



# Plant Pathology Journal

ISSN 1812-5387

**science**  
alert

**ANSI***net*  
an open access publisher  
<http://ansinet.com>

## Leaf Spot in Mulberry Plant (*Morus alba*) in the Lowland Humid Tropics of Southwestern Nigeria

<sup>1</sup>R.A. Baiyewu, <sup>2</sup>N.A. Amusa and <sup>1</sup>J.B. Idowu

<sup>1,3</sup>Institute of Forestry Research, PMB 5054, Ibadan Nigeria

<sup>2</sup>Institute of Agricultural Research and Training, Obafemi Awolowo University, PMB 5029, Moor Plantation, Ibadan, Nigeria

---

**Abstract:** The etiology and epidemiology of leaf spot and seedling blight of mulberry plant (*Morus* sp.) was investigated at Ibadan, South Western Nigeria in the low land humid tropics. *Cercospora moricola* (cooke) was associated with the concentric leaf spot and seedling blight of the mulberry plant. The pathogen was found associated with plant debris and weeds plants namely *Macrophyra longistyla* (Rubiaceae) *Mucuna pruriensis*, *Commelina erecta* and *Chromolaena odorata* (Compositae) found growing within the mulberry plots. Field infections usually began early in the raining season in form of patches later spread causing severe defoliation.

**Key words:** Epidemiology, etiology, leaf spot, *Morus* sp.

---

### INTRODUCTION

Mulberry (*Morus alba* L.), is perennial plant and usually cultivated as monocrop for its leaf to rear silkworm<sup>[1]</sup>. Mulberry leaves are sometimes eaten as vegetables and are useful as a cattle fodder. Being nutritious and palatable, they are said to improve milk yield of dairy animals<sup>[2]</sup>. The fruits are eaten fresh or made into juice, jam and jelly, it also serves as an important ingredient of a particularly seductive drink known as Mulberry Wine<sup>[2]</sup>. Dandin *et al.*<sup>[3]</sup> reported that mulberry wood is a blend of strength and elasticity hence is used in the manufacture of furniture, sport goods such as hockey sticks, cricket bat, racket tool handles and toys, for tanning and coloring purpose. The barks stripped are used in paper industry while the plant can be used for ornamental purposes. Medicinally, fruits are laxative, refrigerant in fevers and used locally as remedy for sore throat, dyspepsia and melancholia<sup>[3]</sup>. The fruit juice is used in folk remedies for tumors of the fauces, the latex is used for dermal cream, while the root extract is used for control of high blood pressure and fruit are used for treating depression, high fever and laxative<sup>[4]</sup>. Roots and barks are purgative, anthelmintic and astringent; leaves are considered as diaphoretic and emollient; a decoction of leaves being used as a gargle for inflammation of throat<sup>[2,4,5]</sup>. While the main plants are used for environmental amelioration to solve drought, erosion and deforestation problems<sup>[6]</sup>.

The introduction of mulberry sericulture to Nigeria started with simultaneous effort to getting an industry gradually put in place, to alleviate poverty among the peasant farmers.

However, recent observation revealed severe spotting of the leaves and wilting of sprouting mulberry plants in both the research and mulberry Agro forestry farms in various location in humid tropics of Southwestern Nigeria.

Leaf spot caused by *Cercospora moricola* and *Cercospora mori* have been reported as major epidemic diseases of mulberry plant, causing between 10-20% leaf yield loss during rainy and winter seasons<sup>[7]</sup>. This leads to a shortage of quality leaf for late-age rearing of silkworm, which results in severe economic loss to farmers.

Despite the much reports on mulberry sericulture and mulberry plant potentials in Nigeria, negligible researches have been done on the occurrence of field diseases of the plant in the South Western Nigeria. Although many researchers<sup>[1,7-11]</sup> reported the occurrence of field disease of mulberry plant in Asian countries like China and India.

This study reports an investigation of the occurrence of leaf spot and wilting of mulberry plant at Ibadan in lowland humid tropics.

### MATERIALS AND METHODS

The mulberry plant fields utilized in this study were located in the mulberry agro forestry plantation plots at

the Forestry Research Institute of Nigeria, Ibadan. Ibadan (7°20' N, 30°5' E, 200 m above sea level), which lies within the lowland humid rain forest zone. The mean annual rainfall of 1150-1500 mm falls mainly between April and October with the major peak in June. Higher relative humidity values (80-95%) are recorded during the rainy season than the dry season (20-50%). The mean maximum and minimum temperatures are 34 and 24°C, respectively.

