



UNIVERSITI PUTRA MALAYSIA

**INDUCTION OF SUPPRESSIVE SOIL IN THE MANAGEMENT OF
FUSARIUM WILT ON BANANA SEEDLINGS**

ADELINE TING SU YIEN

FP 2001 5

**INDUCTION OF SUPPRESSIVE SOIL IN THE MANAGEMENT OF
FUSARIUM WILT ON BANANA SEEDLINGS**

By

ADELNE TING SU YIEN

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master of Agricultural Science in the Faculty of Agriculture
Universiti Putra Malaysia**

April 2001



For my Beloved ones:

*Pa, Ma, Eve, Jarrod,
and, Steve;*

*"Yesterday, it was a wish,
Today, it is a meaningful wonder,
Tomorrow, it will be an inspiration,
and always will be."*

Thank You for Everything.



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

**INDUCTION OF SUPPRESSIVE SOIL IN THE MANAGEMENT OF
FUSARIUM WILT ON BANANA SEEDLINGS**

By

ADELINE TING SU YIEN

April 2001

Chairman: Professor Sariah Meon, Ph.D.

Faculty: Agriculture

This study determined the potential of using artificially 'induced' suppressive soil to suppress the development of *Fusarium* wilt on susceptible banana seedlings (cultivar Berangan). *Trichoderma harzianum* (UPM 40) was selected as the microbial antagonist, and calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) as the soil amendment. Both biotic and abiotic components, respectively, were incorporated into the soil to mimic the contents of naturally existing *Fusarium* suppressive soils. The potential of *T. harzianum* as a biocontrol agent was confirmed from the series of antagonism tests, with positive results in lysis, antibiosis and mycoparasitism tests. *In vitro* tests determined that *T. harzianum* required early establishment prior to challenge with *Fusarium oxysporum* f. sp. *cubense* race 4 (FocR4), to ensure effective antagonistic activity. Both *T. harzianum* and FocR4 tolerated pH 5-8, and Ca^{2+} concentrations within 5–750 ppm. Soil pH was not affected by $\text{Ca}(\text{NO}_3)_2$ application, indicating



compatibility of inoculating *T. harzianum* together with $\text{Ca}(\text{NO}_3)_2$ application. When tested on Berangan seedlings in the glasshouse, treatment with $\text{Ca}(\text{NO}_3)_2$ alone provided better disease suppression compared to treatment with both *T. harzianum* and $\text{Ca}(\text{NO}_3)_2$, and treatment with *T. harzianum* alone. Treatment with $\text{Ca}(\text{NO}_3)_2$ alone recorded low disease incidence (DI) of 51% as compared to 59% and 69% from combined treatments and *T. harzianum* alone, respectively, 8 weeks after inoculation. Calcium reduced the population of FocR4, promoted plant growth, and induced host resistance through increased peroxidase and polyphenoloxidase activity, and phenol content. Increased enzymatic activities and phenol content was related to extensive cell wall lignification as revealed by histological observations, resulting in resistance to FocR4 hyphal penetration. The formation of Ca-pectate also contributed to host resistance. Biocontrol efficiency of *T. harzianum* was dependent on soil environment, as the glasshouse trial did not suppress disease incidence, contrary to its antagonistic effect in *in vitro* tests. *T. harzianum* did not induce host resistance, instead, predisposed the seedlings to infection by increasing root growth and infection sites. Disease suppression achieved through treatment with $\text{Ca}(\text{NO}_3)_2$ was dependent on Ca^{2+} availability in the soil and Ca^{2+} content in the plant tissues. A more frequent application using suitable rates is then suggested as follow-up studies.



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

**INDUKSI TANAH PENINDAS DALAM PENGURUSAN PENYAKIT
LAYU *FUSARIUM* PADA ANAK POKOK PISANG**

Oleh

ADELINE TING SU YIEN

April 2001

Pengerusi: Profesor Sariah Meon, Ph.D.

