P-ISSN: 2305-6622; E-ISSN: 2306-3599
International Journal of Agriculture and Biosciences
www.ijagbio.com; editor@ijagbio.com

Research Article

# Preliminary Observations on *Phytophthora* sp. on Kola (*Cola nitida*) (Vent.) Schott) and Endlicher: Implications in Epidemiology of the Black Pod Disease of Cocoa (*Theobroma Cacao* L.) in Cameroon

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**Article History:** Received: October 12, 2016 Revised: December 23, 2016 Accepted: January 02, 2017

**ABSTRACT**

*Phytophthora* sp. was isolated from naturally infected kola (*Colanitida*) fruits and cocoa (*Theobroma cacao* L.) pods. Artificial inoculations were made on healthy kola and cocoa fruits, using these isolates, grown on an artificial medium. They were compared using morphological and physiological criteria. The mode of infection of kola by this fungus was studied. The physiological isolate from kola is infecting cocoa. It is a secondary parasite on the studied kola tree. All the isolates studied could be *P. megakarya*. The study for disease transmission in kola fruits permitted detection of the parasite on mosses and barks of the kola tree trunk between 0 and 2 meters. Disease transmission is due to piercing and flying insects and water from sporulation, when these are above the kola fruits. In a cocoa plantations, the attacked kola tree is a source of inoculum. Its impact on the epidemiology of cocoa black pod disease needs further investigation by plant pathologists and entomologists.

**Key words:** Kola, Cocoa, Epidemiology, Cameroon, *Phytophthora* sp.

## INTRODUCTION

In Cameroon, cultivated species for fruit production in the Sterculiaceae family respectively include cocoa of genus *Theobroma* and Kola of genus *Cola*. Generally in some African countries, the kola nut plants are planted between cocoa, coffee and food crops (Afolami and Egbe, 1984; Purseglove, 1984). An identical distribution pattern is observed in Cameroon (Nkongmeneck, 1982).

Numerous works on plant protection exist and notably in the field of entomo fauna (Pujol, 1957; Lavabre 1961; Boulard, 1969; Nkongmeneck, 1982) and phytopathology, including some parasites (*Botryodiplodiatheobromae* and *Fomes* spp., *Phellinus* spp., on one hand and *Rigidoporuslignosus* on the other hand). They are respectively responsible for theblack pod fruitdisease (Roger, 1954; Oludemokun, 1979; Olunloyo, 1979) and root decay (Oludemokun, 1979, Purseglove, 1984). However *Phytophthora* sp. is hardly mentioned on fruits(follicles) of *Cola nitida*.

Cocoa black pod disease caused by *Phytophthora* sp. is reported in Cameroon as the main disease affecting this plant, creating production losses of up to 80% or more (Muller, 1969). Its presence on the kola tree may influence cocoa black pod disease because in Cameroon, Kola trees are spread all over cocoa plots.

The aim of this work is first of all to describe this newly identified infection on the kola plant, and to study the laboratory impact this may have on cocoa crop pathology, while attempting to relate the provenance of this parasite to the kola tree crop.

## MATERIALS AND METHODS

### Materials

Field observations and sample collection were at Nomayos II, a village situated at 20 km from Yaounde, in Mbankomo subdivision, in the Mefou and Akono division. Laboratory works were carried out at the Phytopathology Laboratory of the Institute of Agricultural Research for Development (IRAD) at Nkolbisson.

*Phytophthora* isolates used for the studies were sampled from the under-epidermal parts of the follicle (at about four (4) meters above the soil) and from the kola nut seed, as well as from cocoa pods (situated at a meter above the soil). These all showed natural infections. Selected cocoa trees for isolation were amongst those found below and closest to the kola trees (one meter).

**Cite This Article as:** Nyasse S and Efombagn MIB, 2017. Preliminary observations on *Phytophthora* sp. on kola (*Cola nitida*) (Vent.) Schott) and endlicher: implications in epidemiology of the black pod disease of cocoa (*Theobroma cacao* L.) in Cameroon. Inter J Agri Biosci, 6(1): 49-52. www.ijagbio.com 

Two (2) culture media types were used: Agar water medium at 1.5% (EG medium) for isolations, and the basic medium for small weight decoctions (7%) gelose of 1.5% (PP medium) for observations. Artificial inoculations were in the laboratory, using healthy and green Kola fruits (folicles) and cocoa fruits (pods), all respectively from same trees.

**Observation method**

***Observation methods for diseased follicle***

On ten (10) naturally infected fruits, then on six (6) follicles experimentally inoculated in the laboratory, observations made were on aspects of stains seen on follicles (in situ observation for one (1) week).

***Artificial inoculations***

Inoculation technique is that used by Blaha and Lotode (1976): consisting of placement of buckets realized on fruits using a modeling paste from 50 µl of a calibrated suspension of zoospores (8 X $10^5$ spores/ml). Each fruit has five (5) infection points.

