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THE AETIOLOGY, INCIDENCE AND SEVERITY OF MANGO TREE DECLINE DISEASE IN GHANA

J. O. HONGER*, S. R. COLEMAN, F. K. ABLORMETI, E. W. CORNELIUS, E. OWUSU AND G. T. ODAMTTEN

(J.O.H: Soil and Irrigation Research Centre, College of Basic and Applied Sciences, University of Ghana; S. R. C., E. O. & G. T. O.: Department of Plant and Environmental Biology, College of Basic and Applied Sciences, University of Ghana; F. K. A. & E. W. C.: Department of Crop Science, College of Basic and Applied Sciences, University of Ghana)
**Corresponding author's email: johonger@yahoo.com*

Abstract

Mango tree decline was previously an unknown disease in Ghana. In this study, mango trees from all the major agro-ecological zones of Ghana, where mangoes are grown were surveyed for the disease incidence, severity and aetiology of a similar disease affecting the crop. Farm visits were made to some selected farms within the selected agro-ecological zones and both the local and exotic trees were inspected. The percentage of infected trees were calculated while the severity of the disease was rated on a scale of 0-5, where 0 = no symptoms and 5 = death of trees. Samples of the diseased plant parts were collected and the causal agent was isolated on media and identified. The isolated fungus was tested for its pathogenicity using mango seedlings as test crops. The disease, characterised by profuse gumming, bark cracking and die back, was found to be present in all the agro-ecological zones. The disease incidence was higher on the local variety compared to the exotic varieties. *Lasiodiplodia theobromae*, isolated from the diseased plant parts, was able to cause the disease on inoculated mango seedlings. The nature of the disease symptoms and its causative agent in Ghana, confirms the disease as the mango tree decline disease.

Introduction

Mango is a leading crop in most of the international markets and hence an important source of foreign exchange for most developing countries including Ghana. The crop is a major source of income to many people in the mango industry in Ghana (Honger, 2013). The crop is one of the most important non-traditional export crops from Ghana and has been projected to make significant contributions to the export portfolio of Ghana (Zakari, 2012). The potential of mango as an important source of foreign exchange to Ghana is huge. There is an ever increasing demand of the crop in international markets and the country has a potential of increasing the volumes sent to these markets (Zakari, 2012).

Currently, Ghana is one of the few countries worldwide with two mango production seasons per the calendar year and therefore has the potential of supplying fruits throughout the year. Due to the great potential of the crop, several institutions including the Export Development and Investment Fund (EDIF) have invested heavily in the industry in Ghana. EDIF has implemented a US$ 50 million mango plantation project in the Northern Region. Interventions by technical and donor institutions such as United States Agency for International Development (USAID) through the Trade and Investment Project for a Competitive Export Economy (TIPCEE), have led to the structuring of the sector.

Other agencies such as the German International Cooperation (GIZ) from Germany have shown considerable efforts at training mango farmers for improved yield for export. Due to the

potential role mango plays as a food security crop, it was streamlined and strategically selected as an agricultural produce by the Economic Community of West African States (ECOWAS) (Zakari, 2012). However, the important role mango plays in the lives of the people and economy of Ghana is under threat by a new mango disease, similar to mango tree decline disease reported elsewhere (Al-Adawi *et al*., 2006).

The disease is characterized by several symptoms; however, the most destructive nature of the disease is its ability to stagnate the growth and development of the affected trees. Mango tree decline disease was first reported in Brazil in 1945 where about 60% of mango trees were reported to have been affected (Ploetz et al., 1996). It was later reported in several other mango producing countries including India (Al-Adawi *et al*., 2006), Mexico (Alvarez- García & López‐García, 1971), USA (Ploetz *et al*., 1996), Oman (Asad *et al*., 2010), Pakistan (Fateh *et al*., 2006) and Jordan (Al-Adawi *et al*., 2006). Since the disease was first observed in an orchard in the Manya Krobo District of the Eastern Region of Ghana, it appears to be widespread in the country as other farmers from other mango growing areas reports of similar diseases (Adiku, 2014).

