Pathogenicity and fungicide sensitivity of the causal agent of postharvest stem end rot disease of mango in Ghana

J. O. HONGER, C. E. WILLIAM, D. OWUSU-ANSAH, K. SIAW-ADANE, & G. T. ODAMTTEN

(J.O.H.: Soil and Irrigation Research Centre, niversity of Ghana, Legon; C.E.W., D. O.-A. & S.-A.-K.: Crop Science Department, University of Ghana, Legon; G.T.O.: Department of Botany, University of Ghana, Legon)
Corresponding author's email: johonger@yahoo.com

ABSTRACT

Studies were carried out on the stem end rot disease of mango in Ghana. The incidence and severity of the disease were evaluated on mango fruits collected from major mango growing areas of Ghana. The causal agent was isolated on media and identified. The pathogenicity of the fungus and its cross-infection potential were determined on mango, avocado, papaya and banana fruits. The sensitivity of the pathogen to fungicides was determined by assessing radial mycelial growth on potato dextrose agar (PDA) amended with nine different fungicides (Bendazim, Funguran, Ivory, Topsin, Asuoku master, Kocide, Mirage, Sulphur 80 and Copper oxychloride). Stem end rot disease was prevalent in the major mango growing areas of Ghana. Two pathogens, *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides* were isolated from the disease lesions. However, only the former was able to cause stem end rot disease symptoms on the artificially inoculated fruits, confirming it as the causal agent of the disease. It was also found to be highly susceptible to Bendazim, Ivory, Topsin, Asuoku master and Mirage, whilst it was resistant to Funguran, Kocide, Sulphur 80 and Copper oxychloride.

Original sciencitic paper. Received 30 Apr 14; revised 17 Oct 14.

Introduction

Mango (*Mangifera indica* L.) is an important fruit crop grown in Ghana and serves as sources of income to many people in the country. The fruit is one of the cheapest sources of vitamins and minerals in the diet of the populace, and is currently one of the important non-traditional export commodities of Ghana.

Mango cultivation in Ghana is hampered by the incidence of fungal diseases including anthracnose (Oduro, 2000; Offei *et al.*, 2008) and stem end rot (Saeed, 2012). Stem end rot is reported to be the second most important fungal disease of mango in several tropical areas and is very difficult to

control (Huang & Liu, 1995). The disease is specific, begins in the flesh and spreads to the pericarp. Once the fruit is infected by the pathogen, it is no longer edible (Huang & Liu, 1995). Symptoms on the fruit appear as colourless lesions, that sometimes spread downwards in a finger-like projection. As the lesion ages, it turns to dark brown and the affected fruit parts become soft and watery. These watery parts can then burst to release a foul smelling fluid (Johnson, 1998).

The incidence of the disease has been reported to range between 28 per cent and 36 per cent on stored mango fruits in Taiwan (Ko *et al*., 2009), which can be translated to mean high postharvest losses as the disease always renders the fruit unusable in any way. The disease has also been reported as a major contributory factor to postharvest losses of the crop in Ghana (Saeed, 2012). In grocery shops dotted around urban areas in Ghana, symptoms of the disease could be seen on some of the mango fruits displayed on the stalls. Due to the nature of the disease, these fruits are eventually discarded as the rot renders them unmarketable. The economic losses due to the disease could be very enourmous.

Several fungi species have been associated with stem end rot disease worldwide. These include *Botryodiplodia theobromae* Pat (Syn; *Lasiodiplodia theobromae*), *Dothiorella dominicana* Petr. and Cif., *Dothiorella mangifera* H and P Syd. and But. and *Phomopsis mangifera* Ahmad (Chandra & Pathak, 1989; Meah, Plumbley & Jeger, 1991; Johnson *et al*., 1992; Johnson, 1998). Among these fungi pathogens, only *L. theobromae* Pat. has been isolated from mango fruits from Ghana and associated with stem end rot disease of the crop (Saeed, 2012).

Currently, information on the importance, aetiology and control of stem end rot disease of mango in Ghana is scanty. The disease has been reported as contributory factor to food insecurity through postharvest losses in Ghana (Saeed, 2012). However, data to support the level of destruction caused by the disease are scanty. *L. theobromae* has been isolated from mango fruits and associated with the disease in Ghana (Saeed, 2012). However, *Colletotrichum gloeosporioides*, another pathogen which has been isolated from mango by several workers in Ghana has also been associated with stem end rot in north eastern Brazil (Costa *et al*., 2010). It is also common for both *L. theobromae* and *C. gloeosporioides* to be isolated from mango fruits showing stem end rot disease symptoms in Ghana (Honger, 2013).

Inoculation studies with pure cultures of the isolated pathogens, which are necessary to prove their pathogenicity and confirm the aetiology of the disease has not been done in Ghana. Another important information lacking is the sensitivity of the causal agent of the stem end rot disease to some of the common fungicides available in Ghana, which are mainly used for the control of anthracnose disease in Ghana. Knowledge of the sensitivity of the fungus to these fungicides would be useful in formulating a cost-effective control measure against the disease.

In view of the shortfalls in knowledge of the stem end rot disease of mango in Ghana, the study was carried out to 1) determine the incidence and severity of the disease in some of the major mango growing areas of Ghana, 2) confirm the aetiology of the disease, and 3) determine the sensitivity of the causal agent to some of the commonly used fungicides in mango farms in Ghana.

Materials and methods

Collection of fruits for the study

Physiologically matured mango fruits of the Keitt variety aged between 110 and 120 days after flowering were harvested from 12 administrative metropolis/municipals/districts in Ghana (Table 1). An average of 10-year weather data (rainfall, relative humidity and temperature) were obtained from the Ghana Meterological Services Department of Ghana (Fig. 1.).

