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The rot fungus *Botryodiplodia theobromae* strains cross infect cocoa, mango, banana and yam with significant tissue damage and economic losses

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***Botryodiplodia theobromae,* a common rot fungus, causes serious economic losses in cultivation of many different crops such as cocoa, mango, banana and yam. Determination of infectious routes of plant pathogens and their mechanisms of infection are of great importance in any disease control programme. Until this work, no investigation had been made into cross infectivity of different isolates of the fungus in Ghana. The aim of the study therefore was to investigate cross infectivity of various *B. theobromae* isolates from cocoa, mango, banana and yam. Mature fruits of cocoa, mango and banana and yam tubers with symptoms of *B. theobromae* rot were collected from farms within four regions in Ghana for fungal isolation. The isolated fungi from the four crops were developed into pure cultures on potato dextrose agar (PDA). Five-day old pure cultures were separately inoculated into healthy mango fruits and cocoa pods. This was followed by daily measurement of lesion diameter, and observation of the fruit internal tissue damage caused by the fungi infection. The results indicated that the symptoms of rot and mycelia characteristic of the *B. theobromae* were visible within two days. The cocoa, mango, banana and yam isolates of *B. theobromae* were found to be virulent with similar pathological effects in the experimental crops, that is, cocoa and mango. It is concluded from this work that *B. theobromae* isolates from the four different crops, that is, cocoa, mango, banana and yam in Ghana's forest agricultural zones are infectious and have damaging effects on other neighbouring crops with economic consequences. The findings cast doubts on the mixed- or inter-crop system which is usually adopted to control fungal rot in plantation farms.**

Key words: *Botryodiplodia theobromae*, cross infectivity, mixed-crop system, inter-cropping.

INTRODUCTION

Botryodiplodia theobromae (Pat.) Griff. and Maubl. (Syn: *Lasiodiplidia theobromae* Pat.) and its asexual state, *Botryosphaeria rhodina* (Berk and M.A. Curtis) Arx are fungal pathogens of great economic importance. *B. theobromae* is an opportunistic plant pathogen that causes different types of plant diseases with worldwide distribution within tropical and subtropical regions (Faber et al., 2007). It has a wide host range estimated to be more than 280 plant species (Domsch et al., 2007; Khanzada et al. 2006; Sutton, 1980) although with varied

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pathological effects on its hosts.

In the tropics, *B. theobromae* is an economically important fungus known to cause major losses to mango, cocoa, banana and yam farmers (Rieger, 2006; Amusa et al., 2003). The fungus is known to cause tuber rots in yam, root rot in cassava, collar rot in peanuts, crown rot in banana, stem end rot in mango fruits, stem rot in pawpaw and leaf spot in citrus (Sangeetha et al., 2011; Rossel et al., 2008; Khanzada et al., 2004b; Jiskani, 2002; Arjunan et al., 1999; Sangchote, 1988). *B. theobromae* is associated with die-back on mango (Khanzada et al., 2004a, b) and pod rot of cocoa (Phillips, 2007). Onyenka et al. (2005) reported that the fungus is present in more than 70% of farms surveyed in Nigeria and it is linked to colossal yield losses around 80% of crop harvest. Jiskani (2002) and Sangchote (1988) respectively have identified *B. theobromae* to be a virulent fungus and a common isolate found on diseased mango fruits in Pakistan. French in 2006 also reported that the pathogen infects and causes extensive damage to mango, cocoa and banana fruits and yam tubers.

Rots caused by the fungus, particularly in the root and tuber crops often occur underground and so diagnosis of the disease is usually delayed or under repaired. Moreover, the wider host range (Crammer, 1979) and the host non- specificity (Mohali et al., 2005) of *B. theobromae* makes control and management of the disease very difficult. Regrettably, there is limited information about the diversity of *B. theobromae* affecting food and cash crops in Ghana.

