

Host-endophyte-pest interactions of endophytic *Fusarium oxysporum* antagonistic to *Radopholus similis* in banana (*Musa* spp.)

By

Shahasi Yusuf Athman

Submitted in partial fulfilment of the requirements for the degree of

PhD (Plant Pathology)

In the Faculty of Natural and Agricultural Sciences.

University of Pretoria

Pretoria, South Africa

June 2006

Supervisor: Prof. Nico Labuschagne (University of Pretoria), South Africa

Co-supervisors: Dr. Altus Viljoen (University of Pretoria), South Africa

**Dr. Thomas Dubois (International Institute of Tropical
Agriculture (IITA)-Uganda**

Dr. Daniel Coyne (IITA-Uganda)

Dedication

To my family

Declaration

I declare that the thesis which I hereby submit at the University of Pretoria for the award of the degree PhD (Plant Pathology) is my work and has not been submitted by me for a degree to any other university or institution of higher education.

.....

Shahasi Yusuf Athman

TABLE OF CONTENTS

HOST-ENDOPHYTE-PEST INTERACTIONS OF ENDOPHYTIC <i>FUSARIUM OXYSPORUM</i> ANTAGONISTIC TO <i>RADOPHOLUS SIMILIS</i> IN BANANA (<i>MUSA SPP.</i>)	I
DEDICATION	II
DECLARATION	III
ACKNOWLEDGEMENTS	VIII
GENERAL INTRODUCTION	X
PROBLEM STATEMENT	XII
RESEARCH OBJECTIVES	XII
REFERENCES	XIII
CHAPTER 1	1
REVIEW OF THE ROLE OF ENDOPHYTES IN BIOLOGICAL CONTROL OF PLANT-PARASITIC NEMATODES WITH SPECIAL REFERENCE TO THE BANANA NEMATODE, <i>RADOPHOLUS SIMILIS</i> (COBB) THORNE	1
1. INTRODUCTION	2
2. WHY ENDOPHYTES?	4
3. COLONIZATION OF PLANTS BY ENDOPHYTES	5
4. METHODS FOR ISOLATION OF ENDOPHYTES FROM PLANTS	6
5. ENDOPHYTIC FUNGI AND NEMATODE CONTROL	6
6. ENDOPHYTIC BACTERIA AND NEMATODE CONTROL	8
7. MECHANISMS OF ACTION OF ENDOPHYTES AGAINST NEMATODES	9
7.1 PRODUCTION OF NEMATICIDAL SECONDARY METABOLITES	9
7.2 CHANGES IN HOST PLANT PHYSIOLOGY	11
7.3 INDUCED PLANT DEFENSE RESPONSES	13
7.3.1 INDUCED PHYSICAL AND MECHANICAL DEFENSE RESPONSES	14
7.3.2 INDUCED BIOCHEMICAL DEFENSE RESPONSES	15
7.3.3 HOW TO DETECT ISR	15
7.3.4 BENEFITS OF ISR	16
8. BANANA PRODUCTION IN UGANDA	16
9. NEMATODE PROBLEMS OF BANANA	18
10. BIOLOGY OF <i>RADOPHOLUS SIMILIS</i>	18