Incubation of fruits and assessment of disease incidence and severity after harvest

Between 50 and 100 physiologically matured, but unripe fruits of the Keitt variety were harvested at random from each farm. All fruits collected from farms within the same district were bulked and 100 fruits were sampled at random. The sampled fruits were washed with liquid detergent and then thoroughly rinsed in three changes of tap water after which they were air-dried. The fruits were then divided into lots of four with each lot representing a replicate of each district/metropolis/municipality. Fruits were packed into plastic baskets, covered with jute sacks, and left on benches in the laboratory under the prevailing relative humidity of 60 per cent to 65 per cent and temperature

TABLE 1

Administrative Areas of Ghana from where Mango Fruits were Obtained for the Study

Area	*Administrative division*	*Region*	*Agroecological zone*
Ga West	District	Greater Accra	Coastal savanna
Dangme West	District	Greater Accra	Coastal savanna
Manya Krobo	District	Eastern	Coastal savanna
Yilo Krobo	District	Eastern	Coastal savanna
North Tongu	District	Volta	Coastal savanna
Hohoe	Municipal	Volta	Semi-deciduous
Kwaebibirem	District	Eastern	Semi-deciduous
Kumasi	Metropolis	Ashanti	Semi-deciduous
Berekum	Municipal	Brong Ahafo	Transitional
Kintampo	Municipal	Brong Ahafo	Transitional
Savelugu/Nanton	District	Northern	Guinea savanna
Tolon/Kumbugu	District	Northern	Guinea savanna

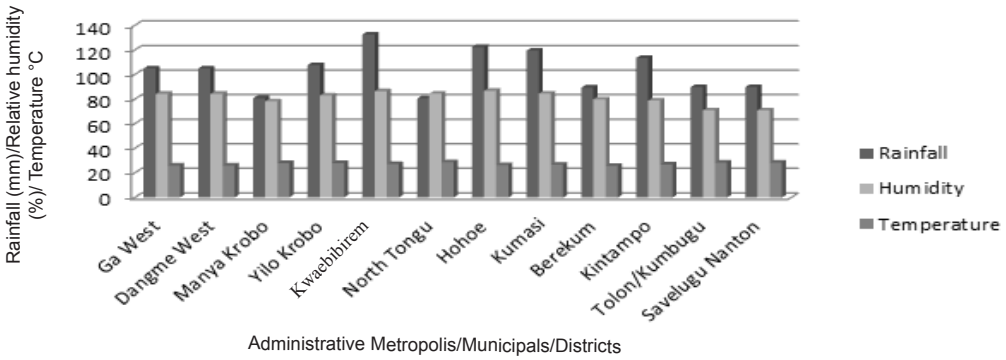

Fig. 1. Ten-year mean rainfall, relative humidity and temperature recorded from the indicated districts/metropolis/municipalities of Ghana.

of 25-27 °C for 2 weeks. During the period, the number of fruits showing stem end rot symptoms before the end of the incubation period and percentage of the surface area of the diseased fruits were recorded and the fruits discarded. The disease incidence (DI) was calculated using the formula (Madden *et al*., 2007):

$$DI = \frac{\text{No. of fruits with disease symptoms}}{\text{Total no. of fruits incubated.}} \times 100$$

Disease severity was also determined by rating the surface area of the fruit covered by the disease lesion on a scale of 0-5 after Lakshmi, Reddy & Prusad, 2011(Table 2) . The disease severity index per replicate was computed using the formula:

$$DSI = \frac{\sum fX}{\sum x}$$

where DSI = disease severity index, f = no. of fruits with a particular rating and x = a particular rate based on the percentage of fruit surface area covered by disease lesion.

Isolation and identification of the causal agent of the disease

Mango fruits showing diseased symptoms of stem end rot were sampled from various grocery outlets and markets in Accra, Ghana. Also, some of the fruits that developed the stem end rot symptoms after incubation of the harvested fruits were included. The isolation and identification of causal agent were carried out at The Plant Pathology Laboratory of the Department of Crop science, University of Ghana, using both the direct method and isolation on media.

TABLE 2

Disease Severity Rating Scale used for the Assessment of Disease Severity in Different Mango Farms in Ghana

Rating	*Meaning*
0	No infection
1	Up to 5% of fruit surface area covered
2	6-10% of fruit surface area affected
3	Between 11 and 20% of fruit surface area covered
4	21-50% of fruit surface area affected
5	More than 50% of the fruit surface area covered

Direct isolation

A flamed sterile scalpel was used to scrape some of the mycelia of fungi growing on the diseased fruit surface and placed on a microscope slide containing a drop of water. The slide was covered with a cover slip and observed under a stereo microscope.

Isolation on culture media

The isolation of causal agents was first done on water agar (WA) and then on potato dextrose agar (PDA). WA and PDA were prepared at rates of 20 gl^{-1} and 39 gl^{-1}, respectively. Each mixture was autoclaved at 121 ºC for 15 min, allowed to cool and poured into clean sterilised plates to set. Pieces of the fruit tissues taken from the advancing edge of the lesion at all parts of the fruits (stem end, middle and bottom portions) were surface sterilised with one per cent sodium hypochlorite for 15 s, rinsed in sterile distilled water and blotted dry with a paper towel. The sterilised pieces of tissues were plated singly on WA plates, and incubated till enough growth of fungal pathogens was observed. The pathogens were then subcultured on PDA to obtain pure cultures.

Identification of the isolated fungal pathogens

The number of days taken by each isolated pathogen to cover the plate, the colour of the mycelium, and the type of fruiting bodies were recorded and used for identification. Mycelium bearing conidia were placed on slides and stained with cotton blue. The morphology of the conidia and growth characteristics of the hyphae were recorded using photomicrography to further aid in the identification of the pathogen. The isolates were identified with the aid of standard reference materials (Johnson, 1998; Barnett & Hunter, 2006). Fungal species associated with stem end rot disease of mango in Ghana and elsewhere were maintained on slants and used for pathogenicity tests.