As a common practice, Ghanaian farmers use mango, cocoa, banana or yam as intercrops or boundary crops in their farms. The rationale behind such farming practice, as widely indicated by the Ghana's Directorate of Agricultural Extension Service, Ministry of Food and Agriculture, is to (1) help provide subsistence to the farmer, (2) reduce spread of infection as the intercrops serve as disease barriers, and (3) to efficiently utilize plant food for crop yield (Osei-Bonsu et al., 2002; ADRA, 2010; Banful, 1998; Cunningham and Smith, 1961; www.mofa.gov.gh). However, it is also believed that such farming practice may facilitate cross infections if strains of pathogens possess broad range infectivity of plant hosts on the farm (Mohali et al., 2005). Information on host range of *B. theobromae* on Ghanaian farms is currently unavailable. Establishment of host range of this important pathogenic fungal species with high economic importance would be useful in designing suitable control measures such as farming practices to reduce yield losses. The outcome of the research findings would help revision or otherwise of the well-known intercropping systems commonly adopted by Ghanaian farmers amidst the recurrence of fungal infection outbreaks on plantation farms.

This study sought to investigate cross infectivity and host range characteristics of four *B. theobromae* isolates from cocoa, mango, banana and yam tested on fresh cocoa and mango fruits.

MATERIALS AND METHODS

Sample collection

The study involved cocoa, mango, banana and yam plantations and farms from four Regions in Ghana. These were the Ashanti, Brong-Ahafo, Central and Northern Regions. From each crop per farm, ten diseased fruits or tubers were collected aseptically into sterilized containers and transported into the laboratory. Subsequent culturing and morphological identification of the fungi were carried out in the Plant Pathology Laboratory of the Crops Research Institute of the Council for Scientific and Industrial Research (CSIR-CRI), Fumesua in Ghana. The inocula of the *B. theobromae* isolates from the four crops, namely cocoa, mango, banana and yam, represent the major treatments.

Preparation of pure inoculum of *B. theobromae*

Cocoa pods, mango and banana fruits, and yam tubers with symptoms of *B. theobromae* rots were aseptically collected and specimens of diseased tissues excised from diseased lesions. The cut pieces were surface sterilized in 5% sodium hypochlorite solution for five minutes and rinsed three times in sterile distilled water, air dried in a sterile hood and plated on Potato Dextrose Agar (PDA). The plates were incubated at 28°C. Fine hyphae that grew from diseased tissue on the culture media were sub-cultured on fresh PDA. Colonies of *B. theobromae* were morphologically identified and subjected to further sub-culturing on PDA amended with chloramphenicol (50 mg/L) to control bacterial contamination. Pure cultures of the isolates were finally maintained on fresh PDA (Waller and Lenné 2002).

Inoculation of fresh mango fruits and cocoa pods with *B. theobromae* isolates

Healthy, freshly harvested uninfected fruits of Kent mango variety and *Akokra Bedi* cocoa pods of similar size, shape and physiological maturity were surface sterilized for five minutes in 5% sodium hypochlorite solution. This was immediately followed by two rinses in sterile distilled water and allowed to dry. The inoculums of the *B. theobromae* isolates from the four crops, namely cocoa, mango, banana and yam, represented the major treatments. The experiment was conducted in a complete randomized design in triplicate.

Each fruit or pod was doubly inoculated at opposite sides using inoculums from five-day old actively growing cultures of *B. theobromae* isolates (that is, cocoa, mango, banana and yam isolates) in a sterile Laminar-Flow hood. The inoculation involved removal of 0.5 cm deep and 5 mm diameter discs of tissues from each of the fresh and uninfected fruits, pod or tuber using hot-oven sterilized 5 mm diameter cork borer. The resulting 'craters' served as sites for inoculation. Each replicate had four fruits or pods per single fungal isolate. The inoculum consisted of mycelia plug of each fungal isolate cut with a 5 mm diameter cork borer. This was inoculated into the craters by inversion, thus plugging the hole (Figures 1 and 2). The removed discs were repositioned and the edges of the wounds sealed with melted wax. The control set ups were treated in a similar manner except that no mycelial inocula or plugs were placed in the craters or hole. The inoculated fruits and controls were incubated at 28°C. The fungal treated fruits and pods were examined daily over four and six-day durations respectively for mango and cocoa to record the disease development. Daily measurements of the longest radial spread of the lesions resulting from the inoculations were recorded. Koch's postulate was completed by re-isolating *B. theobromae* fungus from the fruits and pods that developed symptoms characteristics of *B. theobromae*

Figure 1. Lesions of infections on Kent mango fruits four days after inoculation with *Botryodiplodia theobromae* isolates from banana (A), cocoa (B), mango(C) and yam (D). The control setup (first right panel) was without mycelia inoculation.