11. DAMAGE AND ECONOMIC IMPORTANCE OF <i>RADOPHOLUS SIMILIS</i>	19
12.1 CULTURAL CONTROL	20
12.2 HOST PLANT RESISTANCE	21
12.3 TRANSGENIC BANANA	22
12.4 TISSUE CULTURE BANANA PLANTS	22
12.5 BIOLOGICAL CONTROL.....	23
12.5.1 RHIZOSPHERE BACTERIA AND ACTINOMYCETES	23
12.5.2 MYCORRHIZAE.....	23
13. CONCLUSIONS	26
14. REFERENCES	29
CHAPTER 2	46
<i>IN VITRO</i> SCREENING OF ENDOPHYTIC <i>FUSARIUM</i> ISOLATES AGAINST THE BANANA BURROWING NEMATODE, <i>RADOPHOLUS SIMILIS</i> (COBB)	
THORNE	46
ABSTRACT	47
INTRODUCTION	48
MATERIALS AND METHODS	49
DISCUSSION	59
REFERENCES	63
CHAPTER 3	77
<i>IN VIVO</i> SCREENING OF ENDOPHYTIC <i>FUSARIUM OXYSPORUM</i> ISOLATES FOR ACTIVITY AGAINST <i>RADOPHOLUS SIMILIS</i> IN TISSUE CULTURE BANANA PLANTS	
ABSTRACT	78
INTRODUCTION	79
MATERIALS AND METHODS	80
SITE DESCRIPTION.....	80
TISSUE CULTURE PLANTS.....	80
FUNGAL ISOLATES	81
<i>RADOPHOLUS SIMILIS</i> CULTURES	82
INOCULATION OF PLANTS WITH FUNGAL ISOLATES	83
INOCULATION OF PLANTS WITH NEMATODES	83
EXPERIMENTAL DESIGN AND LAYOUT.....	84
ASSESSMENT OF PLANT GROWTH PARAMETERS	84
ASSESSMENT OF NEMATODE REPRODUCTION AND DAMAGE	84

DETERMINATION OF FUNGAL COLONIZATION	85
DATA ANALYSIS	86
RESULTS	87
RADOPHOLUS SIMILIS REPRODUCTION	87
RADOPHOLUS SIMILIS DAMAGE	88
PLANT GROWTH AND PERFORMANCE	89
ENDOPHYTIC COLONIZATION	91
DISCUSSION	92
REFERENCES	96
CHAPTER 4	114
EFFECT OF ENDOPHYTIC <i>FUSARIUM OXYSPORUM</i> ISOLATES ON HOST PREFERENCE, ATTRACTION, ROOT PENETRATION AND REPRODUCTION OF <i>RADOPHOLUS SIMILIS</i> IN TISSUE CULTURE BANANA PLANTS	114
ABSTRACT	115
INTRODUCTION	116
MATERIALS AND METHODS	117
SITE DESCRIPTION.....	117
FUNGAL ISOLATES AND NEMATODE CULTURES.....	118
TISSUE CULTURE PLANTS.....	118
INOCULATION OF PLANTS WITH FUNGAL ISOLATES.....	119
HOST PREFERENCE AND ATTRACTION EXPERIMENTS	120
DETACHED ROOT BIOASSAY FOR HOST PREFERENCE AND ATTRACTION	120
INTACT PLANT BIOASSAY FOR HOST PREFERENCE AND ATTRACTION	121
ROOT PENETRATION EXPERIMENTS	122
IN VITRO ROOT PENETRATION EXPERIMENTS	122
IN VIVO ROOT PENETRATION EXPERIMENTS	123
<i>RADOPHOLUS SIMILIS</i> REPRODUCTION EXPERIMENTS	123
DATA ANALYSIS	124
RESULTS	126
HOST PREFERENCE AND ATTRACTION EXPERIMENTS	126
ROOT PENETRATION EXPERIMENTS	127
IN VITRO EXPERIMENTS	127
IN VIVO EXPERIMENTS	127
RADOPHOLUS SIMILIS REPRODUCTION.....	127
ENDOPHYTIC COLONIZATION OF BANANA ROOTS.....	129
DISCUSSION	130
REFERENCES	133

