Occurrence, Pathogenicity and Survival of *Macrophoma mangiferae* in Leaves, Branches and Stems of Mango (*Mangifera indica* L.)

RALPH N. OKIGBO

Michael Okpara Federal University of Agriculture – Department of Biological Sciences, Umuahia, Nigeria

Abstract

OKIGBO R.N. (2001): **Occurrence, pathogenicity and survival of *Macrophoma mangiferae* in leaves, branches and stems of mango (*Mangifera indica* L.).** Plant Proctect. Sci., **37**: 138–144.

Frequency of occurrence and persistence of *Macrophoma mangiferae* Higorani & Sharma on 4-year old mango plants (*Mangifera indica* L.) growing under various conditions were investigated over two periods of 6 months in South-Eastern Nigeria. Among the five locations studied, the frequency of occurrence of the disease was highest at Umuahia, with 65% for leaves and 41% for stems. The chances of the fungus to survive adverse conditions in mango tissues were higher in the stems and branches than in the leaves; more fungal colonies were recovered from the stems and branches than from the leaves. The fungus survived adverse conditions, forming structures such as chlamydospores and thick walled hyphae under continuous light. In the dark, the fungus remained in vegetative form and hence did not produce any sexual reproductive structures. When subjected to continuous light, the fungus produced pycnidia. Leaving dried leaves and fragments of dried stems and branches on the farms is likely to raise the level of primary inoculum for mango diseases.

Keywords: *Macrophoma mangiferae*; survival; mango

Mango (*Mangifera indica* L.) belongs to the dicotyledonous family *Anacardiaceae*. It is decidedly the most popular edible fruit among millions of people in the tropical and sub-tropical regions, where it is considered to be the choicest of all indigenous fruits (SINGH 1960). In fact, because of its excellent flavor, attractive fragrance, beautiful shades of colour, delicious taste and healthful value, mango is now recognized as one of the best fruits in the world (SINGH 1960), including Nigeria (OPEKE 1987). The commonly cultivated cultivars of mango in Nigeria are Edward, Early Gold, Local Alphonso, Julie, Haden and Ogbomosho.

Mango is subject to a number of diseases at all stages of its development in Nigeria (OPEKE 1987), including leaf blight of mango caused by *Macrophoma mangiferae* Higorani & Sharma, from the order *Sphaeropsidales*. *M. mangiferae* has been associated with a wide range of diseases of economic crops in Nigeria. APPAH (1983) associated it with mango leaf anthracnose, EKUNDAYO (1984) with basal stem rot, OKIGBO and EMOGHENE (2000) with root rot of pawpaw (*Carica papaya*), and in India with blight of seedlings and young grafted mango (VERMA *et al.* 1991). Another cause of blight in mango is *Nattrassia mangiferae* which causes blossom blight (WYSOKI 1997). *M. mangiferea* is a pycnidia forming fungus. ALASOADURA (1971) and EKUNDAYO (1976) observed that the pycnidia are usually grouped in structures known as stromata; since stromata are spore-bearing structures of *M. mangiferea*, the importance of spores in the spread of the fungus needs no further emphasis.

The attention of mycologists is presently being focused on measures to control mango diseases (VERMA *et al.* 1996a). Although considerable interest has been shown in *M. mangiferea* as an important pathogen, there is little literature on its continued presence in the field after harvest until the next cropping season. The present study is a preliminary investigation on the frequency of occurrence, length and form of survival of *M. mangiferea* in the branches, stems and leaves of mango under different environmental and laboratory conditions in South-Eastern Nigeria.