The first type of inoculations allows reproduction on respective hosts of disease symptoms. Inoculations with and without scarifications were done, with *Phytophthora* isolates originating from their respective hosts, and maintained under small-weight of cultural milieu. Four (4) treatment categories studied included:

- Pods not scarified and inoculated with isolate from cocoa (50 infection points)
- Pods scarified and inoculated with isolate from cocoa (50 infection points)
- Follicles not scarified and inoculated with isolate from the Kola tree (50 infection points)
- Follicles scarified and inoculated with isolate from the kola tree (50 infection points).

For each of the four (4) treatment categories, 10 fruits were inoculated.

Cross inoculation targets were used to verify if isolates from kola tree follicles did attack cocoa pods and vice-versa. Healthy and green kola tree follicles were inoculated with or without scarifications (10 fruits, making 50 infection points for each treatment category) with the cocoa isolate. Healthy and green cocoa pods on their own part inoculated as previously described, with isolate derived from the kola tree.

For both types of inoculations, observations ran from the third to the $10^{th}$day after inoculation and were based on successes or not of this, followed by eventual re-isolation of parasites for Koch postulate verifications.

***Comparative studies of isolates***

To compare *Phytophthora* sp. of the kola tree to that of the cocoa tree, certain morphological and physiological studies were done on 8 isolates; using the following codes:

L2C2: Laboratory reference strain (*P. megakarya* of cocoa)
CAO1: isolate from cocoa pods of trees situated round a kola tree
CAO2: re-isolation of CAO1 after pod inoculations
CAO3: re-isolation of CAO1 after inoculations on follicle
FOL1: isolate from follicle of the kola tree
FOL2: re-isolation of FOL1 after inoculation of follicle
FOL3: re-isolation of FOL1 after pod inoculation
GRAC: isolate from nut of a diseased follicle.

For morphological studies, each isolate (implant of 5 mm diameter) was sown in five (5) replicates, in petri dishes of 90 mm diameter, containing the PP milieu. Incubation was under darkness and at 26°C. On parasite invasion of about 4/5 of the box, the aspects were observed and described. Sporocyst production was induced under white light (18 W neon tubes) for two to three days. Sporocysts were mounted in between slides, for eventual observations under the microscope and for eventual description.

For physiological studies, cultures were as previously described, but incubation under darkness was under a range of temperatures (10, 14, 18, 22, 26, 30 and 34°C).Growth diameters were measured daily as from the third to the $10^{th}$day after planting in order to determine the cardinal growth speeds and temperatures. Statistical analyses of results consist of comparing isolate growth speed sat 26°C. Based on the outcome of this analysis, four (4) isolates (CAO1, FOL1, GRAC and L2C2) are retained for cardinal growth temperature studies. Another set of five (5) replicates were placed under incubation at 26-28°C with alternating light/darkness (12h/12h) while observing their behavior.

***Phytophthora* sp. isolation on mosses and barks**

In order to study the transmission of the disease on to follicles, an attempt was made to isolate the fungus from mosses and barks found on kola tree trunks. For this, moss and bark samples were taken at different heights above the soil (0, 1, 2, 4, 6, 8 and 10 m) up to the branches where most of the follicles were used as samples for laboratory trapping experiments of the parasite. For each sampling height, 50 g of mosses and 20 g of barks were placed separately in beakers of 500 ml containing 200 ml of distilled sterilized water. In each beaker was placed a pod soaked in through the distal end. The used healthy and green pods coming from same tree were not treated with any fungicide. This experiment had three (3) replicates, and ran for ten (10) days. Observations on successful and unsuccessful infection of the fungus were derived from parts of the pods in contact with the receiving solution.

## RESULTS AND DISCUSSION

**Description of symptoms on diseased follicles**

Infected regions had indications of brownish color and a moist nature found generally at the zone of the fruit peduncle. While the consistence of the epidermis of the follicle remained preserved, internal tissues got softened. Dislodged sub-epidermal tissues of the fruit because of parasitic activity, explains observed loss of original tissue consistence. Surface stains increased with time and its periphery presents a net frontline of attack. As from this front, the epidermis remains green in color, while the tissue just under it gets softened systematically. Once necrotic lesions have invaded the quasi-totality of the follicle, on the epidermis is observed fruiting that under the microscope shows many parasitic spores. Within a couple of days, stains end up invading the entire surface area of the follicle. Some rare cases of fungi get to the seeds via the pulp that envelops the seed.

**Table 1:** Successful infection scores (% IR) at three (3) days after simple inoculations from pods and follicles respectively by CAO1 and FOL1

| Treatment categories | Inoculated pods by CAO1 | | Follicles inoculated by FOL1 | |
|---|---|---|---|---|
| | Non wounded | Wounded | Non wounded | Wounded |
| % IR at three (3) days after inoculation | 100 | 100 | 0 | 100 |
| Number of infection points | 50 | 50 | 50 | 50 |

**Table 2:** Successful infection scores (% IR) at three (3) days after crossed inoculations from pods and follicles respectively by CAO1 and FOL1

| Treatment categories | Inoculated pods by FOL1 | | Follicles inoculated by CAO1 | |
|---|---|---|---|---|
| | Non wounded | Wounded | Non wounded | Wounded |
| % IR at three (3) days after inoculation | 100 | 100 | 0 | 100 |
| Number of infection points | 50 | 50 | 50 | 50 |

**Table 3:** Parasite trapping from mosses and barks, relative to sampling height

| Sampling height (m) | 0 | 1 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| Presence of parasite in three (3) replicates | 3/3 | 3/3 | 3/3 | 0/3 | 0/3 | 0/3 | 0/3 |

Symptoms observed on follicles that are artificially inoculated is like that on naturally infected follicles, the only difference being that stains remained localized around the isolation point.

