussi la lutte biologique, les pratiques culturales et l'utilisation de variétés résistantes/tolérantes comme composantes majeures d'une stratégie de lutte intégrée contre cette maladie. Le besoin de recherche sur la gestion intégrée de la maladie avec un accent particulier sur la lutte biologique et l'utilisation de variétés résistantes, ainsi que la mise en application des outils et information génétique de *T. cacao* et autres Oomycètes pour la lutte contre *P. megakarya* ont été aussi discutés.

Mots Clés: maladie de la cosse noire, CODAPEC, indigène, *Phytophthora palmivora*

INTRODUCTION

Five major diseases of cocoa (*Theobroma cacao* L.), *Phytophthora* pod rot (black pod), witches broom, swollen shoot virus, vascular streak dieback, and monilia pod rot account for over 40% annual loss of cocoa (Flood *et al*., 2004). *Phytophthora megakarya*, Brasier & Griffin, is one of the *Phytophthora* species reported on *T. cacao* and is the most virulent of the species, causing black pod disease. Based on chromosome number, sporangial characteristics and pedicel length, *P. megakarya* was first described in 1976 as a new *Phytophthora* species on *T. cacao* in West Africa (Brasier and Griffin, 1979; Sansome *et al.,* 1979). *Phytophthora megakarya* is indigenous to West and Central Africa, and it has become the main yield-limiting factor for cocoa production in affected areas (Opoku *et al*., 2000), rapidly surpassing *P. palmivora*. Under the conditions of high and frequent rainfall in Cameroon, *P. megakarya* can cause yield losses of up to 100% when no control measures are taken (Despre´aux *et al*., 1988). In Ghana, losses ranging between 60 to 100% have been reported (Dakwa, 1987).

The emergence of *P. megakarya* has had dramatic social and economic consequences in cocoa producing countries in West and Central Africa, clearly demonstrating the scale of damage that it may cause in case it spreads into other cocoa producing regions. For example, in Ghana, it was reported that some cocoa farms were neglected or abandoned and, some cocoa farmers switched over to cultivation of other crops as a result of *P. megakarya* (Opoku *et al.*, 2000; Akrofi *et al.*, 2003). Government of Ghana has instituted several national programmes, including the recent national Cocoa Pests and Diseases Control Programme (CODAPEC), in which *P. megakarya* infected farms were sprayed with fungicides at the expense of the government (Opoku *et al*., 2006). Resources invested in these programmes could have been used in enhancing the lives of farmers.

This paper reviews the current state of knowledge on the origin, host range, distribution, taxonomy and biology of *P. megakarya* in West Africa; and also provides an overview of current methods of managing black pod disease and the challenges associated with the available methods.

***Phytophthora* species on cocoa.** Correct identification of plant pathogens is critical and fundamental to population genetics, epidemiological studies and the development of disease control strategies. Due to the similarity in growth patterns of Oomycetes including *Phytophthora* species and fungi, Oomycetes were previously considered as a class within the fungi. Fundamental differences between Oomycetes and fungi have been established (Benson, 1997; Judelson and Blanco, 2005; Fry, 2008) and the two are now known to be taxonomically distinct in spite of their common infection strategy (Latijnhouwers *et al.,* 2003). As a result of the initial consideration of Oomycetes as a class within the fungi, Govers (2001) reported that researchers have for several decades pursued a wrong track in addressing the menace caused by *Phytophthora infestans.* For example, chitin was earlier reported as a minor component of Oomycete cell walls and, therefore, insensitive to chitin synthase inhibitors, but it is now known to be an important component of hyphal tips in Oomycetes (Guerriero *et al.*, 2010).

Classification of species within the genus *Phytophthora* has progressed through the use

of several criteria, including morphological datasets of colony, sporangium and oogonium characteristics, presence or absence of chlamydospores and hyphal swellings, physiology (Waterhouse, 1963; Brasier and Griffin, 1979), isozyme patterns (Oodemans and Coffey, 1991) and lately the combined use of molecular markers and morphological characteristics (Kroon *et al*., 2012). Until 1979, *P. palmivora* was considered the only causal agent of black pod disease. Sansome *et al.* (1975; 1979) suggested a reclassification of some of the isolates previously described as *P. palmivora* into distinct species. Consequently, based on size and number of chromosomes, they introduced the S and L-type designations, which represented isolates having comparatively smaller chromosomes with *n*=9-12 and isolates having large chromosomes with *n*=5, respectively.

The controversy of many variants of *P. palmivora* was settled after a comprehensive study of 950 isolates identified by different researchers as *P. palmivora* at a Cocoa *Phytophthora* Workshop at Rothamsted Experimental Station, Harpenden, UK in 1976 (Brassier and Griffin, 1979). Following that study, the four morphological forms (MF) of *P. palmivora* defined by Griffin (1977), which grouped the species into those with short pedicel (MF1 and MF2), intermediate pedicel (MF3) and long pedicel (MF4) was discontinued. Turner (1960; 1961a; 1961b) had earlier described the existence of two separate *P. palmivora* types based on shape of sporangia and the development of lesions. These two types appeared to correspond to the MF1 and MF3 types, and were designated as *P. palmivora* and *P. megakarya* at the Rothamsted Workshop.