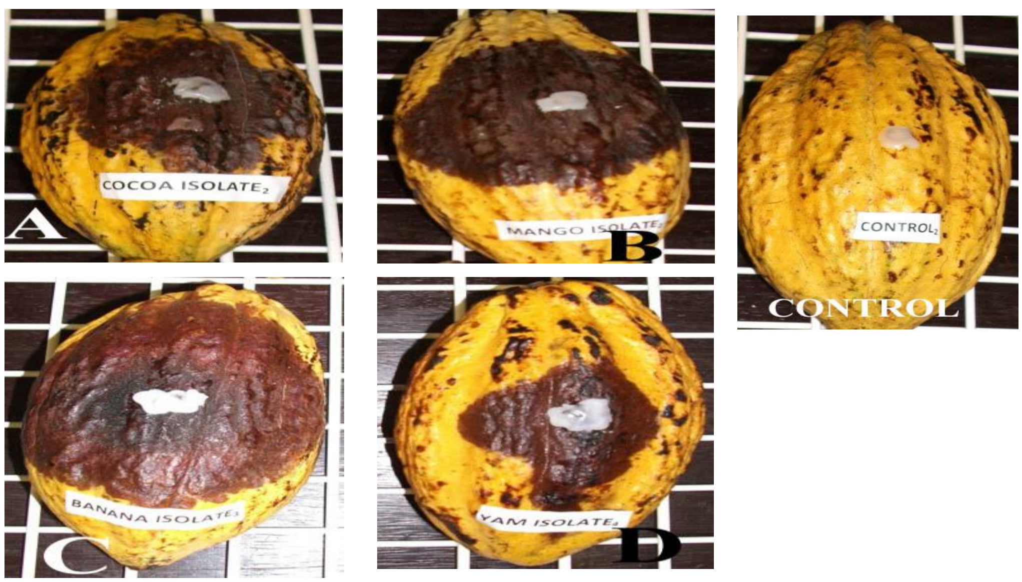

Figure 2. Lesions of infections on *Akokra Bedi* cocoa pods six days after inoculation with *B. theobromae* isolates from cocoa (A), mango (B), banana (C) and yam (D). The control setup (first right panel) was without mycelia inoculation.

infection on mango and cocoa (Cohen, 1994).

RESULTS

Isolation of *B. theobromae* strains

In the inoculation experiments conducted, it was observed that *B. theobromae* isolates, obtained from mango, cocoa, banana and yam were able to induce infections on both inoculated mango fruits and cocoa pods (Figures 1 and 2). The controls were however, not infected.

Colonization of fungal isolates on mango and cocoa fruits

Statistical analysis of the daily measurements of the lesions produced by the four isolates at the end of the

Table 1. Diameter of the lesions of infection by four *B. theobromae* isolates following cross inoculation on *Kent* mango fruits.

Isolates	Radius of infection* (mm)			
	Day 1	**Day 2**	**Day 3**	**Day 4**
C	0.00 ± 0.00	4.17 ± 2.31[c]	26.67 ± 1.59[a]	40.67 ± 0.33[a]
M	0.00 ± 0.00	27.17 ± 1.88[a]	39.67 ± 2.92[a]	51.83 ± 1.59[a]
B	0.00 ± 0.00	17.83 ± 0.67[b]	30.83 ± 2.89[a]	44.17 ± 6.01[a]
Y	0.00 ± 0.00	20.67 ± 3.49[a,b]	27.50 ± 8.89[a]	38.83 ± 12.32[a]

*Values are means of three replications. Different letters indicate significant differences at 5% significant level. C, Cocoa isolate; M, mango isolate; B, banana isolate; Y, yam isolate.