CHAPTER 5	152
MECHANISMS OF ACTION OF ENDOPHYTIC <i>FUSARIUM OXYSPORUM</i> AGAINST <i>RADOPHOLUS SIMILIS</i> IN BANANA PLANTS	152
ABSTRACT	153
INTRODUCTION	154
MATERIAL AND METHODS	156
SITE DESCRIPTION.....	156
FUNGAL ISOLATES, NEMATODE CULTURES AND BANANA PLANTS.....	156
PRODUCTION OF EXTRA CELLULAR ENZYMES ON SOLID MEDIUM.....	156
SPLIT-ROOT EXPERIMENTS FOR ASSESSING INDUCED RESISTANCE	157
ANALYSIS OF PHENOLIC COMPOUNDS IN ENDOPHYTE-TREATED PLANTS	159
HISTOLOGICAL ANALYSIS	160
HISTOCHEMICAL ANALYSIS	162
DATA ANALYSIS	163
RESULTS	165
PRODUCTION OF EXTRA CELLULAR ENZYMES	165
SPLIT-ROOT EXPERIMENTS FOR ASSESSMENT OF INDUCED RESISTANCE.....	165
ANALYSIS OF PHENOLIC COMPOUNDS	166
HISTOCHEMICAL ANALYSIS	167
DISCUSSION	169
REFERENCES	173
CHAPTER 6	191
GENETIC DIVERSITY OF ENDOPHYTIC <i>FUSARIUM</i> SPP. ASSOCIATED WITH CAVENDISH BANANA IN SOUTH AFRICA	191
ABSTRACT	192
INTRODUCTION	193
MATERIALS AND METHODS	195
COLLECTION OF PLANT MATERIAL.....	195
ISOLATION OF ENDOPHYTIC <i>FUSARIUM</i> SPP.	195
MORPHOLOGICAL IDENTIFICATION OF <i>FUSARIUM</i> SPP.	196
STATISTICAL ANALYSIS OF DATA.....	196
MOLECULAR CHARACTERIZATION OF <i>FUSARIUM</i> SPP.	197
RESULTS	201
ISOLATION FREQUENCIES OF ENDOPHYTIC <i>FUSARIUM</i> SPP. FROM CAVENDISH BANANA PLANTS	201
MOLECULAR CHARACTERIZATION OF ENDOPHYTIC <i>FUSARIUM</i> SPP.	202
SEQUENCE ANALYSIS OF THE TEF 1-A REGION OF SELECTED <i>FUSARIUM</i> ISOLATES	202

AFLP ANALYSIS OF FUSARIUM SPP.	202
REFERENCES	209
SUMMARY	221

Acknowledgements

First of all I would like to thank Almighty Allah for giving me the beautiful gift of life and for being the strongest pillar and source of inner strength and resilience through out my entire life.

The research presented in this thesis was conducted with a scholarship from the International Institute of Tropical Agriculture (IITA) through a grant by the German Ministry for International Cooperation (BMZ). My sincere gratitude to these two institutions for providing the financial support to conduct this research.

I am indebted to my supervisors at IITA namely Drs. Cliff Gold, Thomas Dubois, Bjerne Niere and Danny Coyne for giving me a chance to pursue my PhD studies under their leadership. At the University of Pretoria (UP), Prof. Nico Labuschagne and Dr. Altus Viljoen, for accepting to supervise me and for supporting me through out the study. Thank you all for your outstanding and critical editing skills that helped me articulate the complex issues in this thesis.

At UP, Dr. Thierry Regnier and Mr. Rony Gilfillan, thanks for your help with the phenolics and HPLC analysis respectively. Barbara and Susan, for sharing your knowledge on RFLP and AFLP analyses.

Dr. John Kimenju, (University of Nairobi) for all the support and guidance that you have given me since my undergraduate studies in Kabete and especially for introducing me to the world of the ‘unseen worms’. Thanks for believing in me.

Friends and colleagues at IITA-Uganda (Fred, Jully, Baker, Patrick, Pam, Jolly, Frank, Sinnia, William, Agnes, Hussein, Kagezi, Perez and many others) for your inestimable support and encouragement. I really enjoyed working with you all. Phillip Ragama, for providing help with statistical analysis.

At the Faculty of Veterinary Medicine of Makerere University: William, Maria and Monica for your technical assistance during the histological analysis of phenolic compounds, it was fun working with you guys.