Pathogenicity Test

Pathogenicity of the isolated fungi was tested on mango fruits. Freshly harvested untreated, unwaxed and physiologically mature fruits of mango were washed, surface sterilized with per cent sodium hypochlorite and air dried. Plugs (4mm diameter) of mycelia of isolated causative agent were cut from actively-sporulating areas near the edge of an 8-day old culture of each isolate. One 7 mm deep hole was aseptically punched with cockborer (4mm diameter) around the stem end of each fruit, and a plug of the isolate was placed into each of the holes. Fruits inoculated with PDA only served as a control. Fruit plugs removed from punched holes were replaced and covered with parafilm. The inoculated fruits were incubated upright at the prevailing relative humidity and temperature conditions in the laboratory (60-65% RH and 23-25 °C). Inoculated fruits were observed daily till symptoms appeared. The pathogen that was able to induce similar stem end rot symptoms on the artificially inoculated fruits was considered as the causal agent of the disease. Subsequently, it was re-isolated from the disease lesions to confirm Koch's postulates and authenticate the pathogen as the causal agent of the disease.

Cross infection potential

The cross infection potential of the pathogen causing stem end rot on mango was determined on avocado, papaya and banana, which were the major fruit crops observed in most mango farms during the period of the study. Six physiologically matured but unripe fruits of each of the fruit crops were harvested, washed and surface sterilised. A sterilised cork borer (4 mm) was used to wound each of the fruit. Each hole was filled with a mycelial plug of the pathogen and incubated in the laboratory. Five days after incubation, the diameters of the disease lesions that formed on the fruits were measured and the data analysed.

Fungicide selection and sensitivity tests

Based on rapid appraisal method of interviewing the 82 mango farmers, the type of fungicides they use to control diseases on their farms were selected. The selected fungicides (Table 3) were tested on 7-day old culture of the isolated fungus *in vitro*, by determining the radial mycelia growth of the fungus on PDA amended with recommended rates of the fungicides (Khanzada, Lodhi & Shahzad, 2005; Mahmood, Saleem & Akhtar, 2002).

The required amount of each fungicide was measured and mixed thoroughly with 100 ml of autoclaved molten PDA (39 gl^{-1}) and dispensed into three Petri dishes, each

TABLE 3

Fungicides Evaluated for their Effect on the Radial Mycelial growth of Lasiodiplodia theobromae*, the Suspected Causal Agent of Stem End Rot of Mango in Ghana*

Fungicide	*Manufacturer*	*Active ingredient*	*Nature*	*Application rate*
Bendazim	Iprochem	50% Carbendazim	Systemic	2.00 gl^{-1}
Funguran	Makhteshim	50% Copper hydroxide	Contact	2.00 gl^{-1}
Ivory	Calliope	80% Mancozeb	Contact	4.00 gl^{-1}
Topsin	Sino	70% Thiophanate methyl	Systemic	1.25 gl^{-1}
Asuoku Master	Hockley Int.	Copper oxide + Metalaxy	Contact/systemic	3.00 gl^{-1}
Kocide 101	Dupont	Copper hydroxide	Contact	3.00 gl^{-1}
Mirage	Makhteshim	Prochloraz	Systemic	1.5 ml l^{-1}
Sulphur 80	Aryan	80% Sulpur	Systemic	3.00 gl^{-1}
Copper oxychloride	Agricola	Copper oxychloride	Contact	2.00 ml l^{-1}

containing about 30 ml of the fungicide-amended media and allowed to set. Plugs of each isolate taken from the periphery of a 7-day old culture, using a sterile cock borer, were placed in the middle of each plate at one plug per plate. Three replications of each isolate were used. Control plates contained PDA without fungicide amendment. The Petri dishes were placed in polythene bags and incubated in the laboratory at temperature of 23-25 ºC and relative humidity of 60 per cent to 65 per cent using the completely randomised design. Radial mycelial growth of the colonies were measured daily, by measuring the diameter of the colonies along diagonal lines drawn at the reverse side of the Petri dishes with a ruler. Measurement of mycelia growth started a day after incubation, when significant growth was recorded on the control plates to the 3rd day when the growth in the control had covered the entire plate. Per cent inhibition in radial growth over control was calculated using the formula:

$$I = \frac{C-T}{C} \times 100$$

where I = per cent inhibition of growth of test fungi, C = radial growth (mm) in control and T = radial growth (mm) in treatment. After the 4th day, plates without growth were kept for 3 days for further observation before they were finally discarded.

Data analysis

Data on disease severity of postharvest anthracnose disease and diameter of disease lesion on inoculated fruits were directly subjected to ANOVA. Disease incidence of postharvest anthracnose and percentage inhibition of radial growth of the fungus were arcsine transformed before analysis. The ANOVA was performed using Genstat software, version 9.2. Means were separated using LSD at 5 per cent.

Results

Nature of the symptoms of the stem end rot disease observed on the diseased fruits in storage

The disease was characterised by diffused areas of water-soaked tissue that radiated from the stem end in fingerlike projections. The infected areas quickly darkened and coalesced into circumpedicular lesions with

crenate margins. Necrosis remained beneath the fruit cuticle and penetrated all fruit flesh within 5-7 days. As the lesion aged, grey fluffy mycelia of the causal agent began to show on the fruit surface particularly, around the fruit pedicel. A rotten smelling, straw-coloured fluid oozed out of the fruits through broken portions of the fruit surface.

Incidence and severity of postharvest stem end rot disease from different administrative metropolis/municipals/districts of Ghana.

The disease symptoms were found on fruits that were initially harvested without the disease and stored for 14 days. The disease was detected on fruits from all the 12 different mango growing areas of Ghana during the period of the study (Table 4). In 2010, significant difference in the disease incidence among the different areas from which the fruits were obtained was observed. The highest disease incidence of 83 per cent was recorded on fruits obtained from the Kumasi Metropolis, whilst the lowest disease incidence of 20 per cent was recorded on fruits from the Savelugu/ Nanton District. There was no significant difference in the disease incidence recorded on the fruits from the Kumasi Metropolis and the Kwaebibirem District (Table 4). Similarly, the disease incidence recorded on fruits from Savelugu-Nanton, Tolon-Kumbugu, Kintampo and Berekum districts was not significantly different. Also, the disease incidence on fruits from Ga West, Dangme West, Manya Krobo, Yilo Krobo and North Tongu districts was not significantly different.