Consequently, the species were reclassified into three types, based on chromosome number, sporangial characteristics and pedicel length (Brasier and Griffin, 1979). The S-type was regarded as *P. palmivora* sensu Butler (MF1) with 9-12 small chromosomes, papillate sporangia varying from near spherical to ovate-elongate shape, a short pedicel (2-5 µm) and being worldwide in distribution. The L-type was reclassified as *P. megakarya* (MF3), with 5-6 large chromosomes, papillate near spherical sporangia shape, pedicel of medium length (10-30 µm) and found only in West and Central Africa. Thus, the name "megakarya" is derived from the relatively large (mega) chromosomes. The third group classified as *P. capsici* (MF4), with characteristics similar to *P. capsici* from black pepper (Kaosiri *et al.,* 1978; Zentmyer *et al*., 1988), had longer pedicel (20-150 µm). The MF2, however, remains a variant of *P. palmivora.*

The occurrence of hybridisation is an important phenomenon in *Phytophthora*, given that hybridisation may result in genetic variation that will adapt to new hosts or environments. Further confusion in the "*P. palmivora*" complex can occur due to heterothallic mating behaviour of the species. Sexual reproduction in *P. megakarya* and *P. palmivora* results in the production of oospores and this requires the two opposite mating types, A1 and A2. Brasier and Griffin (1979) indicated that the mating types in *P. megakarya* and *P. palmivora* show a curious imbalance, with A1 predominating in *P. megakarya* and A2 in *P. palmivora*. This imbalance in mating types might favour hybridisation between species, but not sexual reproduction within species. In spite of the two species coexisting on cocoa fields, no hybrids have been observed. The differences in chromosome numbers between *P. megakarya* and *P. palmivora* may also hinder hybridisation and, hence, the rare occurrence of oospores in nature.

Other *Phytophthora* species reported on *T. cacao* include *P. botryosa,* causing cacao pod rot in Malaysia (Kroon *et al*., 2004), *P. citrophthora* in Bahia, Brazil (Campelo and Luz, 1981; Kellam and Zentmeyer, 1981), *P. capsici, P. citrophthora* and *P. heveae* in Mexico (Lozano and Romero, 1984), *P. katsurae* in Côte d'Ivoire (Liyanage and Wheeler, 1989), and *P. megasperma* in Venezuela (Zentmeyer, 1988). Apart from *P. palmivora,* which is cosmopolitan, the other species have only been found in certain countries or geographical regions. The factors responsible for this geographical separation of the species are yet to be elucidated, but it is possible that lack of intensive surveys, coupled with isolation of isolates from the same location, and from a few plant species and on a narrow range of media could account for this observation. It is also possible that these species occur rarely on cacao but these needs to be investigated.

Disease cycle. Black pod disease incidence in the field is influenced by environmental conditions. Numerous studies have established the role of climatic factors on the incidence of black pod disease, caused by *Phytophthora* spp. (Dakwa, 1973; Deberdt *et al*., 2008). Rainfall, high relative humidity, and low temperature are known to create favourable humid conditions for the development of the disease (Ndoumbe'-Nkeng, 2002). Dakwa (1973) showed that in Ghana, black pod disease developed when the relative humidity, particularly within the day, remained above 80% under the cocoa canopy and that the rate of disease development was influenced by the frequency and amount of rainfall. Deberdt *et al.* (2008), also reported a significant positive correlation between rainfall when assessed after 1-week lag, and *P. megakarya* pod rot incidence in Cameroon, and emphasized the role of rainfall in the disease epidemics. Dakwa (1987) further showed that the time and/or period for black pod peak infection in Ghana varied annually and also with location depending on the rainfall. In Ghana, it is known that primary infections usually occur around June, but the peak of *P. megakarya* black pod disease generally occurs between August and October (Opoku *et al*., 2000; 2007a). Such information on periods for attaining disease infection peaks is useful in forecasting the pattern of disease development and it is an important tool for disease management since conditions immediately preceding the infection peaks must be favourable for disease development.

Phytophthora megakarya, like *P. palmivora,* undergoes a series of developmental stages throughout the disease cycle (Fig.1). This

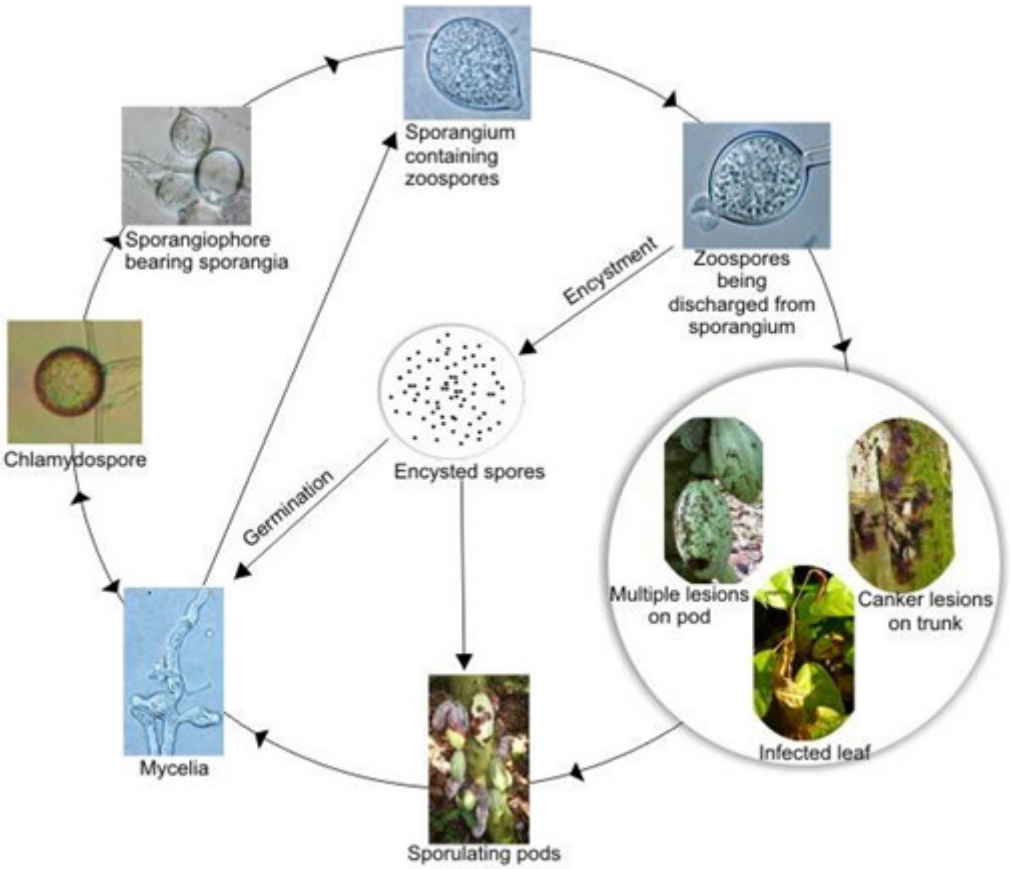

Figure 1. Disease cycle of *P. megakarya* on cacao highlighting the main spore types and infective propagules. In the cycle, sporangiophore bearing sporangia, sporangia containing zoospores, zoospores being discharged from sporangium, infection on cacao pod, infection on tree trunk, infection on leaf, different levels of infection on cacao pods, mycelia and encysted zoospores are shown.