MATERIALS AND METHODS

Disease survey: Pathogen isolation, identification and characterization

Surveys were carried out in 1994–1995 in Umuahia, Enugu, Nsukka, Ojoto and Okigwe (South-Eastern Nigeria) to determine the frequency of occurrence of *M. mangiferea*. Ten mango trees 4 years old were selected randomly at each site. The frequency of occurrence was taken as the number of mango trees affected by the disease and expressed as percentage of the total number of trees at a location. This was done by using a visual assessment technique, which helps to evaluate many plantations in a relatively short period. Students *t*-test was used to compare the occurrence of the disease on the leaves and stems. Diseased leaves, branches and stems were collected and brought to the laboratory for identification of the pathogen. The samples were surface-sterilized with 0.5% mercuric chloride solution for 10 min and rinsed repeatedly with sterile water. Pieces of sterilized leaves, branches and stems were cut into pieces of 5 mm, plated on Petri dishes containing potato dextrose agar (PDA) and these incubated at room temperature (28 ± 1°C). After 4 d incubation, isolates were taken from each Petri dish, subcultured on three replicate plates and identified by standard procedure (DADE & GUNNEL 1969; BARNETT & HUNTER 1972). *M. mangiferae* originating from cv. Ogbomosho has a dark globose pycnidium and the conidiophores are simple and elongated with hyaline conidia. Identified cultures were stored in a refrigerator at 4°C until further use.

Preparation of spore suspension

M. mangiferae (isolate G2), isolated from diseased *Mangifera indica* cv. Ogbomosho in the Umuahia orchard, was grown on PDA for 12 d in Petri dishes at 28°C under continuous light. Pycnidia formed on this culture were picked off by forceps and broken with a sterile spatula in 10ml of sterile distilled water. The suspension was filtered through two thin layers of glass wool. The filtrate, consisting of the spore suspension, was used for pathogenicity tests.

Pathogenicity test

The culture of *M. mangiferae* used for the experiment was incubated for 10–15 days on a laboratory bench at a room temperature of 28 ± 2°C. The spore suspension was prepared by centrifuging and re-suspending the spores in three changes of sterile distilled water. The resulting suspension was adjusted to 20 000 spores per 1 ml.

Healthy leaves on a young mango tree were used for the pathogenicity test, surface sterilized with 0.5% mercuric chloride solution for 10 min and then cleaned with sterile distilled water. The lower epidermis of the leaves (where stomata are located) were sprayed with the spore suspension (20 000 spores per 1 ml) using a rocking sprayer.

The spore suspension was used to spray leaves, branches and stems on 4-year old trees, cv. Ogboomoso. In each experiment, three leaves and three stems or branches on each of 10 trees were sprayed. A healthy branch per tree was sprayed with sterile distilled water and served as control. All branches and leaves were covered with sterile cellophane bags for 2 d to allow the fungus to establish itself. The leaves and stems were then inspected daily from October 1994 to March 1995 for blight symptoms of the disease, or any other effect of the pathogen on the leaves. Pieces of affected tissues were removed from apparently infected areas and were plated on PDA for re-isolation and re-identification of the causal organism (BARNETT & HUNTER 1972; DADE & GUNNEL 1969). The experiment was repeated from October 1995 to March 1996.

The survival of *M. mangiferae* in branches, stems and leaves of mango under three different sets of conditions of storage was studied in the laboratory. Infected tissues were covered with paraffin plastic.

Infected leaves, stems and branches of mango were collected from an orchard at Umuahia. One set each of the pieces of branches and stems was stored in a sterile 250 ml beaker covered with paraffin plastic at room temperature. At 2 weeks intervals, 1g of dried infected tissue was comminuted in 20 ml sterile distilled water, serially diluted, and plated onto PDA. The plates were incubated at 28°C until fungal colonies appeared and these were counted. Student *t*-test was used to compare the recovery rate of the fungus.

Infected tissue stored under dark conditions

Whole leaves, and branches and stem pieces cut into 10 cm pieces were wrapped in black paper and kept inside a dark cupboard for 6 months. At 2 weeks intervals the fungus from these samples was isolated and plated on PDA. The plates were incubated in the dark at 28°C for a period of 14 d.

Infected tissue kept under continuous light

A sterile cork borer of 6 mm diameter was used to remove pieces from lesions on the leaves; branches and stems were cut into pieces of 5 mm. The pieces were placed on PDA in Petri dishes and incubated at 28°C under continuous light. After 12 d the fungus was re-isolated from the pieces of tissue and plated onto PDA to obtain pure cultures. Two sets of the experiments were set up with the re-isolated fungus in a randomised complete block design. One set was kept 40 cm from a fluorescent tube which served as a source of continuous light. The other set was kept on a laboratory bench and 232 cm away from the fluorescent tube. At 2 weeks intervals small blocks of medium and colony were removed and examined microscopically.