TABLE 4

Incidence and Severity Index of Postharvest Stem End Rot of Mango Recorded in the Different Administrative Metropolis/ Municipals/Districts of Ghana in 2010 and 2011

Metropolis/ Municipals/Disricts Index	*2010*		*2011*	
	**Incidence (%)*	***Severity index*	**Incidence (%)*	***Severity*
Ga West	62.0 (51.9)	3.2	65.0 (53.8)	2.8
Dangme West	61.0 (51.4)	3.0	67.0 (55.0)	2.4
Manya Krobo	62.0 (52.00	2.9	66.0 (54.4)	3.1
Yilo Krobo	66.0 (54.5)	2.7	66.0 (54.4)	2.4
North Tongu	65.0 (53.8)	2.9	67.0 (55.0)	2.6
Hohoe	63.0 (52.5)	3.6	70.0 (57.3)	3.6
Kwaebibirem	79.0 (62.7)	4.1	77.0 (61.4)	3.9
Kumasi	83.0 (65.7)	4.2	82.0 (65.2)	3.9
Berekum	20.0 (26.6)	2.0	39.0 (38.7)	1.2
Kintampo	22.0 (27.9)	1.6	21.0 (27.30)	1.3
Savelugu/Nanton	20.0 (26.6)	1.2	19.0 (25.6)	1.1
Tolon/Kumbugu	21.0 (27.3)	1.2	19.0 (25.6)	0.9
L.S.D (5%)	(4.5)	0.8	(6.2)	0.7

*Means with arcsine transformed figures in parentheses; **Severity measured on a scale of 0-5

The disease incidence recorded in 2011 was similar to what was recorded in 2010 (Table 4). There was significant difference in the incidence of the disease on mango fruits obtained from the different mango growing areas of the country. The highest disease incidence of 82 per cent was recorded on fruits from Kumasi Metropolis, which was not significantly different from the 77 per cent recorded on fruits from Kwaebibirem District. The lowest disease incidence of 19 per cent was recorded on fruits from Tolon-Kumbugu and Savelugu-Nanton districts (Table 4). Disease incidence recorded on fruits from Ga West, Dangme West, Manya Krobo, Yilo Krobo and North Tongu districts was not significantly different.

Significant difference in disease severity index was recorded in both 2010 and 2011. In 2010, the highest disease severity index of 4.2 was recorded on fruits from Kumasi Metropolis, whilst the lowest of 1.2 was recorded on fruits from Tolon-Kumbugu District. The difference in disease severity index on fruits from Kumasi Metropolis and Kwaebibirem District was not significant. Similarly, the disease severity index recorded on fruits from Ga West, Dangme West, Manya Krobo, Yilo Krobo and North Tongu districts was not significant. Also, the difference in disease severity on fruits from Tolon-Kumbugu and Savelugu/Nanton, Kintampo and Berekum districts was not significant (Table 4). In 2011, the highest severity index of 3.9 was recorded on fruits from Kumasi Metropolis and Kwaebibirem District, whilst the lowest severity index of 0.9 was recorded on fruits from Tolon-Kumbugu District. Disease severity index on fruits from Tolon-Kumbugu and Savelugu-Nanton, Berekum and Kintampo districts was not significantly different (Table 4).

Identification of isolated fungal species

Two different fungal species were isolated from the stem end rot disease symptoms on the infected mango fruits and identified as *Lasiodiplodia theobromae* (syn = *Botryodiplodia theobromae*) and *Colletotrichum gloeosporioides*. *L. theobromae* was identified with the following cultural and morphological characteristics: Isolates produced mycelium which was initially white and turned dark as the culture aged. Mycelia grew and filled the entire 9 mm plate in 4 days (Fig. 1a). The hyphae were initially hyaline and turned dark, and were septated (Fig. 1b). Two types of conidia, mature and immature were observed. The immature conidia were hyaline, aseptate, granular, ovoid and thick-walled (Fig. 1c). The matured ones were uniseptate, brown walled and had longitudinal striations (Fig. 1d). These conidia were produced in dark coloured pycnidia. The cultural and morphological characteristics connotes the fungus, *L. theobromae*

C. gloeosporioides was identified based on the following cultural and morphological characteristics: Colonies of isolates were initially white, which turned grey or remained white as the culture aged (Fig. 2a). Mycelium grew and filled the entire 9 mm Petri dish in 9 days. Most isolates produced bright orange coloured acervuli in culture. The acervuli contained numerous conidia which were short, conical and rounded at the edges (Fig. 2b).

Pathogenicity test of isolated fungi on mango fruits

Between the two different fungal species isolated, only *L. theobromae* was able to in-

duce the characteristic stem end rot symptoms on the artificially inoculated fruits similar to what was observed on the naturally infected fruits. The initial symptoms consisted of dark necrotic areas around the wound. This was seen on both fruits inoculated with either the mycelial plug of the pathogen and those inoculated with PDA only. However, the lesions on the fruits inoculated with the pathogen continued to expand while those on the control fruits did not expand. Four days after inoculation, the fruits inoculated with the pathogen had begun to show rotted tissue around the areas where the disease lesion had spread.