Table 2. Diameter of the lesions of infection by four *B. theobromae* isolates following cross inoculation on *Akokra Bedi* cocoa variety.

Isolates	Radius of infection* (mm)					
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
C	0.00 ± 0.00	1.50 ± 0.76[a]	3.33 ± 1.69[a]	11.00 ± 5.50[a]	21.83 ± 6.00[a,b]	33.83 ± 10.42[a,b]
M	0.00 ± 0.00	3.17 ± 2.05[a]	7.50 ± 5.20[a]	12.83 ± 11.13[a]	30.00 ± 7.42[a]	42.50 ± 8.26[a]
B	0.00 ± 0.00	3.50 ± 2.56[a]	8.50 ± 6.60	27.83 ± 6.84[a]	38.33 ± 7.84[a]	53.50 ± 8.04[a]
Y	0.00 ± 0.00	0.00 ± 0.00[a]	2.50 ± 2.50[a]	5.83 ± 4.64[a]	17.17 ± 5.51[a,b]	34.17±12.82[a,b]

*Values are means of three replications. Different letters indicate significant differences at 5% significant level. C, Cocoa isolate; M, mango isolate; B, banana isolate; Y, yam isolate.

experiment indicated no significant differences among the isolates on the mango fruits and cocoa pods inoculated (Tables 1 and 2). The lesions on the inoculated mango fruits, from the opposite sides, merged four days after inoculation. A range of 51.83 to 38.83 mm of lesion length was produced by *B. theobromae* isolates from mango and yam respectively on the fungal inoculated mango fruits.

Cross-sections through the infected mango fruits showed a watery, softened pulp (Figure 3). After three days of exposure of the cut surface of fungal inoculated fruits in a sterile environment, a visible growth of mycelia of *B. theobromae* was observed (Figure 4).

The lesions produced on the inoculated cocoa pods, from the opposite sides, merged six days after inoculation. A range of 53.50 to 33.83 mm of lesion length was produced six days after inoculation with *B. theobromae* isolates from banana and cocoa respectively on the cocoa pods.

Ten days after inoculation of the fresh cocoa pods, the pods were totally engulfed by the fungal lesions (Figure 5) and showed visible grayish black mycelial growth and black soot (matured spores) of *B. theobromae*. Cross sections through the inoculated cocoa pods ten days after inoculation with *B. theobromae* revealed that the infection reached the beans (Figure 6). The beans turned black with grayish black mycelia, a characteristic of *B. theobromae* (Figure 6). Koch's postulate involving re-isolation of *B. theobromae* from the inoculated mango fruits and cocoa pods confirmed the fungal presence.

DISCUSSION

The state of the infections and rots produced by mycelial spores of the four isolates of *B. theobromae* establish the infectious nature of spore contaminated fruits and pods on the farm (Opoku et al., 2007). Thus farm crops may serve as alternative host of different strains of *B. theobromae* pathogen and as such contaminating source for other healthy plants (Lichtfouse et al., 2009; Opoku et al., 2007; Philips, 2007; Jones and Stove, 2000; Muirhead and Jones, 2000). Spores and mycelial hyphae on the farm are important inocula for rot disease induction. The consequence of this is high yield loss with great financial burden to the farmers.

The results in this study also confirm the nature of *B. theobromae* as a broad spectrum pathogen with a wide range of hosts as has been reported in several studies (Pitt and Hocking, 2009; Opoku et al., 2007; Domsch et al., 2007; French, 2006; Khanzada et al., 2004a; Sutton, 1980). Broad host specificity of *B. theobromae* isolates has earlier been reported in Sri Lanka (Shanthi et al., 2008) and Venezuela (Mohali et al., 2005), two countries with climates similar to that in Ghana. The results suggest that intercropping cocoa, mango, banana or yam with other food or tree crops infected by *B. theobromae*, as commonly practiced in Ghana (ADRA, 2010; Banful, 1998), may facilitate spreading of the diseases on the farm.