RESULTS

Disease survey and identification

The survey showed that Umuahia had the highest incidence of *M. mangiferae* followed by Nsukka, Ojoto, Enugu and Okigwe, in that order (Table 1). The level of occurrence of the fungus was significantly higher on the leaves ($P > 0.05$) than on the stems (Table 1). The disease was found to be more frequent at the onset of the rainy season, i.e. March–April with a relative humidity of 80%. The fungus, when cultured on PDA, coloured the medium dark blue and sporulated within 14 d under continuous light. The stromata were small and embedded in the medium. Conidiophores were hyaline, and bore single unicellular conidia.

Table 1. Frequency of occurrence (%) of *Macrophoma mangiferae* in leaves, branches and stems of mango at five locations in South-Eastern Nigeria

Location	Latitude N *	Longitude* (E)	Altitude*	Frequency of occurrence (%)	
				leaves	stems
Umuahia	5°29′	7°33′	1005	65.1	40.8
Enugu	6°27′	7°29′	1006	43.7	30.6
Nsukka	6°52′	7°23′	1000	54.6	41.1
Ojoto	6°10′	6°47′	948	52.8	28.4
Okigwe	5°59′	7°21′	1005	28.9	22.8

*From National Root Crop Research Institute, Umudike, Nigeria

Pathogenicity Tests

The results of pathogenicity tests showed that mango leaves, branches and stems were infected by *M. mangiferae* 5 weeks after inoculation with spore suspension. The leaves, branches and stems sprayed with spores of *M. mangiferae* showed characteristic lesions (Figs. 1 and 2). The lesions on the leaves first appeared as small, circular yellow spots, which gradually enlarged, became irregular in shape and involved the entire leaf surface (Table 2).

Table 2. Percentage of leaf area occupied by lesions on the mango tree after inoculation with *Macrophoma mangiferae*

Weeks	Leaf area covered by lesions (%)	Weeks	Leaf area covered by lesions (%)
4	20	9	54
5	35	10	63
6	40	11	78
7	48	12	92
8	49		

Infected stems showed general yellowing with some dark spots. Fruiting bodies were produced mostly on the undersurface of the leaves (Fig. 1). A light brown colour of the fruiting bodies was noticed. Younger leaves, branches and stems were more susceptible than older ones. The leaves and stems sprayed with sterile distilled water as a control did not show any sign of infection throughout the period of observation.

Survival of *Macrophoma mangiferae* in leaves, branches and stems under laboratory conditions

The infected stems, branches and leaves of mango covered with paraffin plastic showed that *M. mangiferae* was present for at least six months (Table 3). The fungus was found to survive longer in stems than in leaves. Recovery from stems was higher than that from leaves at the end of the experiment, but not significantly different ($P > 0.05$). Recovery of the pathogen from infected leaves was high 2 weeks after infection, but a drastic decrease in recovery of fungal colonies followed. It was also noted that the stem encouraged the growth of the organism more than the leaves.

When infected stems and leaves were kept under dark conditions it was found that the fungus was present in a vegetative form throughout the experimental period (Table 4). In culture over a long period under dark conditions it grew profusely, initially producing a brown colony with abundant aerial mycelium which eventually turned violet brown with age. The characteristic stromata produced by the fungus were absent. The hyphae were rather slender and unusually elongated before separation (Fig. 4).

The test with infected leaves, branches and stems subjected to continuous light whose source was 40 cm away

Table 3. Two-weekly sampling of mango tissue covered with paraffin plastic for recovery of *Macrophoma mangiferae* from October 1994 to March 1995

Weeks	Number of fungal colonies from 1 g of infected mango tissue		
	leaves	branches and stems	control
2	40 ± 0.1	29 ± 0.2	0
4	38 ± 0.3	27 ± 0.2	0
6	30 ± 0.1	24 ± 0.1	0
8	24 ± 0.4	21 ± 0.3	0
10	20 ± 0.2	19 ± 0.4	0
12	17 ± 0.8	19 ± 0.2	0
14	11 ± 0.2	20 ± 0.8	0
16	7 ± 1.0	20 ± 0.7	0
18	5 ± 0.3	17 ± 0.2	0
20	3 ± 0.1	14 ± 0.6	0
22	2 ± 0.1	14 ± 0.1	0
24	2 ± 0.1	12 ± 0.2	0