The research plots were planted with 3 different varieties of mulberry plants (S<sub>54</sub>, S<sub>36</sub> and S<sub>30</sub>).

The planted fields were usually manually weeded at six weeks interval till the end of the project, leaves showing leaf spot symptoms from mulberry plots were carefully monitored, excised from the main plant stem, while wilted mulberry seedlings were also uprooted from the mulberry nurseries located at the Institute nursery.

These samples were collected and kept in separate sterile sampling bags. Soil samples were also collected (1-5 cm deep) randomly from the infected plots during the course of the experiment and kept in the sampling bags. The following weeds *Macrophyra longistyla*, *Centosema pubescens*, *Ipomae* sp. and *Chromolaena odorata*, found within the mulberry plots with symptoms of infection were also collected in sterile sampling bags. All the samples were taken to the laboratory for the isolation of the causative pathogen.

**Isolation of the pathogen:** The infected mulberry plant leaves and that of the weed species were excised, cut into 2 mm pieces and surface sterilized with 10% sodium hypochloride for 30 sec and rinsed in 4 successive changes of sterile distilled water. They were then planted on Acidified Potato Dextrose Agar (APDA) and incubated for 6 days at 27°C under 12 h photoperiod. The pathogen was identified by microscopic examination and by comparing with a standard<sup>[12]</sup>.

A gram of ground plant debris (leaves) was placed in 9 mL of sterile water in McCartney bottles, vigorously shaken in a whirl mixer for 10 min and then serially diluted up to 10<sup>-6</sup> after which 1 mL of the suspension from 10<sup>-4</sup> to 10<sup>-6</sup> was plated on APDA. The plates were then incubated for 5 days at 28°C. The pathogens were identified as described above. Plate count i.e. colony forming units-cfu) was used to determine the inoculum load of the isolates.

**Test for pathogenicity of the isolates:** Detached leaf bioassay technique was used to determine the pathogenicity of the isolate. Healthy leaves of mulberry plants were excised at the petioles with sterile razor blade and covered immediately with a moistened sterile filter

paper and transferred to the laboratory in polyethylene bags containing moistened filter paper. The leaves were washed in running tap water rinsed in three changes of sterile distilled water and placed in petri dishes that have been lined with moist filter paper. Ten leaves were spot-inoculated with 0.3 mL of a spore suspension of the fungus inoculated with 2.6×10<sup>5</sup> spore mL<sup>-1</sup>. Petri dishes were incubated at 28-30°C for six days and leaves were observed daily for the development of symptoms. The fungus was re-isolated on PDA in pure culture and compared with the initial culture.

Ten mulberry plant seedlings were also raised in poly-pots filled up with oven-sterilized soil. While another one set of 2 months old mulberry plant seedling were also inoculated by raising them in poly-pots filled up with the sterile soil mixed with corn meal cultures of the pathogen. Ten pots per variety replicated 3 times including the control (i.e. the treatment without pathogen) were used for the experiment.

The experiments were observed from 3 weeks after inoculation until the plants were 6 months old. The developed lesions were excised and plated onto APDA while the wilted seedlings were uprooted and plated onto APDA. The isolated pathogens were compared with the initial isolates.

## RESULTS AND DISCUSSION

The initial observation symptoms of the disease include the appearance of small irregular brownish spots (Fig. 1A), which became enlarged (Fig. 1B), coalesce having shot holes (Fig. 1C). Under severe infections, the affected leaves became yellow and fall off prematurely. The initial symptoms on the field, usually began early in the raining season in form of patches and at the peak of the raining season become severe, with over 20% of the leaves of the infected field becoming blighted (Fig. 1C).

The fungus found associated with the leaf spot and wilting of the mulberry plant was *Cercospora moricola* (Cooke). The pathogen produces a compact mass of interwoven cushion like hyphae bearing conidia on the conidiophores. The conidia are 3-7 celled, hyaline and tapering at one end and 70×30 μ in size. On four occasions, *Colletotrichum* sp. was isolated alongside *Cercospora moricola* from infected plant materials. Weeds found growing in and around the mulberry plot, which harbored fungal pathogen and the rates of occurrence are shown in Table 1. Of the 5 weed plants found in and around the farm, *M. longistyla*, *Centosema pubescens*, *Chromolaena odorata* and *Commelina erecta*, harboured *C. moricola*.