In conclusion, we have demonstrated in this study that *B. theobromae* isolates from the four important crops in

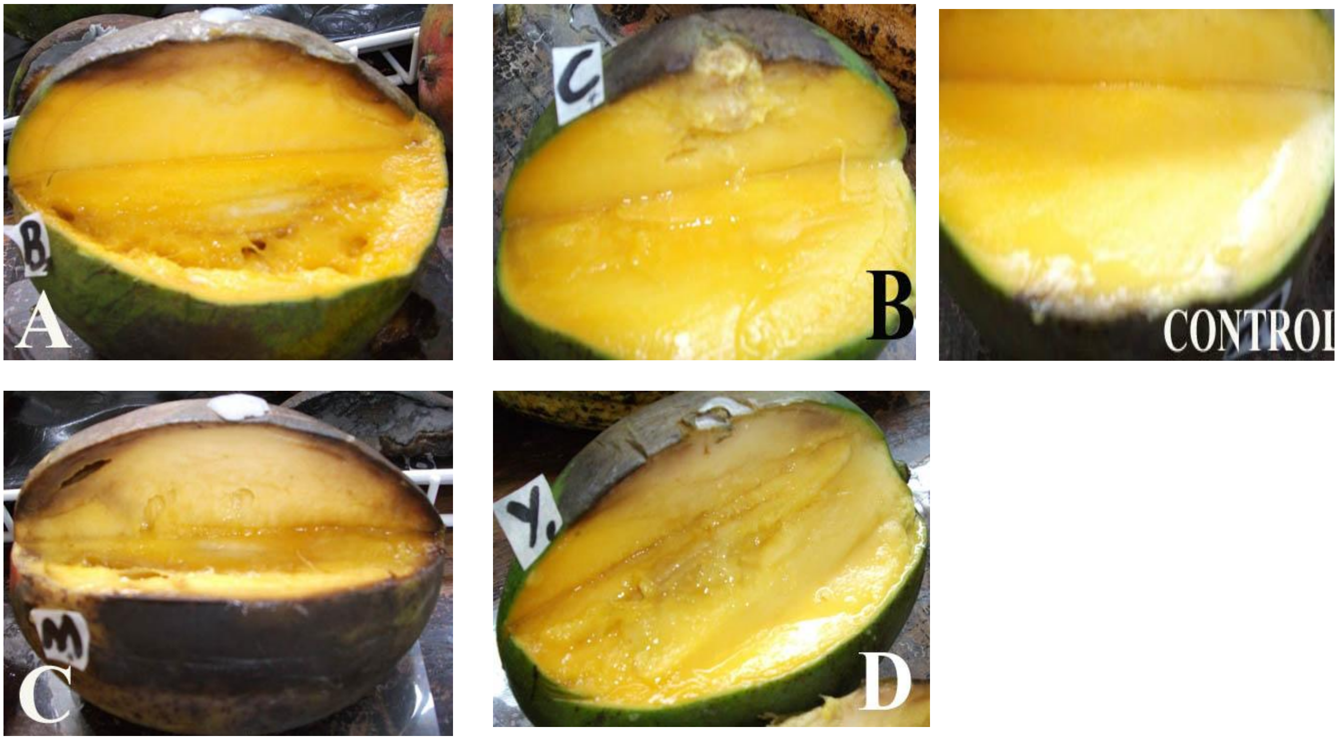

Figure 3. Cross-section of infected Kent mango fruits showing watery softened internal tissues (pulp) four days after inoculation with *B. theobromae* isolates from banana (A), cocoa (B), mango (C), and yam (D). The control setup (first right panel) was without mycelia inoculation.

Figure 4. Mycelial growth from pulp of infected Kent mango fruits four days after inoculation with *B. theobromae* isolates from banana (A), cocoa (B), mango (C) and yam (D) followed by three days of exposing the cut surface.