Currently, information about this new threatening disease is scanty in Ghana. It follows therefore that there is the need for work to be done to gather more information about the disease to facilitate the development of control measures against it. For effective control measures to be formulated against a disease, knowledge of its aetiology and epidemiology is essential. This present research work was carried out to determine the nature and spread of the new disease in Ghana and determine the causal agent responsible for the disease

Experimental

Study area

The study was carried out in 12 selected Metropolis/Municipals/Districts of Ghana from the Greater Accra, Eastern, Volta, Ashanti, Brong Ahafo and Northern Regions. The areas were distributed among the four major agro-ecological zones of Ghana namely the Coastal Savanna, the Semi-Deciduous Forest, Transition and the Guinea Savanna (Table 1).

TABLE 1

Administrative areas of Ghana and their agro-ecological zone selected 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
Kwaebibrem	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
Kumbugu	District	Northern	Guinea Savanna

Determination of the disease incidence and severity

Field survey for the disease incidence and severity was carried out in a total of 36 mango farms from the various districts, Municipals, and Metropolis. In each farm, 10 trees each of the local and exotic varieties were selected at random and used for the determination of the disease incidence. The number of trees showing one or more of the disease symptoms was counted and used to calculate the disease incidence (I) (After Madden *et a*l., 2007) as follows

$$I = \sum (x/N)$$

Where
x = number of diseased plants
N = total number of plants evaluated.

10 trees were further selected at random and each was visually rated for the disease severity on a scale of 0-5 where 0 = no observable disease symptoms and 5 = whole tree death (modified after Cordoso *et al*., 2004) (Table 1). Severity (S) was estimated by the equation:

$$S = \sum(x_i n_i)/n$$

Where,
x_i = disease grade (modified) as per Table 1
n_i = number of diseased plants on the ith grade of the disease scale
n = total number of diseased plants evaluated.

The results obtained for the disease incidence and severity in farms that were found in the same agro-ecological zone were bulked and the data among the different agro-ecological zones were analysed to determine differences. Data on exotic varieties were treated separately from those obtained from local trees. Analysis of variance (ANOVA) was performed on the arcsine-transformed data and directly on the disease severity index, using Genstat statistical software package, V. 11. Means were separated using L.S.D at 5%.

TABLE 2

Disease severity rating scale used for the assessment of disease severity in different mango farms in Ghana

Rating	*Meaning*
0	No symptoms
1	Little wilting of upper tips
2	Wilting and small exudates of gum, yellowing of leaves, little browning of vascular tissues
3	Drying of branches, heavy gum exudation from branches, large-scale browning of vascular tissues
4	Splitting of the bark, gum exudation from branches as well as from main trunk, drying of more than half of the tree
5	Death of plant

Cardoso et al., 2004 (modified)

Isolation of the causal agent

Leaves, twigs, fruits and bark of mango trees showing mango decline symptoms were sampled and collected from randomly selected farms and sent to the Microbiology laboratory of Department of Plant and Environmental Biology and the Plant Pathology Laboratory of The Department of Crop Science, all of the University of Ghana for isolation of the causal agents.

The isolation of causal agent was first done on water agar (WA) and then on Potato Dextrose Agar (PDA). WA (15 g/L) and PDA (39 g/L) were prepared and each mixture was autoclaved,

allowed to cool and poured into clean sterilized Petri dishes and allowed to set. Pieces (1-2 cm) of the sampled plant parts (leaves, twigs, bark and vascular tissues) were taken from the advancing edge of infection with a sterile scalpel. These excised tissues were then surface sterilized in a 1% Sodium hypochlorite for 5 minutes, rinsed twice in sterile distilled water and blotted dry using a sterile tissue paper. The sterilized plant parts were plated singly on water agar plates and incubated for six to eight days when enough growth was observed. The growth were then sub-cultured on PDA and incubated for the same period (six to eight days) to obtain pure cultures.

Identification of the isolated fungal species

The number of days taken by the isolates to cover the plate, the colour of the mycelium and the type of fruiting bodies were recorded to aid in its identification. Bits of mycelium and conidia were placed on slides and stained with cotton blue. The morphology and dimensions of the conidia and the characteristics of the hyphae were recorded using photomicrography to further aid in the identification of the pathogen. The isolates were identified with aid of standard reference materials (Johnson, 1998; Barnett & Hunter, 2006)

Molecular characterisation

DNA was extracted from the isolate using the Sigma's GenFlute Plant Genomic DNA Miniprep Kit, following the manufacturer's instructions. The extracted DNA was used as templates in polymerase chain reaction with the primer pair ITS1/ITS4 (White *et al*., 1990) to amplify the internal transcribed spacer (ITS) region of the isolates. PCR was carried out in a total reaction volume of 50µl. The reaction mixture was made up of 34.25 µl of double distilled water, 5 µl of 10X PCR buffer (Invitrogen, Carlsbad, CA), 2.5 µl of deoxynucleoside-triphosphate (DNTP) mix (2.5 mM each), 0.25 µl bovine serum albumin (20 mg/ml), 2 µl each of the forward and reverse primer, and 0.2 µl of taq polymerase, 1.8 µl of magnesium chloride (50 mM) and 2 µl target DNA.