Also observed in attacked zones of naturally infected follicles, are dark points that were like insect bites or stings. These symptoms resemble those of the black pod disease in cocoa.

**Fungal isolation from mosses and barks**

Trapping of the parasite on moss and bark samples was only possible between 0 and 2 m (table3).In this study, mosses and barks of the kola tree were host to the parasite up to tree heights of 2 m. This inoculum originate from wash offs from sporulating pods closest to the kola tree (during the rainy season), and insects (often rampant) whose frictions against mosses permit retention of infectious propagules that may be transported with them. This reflects a probable mode of transmission by biting or stinging insects – their bites being possible entry points for the parasite – and flying insects. This contamination path seems most probable given that insects of the kolatree most often are the same as those found on cocoa tree (Pujol, 1957; Boulard, 1969; Nkongmeneck, 1985).

This domain needs the attention of entomologists; to carry out an inventory of the entomo fauna of the kola trees, to identify insects intervening in the infection of follicles – either through biting by transporting the inoculum – ending up the transmission chain using adequate insecticides or a mixture of treatments.

The results of our study show that stains observed from rottening on kola tree follicle arise from a fungus of the *Phytophthora* genus. Following this, the cocoa tree in a different way, shows as studied in the kola tree a secondary parasite; making the kola tree plant a source for potential inoculum for cocoa trees.

The different isolates show morphological and physiological characteristics that resemble those of *P. megakarya*of the cocoa tree.

Transfer of the parasite up to the follicles is by insects that bite, sting and fly. It may also be from sporulating pod wash when these overly follicles. Insecticide applications to control the cocoa black pod disease are therefore necessary. In perspective, in-depth study should be done on follicle decay in the kola tree due to *Phytophthora sp.* The aim of this is to bring out the evolution of symptoms and the importance of damages caused. Inoculations involving many kola tree genotypes and *Phytophthora* sp. pathotypes seen on this plant is necessary to bring out a better understanding of the nature of host/parasite relationship. The role of insects in the contamination chain needs to be investigated.

Similar investigations are advisable on other shade trees being used in cocoa cultivation, so as to bring out species to be eliminated and those to be retained for farmers. This will permit precision on the impact of shade trees as sources of inoculum in the epidemiology of the black pod disease in cocoa, to add to their microclimatic influence (hygrometry, temperature regulation) in cocoa production.

**Acknowledgements**

We will attempt to thank all those that made contributions towards preparing this article through encouragements, advices and remarks. We finally wish to recognize Ntonga Atangana Gallus who made available a cocoa farm that had in association kola trees, and Atangana Joseph for collecting samples of mosses and barks from the kola tree.

**REFERENCES**

Afolami CA and NE Egbe, 1984. Yield response of Kola (*Cola nitida*) (vent) Scott) and Endlicher to N, P and K fertilizer application: A case study of preliminary trial. Café Cacao The, 28: 13-16.

Blaha G and R Lotode, 1976. Un critère primordial de sélection du cacaoyer au Cameroun : la résistance a la pourriture brune des cabosses (*P. palmivora*). Café Cacao The, 20: 97-114.

Boulard M, 1969. Homoptères jassidomorphes nouveaux liés aux colatiers et aux autres plantes stimulantes cultivées en Afrique central. Café Cacao The, 16: 151-156.

Lavabre EM, 1961. Protection des cultures de caféiers, cacaoyers et autres plantes pérennes tropicales. IFCC, 268p: Jouve, paris.

Muller RA, 1969. Contribution àla recherche des fongicides éfficaces contre *p. p*almivora (Butl.) Butl.

Au Cameroun. 3eme Conf Inter Sur la Rech Cacaoyere Accra, pp: 439-446.

Nkongmeneck BA, 1982. Contribution à l'étude du genre cola au Cameroun. The se de Doctorat 3eme cycle, 198p. Université de Yaoundé.

Nkongmeneck BA, 1985. Le genre Cola au Cameroun. Rev Sci Tech Ser Sci Agron, 1: 57-74.

Oludemokun AA, 1979. A review of Cola diseases. PANS, 25: 265-269.

Olunloyo AA, 1979. Fungi associated with deterioration of stored kola nuts. Niger J AgricSci, 1: 51-59. Cocoa Research Institute, Nigeria.

Pujol R, 1957. Etude préliminaire des principaux insectes nuisibles aux colatiers. J Agr Trop Bot Appl, 4.

Purseglove JW, 1984. Tropical crops: Dicotyledons. London: Longman. 719p.

Roger L, 1954. Phytopathologie des pays chauds. Tome III, p: 2723. Paul Chevalier, éditeur. Paris Vieme.