C. gloeosporioides was also found to be pathogenic to the mango fruit by inducing disease symptoms on the inoculated fruit. However, the disease symptoms were different from that of the stem end rot. It was made up of a dark areas around the point of inoculation. The lesions expanded and became slightly sunken. The disease progression was much slower compared to the symptoms of stem end rot, and 7 days after inoculations, the acervuli of the causal agent was found accompanying the lesions. These symptoms were similar to what has been

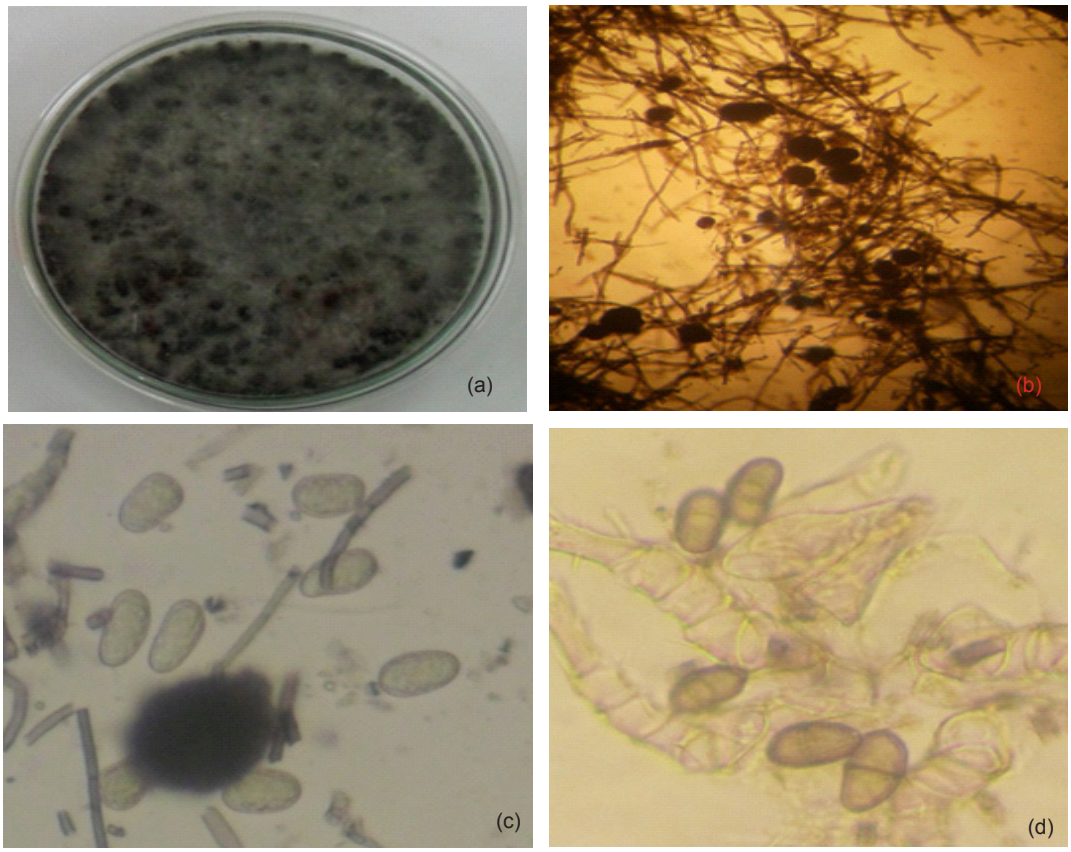

Fig. 1. Cultural and morphological characteristics of Lasiodiplodia theobromae isolated from the diseased fruits. A = cultural growth on PDA showing pycnidia (arrowed); B = micrograph of hyphae and pycnidia C = micrograph of immature conidia; D = micrograph of immatured matured conidia (arrowed). Micrograph magnification = x400.

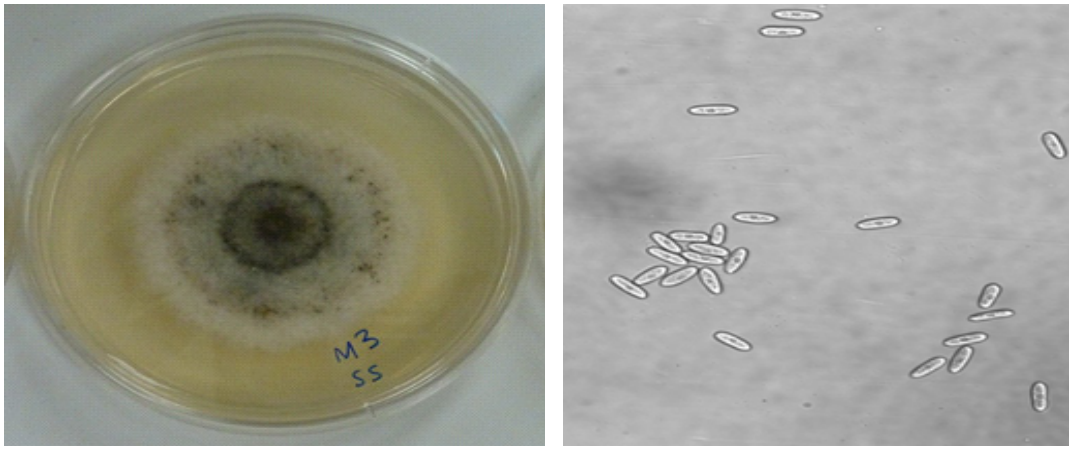

Fig. 2. Cultural and conidial morphology of *Colletotrichum gloeosporioides* isolated from stem-end rot disease.

described as anthracnose, attributed to the pathogen.

Cross infection potential of L. theobromae *on avocado, papaya and banana*

The nature of the disease symptoms observed on banana, papaya and avocado fruits that were inoculated with the mycelia plugs of *L. theobromae* were the same as what was found on the mango fruit. The largest disease lesion diameter of 193.2 mm was recorded on avocado, whilst the smallest, 145.5 mm was recorded on banana. However, there was no significant difference in lesion diameter recorded on avocado and mango. Similarly, there was no significant difference in lesion diameter between banana and papaya (Table 5).