Fakulti: Pertanian

Kajian ini menentukan potensi menggunakan tanah penindas 'buatan' untuk menindas kejadian penyakit layu *Fusarium* pada anak pokok pisang yang peka (kultivar Berangan). *Trichoderma harzianum* (UPM 40) dipilih sebagai mikrobial antagonistik, manakala kalsium nitrat ($\text{Ca}(\text{NO}_3)_2$) dipilih sebagai bahan pemulih tanah. Kedua-dua komponen biotik dan abiotik ini ditambah ke dalam tanah untuk meniru komposisi sebenar tanah penindas *Fusarium* yang sediaakala. Potensi menggunakan *T. harzianum* sebagai agen kawalan biologi terbukti dari siri ujian antagonistik yang dijalankan, dengan keputusan positif dalam ujian lisis, antibiosis dan parasitism. Ujian *in vitro* telah mengesahkan pentingnya *T. harzianum* diinokulat lebih awal sebelum didedahkan kepada *Fusarium oxysporum* f. sp. *cubense* ras 4 (FocR4), supaya efisiensi aktiviti antagonistiknya terjamin. *T. harzianum* dan FocR4 diperhatikan mempunyai toleransi terhadap pH 5-8, serta kepekatan Ca^{2+} 5-750 bsj. Aplikasi $\text{Ca}(\text{NO}_3)_2$ tidak mempengaruhi pH tanah, sekaligus membuktikan



kesesuaian menginokulat *T. harzianum* bersama rawatan $\text{Ca}(\text{NO}_3)_2$. Apabila diuji pada anak pokok pisang Berangan, rawatan dengan $\text{Ca}(\text{NO}_3)_2$ sahaja menunjukkan potensi paling baik untuk menindas insiden penyakit *Fusarium*, berbanding rawatan dengan kedua-dua *T. harzianum* dan $\text{Ca}(\text{NO}_3)_2$, dan rawatan menggunakan *T. harzianum* sahaja. Rawatan dengan $\text{Ca}(\text{NO}_3)_2$, mencatatkan insiden penyakit yang rendah sebanyak 51% berbanding 59% dan 69% oleh rawatan kombinasi dan *T. harzianum* sahaja, 8 minggu selepas rawatan. Kalsium juga mengurangkan populasi FocR4 dalam tanah, mengalakkan pertumbuhan anak pokok pisang, dan mengalakkan sistem pertahanan teraruh melalui peningkatan aktiviti enzim *peroxidase*, *polyphenoloxidase* dan kandungan fenol. Peningkatan aktiviti enzim ini dikaitkan dengan lignifikasi pada dinding sel sebagaimana yang diperhatikan dari ujian histologi, yang meningkatkan keroskatan kepada penembusan hifa FocR4. Pembentukan Ca-pektat dalam sel juga memperkukuhkan lagi ketahanan hos terhadap serangan penyakit. Efisiensi *T. harzianum* sebagai agen kawalan biologi terhadap FocR4 dipengaruhi oleh keadaan tanah, kerana ujian di rumah kaca membuktikan *T. harzianum* tidak menindas insiden penyakit *Fusarium*, yang berlawanan dengan keputusan dari ujian *in vitro*. *T. harzianum* juga tidak mengalakkan ketahanan teraruh, malah mengalakkan kejadian penyakit melalui pertumbuhan akar dan tapak jangkitan. Kesan penindasan penyakit dipengaruhi oleh kepadatan Ca^{2+} dalam tanah dan kandungan Ca^{2+} dalam tisu tumbuhan. Dengan itu, kawalan yang lebih berkesan dijangka dicapai jika kekerapan aplikasinya ditambah, dengan menggunakan kadar yang sesuai, dan penentuan ini memerlukan kajian yang selanjutnya.

ACKNOWLEDGEMENTS

I thank the good Lord for His providence and immense strength in the many moments of my life. Putting Him in the center has made all things come together.

I also wish to express my sincerest gratitude and appreciation to Prof. Dr. Sariah Meon, who played the many roles of a mentor, supervisor, teacher and advisor to perfection. Not forgetting, Dr. Jugah Kadir and Dr. Anuar Abdul Rahim, whose continuous guidance has helped me tremendously in the on-going of my research work and the preparation of my thesis.

Many thanks too, to the staffs in the Pathology Laboratory; Mr. Khir, Mr. Johari and Mr. Nazri, for their advice and assistance in the preparation of materials. To my friends; Siew Eim, Ee Fong, Kam Loong and Khairul, thank you for sharing and making these two years memorable.

To my dearest Pa, Ma, Eve and Jarrod, thank you for your love, support and encouragement. And, for my dear Steve, thank you for always inspiring, and putting colours into my life.



I certify that an Examination Committee met on 19th April 2001 to conduct the final examination of Adeline Ting Su Yien on her Master of Agricultural Science thesis entitled “Induction of Suppressive Soil in the Management of *Fusarium* Wilt on Banana Seedlings” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

HIRYATI ABDULLAH, Ph.D

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

SARIAH MEON, Ph.D.