includes the formation of mycelium and three main spore types, i.e., sporangia, zoospores and chlamydospores that may directly or indirectly cause infection. Primary inoculum in the form of mycelium in soil and bark cankers develop into sporangia, which germinate during wet and humid conditions to establish an infection (Luterbacher, 1994).

A successful infection results in the generation of secondary inoculum of sporangia containing motile biflagellate zoospores. The most important developmental factor in *P. megakarya* is its ability to emit zoospores earlier and also two times more than *P. palmivora* (Brasier *et al*. 1981). Zoospores actively detect and swim toward cacao plant tissue to infect it (direct infection) or encyst in the absence of free water, and germinate later to infect susceptible plant tissue (indirect infection). Under humid conditions a single pod may produce up to 4 million sporangia (containing motile zoospores), that are disseminated by rain, movement of planting materials, insects and rodents, and contaminated harvesting tools and pruning implements (Brasier *et al*., 1981). Chlamydospores are the principal long-term survival structures of *P. megakarya* in soils (Brasier *et al*., 1981). These chlamydospores develop into mycelia and infect cacao tissue. In determining the survival of *P. palmivora* and *P. megakarya* in soils, the two species were introduced into plantation soil before the dry season. *Phytophthora palmivora* could be recovered for ten months and *P. megakarya* for 18 months after the introduction (Brasier *et al*., 1981). The long time survival of *P. megakarya* in soil and infected debris, and evidence of its adaptation in soil and survival on roots of cacao and other forest trees (Opoku *et al*., 2002) makes the control of *P. megakarya* difficult.

Brasier *et al.* (1981) attributed movement of *P. megakarya* inoculum into the cacao canopy to rainsplash, aerosols, contaminated equipments, rodents and insects, mainly ants, but further pointed out that rainsplash activity was restricted to 75 cm from the ground. They also pointed out that the relatively protected canopy of cacao trees limits aerosols as means of dispersal of *Phytophthora* inoculum on cacao plantations. The role of invertebrate vectors, including ants and termites in the spread of *P. palmivora* is well documented (Evans, 1973a; 1973b; Taylor and Griffin, 1981). Evans (1973a) found viable zoospores of *P. palmivora* in the faeces of insects living in cocoa plantations.

A decade ago, Scolytid and Nitidulid beetles were reported to spread *P. palmivora* inoculum in the *T. cacao* canopy in Papua New Guinea (Konam and Guest, 2004). Konam and Guest (2004) indicated that the longer a *P. palmivora* infected pod remained in the canopy the more beetles it attracted and the more inoculum it dispersed. However, the role of beetles in the spread of black pod disease, caused by *P. megakarya* has not been studied. While evidence for infection from *P. megakarya* diseased pods left on trees is conflicting, Dennis and Konam (1994) reported that *P. palmivora* infected pods shrivel to form mummified pods, which provide a reservoir of inoculum for at least 3 years, and necessitating the removal of mummified pods during routine sanitary pruning. Mummified pods on tree trunks and branches are common on *P. megakarya* infected farms and these pods may serve as potential sources of inoculum and possibly account for some of the "unknown" sources of inoculum in *P. megakarya* infected fields reported by Brasier *et al.* (1981). The role of mummified pods in *P. megakarya* epidemics needs to be studied.

In a study of the spatial and temporal development of a *P. megakarya* epidemic in a plantation in the Central region of Cameroon, ten Hoppen *et al*. (2011) observed a spatial dependence of pod rot distribution on cocoa trees, simultaneous appearance of multiple infection points and numerous infection foci. Multiple infections are common phenomena associated with *P. megakarya,* resulting from rain splashing sporangia from sporulating pods onto healthy ones. ten Hoppen *et al*. (2011) also found more infection foci at the bottom of the plantation and in areas with heavy shade. These areas are humid and favour disease development. They, therefore, hypothesized that primary inoculum was the main determinant for the spatial and temporal development of an epidemic at the plantation level, and that secondary inoculum was mainly responsible for the within-tree temporal development of the black pod epidemic. They

further suggested that more attention should be given to reducing primary inoculum levels of *P. megakarya* in order to improve control efficacy.

Symptoms. *Phytophthora* pathogens, including *P. megakarya* infect every developmental stage and every part of the cacao plant (Appiah, 2001; McMahon and Purwantara, 2004) under wet and humid conditions. Infection of seedlings leads to blight and root rot in nurseries, while infections of stem, chupons and branches lead to cankers (Brasier *et al*., 1981; Guest, 2007). Pod infection leads to pod rot (black pod) and any stages of pod development and parts of all the pod are susceptible to infection (Guest, 2007). Immature pods between 10 and 20 weeks were reported to have the highest disease incidence when pod production dynamics and black pod disease were studied in relation to impact of environmental factors, chemical fungicide and biological control in Cameroon (Deberdt *et al*., 2008). According to Hebbar (2007), such infected immature pods are rendered useless, while for ripe pods, it results in a reduction in bean quality.