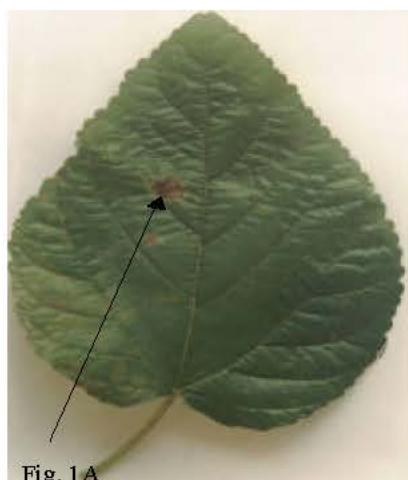


Fig. 1A



Fig. 1B



Fig. 1C

Fig. 1: Mulberry plants showing increase rate of leaf spot severity (A) showing initial leaf spot symptoms after infection, (B) showing blight symptoms and (C) showing blight symptoms with pot holes

Table 1: Weeds and their fungal pathogen at various percentage frequency of occurrence

Weed plant	Fungi pathogen	Frequency of occurrence % on weed plants
<i>Chromolaena odorata</i>	<i>C. moricola</i>	60.0
	<i>Colletotrichum</i> sp.	14.0
	<i>Cerotilium fici</i>	26.0
<i>Mucuna pruriensis</i>	<i>Colletotrichum</i> sp.	25.0
	<i>C. moricola</i>	60.0
<i>Commelina erecta</i>	<i>Fusarium</i> sp.	20.0
	<i>C. moricola</i>	75.0
<i>Macophyra longistyla</i>	<i>C. moricola</i>	60.0
	<i>Curvularia</i> sp.	21.0
<i>Ipomoea</i> sp.	<i>Curvularia</i> sp.	14.0

The plant debris assay of the pathogen revealed that *C. moricola* had an inoculum density of  $2.8 \times 10^4$  cfu  $g^{-1}$  debris,  $2.7 \times 10^4$  cfu  $g^{-1}$  debris and  $2.7 \times 10^4$  cfu  $g^{-1}$  debris of mulberry varieties  $S_{54}$ ,  $S_{36}$  and  $S_{30}$ , respectively.

The pathogenicity test revealed that *C. moricola* induced both leaf spot and wilting of mulberry plant leaves on the test plants. The cultural and morphological features of the isolates when re-isolated were the same as the initial inoculum. The control mulberry plant had no symptoms of infection.

The pattern of infection in the study, namely, the appearance of small irregular brownish spots on leaves, which became enlarge, coalesce having shot holes and under severe infection affected leaves becoming yellowish and falling off prematurely, is similar to the initial symptoms reported by Siddaramaiah *et al.*<sup>[8]</sup>, Sukumar and Ramalingam<sup>[9]</sup>. Sengupta and Pradip<sup>[7]</sup> identified *C. moricola* as the fungal responsible for shoot wilting and leaf spots in mulberry plants. This is the first reported case of leaf spot of mulberry caused by *C. moricola* in Nigeria. *C. moricola* was isolated from *M. longistyla*, *Centosema pubescens*, *Chromolaena odorata* and *Commelina erecta* which grows in and around the mulberry plots and may serve as the reservoir for the pathogen. Weeds have been previously implicated as potential sources of inoculum of many leaf spot/ blight inducing pathogens<sup>[13-16]</sup>.

The occurrence of *Collectotrichum* sp. at a low rates on the infected mulberry leaves might suggest that the fungus is either as a transient resident on the plant or is a secondary invader. However, in Japan, *Colletotrichum dematium* has been reported as an important pathogen inducing anthracnose disease of mulberry<sup>[10]</sup>.

The rapid spread of the disease at the peak of the raining season could be due to the humid condition prevailing at such period, which usually supports profuse growth of the fungal mycelium. At the peak of the raining season the leaf spot enlarges rapidly and coalesce leading to extensive blighting and defoliation. Similar observation

has been reported by Siddaramaiah *et al.*<sup>[8]</sup> on leaf spot of mulberry in the humid condition. Sukumar and Ramalingam<sup>[9]</sup> had also reported that the disease spread primarily with rain droplets through conidia. While Emechebe and Soyinka<sup>[17]</sup> have previously reported that rapid development of lesion and its coalescing leading to extensive blight and defoliation were usually observed during the peak of raining season.