The reaction was carried out in a Thermo Hybaid PXE Thermal Cycler. The reaction cycles were denaturing for 2 min at 94°C followed by 35 cycles of 1min at 94°C, 1 min at 55°C, 2 min at 72°C and a final of 10 min at 72°C. Amplification products were separated by 1.5% w/v agarose gel stained with Ethidium bromide alongside 1.0 kb marker at 80 V for about 1 hour. Bands were observed under UV light and Polaroid photographs taken or viewed using the Gene Flash Documentation System (Snygene Bio Imaging). The PCR amplified product of the ITS region was sent to ATGC in Germany for purification and sequencing. 10 picomole of each primer was used to sequence the products directly from both directions. The sequences were entered into BioEdit and consensus strands were generated. The assembled sequences of ITS region of the isolates were used in a Basic Local Alignment Search (BLAST) (http://blast.ncbi.nlm.nih.gov/BLAST.cgi). Sequences obtained were deposited in the GenBank with the accession numbers KY657456 - KY657465.

Pathogenicity test

Pathogenicity test was carried out on10 month old, nine potted exotic (Keitt) mango variety and nine local mango variety seedlings, obtained from Kpong (certified seedlings grower). All the exotic mango seedlings were grafted on local mango variety stock. This experiment was set up in Completely Randomized Block Design (CRBD) in a screened house at the Department of Plant and Environmental Biology, University of Ghana. Test plants were inoculated artificially by cutting a flap on the basal portion of the stem using a sterilized knife and inserting a 3 mm agar disc obtained from the advancing zone of the test fungus in Petri plates. With the grafted exotic mango seedlings, flaps were cut above the root stock. In the control trials, plants were inoculated with plain sterile agar disc without

the test fungus. The inoculated portions were wrapped with a plastic paraffin film (parafilm). Plants were irrigated after inoculation and the wrapping material was removed from the stems after two weeks of inoculation. Plants were then monitored for possible developments of the decline syndrome symptoms for two months. The pathogen was re-isolated from the test plants to confirm the pathogenicity. Disease incidence and severity in symptomatic plants were determined with aid of the modified disease hedonic score scale (Cardoso *et al.,* 2004).

Results

Nature and typical symptoms of the disease observed in the field

The disease was not restricted to any particular variety of mango found in the surveyed farms as all the different types of cultivars including the local ones were susceptible. In some farms, trees close to the edges of the roads and those close to pits where crop debris are incinerated appear to be highly diseased. During the survey, trees in farms that had been ravaged by bushfires were found showing most of the disease symptoms. Infected plants showed wilting of upper tips (tip dieback); shoot dieback as shown in Fig. 1A; drying of leaves and rolling up of their margins. Affected leaves were scorched and fell leaving dead branches. As the disease advanced, exudates of gum were seen (gummosis) which became heavy as disease severity aggravated, this can be seen in Fig. 1B. Fig 1C shows infected branches of the plant became discoloured in the vascular tissues while the uninfected branch remained fresh and whitish. With time, the bark cracked as gummosis intensified in severe conditions. The branch formed tissues beneath the stem/branch. In extreme cases of pathogenesis, the death of entire plant was observed as shown in Fig. 1D.