Inhibition of mycelia growth of L. theobromae *by different fungicides*

The inhibition of the mycelial growth of *L. theobromae*, the causal agent of the stem end rot disease of mango in Ghana is shown in Table 6. The pathogen grew rapidly on the plates containing PDA only (control) covering the entire plate in 3 days. However, the growth was inhibited at various levels by the different fungicides. At Day 1 after incubation, there was significant difference in percentage inhibition of the mycelia growth of the fungus by the different fungicides. The highest percentage inhibition of 100 per cent was recorded on the PDA amended with Asuoku Master, Bendazim, Mancozeb and Mirage and Topsin M, whilst the lowest of 27.5 per cent was recorded on media amended with Sulphur 80. The percentage inhibition recorded on PDA amended with Fungura and Kocide was not significantly different. On Day 2, similar trend was recorded with the highest percentage inhibition of 100 per cent being recorded on Asuoku Master, Bendazim, Mancozeb and Mirage and Topsin M. However, the least inhibition of 11.4 per cent was recorded on PDA amended with Copper oxychloride (Table 6). In Day 3 after inhibition, Asuoku Master, Bendazim, Mancozeb and Mirage and Topsin M still maintained their 100 per cent inhibition of the mycelia growth of the pathogen. During the period, Copper oxychloride and Sulphur 80

Table 5

Diameter of Lesion Induced on Different Types of Fruits by Lasiodiplodia theobromae *Obtained on Mango.*

Type of fruit inoculated	*Number of fruits inoculated*	*Percentage of fruits diseased*	*Mean diameter of lesion (mm)*
Banana	6	100	145.5
Avocado	6	100	193.2
Papaya	6	100	147.9
Mango	6	100	185.8
LSD (5%)	-	-	11.9

could not inhibit the growth of the pathogen.

The results also showed a gradual reduction in the percentage inhibition of the mycelia growth of the fungus from Day 1 to Day 3 by some of the fungicides. Copper oxychloride was able to inhibit the growth at Day 1 by 52.2 per cent which decreased to 11.45 in Day 2 and eventually to 0 in Day 3 after incubation (Table 6). Similarly, the percentage inhibition reduced from 27.5 per cent at Day 1 to 25.4 per cent at Day 2 and then to 0 at Day 3 in the media amended with Sulphur 80. Similar trends were also recorded on media amended with funguran and kocide with the percentage inhibition on the media amended with both fungicides reducing from 67.8 per cent at Day 1 to 24.8 per cent at Day 3 after incubation (Table 6).

Discussion

Fruits infected by the stem end rot pathogen in the study deteriorated rapidly such that they were not fit for consumption and had to be discarded. In most cases, by the time the disease symptoms were observed on the fruit surface, the internal parts of the fruit

Table 6

Inhibition of Mycelia Growth of Lasiodiplodia theobromae *by Different Fungicides.*

Fungicide	*Testing concentration (g or ml l^{-1})*	**Percentage inhibition after*		
		Day-1	*Day-2*	*Day-3*
Asuoku Master	3.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Bendazim	2.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Copper oxychloride	2.0	52.2 (46.2)	11.4 (19.8)	0.0 (4.1)
Funguran	2.0	67.8 (55.5)	53.2 (46.7)	24.8 (29.8)
Kocide	2.0	67.8 (55.5)	53.2 (46.7)	24.8 (29.8)
Mancozeb	4.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Mirage	1.5	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
Sulphur 80	3.0	27.5 (31.6)	25.4 (30.0)	0.0 (4.1)
Topsin M	1.25	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
LSD (5%)		(1.8)	(0.6)	(0.5)

*Means with transformed data in parenthesis.

had rotten. This corroborates the reports by Huang & Liu (1995) that the disease symptoms begin from the flesh and spreads to the pericarp. The nature of the disease makes it impossible for fruits showing even minimal area of the surface diseased, to be utilised in any way. Other mango fruit spot diseases such anthracnose, begin on the pericarp and penetrates the flesh with time. In such cases, some of these fruits can be utilised when the disease has not yet penetrated the flesh. This makes the stem end rot a very serious disease, and in some places may be more important than anthracnose caused by *C. gloeosporioides*.

Collection and subsequent incubation of healthy mango fruits from different farms located in the different Metropolis/Municipals/Districts of Ghana showed that, postharvest stem end rot disease was prevalent in the country. In both 2010 and 2011 mango production seasons, the disease was detected on the mango fruits which were harvested initially without the disease, confirming the latent nature of the disease. The disease incidence in 2010 ranged from 20 per cent to 83 per cent whilst in 2011 it ranged from 19 per cent to 82 per cent. Since mango fruits infected with the disease cannot be utilised in any way (irrespective of the severity of the disease), the disease incidences recorded in the study showed how devastating the disease could be.

Variations in the incidence and severity of the disease were recorded among the different mango growing districts of Ghana. Generally, the highest incidence and severity were recorded in the Kumasi Metropolis and Kwaebibirem District. These areas lie in the semi deciduous forest agro-ecological zone of Ghana. The zone is characterised by high rainfall and temperatures for most part of the year. This type of climate is known to increase relative humidity (Chala, Burberg & Tronso, 2010). Being a postharvest disease which begins in the field, the development of the stem end rot disease is affected by rainfall and humidity, which have been known to affect the incidence and severity of plant diseases (Agrios, 2005; Trigiano, Windham & Windham, 2008). Therefore, the high incidence and severity of the disease in these areas may be attributed partly to the type of climate pertaining in those areas.

In contrast, the Tolon-Kumbugu and Savelugu-Nanton districts, characterised by the Guinea savanna agro-ecological zone, have the lowest amounts of rainfall in Ghana in most parts of the year. This, coupled with high temperatures all year round, might result in lower relative humidity in these areas. Consequently, the lowest incidence and severity of the disease were found in these districts. Despite the low disease incidence and severity in these areas, the disease could cause significant losses to commercial mango farmers located in those areas. This is because it could develop symptoms on fruits in storage or in transit, which could result in rejection of such fruits on the market. This assertion is given credence by Saeed (2012) that the disease is a contributory factor to postharvest losses of mango in that part of the country.

The other districts namely, Ga West, Dangme West, Manya Krobo, Yilo Krobo and North Tongu districts are in the coastal savanna agro-ecological zone of Ghana. The climatic conditions in these parts of the country are similar to that of the semi-deciduous forest zone with high humidity,

especially during mango production season. Consequently, the disease incidence and severity were also found to be high in these areas. Currently, these areas contain the largest density of mango farms in Ghana. The high disease incidence and severity of the disease in these areas could pose a serious threat to the mango export industry in Ghana.

