UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND PATHOGENICITY OF CLADOSPORIUM MUSAE MASON FROM BANANA LEAF SPECKLE

SAHLAN

IDENTIFICATION AND PATHOGENICITY OF *CLADOSPORIUM MUSAE* MASON FROM BANANA LEAF SPECKLE

By

SAHLAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfillment of the Requirement for the Degree of Master of Agricultural Science

April 2003

DEDICATION

to my wife Atun,
to my daughters and sons Agung, Ageng, Hakim, and Khasanah,
you are really spirit, supporters,
without your patience, understanding, supporting, and endless praying,
this work could never have been completed.

to my parents-in law, and to my family members,
without your endless praying,
many problems faced during this study might not have been solved.

finally, to my parents in memory,
you are still my spirit, nothing is going to change my love and honor
for you forever

it is with my appreciation that this work be dedicated to you all.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Master
of Agricultural Science

IDENTIFICATION AND PATHOGENICITY OF *CLADOSPORIUM MUSAE* MASON FROM BANANA LEAF SPECKLE

By

S A H L A N

April 2003

Chairman : Dr. Zainal Abidin bin Mior Ahmad

Faculty : Agriculture

A study was undertaken to isolate and identify *Cladosporium* sp. from banana leaves associated with a speckle disease which is considered a serious problem especially in 'Berangan' and 'Mas' cultivars planted in Teluk Intan, Perak (West Malaysia).

Direct plating of infected 'Berangan' banana leaves surface sterilized in 5% sodium hypochlorite (NaOCl) solution on Potato Dextrose Agar (PDA) failed to isolate *Cladosporium* sp. However, pure cultures were successfully obtained by a single spore isolation method. Conidia from diseased leaves were first scrapped using a fine paint brush into sterile water. The conidial suspension was then poured over 2% water agar (WA) incorporated with antibiotics (streptomycin and tetracycline sulphate) and incubated for 24 hours at ambient temperature. A single germinated

conidia was picked with an inoculation needle and transferred onto a PDA plate similarly amended with antibiotics.

The fungus produced erect conidiophores and conidia which were mainly fusiform and aseptate or 1-septate on banana leaves. Conidiophores on Malt Extract Agar (MEA) were straight and flexuous, and produced conidia and ramoconidia which were very variable in shape and size.

A comparison between the morphological characteristics and dimensions of the Teluk Intan fungus on banana leaves and culture medium with those previously described in literature suggest its close similarity and synonymous identification with *Cladosporium musae* Mason.

The best culture medium for growth of *C. musae* Mason was on Banana Leaf Extract Agar (BLEA), while MEA and PDA were the best culture medium for supporting maximum conidia production. In culture, it can grow between a temperature range of 18 ^{o}C to 30 ^{o}C, but the optimum temperatures for mycelial growth and sporulation were 22 ^{o}C and 26 ^{o}C respectively. The optimum temperature for conidial germination was 26 ^{o}C combined with relative humidity (RH) of between 99% to 100%.

Inoculation of conidial suspension onto four-month-old seedlings of 'Berangan' (AAA) failed to produce symptoms of speckle disease at three weeks after inoculation. However, evidence of pathogenicity was observed in the inoculation of detached banana leaves. Necrosis of leaves of

'Berangan' incubated at 22 ± 1 ^{O}C occurred at 14 days after inoculation. Penetration of leaves was observed only via the stomata. Subsequent hyphal colonization of leaf cells occurred intracellularly or intercellularly.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian.

PENCAMAN DAN KEPATOGENAN *CLADOSPORIUM MUSAE* MASON DARIPADA PENYAKIT SPECKLE DAUN PISANG

Oleh

S A H L A N

April 2003

Pengerusi : Dr. Zainal Abidin bin Mior Ahmad

Fakulti : Pertanian

Satu kajian telah dijalankan untuk memencil dan mengenalpasti *Cladosporium* sp. daripada daun pisang berpenyakit "speckle" yang merupakan masalah serius pada pokok pisang 'Berangan' dan 'Mas' yang ditanam di kawasan Teluk Intan, Perak (Malaysia Barat).