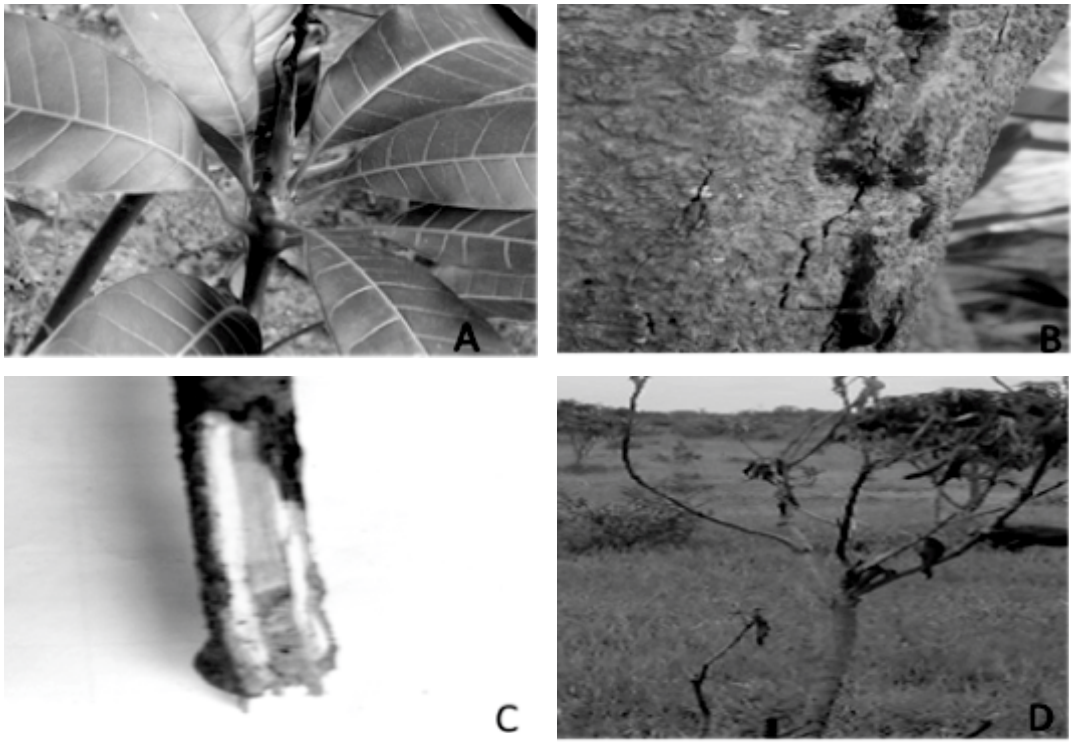

Fig. 1. Typical symptoms of tree decline disease observed in the field. (A=Twig die back, B=gumming, C=vascular disclouration, D=death of entire foliage).

Disease incidence and severity index of the mango tree decline disease in the different agro-ecological zones of Ghana

Table 3 shows the percentage disease incidence and severity indices on either the local or the exotic mango variety plants. Clearly, percentage incidence was higher in the local mango crop stands than in the exotic mango stands in all the agro-ecological zones. Table 3 also The disease incidence was almost four times more in the local variety mango crop stand than in the exotic mango crop stands in almost the agro-ecological zones. The disease incidence in the local mango variety stands varied from 60.0-74.0% as compared to 13.33-20.0% in the exotic mango crop stands. The same trend was true for the record of the severity index; it was higher in the local mango crop stand (2.28-2.70) than in the exotic crop stands (0.81-1.73).

The disease occurred in all the surveyed agro-ecological zones (Guinea savanna, Semi-deciduous forest; Transition, and Coastal savanna) in Ghana. There was no statistical difference ($p \geq 0.05$) between the records of percentage disease incidence on the exotic variety in all the agro-ecological zones surveyed. However, the disease incidence was higher in the Semi-deciduous forest compared to the other agro-ecological zones as far as the local varieties were concerned. On the other hand, the disease was severer in Semi-deciduous, Transitional and Guinea Savanna zones compared to the Coastal Savanna Zone, as far as the exotic varieties were concerned. However, there was no statistical difference in disease severity on the local varieties from the different agro-ecological zones (Table 3)

TABLE 3

Mean percentage incidence and severity index of mango tree decline disease in 36 mango farms in the indicated four agro-ecological zones of Ghana in 2015

Agro-ecological zone	*Disease incidence (%)*		*Disease severity index*	
	Exotic variety	*Local variety*	*Exotic variety*	*Local variety*
Coastal savanna	17.50[b]	60.00[b]	0.81[a]	2.28[b]
Semi-deciduous forest	15.71[b]	74.00[a]	1.71[b]	2.70[b]
Transitional	20.00[b]	65.00[b]	1.73[b]	2.35[b]
Guinea savanna	13.33[b]	65.00[b]	1.11[b]	2.39[b]
Overall mean	16.64	66.00	1.34	2.43

Means in the same column with the same alphabets are not statistically significant ($p \geq 0.05$)

Cultural and morphological characterisation of the isolated fungus

The isolated fungus initially produced a thin mycelium which covered the entire 9 mm plate within three days. The mycelium grew fluffy within five days. The colour of the mycelium was initially white but changed to grey and finally black within seven days as shown in Fig. 2A. The hyphae were initially hyaline and non-septate with a diameter of ≤2.0±0.5µm this can be seen in Fig. 2B. The diameter increased with age from about ≤2.0±0.5 µm in two days to about ≤8.0±1.2 µm in 21 days.