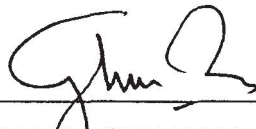
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

JUGAH KADIR, Ph.D.

Faculty of Agriculture
Universiti Putra Malaysia.
(Member)

ANUAR ABDUL RAHIM, Ph.D.


Faculty of Agriculture,
Universiti Putra Malaysia.
(Member)



MOHD. GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 04 MAY 2001.

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science.



AINI IDERIS, Ph.D,
Professor
Dean of Graduate School,
Universiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ADELINE TING SU YIEN

Date: 03.05.2001.

TABLE OF CONTENTS

	Page
DEDICATION	2
ABSTRACT	3
ABSTRAK	5
ACKNOWLEDGEMENTS	7
APPROVAL	8
DECLARATION	10
LIST OF TABLES	13
LIST OF FIGURES	17
LIST OF ABBREVIATIONS	21
 CHAPTER	
I INTRODUCTION	22
II LITERATURE REVIEW	27
Banana Plant	27
<i>Fusarium</i> wilt	29
The Disease	29
The Pathogen <i>Fusarium oxysporum</i>	
f sp <i>cubense</i>	31
Disease Symptoms	35
Disease Epidemiology	38
Factors Affecting Disease Spread	
and Development	41
Control Measures	43
Suppressive Soil	47
Components of Suppressive Soil	49
Soil Physical and Chemical Characteristics	49
Soil Amendments	50
Antagonistic Microorganisms	56
Host Induced Defense Mechanisms	59
III MATERIALS AND METHODS	63
Preparation of Fungal Isolates	63
Morphological Identification of Fungal Isolates	63



Development of Mechanisms of Suppressiveness	65
Establishment of the Mode of Antagonism	65
Effect of pH on the Growth of UPM 40 and FocR4	69
Effect of Ca ²⁺ on the Growth of UPM 40 and FocR4	71
Uptake of Ca ²⁺ by Banana Seedlings	73
Glasshouse Trials	74
Soil Mixture	74
Planting Material	74
Inoculum Production	74
Experimental Layout and Design	75
IV RESULTS AND DISCUSSION	87
Characterization and Identification of Fungal Isolates	87
Development of Mechanisms of Suppressiveness	90
Mode of Antagonism of <i>T. harzianum</i> against FocR4	90
Effect of pH on the Growth of <i>T. harzianum</i> and FocR4	98
Effect of Ca ²⁺ on the Growth of <i>T. harzianum</i> and FocR4	101
Uptake of Ca ²⁺ by Banana Seedlings	104
Efficiency of 'Induced' Suppressive Soil	106
Effect of 'Induced' Suppressive Soil on Seedling Growth	137
'Induced' Systemic Resistance Biochemical Responses	140
V CONCLUSION	148
BIBLIOGRAPHY	152
APPENDICES	164
BIODATA OF THE AUTHOR	190



LIST OF TABLES

Table		Page
1	Treatments used in glasshouse trial	76
2	Type of stains used for detection of lignin, suberin and Ca-pectate	83
3	Antagonistic activity of <i>T. harzianum</i> (UPM 40) against <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> race 4 (FocR4)	91
4	The effect of volatile inhibitors produced by <i>T. harzianum</i> towards growth of FocR4	95
5	Effect of pH on growth of <i>T. harzianum</i> and FocR4 on agar (aerial growth) and in broth (mass weight)	99
6	Effect of different Ca ²⁺ concentration (ppm) on the growth of <i>T. harzianum</i> and FocR4 on agar (aerial growth) and in broth (mass weight)	102
7	Area Under Disease Progress Curve (AUDPC) of <i>Fusarium</i> wilt under different treatment	109
8	Disease Index of seedlings according to weeks	116
9	pH of root tips, rhizosphere and bulk soil under the influence of various treatments	133



10	Effect of different treatments on mean pseudostem length, diameter and root growth	139
11	ANOVA Table for Double Plate Test	165
12	ANOVA Table for Culture Filtrate Test	165
13	ANOVA Table for Ca ²⁺ Test	165
14	ANOVA Table for cfu of isolates in Ca ²⁺ treated soils	166
15	ANOVA Table for Disease Incidence	166
16	ANOVA Table for Ca ²⁺ Content	167
17	Table for contrast comparison for Ca ²⁺ Content using GLM	167
18	ANOVA Table for cfu of FocR4 at root tips, rhizosphere and soils	168
19	Table for contrast comparison for pH values using GLM	168
20	Table for contrast comparison on seedling growth using GLM	169
21	ANOVA Table for seedling growth	169