The initial symptom observed for all *Phytophthora* species on cocoa pods is the appearance of a small translucent spot (Guest, 2007). The appearance of the spot takes about 2-3 days after infection, to manifest. The spot then turns brown and eventually darkens. Under humid conditions, the spot spreads rapidly to cover the entire pod in 7-14 days. Three to five days after the appearance of the first symptom, whitish spores are produced. Pod rot symptoms due to *P. megakarya* (Fig. 2), however, are characterised by multiple lesions (Fig. 2a), which spread fast and coalesce (Fig. 2b) with an abundant bloom of white zoosporangia on the lesion; except for about a centimeter from the advancing margin (Fig. 2c-arrowed). Pods at every stage of development may be infected (Fig. 2d), and infection may start from the distal (Fig. 2e), proximal (Fig. 2f) or lateral (Fig. 2g) portion of the pod.

Canker symptoms of *P. megakarya* and *P. palmivora* are similar, but *P. megakarya* often causes multiple cankers (Appiah *et al*., 2004), which coalesce to form large lesions, usually at the collar region of the stem. In a study of the natural occurrence and distribution of stem cankers caused by *P. megakarya* and *P. palmivora* on cocoa in Ghana, *P. megakarya* was frequently isolated from cushions showing that *P. megakarya* readily causes stem canker on cocoa (Appiah *et al*., 2004), contrary to previous views that *P. megakarya* is less able to infect woody tissue (Gregory and Maddison, 1981; Maddisson and Griffin, 1981). The first sign of the canker is a greyish brown or reddish-brown water-soaked lesion with dark brown to black margins on the bark; and exudation of reddish-brown resinous liquid (bleeding canker), usually through cracks in the bark (Fig. 2h). After scraping the lesion, a distinct spreading scarlet coloration of the cortical tissues is observed (Fig. 2i). Expanding lesions restrict the flow of water and nutrients to the branches leading to wilting, defoliation and die-back. Deaths of cankered trees results in broken canopies in *T. cacao* plantations and facilitate capsid attack. Cankers also serve as source of inocula (Brasier *et al*., 1981; Guest *et al.*, 1994) and play a major role in primary infection of cocoa pods.

Origin, host range and distribution. Nyasse *et al.* (1999a) used isozyme and RAPD markers to estimate the genetic diversity and structure among *Phytophthora* isolates from Ghana, Togo, Nigeria, Cameroon, Gabon and Sao Tome. The two markers separated the isolates into two different genetic groups, one located in Central Africa and the other in West Africa, with the two centres of major diversity located in Cameroon and on the Cameroon/Nigeria border region. This distribution, according to Nyasse *et al*. (1999a), coincides with two major biogeographical domains, reflecting an ancient evolution of *P. megakarya.* Based on RAPDs, they also found a lower genotypic diversity in the West African (Ghana, Togo and Nigeria) isolates compared with those of Central Africa (Gabon and Sao Tome). Furthermore, they observed four intermediate-marker patterns, corresponding to isolates sampled near the border between Nigeria and Cameroon, and assumed that this resulted from genetic exchanges between the Central and West African groups, and purported that the centre of diversity of *P. megakarya* lies on the Cameroon-Nigeria border.

Figure 2. Symptoms of *Phytophthora megakarya* infection on *Theobroma cacao*: (a) multiple lesions on cocoa pod, (b) coalescing lesions, (c) abundant sporangia (arrowed), (d) different stages of infection on cocoa tree, (e) distal infection, (f) proximal infection, (g) lateral infection, (h) canker lesions before scraping and (i) canker lesions after scraping showing scarlet coloration.

In a recent population genetic study to elucidate genetic diversity of *P. megakarya* and how the pathogen emerged, Mfegue *et al*. (2012) used 12 novel polymorphic microsatellite markers to characterise 652 isolates from three populations (Cameroon, Central Africa and West Africa) and also tested the markers for cross amplification in 15 *P. palmivora* isolates. They detected significant heterozygosity within the genotypes, consistent with diploidy. Furthermore, they found highly significant linkage disequilibrium among the pairwise comparisons of loci within the three populations studied. They, therefore, inferred a clonal mode of reproduction in *P. megakarya*. The occurrence of cross amplification between *P. palmivora* and *P. megakarya* observed in their study, had earlier been reported in other *Phytophthora* species (Ivors *et al*., 2006).

Cacao growing in West Africa spread rapidly following the introduction of the crop from Brazil to Principe in 1822, and from there to São Tomé in 1830, Fernando Pó in 1854, Ghana in 1861, Nigeria in 1874, Côte d'Ivoire in 1919 and Cameroon in 1876 (CacaoNet, 2012). Since its introduction, the crop has been affected by many diseases. Two

of such diseases, black pod disease caused by *P. megakarya* and Cocoa swollen shoot virus disease (CSSVD) are confined to Africa. This susceptibility of the crop to new encounter disease has been established for CSSV, where several alternative hosts have been identified (Posnette, 1981). On the contrary, *T. cacao* is the only economic crop with which *P. megakarya* has been associated even though the pathogen has been isolated from other tree species. For example, *P. megakarya* was isolated from *Cola nitida* in Cameroon (Nyasse *et al*., 1999a) and subsequently from the rootlets of *Funtumia elastica*, *Sterculia tragacantha* (Malvaceae), *Dracaena mannii* and *Ricinodendron heudelotii* (Euphorbiaceae) on a cacao farm in Ghana (Opoku *et al*., 2002). These isolations suggest that the pathogen survives on roots of these trees, a finding consistent with that of Opoku (1994) who reported of the survival of *P. megakarya* in cacao roots. *Phytophthora megakarya* was isolated from fallen fruit of an *Irvingia* sp. closely related to *Irvingia gabonensis* from forest soil outside a cacao farm (Holmes *et al*., 2003), but the isolation could have resulted from infection from the soil. Isolation of *P. megakarya* from trees outside cocoa farms need to be studied to give conclusive evidence of alternative hosts of the pathogen.