In the study, the disease symptoms observed on the diseased fruits were similar to stem end rot disease attributed to both *L. theobromae* and *Dothiorella* spp. According to Johnson (1998), the stem end rot symptoms caused by these fungi are diffused areas of water-soaked tissue that radiates from the stem end in finger-like projections and quickly darkens. Necrosis remains beneath the cuticle and may penetrate fruit flesh within 7 days or less. Using morphological characteristics, particularly conidial morphology and pathogenicity tests, the causal agent of the stem end rot disease in Ghana was identified as *L. theobromae*.

L. theobromae has been associated with postharvest mango stem end rot in China (Huang & Liu, 1995). It has been reported as prevailing in hot regions (Johnson, 1998) such as Ghana, and had been identified as the causal agent of mango tree decline in many tropical countries (Khanzada, Lodhi & Shahzad, 2005; Mahmood, Saleem & Akhtar, 2002). The pathogen has also been reported on mango in Ghana and associated with the stem end rot disease.(Saeed, 2012). These reports, therefore, give credence to the identification of the pathogen on mango in the study.

Stem end rot is a specific disease quite different from anthracnose attributed to *C. gloeosporioides,* which can also produce disease lesions at any part of the fruit including the stem end portion. In the study, it was observed that both anthracnose and stem end rot diseases could occur simultaneously on the same fruit. In most of such cases, the stem end rot symptoms were found to have expanded and masked the lesions produced by the anthracnose agent. Isolation of the causal agent from the disease lesions on such fruits yields both *L. theobromae* and *C. gloeosporioides*. This could lead to wrong diagnosis and may be the reason why *C. gloeosporioides* has been associated with stem end rot disease elsewhere (Costa *et al*., 2010). However, the use of inoculation studies was found to be very useful in clarifying the aetiology of the stem end rot when only *L. theobromae* in pure culture induced the stem end rot disease symptoms on artificially inoculated mango fruits. The study has, therefore, confirmed without doubt that stem end rot in Ghana was caused by *L. theobromae*.

L. theobromae is an ubiquitous pathogen and has a wide host range. It is the cause of fruit and stem end rot of papaya in Hawaii (Nishijima, 2000). In Ghana, the pathogen has been associated with pod rot of cocoa, small pox of kola and storage rot of sweet potato (Offei *et al*., 2008). In the study, the pathogen was found to be pathogenic to papaya, avocado and banana. Despite the fact that the pathogen was isolated from mango, it appeared to be even more virulent on avocado than on mango *in vitro*. This confirmed that the pathogen may have a wide host range, since it has been identified on several crops in Ghana and other tropical areas (Ploetz *et al*., 1998; Oduro, 2000). It also meant that fruit crops such as avocado, papaya and banana, which are common in most mango plantations in Ghana, may

serve as alternative host to the pathogen. Therefore, in such farms, any control measure aimed at the disease on mango must be extended to cover these other fruit crops also. A complete removal of such fruit crops from mango farms would also be a desirable means of controlling the disease.

Stem end rot is a latent disease which begins in the field and, therefore, good control of the disease could be achieved with good fungicide applications in the field. Currently, no fungicide has been labelled for the control of the stem end rot disease in Ghana. In contrast, several fungicides have been labelled for the control of mango anthracnose. Out of the nine of these fungicides evaluated in the study, the pathogen was able to grow on the PDA amended with the recommended rates of Funguran, Kocide and Copper oxychloride (all of them copper-based) and Sulfa 80 (sulphur-based). This may be that the pathogen was either tolerant or resistant to these fungicides. These results were similar to the report by Khanzada, Lodhi & Shahzad, (2005) that fungicides such as Thiovit (sulphur), Copxkil and Cupprocaffaro (both copper based) were found to be ineffective against *L. theobromae* also isolated from mango. It can be conjectured that copper based fungicides may not be effective against the pathogen and, hence, against the disease in the field.

On farms where both pathogens exist, the use of copper-based fungicides is likely to reduce the populations of the *C. gloeosporioides* whilst the *L. theobromae* is allowed to flourish. This may be the reason why it has been observed that on farms where mango anthracnose is controlled very well, the stem end rot disease caused by *L. theobromae* is exacerbated (Johnson, 1998). In the Hohoe District, most of the farms exporting fruits to Europe apply Funguran as the main fungicide to control anthracnose. The high incidence of stem end rot from that district may confirm the ineffectiveness of copper-based fungicides against the disease.

Among the fungicides evaluated in the study, five of them namely Bendazim (carbendazim), Topsin (Thiophanate methyl), Asuoku Master (Copper oxide + Metalaxyl), Ivory (Mancozeb) and Mirage (Prochloraz) were able to completely suppress the mycelial growth of the pathogen within the period of the experiment. Elsewhere, Thiophanate methyl and Carbendazim have been evaluated both *in-vitro* and *in-vivo* against the *L. theobromae* and found to be very effective against the pathogen and the disease it causes on the field (Sheler *et al*., 1997; Banik, Kaiser & Dhua., 1998; Mahmood, Saleem & Akhtar, 2002). This, therefore, gives credence to the findings in the study. In addition to these two fungicides, Mancozeb, a very popular fungicide currently being used for the control of mango anthracnose in Ghana was also found to be very effective against the pathogen. Being prophylactic rather than curative in nature, it must be sprayed at very early stages of fruit formation to prevent infection.

Despite the observation that copper-based fungicides were found to be ineffective against the pathogen, Asuoku Master was found to be effective against the pathogen. This could be attributed to the presence of an additional active ingredient, Metalaxyl present in the Asuoku Master, making it a potential fungicide for the control of the disease on the field. However, Metalaxyl has not been labelled to be used on mango, and until it is permitted the use of the fungicide

is not recommended. Therefore, fungicides such as Carbendazim, Mancozeb and Thiophanate methyl are highly recommended for the control of fungal diseases on mango, as it would serve a dual purpose of controlling both mango anthracnose and stem end rot diseases.