Pemencilan secara terus daripada daun pisang 'Berangan' yang berpenyakit dengan rawatan permukaan 5% larutan natarium hipoklorit (NaOCl) atas Agar Kentang Dekstrosa (PDA) gagal menghasilkan *Cladosporium* sp. Walau bagaimanapun, kultur tulen telah berjaya diperoleh melalui kaedah pemencilan spora tunggal. Konidium dari daun berpenyakit mulanya digores dengan menggunakan berus cat halus ke dalam air steril. Ampaian konidium dituang ke atas 2% agar air (WA) yang telah dicampur dengan antibiotik (streptomycin dan tetracycline sulfat) dan dieram selama 24 jam pada suhu bilik. Satu konidium yang telah

bercambah diambil dengan menggunakan jarum inokulasi dan dipindahkan ke atas satu piring PDA yang telah juga ditambah dengan antibiotik.

Kulat daripada daun pisang mengeluarkan konidiofor yang tegak dan konidium yang kebanyakannya berbentuk fusiform dan tanpa berseptum atau mempunyai satu septum. Konidium di atas Agar Ekstrak Malt (MEA) adalah lurus dan fleksuos dan menghasilkan konidium dan ramokonidium yang sangat bervariasi dalam bentuk dan saiz.

Satu perbandingan di antara ciri-ciri morfologi dan ukuran pencilan kulat Teluk Intan daripada daun pisang dan di atas medium kultur dengan yang telah dahulunya dilaporkan dalam bahan bertulis mencadangkan persamaan yang rapat dan pencaman yang selari dengan *Cladosporium musae* Mason.

Medium kultur pertumbuhan yang terbaik bagi *C. musae* adalah Agar Ekstrak Daun Pisang (BLEA), sementara MEA dan PDA adalah medium kultur terbaik untuk pengeluaran konidium yang tertinggi. Dalam keadaan kultur, kulat didapati boleh tumbuh antara julat suhu 18 ^{O}C hingga 30 ^{O}C, tetapi suhu optimum untuk pertumbuhan miselium dan pensporulaan masing-masing adalah 22 ^{O}C dan 26 ^{O}C. Suhu optimum untuk per-cambahan konidium adalah 26 ^{O}C yang digabungkan dengan kelembapan bandingan (RH) antara 99% dan 100%.

Inokulasi ampaian konidium kepada anak benih pisang 'Berangan' yang berumur empat bulan gagal menunjukkan simptom penyakit "speckle" tiga minggu selepas diinokulasi. Walau bagamainapun, bukti kepatogenan telah dapat dilihat pada inokulasi ke atas potongan daun pisang. Nekrosis pada daun pisang 'Berangan' yang dieram pada suhu 22 $\pm$ 1 ^{o}C telah dicatatkan 14 hari selepas diinokulasi. Penembusan daun telah dilihat hanya melalui stomata. Kolonisasi seterusnya oleh hifa di bahagian sel daun berlaku secara intersel atau intrasel.

ACKNOWLEDGEMENTS

Alhamdulillah, be praised to Allah SWT, the most beneficent, the most merciful, and the most compassionate for giving me the physical and physiological strength and comfort to finished this study.

I wish to express my deepest personal appreciation and sincere thanks to Dr. Zainal Abidin bin Mior Ahmad, the chairman of the supervisory committee, for his excellent guidance, encouragement and constructive suggestions throughout the undertaking of this study. The constructive suggestions from Prof. Dr. Sariah Meon and Dr. Zakaria bin Wahab, members of the supervisory committee are also acknowledged. I wish also to specially acknowledge my gratefulness to Dr. Gurmit Singh, also a member of the supervisory committee, for his constructive guidance and for his collaborative support (especially in kind and materials) throughout this study. The support by all laboratory staff of Plant Pathology Section, Department of Plant Protection, Faculty of Agriculture UPM, as well as others who may have contributed assistance either directly or indirectly is also acknowledged with thanks.