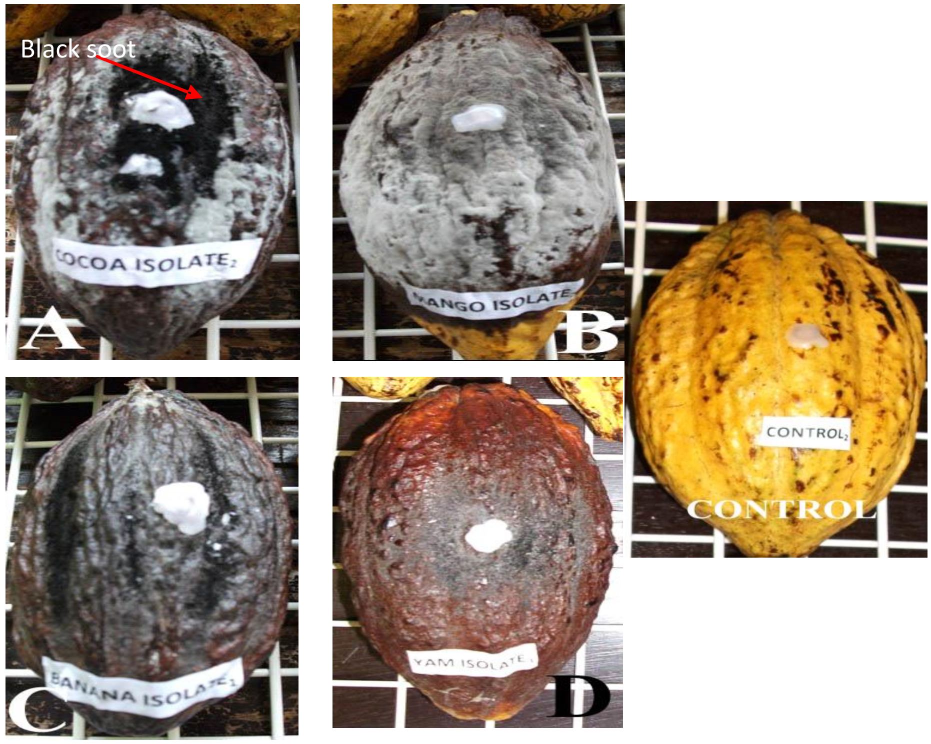

Figure 5. *Akokra Bedi* cocoa pods ten days after inoculation with *B. theobromae* isolates from cocoa (A), mango (B), banana (C) and yam (D). The control setup (first right panel) was without mycelia inoculation.