Phytophthora megakarya is indigenous and limited to West and Central Africa, and has been described as an invasive pathogen on *T. cacao* in this region (Holmes *et al*., 2003; Evans, 2007). It has spread westwards from Cameroon through Nigeria, Togo to the major cocoa producing countries of Ghana and Côte d'Ivoire, and southwards to Gabon and Equatorial Guinea *Phytophthora megakarya* was originally identified in Nigeria in 1979 (Brasier *et al*., 1981), Togo in 1982 (Djiekpor *et al.,* 1982) and later in Ghana in 1985 (Dakwa, 1987). The pathogen was found on *T. cacao* at the border of Côte d'Ivoire in 1993 (Luterbacher and Akrofi, 1994) and in Côte d'Ivoire in 2003 (Risterucci *et al*., 2003). Presently, *P. megakarya* is the predominant species responsible for black pod disease of cocoa in the West Africa (Opoku *et al.,* 1997) and the only species on cacao in Cameroon (Nyasse *et al*., 1999b).

Strategies for managing *P. megakarya.* Crop losses and cost of controlling *Phytophthora* diseases constitute a significant financial burden on agricultural enterprises and has serious socio-economic and environmental consequences wherever these pathogens are found. Neglect of cocoa farms infected with *P. megakarya*, cultivation of crops other than *T. cacao* in infected areas (Opoku *et al*. 2000), and establishment of *T. cacao* in *P. megakarya*-free forest areas have significant impacts on the economies of the cocoa producing countries in West Africa. It also has effects on biodiversity and functioning of the natural ecosystems. Consequently, there is an urgent need for effective and sustainable control of *P. megakarya*. The effective and sustainable management of black pod disease, caused by *P. megakarya,* requires integrated approach of several methods, including quarantine, cultural, chemical and biological control and use of resistant cocoa varieties.

Quarantine. The exchange of cocoa germplasm between countries carries the risk of introduction of pathogens and pests, along with the host plant material. The need to minimise such a risk is important given the fact that the major cocoa diseases and pests are restricted to particular geographical locations. For example, *P. megakarya* is presently confined to West and Central Africa; while witches broom and monilia pod rot are found only in South and Central America. It is, therefore, essential for exchange of materials to occur *via* intermediate quarantine stations to restrict geographical spread of these major diseases. Furthermore, as new locations are developed for cocoa growing, it is quite possible that new or hitherto unimportant diseases will become significant.

Phytophthora megakarya has spread within the West and Central African subregions and it is still in its invasive phase. In Ghana, the spread of *P. megakarya* from one location to the other has been linked with the movement of planting materials (Opoku *et al*., 1997; 2000; Akrofi *et al*., 2003). With faster communication and travel, trade links and the relatively free movement of people and commodities all over the world, there is a

serious and real risk of introducing *P. megakarya* to other cacao growing regions; a situation which will impact negatively on world cocoa production. Similarly, the introduction of the other major cocoa diseases with high risks such as witches broom, monilia pod rot and vascular streak dieback (End *et al*., 2010), from other cocoa producing areas into West and Central Africa, would present a devastating impact on the world's cocoa supply and cause extremely serious social, economic and environmental problems. To minimise such risks, preventive measures and effective testing procedures and exchange of materials through intermediate quarantine facilities must be enforced.

Cultural control. Cultural control is one of the first approaches in plant disease control (Sitapai, 1989). It involves practices that promote crop growth and inhibit, and obstruct pathogen establishment, growth and development. Cultural practices are not only essential for increasing yield, but also providing the right environment for the efficient performance of fungicides (Akrofi *et al*., 1997). With the small holdings and low input cocoa farming and the low income of cocoa farmers, the least expensive disease control option for managing *Phytophthora* diseases on cocoa farms is the use of cultural practices. Epidemiological studies to date provide adequate information to endorse some recommended methods for reducing inoculum. For instance, frequent harvesting saves partly infected mature pods, removes infected pods and reduces sources of sporangial inoculum and also reduces cushion cankers. In Nigeria, frequent removal of diseased pods complemented sprayed programmes in controlling *P. megakarya,* but often, excessive tree heights hampered the effectiveness of the technique (Maddison and Idowu, 1981). Similarly, in Togo, *P. megakarya* diseased pod removal was recommended as part of a package to reduce disease incidence (Djiekpor *et al*., 1982). In Cameroon, innoculum levels were successfully reduced by the pruning and weekly removal of pods, but only in concert with spraying (Tondje *et al.*, 1993).

Another cultural method occasionally recommended, is the removal or spraying of pod husk piles where they occur on farms. It is known that these pod husk piles serve as disease foci on *P. megakarya* farms (Maddison and Griffin, 1981). In Nigeria and Sao Tome, burying of husks was recommended, but its limited effectiveness and expense caused this option to be dropped (Wood and Lass, 1985). However, in Ghana the husk are burnt into potash and used in the production of soap.

Pruning and appropriate tree spacing increases aeration and reduces canopy humidity, thus reducing sporulation. Maintenance of leaf litter or mulches to prevent soil inoculum of *P. megakarya* reaching pods was suggested by Gregory *et al*. (1984), but Luterbacher (1994) found out that leaf litter had a limited effect in reducing pod infection from soil inoculum.

Cultural practices on cacao farms are labour intensive and inadequate when applied alone for *P. megakarya* control. They need to be supplemented with other control methods, such as spraying of fungicides to reduce losses on farms (Akrofi *et al*., 2003; Ndoumbe-Nkeng *et al*., 2004; Opoku *et al*., 2007a; 2007b).

Chemical control. Fungicides have been used to control *Phytophthora* pod rot of cocoa for over a century, and several experiments on different chemical control measures have been done in all cocoa growing countries. The history of the development of fungicides on cocoa has been extensively reviewed (Hidalgo *et al.,* 2003; Bateman *et al*., 2004; Russell, 2005; Norgrove, 2007). The recommendations adopted in the different countries are based on local factors, such as specie of pathogen, climatic conditions, cocoa variety, planting density, and social and economic considerations (Wood and Lass, 1985).