22	Table for contrast comparison for enzyme activity using GLM.....	170
23	Table for contrast comparison for phenol content using GLM.....	170
24	ANOVA Table for Enzyme Activity.....	170
25	ANOVA Table for Phenol Content.....	171
26	ANOVA Table for Enzyme Activities according to weeks.....	171
27	ANOVA Table for Phenol Content according to weeks.....	172
28	Means Comparison Table for Ca ²⁺ Test.....	172
29	Means Comparison Table for cfu of isolates in Ca ²⁺ treated soils.....	173
30	Means Comparison Table for Disease Incidence.....	173
31	Means Comparison Table for Ca ²⁺ Content.....	175
32	Means Comparison Table for cfu of FocR4 at root tips, rhizosphere and soil.....	178
33	Means Comparison Table for Seedling Growth.....	179



34	Means Comparison Table for Enzyme Activity.....	180
35	Means Comparison Table for Enzyme Activity according to weeks.....	181
36	Means Comparison Table for Phenol Content according to weeks.....	187



LIST OF FIGURES

Figure		Page
1	Diagrammatic representation of application of treatments according to time frame	76
2	<i>Trichoderma harzianum</i> (Rifa'i), (A) 3 day old culture on PDA, and SEM micrographs showing (B) conidiophores, (C) swollen phialides and (D) phialospores	88
3	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> (FocR4), (A) 7 day old culture on PDA, and SEM micrographs showing (B) a microconidia and (C) a macroconidia	89
4	Dual culture test, (A) formation of inhibition zone showing inhibitory effect by <i>T. harzianum</i> towards FocR4 and eventual overgrowth (B)	92
5	Failure to produce normal colonies by mycelial plugs from inhibition zone (0 cm) as compared to control and from 2 cm-zone	92
6	Diameter of FocR4 single colony according to different filtrate sampling time	95
7	Initiation of mycoparasitism by growth of <i>T. harzianum</i> hypha alongside the hypha of FocR4	96
8	Antagonistic effect of <i>T. harzianum</i> towards FocR4 at (A) pH 7, (B) pH 4 and (C) pH 9	100

9	Colony forming units (cfu) of <i>T harzianum</i> and FocR4 recovered from soils treated with various Ca ²⁺ concentrations	103
10	Longitudinal root sections from treated seedlings showing presence of Ca-pectate (A) as compared to untreated root sample (B)	105
11	Disease assessment scale (0-4) was developed based on foliar-associated symptoms	107
12	Effect of different treatments (T1-T4) on disease progress of <i>Fusarium</i> wilt on banana seedlings of the Berangan cultivar	108
13	Infected pseudostems were discoloured (A) and split at the base (B), as compared to healthy uninfected pseudostem (C) and (D)	112
14	Mycelium of FocR4 on the surface of dead pseudostem tissues	113
15	Infected seedlings (A) rhizomes with reddish vascular strands, (C) roots with yellowish reddish streaks, and (D) pseudostem with visible reddish streaks, uninfected rhizome (B) and pseudostem (E)	115
16	Development of internal symptoms in the rhizome (A) 25%, (B) 50% and complete discolouration of vascular tissues (C), uninfected rhizome (D)	118
17	Cross section of a portion of a root tissue showing hypha of FocR4 penetrating through cell walls into adjacent cells	119

18	SEM micrograph showing infected root tissues with gel formation in the xylem vessels	120
19	SEM micrograph showing uninfected tissues	120
20	Lignification thickened cell walls to resist hypha penetration	122
21	Uninfected tissues were not lignified but retained its shape and turgor	122
22	Presence of Ca-pectate in tissues stained reddish brown with Alizarin Red S (A), as compared to tissues without Ca-pectate (B)	123
23	Ca ²⁺ content in root tissues in relation to DI	124
24	Ca ²⁺ content in root of seedlings treated with various treatments, 8 weeks after treatment	125
25	Mean colony forming units (cfu) count of FocR4 recovered from root tips, rhizosphere and soil 8 weeks after inoculation	126
26	SEM micrograph showing spores of <i>T harzianum</i> attached to conidia of FocR4 Failure to germinate affected the antagonistic activity of <i>T harzianum</i>	128
27	SEM micrograph showing root colonization by <i>T harzianum</i> which provided 'protection' from FocR4 colonization	128