My friends in Pretoria, Doris, Elizabeth, Jessica, Evonne, John and Yusuf for your friendship and prayers.

The Mathenge family in Kampala for the many ways in which you supported me, the weekend getaways to your home gave me a new lease on life every single week.

Lastly and most specially, my deepest appreciation goes to my dear family, mom and dad, for giving the gift of education, instilling confidence and for believing in me. My sisters, Amina, Hidaya, Asha and Kinya and brothers Kaka, Abdi, Sultan and Farid for your motivation and prayers. Especially my small sister Kinya, nieces and nephews for whom unlimited opportunities lie ahead. Your unending support gave me the strength to live my life to the fullest and persevere through the lonely and difficulty times. May Allah bless you all abundantly 'barakALLAHufika'.

General introduction

Bananas (*Musa* spp.) are among the most important tropical fruits in Sub-saharan Africa, providing the bulk of dietary carbohydrates and daily calorie intake for millions of people. Highland cooking banana (*Musa* spp. group AAA-EA) is the most important staple food crop in the Lake Victoria basin (INIBAP, 1986). Uganda is one of the largest banana producing and consuming countries in the world, and a secondary center of diversity of the highland bananas of the *Musa* AAA group. The primary center of diversity for AAA banana group members is the Malaysian region from where the plants were introduced to the East African Highlands (Simmonds, 1987). The dominant cultivars in Uganda belong to the East African highland cooking bananas, which comprise over 76% of banana production in the country (Karamura, 1993). Banana production is primarily undertaken by semi-subsistent, small-scale households, and most bananas are locally consumed as a starch staple after cooking.

In recent years, there have been marked changes in the location and intensity of banana production in Uganda. Highland banana production in the country has declined sharply. Karamura (1993) reported a production decline of more than 25% from more than 8 tons/ha in the 1970s to less than 6 tons/ha in the 1990s. The major constraints to banana production in the region are mainly pests and diseases (Gold *et al.*, 1993). This has led to the replacement of highland cooking bananas in some traditional growing areas in Central Uganda with more pest and disease tolerant brewing and dessert bananas (Gold, *et al.*, 1999). A complex of plant-parasitic nematodes affect banana production: the burrowing nematode *Radopholus similis* (Cobb) Thorne, the spiral nematode *Helicotylenchus multicinctus* (Cobb) Golden, the lesion nematodes *Pratylenchus goodeyi* (Sher and Allen) and *Pratylenchus coffeae* (Goodeyi), and the root-knot nematodes *Meloidogyne* spp. (Goeldi). These nematodes, together with the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), are the primary banana pest constraints in East Africa (Gold *et al.*, 1993; Karamura, 1993; Speijer *et al.*, 1999a).

Control measures against *R. similis* include the use of (uninfested) planting material such as pared and hot water treated suckers and tissue culture plants (Stanton, 1999; Speijer *et al.*, 1999b). Clean planting material may provide adequate control in the first crop cycle, but the problem of re-infestation of plants in the field is a major disadvantage (Speijer *et al.*, 1995; Speijer *et al.*, 2001). Host plant resistance would offer a safe and long-term intervention

strategy against *R. similis*. Resistance to *R. similis* is, however, yet to be identified in East African highland cooking banana cultivars. Thus, research is currently focusing on several alternative ways, including the use of microbial antagonists such as endophytes, for controlling banana pests and diseases (Sikora and Schuster, 1999; Pocasangre, 2000; Niere, 2001).

Endophytes are microorganisms that spend some time in their life cycle living symptomlessly within plant tissues (Petrini, 1991). Although tissue culture plants may provide healthy, nematode-free planting material, the effects may offer only a temporary solution to nematode problems in banana, necessitating the need for affordable, sustainable and environmentally friendly management strategies that complement the benefits of clean planting material. Due to the sterile conditions under which tissue culture plants are produced, the plants lose naturally beneficial microorganisms such as endophytes (Pereira *et al.*, 1999). The artificial introduction of endophytic fungi in these sterile plants at the hardening phase may offer protection against pests and diseases to the young plants in the early growth stages and extend the life of planting material (Sikora and Schuster, 1999). This strategy would form part of an integrated pest management approach consisting of biological control and clean planting material.