The relative effectiveness of certain treatments and inconsistencies in results between countries and locations depend on the different combinations of these factors. For example, while fungicides are applied at two weekly intervals in Cameroon to control black pod disease, due to the relatively high and frequent rainfall, fungicides are applied at 3-4-weeky intervals in Ghana (Opoku *et al*., 2000). The reason for the difference between the two countries is that Ghana has relatively lower amount and frequency of rainfall than Cameroon.

Life cycles of species may also influence the efficacy of fungicide treatment. For example, the root/soil environment plays an important role in the epidemiology of *P. megakarya* (Gregory *et al*., 1984; Opoku, 1994); while on-tree sources are more significant for *P. palmivora* infections (Brassier *et al*., 1981). In spite of these differences, factors affecting the spread and modes of infection of *P. megakarya* and *P. palmivora* are similar. Therefore, chemical control strategies recommended and used for *P. palmivora* and other *Phytophthora* species can be adopted for *P. megakarya*.

In West Africa, protectant fungicides that are mainly "fixed" copper compounds e.g. copper hydroxides and copper oxides, or systemic fungicides containing copper and metalaxyl as mixtures are routinely sprayed onto pods with lever-operated knapsack sprayers for Phytophthora pod rot disease control. These fixed copper compounds are finely divided molecules that are readily mixed and easy to apply at low volumes. This is in contrast to earlier products such as Bordeaux mixture, which had to be applied in relatively large volumes. These copper fungicides form a chemical barrier on the surface of the pod and guard against infection (Shripat, 1999; Akrofi *et al*., 2003). The spraying of copper and metalaxyl mixtures is to take advantage of multi-site action of the different active ingredients, and to reduce the possible build-up of metalaxyl resistance in *Phytophthora* species on cocoa. Furthermore, it must be emphasized that correct dosage of fungicides, timing of initial application in relation to the epidemic, frequency and target of application are all critical factors to ensure successful and economic chemical control.

The continuous release of copper ions in rain water was used as a basis for the successful application of high doses of cuprous oxide into fewer sprays per year, against *P. palmivora* in Brazil (Pereira, 1985). However, single application of high doses of cuprous oxide was not effective against *P. megakarya* in Ghana (Luterbacher, 1994). Thus, frequent applications of copper or copper-metalaxyl mixtures are necessary to effectively control *P megakarya* infections, a practice that is too expensive for local farmers in Ghana and elsewhere in sub-Saharan Africa (Opoku *et al*., 2000, 2007b; Sonwa *et al*., 2008), not environmentally friendly and unsustainable.

Addo-Fordjour *et al*. (2013) reported copper accumulation and contamination of soils and also detected copper residues in cocoa leaves and beans, resulting from copper-based fungicide sprayed on cocoa plantations in Ghana. Targeting disease foci and using information on disease dynamics to plan for spraying regimes can limit the amount of fungicides sprayed on farms and, thereby, reduce copper accumulation and contamination in the production chain. A novel method of injecting phosphonic acid into trunks of cocoa, developed in Australia and successfully used in controlling *P. palmivora* pod rot and cankers in Papua New Guinea (Guest *et al*., 1994), was found to be equally effective against *P. megakarya* in Ghana (Opoku *et al*., 1998) . However, the method could not be recommended for the disease control in Ghana because the product caused scorching of the internal tissues of injected trees (Opoku *et al*., 1998).

Chemical control of black pod disease is cost-effective when the price of cocoa is high and the crop is under high disease pressure. Even then, the fungicides may not save more than 30% of the crop infected with *P. megakarya* (Akrofi, 2003). However, in a more recent study of the cocoa agroforestry sytem (CAF) in Southern Cameroon, Gockowski *et al*. (2010) found out that intensified use of cocoa fungicides, improved market institutions and expansion of the CAF area cultivated per household reduced rural poverty in Southern Cameroon. This finding emphasizes, that several factors determine the effectiveness and socioeconomics of fungicide use on cocoa.

Fungicides and the use of broad spectrum pesticides, have public health and environmental implications. There has always been a clear appreciation of the potential deleterious effects of the chemicals used in the cocoa industry since the 1960s by consumers of cocoa products. Consequently, standards have been set by the Codex Alimentarius Commission (CAC), a committee on Pesticide Residue of FAO/WHO for acceptable levels of residues in cocoa beans to protect the health of consumers and ensure fair trade practices in the international food trade (Moy and Wessel, 2000).

Many importing countries of cocoa and cocoa products have introduced maximum residue limits (MRLs) allowable in cocoa beans and cocoa products. Japan, for instance, introduced a new legislation on MRLs in 2006; the European Union (EU) has since September, 2008 legislated new MRLs (EC 148/2008). On the other hand, tainting resulting from the accumulation of any chemical in cocoa fat may change the taste of the beans, and eventually that of the chocolate made from them. It is, therefore, the task of cocoa crop protectionists to ensure that recommended pesticides, including fungicides, do not leave any residues. These and other stringent quality control measures on pesticides required by cocoa importing countries, mean that efforts must be intensified to ensure strict compliance to good agricultural practices (GAP) with respect to pesticide use in cocoa. However, introducing GAP to the more than three million (often illiterate) smallholder farmers is a major challenge. Basing spraying schedules on the disease dynamics and targeting disease foci on farms can limit the amount of fungicides sprayed on farms.

The increasing cost of spraying inputs, adverse environmental effects of pesticides and consumers' demand for pesticide-free cocoa products, have led to increased demand for more sustainable and alternative disease control strategies.