It took 12 days for pycnidia to form on PDA kept under continuous darkness and 25 days to form conidia. The conidia were initially unicellular subovoid to ellipsoidal in shape. Conidia were bi-celled, thick walled and ellipsoidal in shape. Dimensions of the conidia on PDA were 10.0-12.0±1.5 µm wide and 20.0-30.0±2.0 µm long. The immature conidia initially had no septum, were subovoid to ellipsoidal in shape but with maturity acquired a single septum (bi-celled) which was thick walled (Fig. 2C).

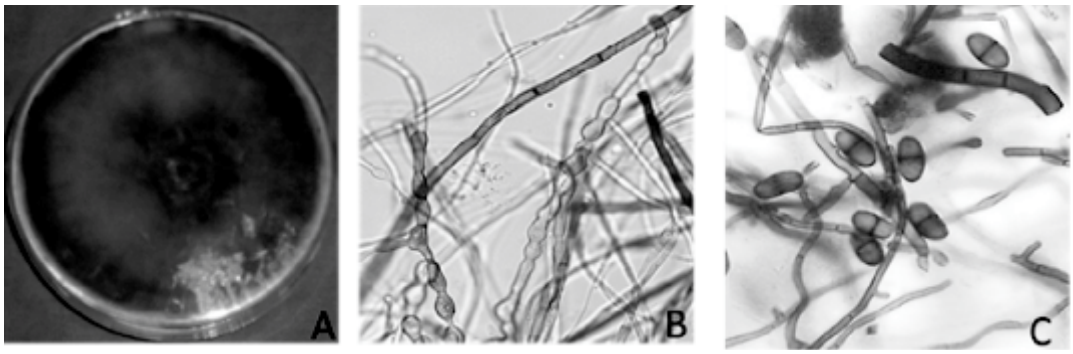

Fig. 2. Cultural and morphological characteristics of Lasiodiplodia theobromae isolated from diseased mango barks. (A=Mycelium growth on PDA, B=Nature of the hyphae, C=bi-celled conidia). (Micrograph magnification=x400)

Basic local alignment search (BLAST)

Blast search using the assembled nucleotide sequences of the rDNA-ITS region of the isolates obtained in thus study showed they were similar to *Lasiodiplodia theobromae*. The percentage similarity was 100% between isolates obtained from this study and the *L. theobromae* isolates L3 (accession number KR 260793.1), and RSGV/PD02 (accession number HM 466960.2) and *Lasiodiplodia parva* type strain CBS 456.78 (accession number NR111265.1).

Pathogenicity test of the causative pathogen of mango tree decline syndrome on the host plant

Host plants of the mango tree decline disease namely local mango variety and exotic Keitt variety were artificially inoculated with the suspected causative pathogen, *L. theobromae* and were observed for the presence of typical symptoms including discolouration of vascular tissues, terminal dieback, exudation of gum from stem and bark of intact tissue, browning of affected leaves and upward rolling of the margins of the affected leaves.

All the typical symptoms mentioned above were observed on both the local and exotic Keitt mango plants after 40 days of incubation in the screen house. These include vascular discolouration, terminal dieback and gummosis. About 37% of the symptomatic plants showed the tip dieback symptom. Some of the inoculated plants (about three of the local and exotic Keitt variety) were asymptomatic suggesting some degree of tolerance of the pathogen, especially in the exotic species. Disease incidence (%) and severity index (on a scale of 0-5) was higher (Disease incidence, 71.0%; Severity, 2.0) in the local mango variety than in the Keitt exotic variety (Disease incidence, 57.0%; Severity, 1.50) although the difference in severity index of the two varieties was not statistically significant ($p > 0.05$) as seen in Table 15. The fungus *L. theobromae* was re-isolated from the infected inoculated plants to conclude Koch's postulates and yielded a recovery of 93.0%.