Fig. 1. Lower epidermis of a noninfected mango leaf (a), and infected leaf (b)

Fig. 2. Healthy stem with healthy petiole (a), diseased stem with infected petiole (b)

Table 4. Development of colonies from infected mango tissue kept in darkness or continuous light; checked every 2 weeks

Weeks	Dark		Continuous light	
	leaves	stems	leaves	stems
2	60.3 ± 0.2	40.1 ± 0.9	54.1 ± 0.1	28.2 ± 0.3
4	60.8 ± 0.3	40.2 ± 0.8	51.4 ± 1.0	28.1 ± 0.9
6	48.1 + 0.1	31.1 + 0.6	52.3 + 0.8	27.7 + 0.1
8	45.0 ± 0.5	31.0 ± 0.1	40.6 ± 0.3	24.3 ± 1.1
10	40.6 ± 0.2	29.1 ± 0.3	40.5 ± 1.0	22.2 ± 0.1
12	32.4 ± 2.3	22.1 ± 1.2	31.8 ± 0.4	21.2 ± 0.1
14	30.2 ± 2.0	20.4 ± 0.1	30.1 ± 0.03	18.1 ± 2.1
16	20.8 ± 0.1	21.0 ± 0.2	15.2 ± 2.1	14.1 ± 0.2
18	14.6 ± 1.0	18.0 ± 0.6	4.1 ± 0.2	14.1 ± 0.6
20	8.1 ± 0.4	18.0 ± 0.6	4.1 ± 02	14.1 ± 0.3
22	6.2 ± 0.3	16.1 ± 0.3	1.0 ± 0.3	13.6 ± 0.7
24	6.1 ± 0.2	15.1 ± 0.8	2.1 ± 0.3	11.1 ± 0.1

Fig. 3. Cluster of thick walled hyphae and chlamydospores of *Macrophoma mangiferae* formed after 2 months (1000×)

Fig. 4. Mycelium of *Macrophoma mangiferae*, characteristic fruiting bodies found when cultured in the dark (1000×)

showed that the fungus survived the condition by producing fruiting bodies and forming thick walled hyphae (Table 4). The result was similar when the source of light was 232 cm away (Table 4). There was no significant difference in the number of colonies recovered from leaves and stems ($P > 0.05$). The culture of *M. mangiferae* kept under continuous light for 10 to 14 d produced numerous stromata that were spherical to globose, dark brown to black. The hyaline spores are cylindrical to ovate and were found on undifferentiated cells within the pycnidium. The number of stromata was higher in cultures 40 cm away from the source of light. Cultures kept 232 cm from the source of continuous illumination produced chlamydospores. These are enlarged hyphal cells occurring singly or in groups along the hyphae, with a thick wall (Fig. 3). When the spore suspension of the fungus was grown 232 cm from the light source and then used as inoculum, symptoms were induced and they were more severe and appeared earlier than from cultures grown 40 cm from the light source (Fig. 3).

The mycelium of *M. mangiferae* grew fast at 28°C on PDA and had covered 9 cm diameter in a Petri dish in 4 d. Hyphae were initially hyaline and later became dark brown. The medium became dark blue, probably due to diffusion of a stain from the mycelium into the medium. Stromatal primordia appeared on the mycelium close to the original inocula on the 3rd day, and by the 4th day on all parts of the mycelium covering the medium. There was a rapid increase in size of the primordia per day, while the pycnidia increase in size and number rapidly between the 3rd and 4th day. By the 7th day the pycnidiospores were already mature and oozed out through ostiolar pores on each stroma as a moist sticky mass of hyaline spores. The spores later became two-celled and dark brown pigmented after 10 weeks in old culture. There was no significant difference in the recovery of the fungus between the combination of the leaves and stems of the fungus kept in the dark and the leaves and stems kept under continuous light.