Breeding for resistant varieties. Breeding programmes for *T. cacao* have been hampered by long generation times, long periods of establishment before fruit production, long periods before attaining maximal fruit production, and a requirement for large planting areas. Genetic variability exists in *T. cacao*, but most breeding work for improved disease resistance and suitable commercial characteristics have utilised materials of only a narrow genetic base. These materials consist mostly of traditional populations of Trinitario, Amelonado and F3 Amazon cocoa; and of open-pollinated populations of selected hybrids (N'Goran and Eskes, 2006). Furthermore, farmers often use seeds from their preferred trees of these traditional populations and selected hybrids, a practice that results in mixed populations, partial inbreeding and loss of vigour. These mixed populations also result in variation in yield and responses to pests and diseases (N'Goran *et al*., 1994). In spite of these variations, some selective improvements of *T. cacao* have been made, providing farmers with materials that give greater returns without major changes in their farming practices. For example, *T. cacao* with various degrees of resistance to *Phytophthora* pod rot caused by *P. palmivora* is available for farmers in West Africa. Furthermore, black pod resistant trees identified on farmers' fields, based on farmers' knowledge in the selection process in Côte d'Ivoire and Cameroon (Efombagn *et al.,* 2007; Pokou *et al.,* 2008) and from wild *T. cacao* from French Guyana (Paulin *et al.,* 2008), are being evaluated for resistance to *P. megakarya* and other agronomic traits.

Several international efforts aimed at improving disease resistance and crop yield in *T. cacao* have also been undertaken. For example, the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilisation and Conservation: A Global Approach", significantly increased international collaboration on germplasm selection, distribution, evaluation, utilisation and conservation (N'Goran and Eskes, 2006). The project emphasized disease and pest resistance, standardised working procedures and succeeded in identifying new sources of resistance to *Phytophthora* pod rot. The resistance identified was enhanced using the genetic diversity present in the International Cocoa Genebank in Trinidad.

Furthermore, an international working collection of 110 accessions, with valuable agronomic traits and wide genetic diversity, was distributed to user countries. In a similar project, the United States Department of Agriculture (Agricultural Research Service in collaboration with Masterfoods Inc.) and national research institutions in *T. cacao* producing countries, identified new sources of resistance in unexploited germplasm and genes involved in resistance to *Phytophthora* and *Moniliophthora* diseases (Schnell *et al.,* 2007a; 2007b). In spite of the progress made in these international collaborative efforts, materials wholly resistant to *Phytophthora* pod rot disease, and particularly to *P. megakarya* or to Witches broom disease and monilia pod rot are commercially unavailable to farmers.

The identification of genetic markers linked to disease resistance has been a major component in cacao improvement programmes (Eskes *et al.,* 1998; Efombagn *et al*., 2006). Efombagn *et al.* (2006) used SSR markers to assess the genetic diversity, genetic differentiation and genetic similarities in cocoa accessions from farmers' farms in Southern Cameroon. They further assessed the genetic diversity of Trinitario and Upper Amazon clones in genebanks and found out that the farmers' planting material had a narrow genetic base and were close to genotypes available in the genebanks. Resistance to *Phytophthora has* been identified as additive and polygenic (Iwaro *et al.,* 1997; Flament *et al.,* 2001), and not specific for *P. palmivora* or *P. megakarya*. Thus, continuous selection and manipulation of materials showing various degrees of resistance, to either *P. palmivora* and/ or *P. megakarya,* could lead to materials with appreciable level of resistance to *P. megakarya*. Pods are the main economic parts of the *T. cacao* plant; hence, pod rot in the field is considered the best criterion for assessing black pod disease resistance (Efombagn *et al.*, 2007). However, the long period of cacao establishment before pod production necessitates alternative methods of assessing resistance. The positive correlation established in the field, between resistance of leaves and pod rot is, therefore, being used to facilitate and speed-up resistance screening for *Phytophthora* in *T. cacao* (Tahi *et al*., 2006a; 2006b; 2007).

Genome mapping has been used to identify and localise QTLs involved in disease resistance (Lanaud *et al.,* 2004), and multiple QTLs have been identified to be involved in resistance to *P. palmivora, P. megakarya* and *P. capsici* (Clement *et al*. 2003; Risterucci *et al.,* 2000; 2003). These tools offer the possibility of improving durability of resistance in *T. cacao* to *P. megakarya* by a possible accumulation of many different resistance genes located in different chromosome regions.

The International Cocoa Genome Sequencing Consortium (IGCS), a collaborative partnership representing 20 institutions from 6 countries, sequenced and analysed the genome of a Beleizian Criollo genotype of *T. cacao* (B97-61/ B2) (Argout *et al*., 2010). The assembly corresponds to 76% of the estimated genome size of *T. cacao* and contains almost all previously described genes, with 82% of the protein-coding genes, anchored on the ten *T. cacao* chromosomes (Argout *et al.,* 2010). Hitherto, the genome sequence of the Amelonado cultivar, Matina 1-6 covering 92% of the *T. cacao* genome with approximately 35,000 genes have been released by the Cocoa Genome Sequencing Group (Schmutz *et al*., 2011). Analysis of these sequences by the two groups is expected to provide major sources of candidate genes for disease resistance and quality improvement to impact positively on cocoa production.

For a long time, the diploid vegetative stage and lack of homologous recombination made the Oomycetes less amenable to genetic manipulation. However, recent technological advances has made it possible to generate genetic linkage maps, bacterial artificial chromosome (BAC) libraries and expressed sequence tags (ESTs) of different developmental stages of some *Phytophthora* species (Tyler *et al*., 2006). DNA transformation methods, including zoospore electroporation, microprojectile bombardment, and *Agrobacterium tumefaciens*-mediated transformations have been developed and used (Cvitanich and Judelson, 2003; Vijn and Govers, 2003). Gene silencing technology was also adopted to circumvent the need for homologous recombination to obtain targeted gene-knockdown strains in *Phytophthora* (van West *et al*., 1999). This technology is being widely exploited to investigate the molecular mechanisms underlying growth, development and pathogenicity of *Phytophthora infestans,* and these tools can be used to uncover new potential targets for disease control in other *Phytophthora* species (Latijnhowers *et al.,* 2003; Govers, 2005; van West *et al*., 2008) including *P. megakarya*.