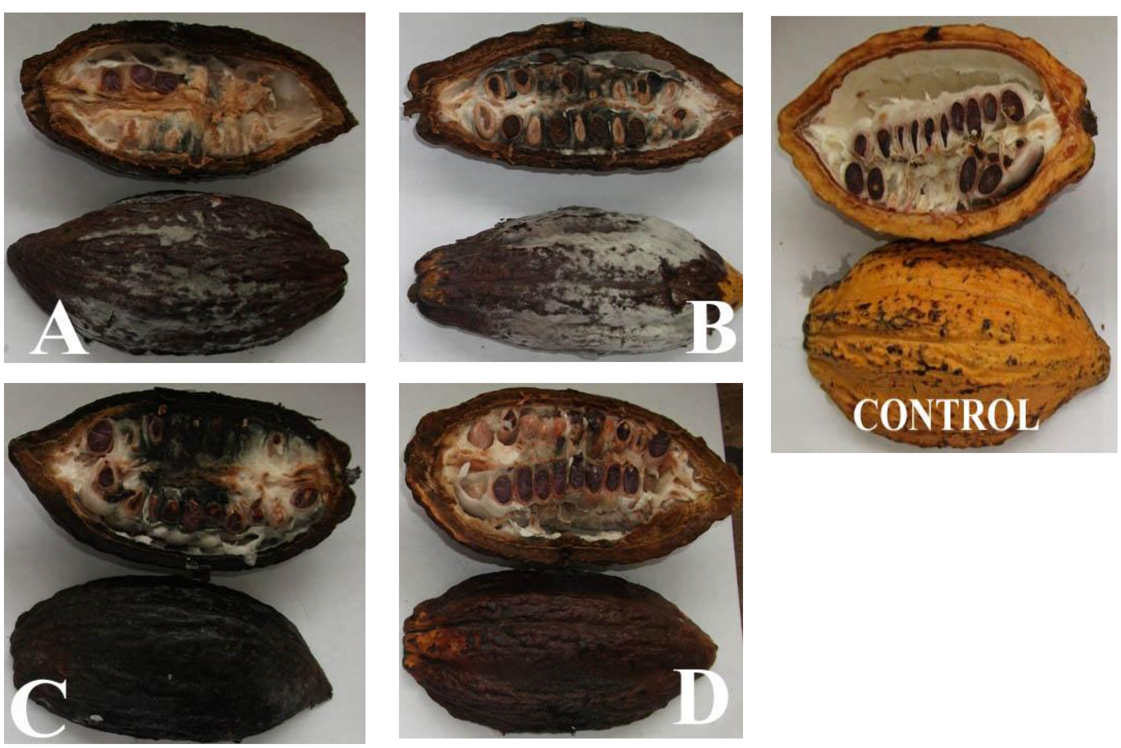

Figure 6. Cross-section of Akokra Bedi cocoa pods showing blackened internal tissues and grayish black mycelia ten days after inoculation with *B. theobromae* isolates from cocoa (A), mango (B), banana (C) and yam (D). The control setup (first right panel) was without mycelia inoculation.

Ghana, that is, cocoa, mango, banana and yam, have broad host specificity. Therefore, the pathogen is capable of infecting several food crops cultivated on a single piece of land. The findings question the current intercropping system adopted by Ghanaian farmers and show that this practice may enhance spread of *B. theobromae* from diseased plants to other plant species with consequent crop yield loses. It is thus suggested that if possible intercrops with cocoa, mango, banana, yam and other *B. theobromae* susceptible crops should be well thought through to ensure that non *B. theobromae* susceptible plant are used.

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REFERENCES

ADRA International (2010). Sustainable Rural livelihood enhancement and poverty reduction through the establishment of perennial tree crops; Best Practices Award for Agroforestry, Extension Services/Farmer Training and Horticulture Production. Best Practices and Innovations (BPI) Initiative, December 2010. Available from: http//www.adraghana.org.

Amusa NA, Adegbite AA, Muhammed S, Baiyewu RA (2003). Yam disease and its management in Nigeria. Afr. J. Biotechnol. 2(12):497-502.

Arjunan G, Karthikeyan G, Dinakaran D, Raguchander T (1999). Diseases of horticultural crops, AE Publications, pp. 56-92.

Banful B (1998). Production of plantain, an economic prospect for food security in Ghana. In: Bananas and Food Security. (Picq C, Foure E, Frison EA). International Symposium, 10-11 November, 1998, Douala, Cameroon, pp. 151-160.

Cohen J (1994). Fulfilling Koch's Postulates. Science 266:1647.

Crammer J (1979). *Botryodiplodia theobromae*. Commonwealth Mycological Institute, Kew, Surrey. P. 123.

Cunningham RK, Smith RW (1961). The Significance of recent work in Ghana on the shade and nutrient requirements of cocoa. 1961 cocoa conference proceedings, pp. 119–122.

Domsch KH, Gams W, Anderson TH (2007). Compendium of Soil Fungi. 2nd Ed. Cornell University. England. ISBN 3930167697, 9783930167692.

Faber GA, Bender GS, Ohr HD (2007). Diseases. UC IPM Pest management Guidelines. UC ANR publication. P. 3436.

French BR (2006). Diseases of food plants in Papua New Guinea- A compendium, 38 Australia 4(41):235-265.

Jiskani MM (2002). Dying of mango orchards needs special attention, Sindh Agriculture University, Pakistan.