28	Parasitization of FocR4 conidia by <i>T. harzianum</i> (A) resulted in growth of abnormal colonies as observed on FSM, 7 days after incubation	130
29	pH value of root tips, rhizosphere and bulk soil	132
30	The relation between disease incidence and pH of (A) root tips, (B) rhizosphere and (C) soil	135
31	Effect of FocR4 on mean (A) pseudostem length and diameter, and (B) root weight of banana seedlings, 8 weeks after treatment	138
32	Mean activity for soluble and ionically bound PO and PPO, and phenol content in infected and uninfected seedlings	142
33	Activity of (A) soluble and (B) ionically bound PO in root tissues sampled from 0-8 weeks in comparison to DI (%)	145
34	Activity of PPO (C) and the phenol content (D) in root tissues sampled from 0-8 weeks, with comparison to DI (%)	146
35	Standard curve for the determination of Ca^{2+} content in root tissues	188
36	Standard curve for the determination of phenol content in root tissues	189

LIST OF ABBREVIATIONS

DOA	Department of Agriculture
FAMA	Federal Agriculture Marketing Authority (FAMA)
FAO	Food and Agriculture Organization
INIBAP	International Network for the Improvement of Banana and Plantain
MARDI	Malaysian Agricultural Research and Development Institute
SIRIM	Standards and Industrial Research Institute of Malaysia



CHAPTER I

INTRODUCTION

The banana industry is a growing fruit industry in most countries worldwide, due to its increasing market demand and relatively low production costs. Most of the bananas produced are mainly for fresh consumption, eaten as dessert fruit or, as staple food because of its high starch content (Valmayor, 1987). Bananas are also commonly used in beer brewing, in vinegar production, in confectioneries to flavour cakes, puddings and muffins, and also as fibre material, wrappers or vegetables (Thurston, 1984; Valmayor, 1987).

As one of the most important fruit crops in many agricultural countries, bananas are produced extensively in Asia (India, Philippines, Thailand, Indonesia, Taiwan), Africa and, South and Central America (Honduras, Panama, Costa Rica, Guatemala) (Hassan and Pantastico, 1990). In Malaysia, it is the second most important fruit crop, accounting for 20 % of the total hectareage of fruit plantations (Yaacob, 1991). However, land used for banana cultivation has declined over the years from 40 000 ha in 1993 to 39 000 ha in 2000 (Loh, 2000).



The trading of banana grew significantly in the early 1870's, beginning with the trading of the Gros Michel (AAA) variety (Ploetz, 1994). This first commercially cultivated variety was considered as an "ideal variety" because of its large fruit, smooth skin texture, and cream coloured flesh that is moderately firm, slightly aromatic and sweet. Most importantly, it has excellent keeping quality and produces high yields (Hassan and Pantastico, 1990). Thus, the Gros Michel variety was extensively cultivated in new plantations, or in plantations of another banana variety known as the Silk variety (AAB) (Snyder and Smith, 1981).

The emergence of *Fusarium* wilt disease (Panama disease), in the late 1890's, threatened to diminish the banana industry. This disease caused severe losses in fruit yield, and death of plants. In just over 50 years since its first occurrence, it has destroyed more than 40 000 ha of banana plantations in Central and South America (Su et al., 1986).

Initially, only race 1 of the pathogen, *Fusarium oxysporum* f. sp. *cubense* (FocR1) was identified to be pathogenic towards Gros Michel and Silk varieties (Snyder and Smith, 1981). Race 2 (FocR2) and 3 (FocR3) only infect plantain (cooking) varieties, like Bluggoe (ABB), and *Heliconia* spp., respectively (Su et al , 1986).



Most of the Gros Michel plantations infested with FocR1 then, were successfully replanted with the resistant Cavendish variety. However, resistance to disease development soon vanished with the emergence of race 4 (FocR4), which caused destruction in the Cavendish plantations.

FocR4 spread rapidly and vastly. By 1955, banana varieties of Williams (AAA 'Cavendish') in Australia, have succumbed to FocR4 (Ploetz, 1994). In 1974, Cavendish varieties in the Philippines were severely infected (Snyder and Smith, 1981), and by 1977, FocR4 were reportedly recovered from numerous soils in Taiwan and Canary Islands.

The sudden emergence of FocR4 was believed due to independent mutation that may have occurred in the different Asian regions, as suggested by Su et al. (1986). However, Snyder and Smith (1981) claimed that FocR4 was just one of the many races of Foc, which originated from the Southeast Asian region, which remained undetected, as the discovery of some races of Foc in Vietnam that was undiscovered in other parts of the world. Furthermore, banana is a native of the Indo-Malaya countries, and has long established its existence, together with its diversified pathogens.