Special thanks are due to Participatory Development of Agricultural Technology Project/PAATP (ADB Loan No. 1526-INO), the Agency for Agricultural Research and Development (AARD), Government of Indonesia, for the opportunity and financial support rendered to me to pursue a postgraduate study at the Department of Plant Protection, Faculty

of Agriculture, Universiti Putra Malaysia and also to Head of PAATP Project and Ir. Hartiningsih M. Sc. together with her staff for their generosity and help in overcoming living cost problems during the period of my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(continued)

CHAPTER I

INTRODUCTION

Bananas and plantains (*Musa* sp) are extremely important in developing countries and are cultivated in over 100 tropical and subtropical regions of the world. The harvested area estimated is approximately 10 million ha, with an annual production of about 88 million metric tonnes. Hence, they are considered the most important global food crop after rice, wheat and maize in terms of gross value of production (Sharrock and Frison, 1998).

Local consumption, mainly in the poorest countries of Africa, Latin America and Asia accounts for 90% of total production. In China, banana consumption was estimated at 2 kg per person a year. This was 50 kg per person a year in the Oceania, while in eastern Africa (Uganda, Burundi and Rwanda) the estimate was 250 kg per person a year mainly as a cooked dish, or eaten in beer (Frison and Sharrock, 1998). In Indonesia, the average banana consumption estimate per person in 1996 was 8.72 kg (Setyobudi and Purnomo, 1998).

In Asia, bananas are the most widely produced fruit in India, Indonesia, Philippines, China and Thailand (Sharrock and Frison, 1998). Even though banana production appears to be relatively less important in Malaysia, the export equivalent of 3.9 million boxes in 1997 was greater

than Indonesia (3.2 million boxes) (Molina and Valmayor, 1998). About 95% of Asia's annual bananas production (about 27 million tonnes) is consumed or marketed locally (Frison and Sharrock, 1998). The overall ranking of some Asian countries in terms of total banana production estimates in 1998 compared to other countries in the world is shown in Table 1.1.

Table 1.1: The 20 largest banana and plantain producers in the world (Frison and Sharrock, 1998).

Rank	Country	Production (Metric tonnes)	Rank	Country	Production (Metric tonnes)
1	India	9,934,600	11	Rwanda	2,248,000
2	Uganda	9.893,000	12	Mexico	2.063,860
3	Ecuador	6,622,362	13	Cameroon	2,016,000
4	Brazil	5,779,120	14	Ghana	1,804,000
5	Colombia	4,797,300	15	Nigeria	1,750,000
6	Indonesia	4,767,520	16	Thailand	1,700,000
7	Philippines	3,500,000	17	Venezuela	1,626,799
8	China	3,141,000	18	Tanzania	1,538,000
9	Zaire	2,700,000	19	Burundi	1,507,000
10	Costa Rica	2,505,000	20	Peru	1,390,700

As any other commercial crops, bananas are subjected to various pest and disease problems. Banana leaf speckle disease caused by *Cladosporium musae* Mason has been considered as a serious problem that can cause very severe leaf defoliation. There are no estimates of

damages and losses by leaf speckle disease but banana growers predict more severe infections causing appreciable losses in the future. Although the disease did not appear to cause plant death, severe leaf necrosis can reduce fruit production and quality (Stover, 1972). *Cladosporium* leaf speckle disease was reported to reduce the life span of leaves by 30 days (Holderness *et al.*, 1998).

In Africa, *Cladosporium* leaf speckle is reported to be dominant in several regions. It was considered to be a major threat to banana production in Uganda (Tushemereirwe and Waller, 1993; Holderness *et al.*, 1998) and Malaysia (G. Singh, pers. comm.). *Cladosporium* leaf speckle disease was estimated to cause about 95% of banana leaf defoliation (Tushemereirwe and Bagabe, 1998).