Mirage (Prochloraz) was the only fungicide labelled for the control of postharvest diseases of mango destined for the international markets (Arauz, 2000). The fungicide was evaluated against postharvest anthracnose of mango and found to be effective (Arauz, 2000). In the study, the fungicide was found to be very effective against the causal agent of stem end rot. This was an indication that the fungicide has a potential of being used to prevent the development of postharvest stem end rot disease symptoms in Ghana.

Conclusion

L. theobromae was isolated from diseased mango fruits showing stem end rot disease symptoms, and was confirmed as the causal agent of the disease when it was able to induce similar disease symptoms on artificially inoculated mango fruits. This is the first time the pathogenicity of the fungus has been confirmed on mango in Ghana. The high incidence and severity of the disease across the mango growing areas of Ghana showed that the disease was of economic importance. It was also demonstrated that some of the fungicides currently available for the control of mango anthracnose could also be effective for the control of the disease. However, further studies aimed at formulating control measures against the disease in the field are recommended.

REFERENCES

Agrios, G. M. (2005) *Plant pathology*. 5th edn. Academic Press, New York. 952 pp.

Arauz, L. F. (2000) Mango anthracnose: Economic impact and current options for integrated management. *Plant Disease* **84**(6), 600 – 611.

Banik, A. K., Kaiser, S. A. K. M. & Dhua, R. S. (1998) Evaluation of some systemic and non-systemic fungicides against *Botryodiplodia theobromae*, the cause of dieback disease of mango (*Mangifera indica* L.). *Journal of Soils and Crops* **8**, 199 – 222.

Chala, A., Burberg, M. B. & Tronso, A. M. (2010) Incidence and severity of sorghum anthracnose in Ethiopia. *Plant Pathology Journal* **9**(1), 23 – 30.

Chandra, J & Pathak, M. (1989) Studies on the stem end rot (*Diplodia natalensis* pole Evans) disease of postharvest mango (*Mangifera indica* L.) fruits. *India Journal of Mycology and Plant Pathology* **19**, 37 – 43.

Costa,V. S. O., Michereff, S. J., Martins, R. B., Gava, C. A. T., Mizubuti, E. S. G. & Camara, M. P. S. (2010) Species of *Botryosphaeriaceae* on mango in Brazil. *European Journal of Plant Pathology* **127**, 509 – 519.

Honger, J. O. (2013) *Characterisation of the causal agent of mango anthracnose disease in Ghana* (PhD Thesis). University of Ghana, Legon.

Huang, S. & Liu, X. (1995) Control of postharvest stem end rot of mango. *Tropical Science* **35**, 321 – 326.

Johnson, G. I., Mead, A. J., Cooke, A. W. & Dean, J. R. (1992) Mango stem end rot pathogen-fruit infection by endophytotic colonization of the inflorescence and pedicel. *Annals of Applied Biology* **120**, 225 – 234.

Johnson, G. I. (1998) Stem end rot. In *Comendium of tropical fruit diseases* (R. C. Ploetz, G. A. Zetmeyer, W. T. Nishijima, K. G. Rohrbach and H. D. Ohr, eds) pp. 39 – 40.

Khanzada, M. A., Lodhi, A. M. & Shahzad, S. (2005) Chemical control of *Lasiodiplodia*

*theobroma*e, the causal agent of mango decline in Sindh. *Pakistan Journal of Botany*. **37**(4), 1023 – 1030.

Ko, Y., Liu, C. W., Chen, C. Y., Maruthasalam, S. & **Lin, C. H.** (2009) First report of stem-end rot of mango caused by *Phomopsis mangiferae* in Taiwan. *Plant Disease* **39**(7), 764 – 765.

Lakshmi, B. K. M., Reddy, P. N. & **Prasad, R. D.** (2001) Cross infection potential of *Colletotrichum gloeosporioides* Penz, isolates causing anthracnose in sub-tropical crops. *TropicalAgricultural Research* **2**,183 – 193.

Madden, L. V., Hughes, G. & **van den Bosch, F.** (2007) *The study of plant disease epidemics.* The American Phytopathological Society, Minnesota, USA.

Mahmood, A., Saleem, A. & **Akhtar, K. M.** (2002) Mango decline in Pakistan and its management. *Pakistan Journal of Phytopathology* **14**, 40 – 43.

Meah, M. B., Plumbley, R. A. & **Jeger, M. J.** (1991) Growth and infectivity of *Botryodiplodia theobromae* causing stem end rot of mango. *Mycological Research* **95**, 405 – 408.

Nishijima, W. (1993) Fruit and stem-end rot of papaya URL:*http://www.extento.hawaii.edu/kbase/crop/Type/phomopsi.htm (retrieved in May, 2014).*

Oduro, K. A. (2000) *Checklist of plant pests in Ghana. Volume 1. Diseases.* Plant Protection and Regulatory Service Directorate , Ministry of Food and Agriculture. Accra, Ghana. 105 pp.

Offei, S. K., Cornelius, E. W. & **Sakyi-Dawson, O.** (2008) *Crop diseases in Ghana and their management*. Smartline Publishing Limited, Ghana.

Ploetz, R. C., Zetmeyer, G. A., Nishijima, W. T., Rohrbach, K. G. & **Ohr H. D.** (eds) (1998) *Compendium of tropical fruit diseases.* The American Phytopathological Society, Minnesota, USA.

Saeed, K. (2009) *Causes of postharvest losses of mango and sheabutter fruits in northern Ghana, West Africa* (MSc Thesis). Universiteit of Gent, Belgium. 63 pp.

Sheler, S. A., Padule, D. N., Sawant, D. M. & **Konde, B. K.** (1997) *In vitro* evaluation of fungicides against *Botryodiplodia theobromae*, the cause of dieback disease of mango. *India Journal of Plant Protection* **25**, 118 – 120.

Trigiano, R., Windham, M. T. & **Windham, A. S.** (2008) *Plant Pathology; Concepts and Laboratory Exercises.* 2nd edn. Taylor and Francis Group. Florida. 558 pp.