The presence of the pathogen inoculum in the plant debris suggests that the debris may be a potential source of primary inoculum and probably responsible for the manifestation of patches of infection that were scattered over the fields in the early stages of infection. *Cercospora canescens* and *Cercospora cruenta*, causing leaf spot disease of cowpea has been reportedly harbored in plant debris for a period of over 2 years<sup>[16]</sup>.

From the result of the study, the severity of *Cercospora moricola* disease of mulberry plant leaf can be reduced by thorough general sanitation and proper management, which includes, elimination of susceptible weeds and infected debris around the mulberry plot, could also help reduce the chances of infestation. Regular pruning of the affected plant parts will reduce the inoculum load in the field. Also soil fertility reduces the activities of the pathogen in the soil. The use of systemic fungicide such as Bavistin 50 WP at 0.1% concentration as been suggested<sup>[8]</sup>. Wide range of mulberry plant accessions needs to be evaluated to identify those that are resistant or tolerant to leaf spot in the humid agro-environment. Economic control measures of the disease also need to be investigated so that the acclaimed potential of the mulberry in the humid tropics can be fully exploited.

#### REFERENCES

1. Gunasekhar, V., V.P. Gupta and K. Srikantaswamy, 1998. Linear models for the prediction of leaf rust and leaf spot diseases of mulberry, Paper Number 2\_1\_18 Mullberry.Htm.
2. Reed, C.F., 1976. Information summaries on 1000 economic plants. Typescripts Submitted to the USDA.
3. Dandin, S.B. and S.R.R. Akalpa, 1987. Vruksha called Mulberry. Ind. Silk, 26: 49-53.
4. Bose, P.C., 1987. Mulberry is the medicine. Ind. Silk, Nov., 26: 53-54.
5. Hartwell, J.L., 1971. Plants used against cancer. A survey. 1967-1971, Lloydia, pp: 30-34.
6. Ashiru, M.O., 1996. Mulberry Sericulture in Nigeria. In: NARP/NCRP Terminal Report Forestry Research Institute of Nigeria, Ibadan, pp: 68.
7. Sengupta Govindaiah, K. and P. Kumar, 1991. Diseases and Pests of Mulberry and their Control. (Eds.) Gangluy, A.K. Published by Central Sericultural Research and Training Institute Sriampura, Mysore, pp: 45.
8. Siddaramaiah, A.L., K.S. Krishnaprasad and R.K. Hegde, 1978. Epidemiological studies of mulberry leaf spot caused by *Cercospora moricola* Cooke. Ind. J. Seric., 16: 44-47.
9. Sukumar, J. and A. Ramalingam, 1981. Splash dispersal in *Cercospora moricola*. Sci. Cult., 47: 173-175.
10. Yoshida, S. and A. Shirata, 1999. Survival of *Colletotrichum dematium* in soil and infected mulberry leaves. Plant Dis., 83: 465-468.
11. Gunasekhar, V., 1994. Seasonal occurrence of foliar fungal and bacterial diseases of mulberry in South India. Ind. Phytopathol., 47: 72-76.
12. Barnett, H.C. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. 3rd Edn., Burgess Publishing Co., pp: 209.
13. Onasirosan, P.T., 1975. Seedborne and weed-borne inoculum of web blight of cowpea. Plant Dis. Rep., pp: 338-339.
14. Emechebe, A.M. and D. McDonald, 1979. Seed-borne pathogenic fungi and bacterial of cowpea in northern Nigeria, PANS., 25: 401-404.
15. Oben, V.O., Amusa, N.A. and I. Ezenwa, 1997. Foliar blight in *Centrosema pubescens* (Benth.) in southwestern Nigeria. Mycopathologia, 138: 47-50.
16. Singh, S.R. and J.D. Allen, 1979. Cowpea Pests and Diseases. IITA Ibadan, Nigeria, pp: 75.
17. Emechebe, A.M. and S.O. Soyinka, 1997. Fungal and Bacterial Diseases of Cowpea in Africa. In: Singh, S.R. and K.O. Rachie, (Eds.). Cowpea Reaserch, Production and Utilization. New York, Wiley and Sons, pp: 174-192.