In the past, one of the major constraints to banana production worldwide is Black Sigatoka disease caused by the fungus *Mycosphaerella fijiensis* Morelat. *Cladosporium* leaf speckle disease has been assumed as a minor disease problem affecting the older, mature leaves of banana and plantain growing in a humid climate (Stover, 1972). As such, not much attention was given to this disease and thus, there is a serious lack in basic information on pathogen biology, disease aetiology, host resistant, and aspects on disease management.

Bearing in mind the current interest in seeking new information on *Cladosporium* leaf speckle disease with a view of improving disease management, this study was initiated with the following objectives:

1 To isolate and identify (based on morphological and cultural characteristics) *Cladosporium* sp. from banana leaves associated with speckle disease.

2 To study the direct or interactive effects of media, temperature and relative humidity on growth and sporulation of *Cladosporium* sp.

3 To develop methods for establishing pathogenicity of *Cladosporium* sp. on banana.

CHAPTER II

LITERATURE REVIEW

2.1 Occurrence, Distribution, and Economic Significance of Leaf Speckle Disease of Banana

Banana leaf speckle, caused by *Cladosporium musae* Mason is one of several leaf spot diseases of banana found distributed in all of banana growing regions in the world. Stover (1972) and Jones (2000) reported that *Cladosporium* leaf speckle is usually found on the older leaves of banana plants growing in the humid tropical environments. Although *Cladosporium* leaf speckle has been regarded only as minor disease problem, certain cultivars are known to be very susceptible in specific locations. No serious attempts have been made to calculate yield losses due to *Cladosporium* leaf speckle disease (Jones, 2000).

The first report of *Cladosporium* leaf speckle disease was recorded from Jamaica and West Africa (Stover, 1972). Later, it was reported in other countries in Asia (Bangladesh, Hong Kong, Indonesia, Malaysia, Nepal, Sri Lanka, Thailand and Vietnam), Australia-Oceania (Papua New Guinea, Solomon Islands and Western Samoa), Africa (Burundi, Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Egypt, Ethiopia, Ghana, Guinea, Rwanda, Sierra Leone, South Africa, Sudan, Togo, Uganda and Zimbabwe) and the Latin American-Caribbean regions (Cuba,

Ecuador and Honduras and Jamaica (Frossard, 1963; David, 1988; Sebasigari and Stover, 1988; Jones, 1993a; 1994; 2000).

Tushemereirwe and Bagabe (1998) reported that in Africa, *Cladosporium* leaf speckle is dominant in several regions and distributed in almost all areas from low altitude to the highlands. More recently, this disease was identified as a major threat to banana production in Uganda (Tushemereirwe and Waller, 1993; Holderness e*t al.*, 1998) and Malaysia (G. Singh, pers. comm.). In Uganda *Cladosporium* leaf speckle disease can cause up to about 95% of leaf necrosis, while in Malaysia severe leave necrosis can occur in certain cultivars.

Like Sigatoka leaf disease, *Cladosporium* leaf speckle also caused significant leaf necrosis in banana. Studies have shown that leaf spot defoliation enhances fruit ripening and shortens fruit maturity time. Leaf necrosis was observed to accelerate physiological maturity of fruits both in the field and after harvest and the effect increases with severity of defoliation (Stover, 1972). Beside this, Tushemereirwe and Bagabe (1998) reported that *Cladosporium* leaf speckle reduces the life span of leaves, causing leaf drying and senescence in unprotected plants. As a result of the increasing rate of leaf emergence, the crop cycle was significantly accelerated in unprotected plants, with both flowering time and fruit maturity being hastened although a similar number of fruit were developed. The net effect of less photosynthetic area and a shorter plant maturation period was that the leaf spot complex significantly reduced fruit yield and