DISCUSSION

Among the sites surveyed, Umuahia and Nsukka were seriously affected areas. In some mango orchards there was considerable damage of the leaves, branches and stems. OKIGBO and EMOGHENE (2000) observed that the manifestation of a plant pathogen in an area depends on its ability to survive, not only during its parasitic relations with its host, but also during the period in which the host is not growing. The symptoms observed after the inoculation of *M. mangiferae* on the undersurface of healthy mango leaves, branches and stems is consistent with the report of COOK (1975). Possible depletion of some of the essential chemical elements used for the syn-

thesis of chlorophyll in the leaves infected by the pathogen may account for the observed general chlorosis of the leaves (COOK 1975). The distribution of the fruiting bodies of the fungus on the infected leaves was in accordance with the widespread nature of the chlorotic symptom on the infected leaves. This was 5 weeks after inoculation with a conidial suspension of the fungus and agrees with the report of COCHRANE (1958).

Almost all environmental conditions are known to influence *M. mangiferae* development. The effects of light, darkness and nutrients have been extensively investigated as well as temperature and rainfall conditions which particularly favour the development of symptoms of the disease (VERMA *et al.* 1996b). The conditions for vegetative growth and for reproduction were different. Some minimum period of vegetative development was required before the organism could reproduce. It was during this period that it synthesized specific metabolites such as enzymes or food substances essential for its reproduction. This is supported by COCHRANE (1958) who observed that the ability of the fungus to change from a vegetative growth form to a reproductive form is the result of a combination of genetic competence to produce the new form and the effect of environmental factors. A fungal mycelium which is genetically unable to undergo the change, will remain sterile under all environmental conditions. A mycelium with low potential for differentiation will change only when the external conditions are particularly suitable. Conversely, a mycelium with a high genetic potential for differentiation may be prevented to do so by unsuitable environmental conditions.

M. mangiferae survived in the leaves and stems for a period of 6 months. SHARMA and SMITH (1999) reported that fungi preserved in oil have a changed form of growth and this was observed with *M. mangiferae*. Abundant pycnidia that developed on the infected bark of the stems and twigs of young mango plant were the best means of survival of *M. mangiferae* (VERMA *et al.* 1996b). The fungus also survived in blighted leaves and as dormant mycelium in the wood (VERMA *et al.* 1996a). Consequently, it could be argued that the survival of *M. mangiferae* depends on the surface area and the thickness of host tissues traversed by the pathogen. SUSSMAN (1968) noted that a widely spread mycelium will decompose cellulose at a rate in excess of that required for survival. The growth of *M. mangiferae* within the leaf was more wide-spread than on the stem. The tissues of the leaf were degraded by the fungus at a relatively fast rate.

When a fungus is not actively colonizing a substrate, it may survive in the form of dormant structures, such as spores, resting hyphae or sclerotia. *M. mangiferae* produced chlamydospores which were round or subglobular in shape. The longer ones had thick rough walls. STAKMAN and HARRAR (1956) observed that resistant structures such as chlamydospores of the mango pathogen are often capable of survival over a long period. Chlamydospore formation can be considered to have ecological significance, since it allows the fungus to form a resistant structure under conditions that limit or inhibit macromolecular synthesis.

M. mangiferae was found to survive longer in the stem and branches than in the leaf, although all three were subjected to the same treatment. Furthermore, more isolates of the mango pathogen were obtained from stems in the laboratory and even on the field than from leaves. This result emphasizes a practical point: leaving fallen, dried leaves and fragments of dried branches and stem in the orchards is likely to raise the level of primary inoculum of this mango pathogen.

References

ALASOADURA S.O. (1971): Culture studies on *Botryodiploidia theobromae*. Pathol. Mycopath. Appl., **42**: 158–160.

APPAH J. (1983): The leaf anthracnose of mango. [BSc thesis.] Univ. Benin, Nigeria.

BARNETT H.L., HUNTER B.B. (1972): Illustrated Genera of Imperfect Fungi. 3rd ed. Burgess Publ. Com.

COCHRANE V.W. (1958): Physiology of Fungi. John Wiley and Sons Inc., London.