Discussion

The mango tree decline disease has been reported to be a very devastating disease in several mango growing areas of the world (Ploetz *et al*., 1996; Al-Adawi *et al*., 2006). In this current study, mango trees surveyed in most of the mango growing districts in Ghana, exhibiting similar symptoms, ascribed to the tree decline disease, an indication that the disease under study was the same tree decline disease reported in several parts of the world.

The fungus, *Lasiodiplodia theobromae* was consistently isolated from symptomatic plant

tissues collected from the different locations in Ghana. The identification of the fungus was primarily based on its unique cultural and morphological characteristics. Isolates produced mycelia which were initially white but darkened with time. They produced conidia that were initially hyaline and aseptate, but with time became dark coloured and septated with longitudinal striations.

These morphological features of the fungus, together with the presence of paraphyses within the conidiomata, have been described as a reliable diagnostic feature for the fungus *L. theobromae* (Phillips *et al*., 2008). The cultural and morphological characteristics identification of the fungus was confirmed with the BLAST search. *L. theobromae* has been described as a ubiquitous pathogen with a wide host range (Hunter & Buddenhagen, 1972; Alvarez & Nishijima, 1987). It has been identified in Ghana as a pathogen on several crops including cocoa, kola and almost all the major tropical fruits in the country (Oduro, 2000; Offei *et al*., 2008; Honger *et al*., 2015). In most parts of the world, where the mango tree decline disease has been reported, *L. theobromae* has either been consistently isolated from symptomatic plant parts or was confirmed as the causal agent using pathogenicity tests (Saeed *et al*., 2011; Al-Adawi *et al*., 2006).

In this study, inoculation studies with the isolated *L. theobromae* confirmed the pathogen as the causal agent of the mango tree decline disease when it was able to elicit similar disease symptoms on the tests plants. These observations and reports according to Saeed *et al*. (2011), give credence to the findings in this study that the tree decline disease was caused by *L. theobromae.*

The mango tree decline disease was found in all agro-ecological zones of Ghana, where the study was carried out. This was an indication of how widespread the disease was and therefore poses a danger to the mango industry. Data on the disease incidence and severity of the mango tree decline disease in the different agro-ecological zones in Ghana showed that in each agro-ecological zone, the local mango crop stands had the higher percentage disease incidence than in the exotic Keitt mango stands. Table 3 shows that disease incidence was more than three times higher in the local mango variety crop stand than the exotic Keitt mango variety stands in all the surveyed agro-ecological zones. The same was true for the record of severity index.

Though this observation was not clearly understood, it could be due to the differences in the genetic constitution of the two varieties. The current exotic mango varieties accepted in the export markets were developed in Florida, as a result of hybridisation between different types of the local varieties from different parts of the world (Litz, 1997). This hybridisation may have resulted in changes in the genetic constitution of the progenies thereby conferring on them some resistant factors against certain pests and diseases, which may be absent in the pure-stand local varieties.

The fact that the exotic and local varieties have different susceptibilities to different diseases in Ghana is amply demonstrated by the high susceptibility of the exotic varieties to the mango bacterial black spot disease as contrasted by the almost immunity of the local varieties to the same disease in the country (Honger, 2017). In fact, elsewhere where mangoes are grown in the world, such as Pakistan and Oman, the local varieties have been reported to be highly affected by the mango tree decline disease than the exotic ones imported into those countries (Panhwar *et al*., 2005; Al Yahani *et al*., 2005).

The destructive nature of the disease on the local variety of mango poses a great danger to the mango industry in another respect. These local varieties serve as the main source of rootstock for the raising of budded materials for transplanting. Destruction of this variety would, therefore, lead to a shortage of rootstocks for mango production in the country in the near future if control measures are not instituted immediately to curb the spread of the mango tree decline disease.

Conclusion

The mango tree decline disease was identified for the first time in Ghana and confirmed as a disease of importance as it occurred in all the major mango growing ecologies in the country. Secondly, it was found to retard growth and development of affected trees which would eventually impact negatively on the performance of the tree in terms of final yield. The ubiquitous fungus, *Lasiodiolodia theobromae* was consistently isolated and confirmed as the causal agent of the disease, a finding which is supported by similar reports in other mango growing areas of the world. The confirmation of its pathogenicity on the mango tree in this study provides important information for the management of the disease which could be very devastating. It is therefore recommended that future studies are carried out to formulate good control measures against the disease based on this accurate knowledge of the aetiology of the disease.

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