The genomes of six Oomycetes, four *Phytophthora* species, (*P. sojae, P. ramorum, P. infestans and P. capsici*), a downy mildew, *Hyaloperonospora arabidopsidis* and a *Pythium* species and *Pythium ultimum,* have been sequenced (Kamoun *et al*., 1999; Govers and Gizen, 2006; Grünwald, 2012). The advantages and disadvantages of the sequenced species as model

organisms for Oomycete research have also been extensively reviewed by Lamour *et al.* (2007).

The increased use of genomics has dramatically transformed the phase of Oomycete research and has uncovered many secrets about the biology, pathology and evolution of Oomycetes. Data obtained from various genomic studies are being exploited for different purposes, including specialisation of isolates. The genome of *P. megakarya* that is found mainly on cacao in West and Central Africa, and posing a threat to cocoa production, is yet to be unraveled. *Phytophthora* species produce a protein that has a similar sequence to the necrosis and ethylene inducing protein (NEP1) of *Fusarium oxysporum.* Bae *et al*. (2005) identified multiple copies of NEP1 orthologs (PmegNEP) in *P. megakarya* and in *P. citrophthora, P. capsici, P. palmivora*, and *P. sojae*.

Sequence analysis of nine different PmegNEP orthologs from *P. megakarya* strain Mk-1 revealed that six of these were organised in two clusters of three orthologs, each in the *P. megakarya* genome. They also presented evidence for the instability in the *P. megakarya* genome resulting from duplications, inversions, and fused genes. More studies into the genome of *P. megakarya* will provide opportunities to manage this important cacao pathogen.

Biological control. Several microorganisms, including fungal and bacteria isolated from the surfaces of healthy and infected cacao pods have been reported to be antagonistic to *P. palmivora* (ten Hoopen *et al*., 2003). *Trichoderma virens, T. harzianum*, *Pseudomonas putida* biotype A*, P. aeruginosa, P. spinosa, Burkholderia gladioli, Burkholderia* sp*., Bacillus sphaericus, B. polymyxa,* and *Serratia marcescens* were antagonistic to *P. palmivora* in *in-vitro* experiments (Hanada *et al*., 2009; Mpika *et al*., 2009), but none of these microorganisms has been further developed for commercial application in *T. cacao* fields. Microbial control of *P. megakarya* in Cameroon, with *Trichoderma asperellum* isolate PR 11, was found promising, but not as effective as chemical control (Tondje *et al*., 2007a).

In colonised plate and detached pod assays, Tondje *et al*. (2007a) reported that *T. asperellum* exhibited mycoparasitic activities on *P. capsici, P. citrophthora,* and *P. palmivora*. Furthermore, culture filtrates of the *Trichoderma* isolate showed substantial laminarinase and cellulase activities; the two enzymes that may adversely affect the cell walls of *Phytophthora* (Tondje *et al*., 2007b). The effects of three endophytic fungi, *Colletotrichum gloeosporioides, Clonostachys rosea* and *Botryosphaeria ribis,* on *T. cacao* pod loss due to *Moniliophthora roreri* and *Phytophthora* species was assessed in Panama. The result showed a significant decline in losses due to *Phytophthora pod* rot from treatment with *C. gloeosporioides* and reduced incidence of sporulating lesions by *M. roreri* after treatment with *C. rosea.* The decline in pod losses due to *Phytophthora* and sporulation by *M. roreri* supports the potential of fungal endophytes as biological control agents (Meija *et al.,* 2008; Hanada *et al.,* 2010).

Several natural substances, including plant extracts and bioactive compounds produced by microorganisms, have been evaluated for the control of *Phytophthora* on cacao (Awuah, 1994; Widmer and Laurent, 2006). For example, Widmer and Laurent (2006) showed that rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula officinalis*) leaf extracts reduced germination of *P. capsici, P. megakarya* and *P. palmivora* zoospores, when supplemented to agar plates at different dilutions. Rosemary extracts, containing caffeic acid, rosmarinic acid or derivatives thereof, reduced necrosis of cacao leaf discs caused by *P. megakarya* zoospores.

One other promising class of natural microbial compounds with activity against *Phytophthora* species are the cyclic lipopeptides (CLPs) (de Souza *et al.,* 2003, Raaijmakers *et al.,* 2006, 2010; Tran *et al.,* 2007). de Souza *et al.* (2003) and de Bruijn *et al.* (2007) showed that Massetolide A (MassA) produced by *P. fluorescens* strain SS101 causes zoospore lysis through induction of pores, reduces sporangium formation and increases branching and swelling of hyphae of *P. infestans*. It also induces systemic resistance in tomato plants and reduces the number and expansion of late blight lesions on tomato caused by *P. infestans* (van de Mortel *et al*., 2000; Tran and Raaijmakers, 2007). Given that hyphae, sporangia and zoospores are important sources of inoculum and play major role in cacao black

pod epidemic, there is the need to investigate if CLPs or CLP-producing microorganisms can be exploited for the management of black pod disease caused by *P. megakarya*.

CONCLUSION

Phytophthora megakarya infestation of cacao is a threat to the economies of countries in in West Africa. It is spreading fast in the sub-region, displacing the original populations of the less severe *P. palmivora*. The mechanisms for this shift in population composition of the black pod disease complex remain unknown, although the possibility of further spread to other cacao producing regions is a great concern to all chocolate industry participants. Current methods of control through routine spraying of inorganic fungicides is expensive and environmentally unfriendly. The available and fast emerging genomic and genetic information on Oomycete pathogens and their hosts, including *T. cacao*, should be utilised for the development of new sustainable management practices for *P. megakarya*.

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