COOK A.A. (1975): Diseases of Tropical and Subtropical Fruits and Nuts. New York, Hafner Press.

DADE H.A., GUNNEL J. (1969): Class Work with Fungi. Commonwealth Mycol. Inst., Kew, Surrey, England.

EKUNDAYO C.A. (1976): Studies on the development of reproductive structures in *Sphaeropsidales*. [PhD thesis.] Univ. Ibadan, Nigeria.

EKUNDAYO C.A. (1984): Effect of some fungicides on the mycelial growth and stomatal production of *Macrophoma mangiferae*. Macrobios Lett., **25**:13–15.

OKIGBO R., EMOGHENE A. (2000): Root and butt rot of orange trees in South-Western Nigeria. Fruits, **55**: 309–314.

OPEKE L. K. (1987): Tropical Tree Crops. Woye and Sons Ltd., Ilorin, Nigeria: 105–108.

SHARMA B., SMITH D. (1999): Recovery of fungi after storage for over a quarter of a century. World J. Microbiol. Biotech., **15**: 517–519.

SINGH L.B (1960): The mango, Botany, Cultivation and Utilization. Leonard Hills Ltd. London.

STAKMAN E.C., HARRAR J.G. (1957). Principles of Plant Pathology. The Ronald Press Co. New York: 258–300.

SUSSMAN A.S. (1968): The Fungi. Vol. 3. Academic Press, New York : 447–486.

VERMA K.S., CHEEMA S.S., KANG M.S., SHARMA A.K. (1991): Hitherto unrecorded disease problems of mango from Punjab. Plant Disease Res., **6**: 141–142.

VERMA K.S., JUGINDER S., SINGH J. (1996a): Perpetuation and management of *Macrophoma mangiferae* causing blight of mango. Indian J. Mycol. Plant Pathol., **26**: 75–78.

VERMA K.A., JUGINDER S., SINGH J. (1996b): Pathology and epidemiology of Macrophoma blight of mango. Indian J. Mycol. Plant Pathol., **26**: 98–100.

WYSOKI M. (1997): Biology and control of *Nattrassia mangiferae*. In: SAAIMAN W.C, LAVI U., TOMER E. (Eds): Proc. 5th Int. Mango Symp., Tel Aviv, Israel, 1–6 Sept. 1996, Vol. 2. Acta Horticult., **455**: 558–568.

Received for publication March 22, 2001
Accepted for publication August 1, 2001

Abstrakt

OKIGBO R.N. (2001): **Výskyt, patogenita a přežívání houby** *Macrophoma mangiferae* **na listech, větvích a kmenech mangovníku** (*Mangifera indica* L.). Plant Protect. Sci., **37**: 138–144.

V jihovýchodní Nigérii jsme ve dvou půlročních obdobích sledovali četnost výskytu a persistenci houby *Macrophoma mangiferae* Higorani & Sharma na čtyřletých rostlinách (*Mangifera indica* L.) pěstovaných za různých podmínek. Z pěti sledovaných lokalit byla četnost výskytu choroby nejvyšší v lokalitě Umuahia (65 % na listech a 41 % na kmenech). Šance houby na přežití v pletivech mangovníku za nepříznivých podmínek byly vyšší na kmenech a větvích než na listech; větší počet kolonií této houby jsme získali z kmenů a větví než z listů. Houba v nepříznivých podmínkách přežívala, při nepřetržitém osvětlení vytvářela útvary jako chlamydospory a silnostěnné hyfy. Ve tmě houba zůstávala ve vegetativním stavu a nevytvářela žádné pohlavní rozmnožovací orgány. Po vystavení nepřetržitému osvětlení houba tvořila pyknidy. Ponechávání uschlých listů a úlomků suchých kmenů a větví na farmách pravděpodobně zvyšuje hladinu primárního inokula pro infekci mangovníku.

Klíčová slova: *Macrophoma mangiferae*; přežívání; mangovník

Corresponding author:

Dr. RALPH N. OKIGBO, Department of Biological Sciences, Michael Okpara Federal University of Agriculture, Umudike, PMB 7267, Umuahia, Nigeria
e-mail: okigborn17@yahoo.com