Jones DR, Stove RH (2000). Fungal diseases of banana fruit-preharvest disease. CABI Publishing. UK, pp. 173-190.

Khanzada MA, Rajput QA, Shahzad S (2006). Effect of medium, temperature, light and inorganic fertilizers on *In Vitro* Growth and sporulation of *Lasiodiplodia theobromae* isolated from mango. Pak. J. Bot. 38(3):885-889.

Khanzada MA, Lodhi AM, Shahzad S (2004a). Mango dieback and gummosis in Sindh, Pakistan caused by *Lasiodiplodia theobromae*. Plant Health Progress. Doi: 10.1094/ PHP- 2004- 0302- 01- DG.

Khanzada MA, Lodhi AM, Shahzad S (2004b). Pathogenicity of *Lasiodiplodia theobromae* and *Fusatium solani* on mango. Pak. J. Bot. 36(1):181-189.

Lichtfouse E, Navarrete M, Debaeke P, Souchère V, Alberola C (2009). Sustainable Agriculture. London, Springer. P. 455.

Mohali S, Burgess TI, Wingfield MJ (2005). Diversity and host association of the tropical Tree endophyte *Lasiodiplodia theobromae* revealing using simple sequence repeat markers. Berlin Forest Path. 35:385-396.

Muirhead IF, Jones RH (2000). Fungal diseases of banana fruit-postharvest disease. UK, CABI Publishing: pp. 190-206.

Onyenka TJ, Dixon AGO, Ekpo EJA (2005). Identification of levels of resistance to cassava root rot disease (*Botryodiplodia theobromae*) in Africa landrace and improved germplasm using *in vitro* inoculation methods. Euphytica 1457(3), Netherlands, Springer. ISSN 1573-5060:281-288.

Opoku IY, Assuah MK, Domfeh, O (2007). Manual for the identification and control of diseases of cocoa. Cocoa Research Institute of Ghana-Ghana Cocoa Board, Ghana. Technical Bull. 16:18-19.

Osei-Bonsu K, Opoku-Ameyaw K, Amoah F, Oppong F (2002). Cacao-coconut intercropping in Ghana: agronomic and economic perspectives. Agrofor. Syst. 55(1):1-8.

Phillips AJL (2007). *Lasiodiplodia theobromae*. Portugal Centro de Recursos Microbiológicos, Faculdade de Ciênciase Tecnologia, Universidade Nova de Lisboa. P. 2.

Pitt JI, Hocking AD (2009). Fungi and Food Spoilage. 3rd Edn., Springer, USA., ISBN-10: 0387922067, pages 519.

Rieger M (2006). Introduction to fruit crops. The Haworth Press. NewYork. P. 87.

Rossel G, Espinoza C, Javier M, Tay D (2008). Regeneration guidelines: sweet potato and yam. In: Dulloo ME, Thormann I, Jorge MA, Hanson J. Crop specific regeneration guidelines [CD-ROM]. CGIAR System-wide Genetic Resource Programme, Italy. P. 9.

Sangeetha G, Anandan A, Rani SU (2011). Morphological and Molecular characterization of *Lasiodiplodia theobromae* from various banana cultivars causing crown rot disease in fruits. Archives of Phytopathology and Plant protection, pp. 1-12.

Sangchote S (1988). Botryodiplodia Stem End Rot of Mango and Its Control. Kasetsart J. Natl. Sci. Supply 22:67-70.

Shanthi WW, Yasodha D, Deepthi, W (2008). Host Specificity of Colletotrichum gloeosporiodes and Botryodiplodia theobromae isolates from mango, papaya and rambutan and their response to *Trichoderma harzianum*. Tropentag. Conference on International Research on Food Security, Natural Resource Management and Rural Development. University of Hohenheim, October 7-9.

Sutton BC (1980). The Coelomycetes. Common wealth Mycological Inst. Kew, Surrey.

Waller JM, Lenné JM (2002). Detection and isolation of fungal and bacterial pathogens. Plant Pathologist's pocketbook (3):208. www.mofa.gov.gh