Research on the use of endophytes for the biological control of the main banana pests in Uganda was initiated at the International Institute of Tropical Agriculture (IITA) in the late 90s. This led to the isolation of hundreds of endophytic fungal isolates from apparently healthy banana plants growing in nematode and banana weevil infested plantations (Griesbach, 2000; Niere, 2001). These isolates are being preserved at the IITA laboratory in Namulonge, Uganda. Although various fungal endophytes were isolated, *Fusarium* spp. and especially *Fusarium oxysporum* Schlecht.: Fries were the dominant endophytic taxa. Research aimed at identifying fungal isolates with nematode-controlling activities has since focused on *F. oxysporum* isolates against the most economically important nematode species *R. similis*. Preliminary results have shown that some fungal isolates tested against *R. similis* possessed *in vitro* nematicidal activity and also led to a reduction in nematode populations in tissue culture plants under screen house conditions (Niere, 2001).

Problem statement

Preliminary results involving *R. similis* control with endophytic *F. oxysporum* isolates have been very promising (Niere, 2001). The isolates tested have shown the potential to kill *R. similis in vitro* and also reduce *R. similis* populations in endophyte-treated tissue culture plants. Despite these exciting results, information on the interactions between the host plant, the endophyte and the nematode, and the mechanisms involved in nematode suppression, remains limited. Little is known on the effects of the endophyte on host preferences, invasion and root penetration, and population build-up of *R. similis* in banana plants. The role of induced resistance by endophytes against *R. similis* has also been postulated but not confirmed. Knowledge of these interactions is essential in elucidating the mechanisms involved in nematode suppression. Understanding how endophytes control *R. similis* in banana plants would further be useful in designing appropriate nematode control strategies and also in maximizing benefits of endophyte inoculation in plants.

Research objectives

The main objective of this research was to identify endophytic *Fusarium* spp. isolates for use as biological control agents against the major banana nematode *R. similis* and to study the interactions and mechanisms of control involved.

Specific objectives:

- To screen endophytic *Fusarium* spp. for *R. similis* suppression both *in vitro* and *in vivo* in tissue culture banana plants.
- To determine the effect of endophytic *F. oxysporum* isolates on the *R. similis*-banana plant association.
- To determine mechanisms involved in *R. similis* control by endophytic *F. oxysporum* isolates.

References

- Gold, C.S, Ogenga-latigo, M.W., Tushemereirwe, W., Kashiya, I. and Nankinga, C. (1993). Farmer perceptions of banana pest constraints in Uganda: results from a rapid rural appraisal. In: Gold, C.S. and Gemmill, B. (Eds). Biological and integrated control of highland banana and plantain pests and diseases. IITA, Ibadan, Nigeria. pp. 3-24.
- Gold, C.S., Karamura, E.B., Kiggundu, A. Bagamba, F. and Abera, A.M.K. (1999). Geographic shifts in highland cooking banana (*Musa* spp., group AAA-EA) production in Uganda. International Journal of Sustainable Development and World Ecology 6: 45-59.
- Griesbach, M. (2000). Occurrence of mutualistic fungal endophytes in bananas (*Musa* spp.) and their potential as biocontrol agents of banana weevil *Cosmopolites sordidus* (Germar) in Uganda. PhD thesis. University of Bonn, Bonn, Germany. p129.
- INIBAP, (1986). A preliminary study of the needs for banana research in Eastern Africa. INIBAP doc. EA-001e.
- Karamura, E.B. (1993). The strategic importance of bananas/ plantains in Uganda. In: Gold, C.S. and Gemmill, B. (Eds). Biological and Integrated Control of Highland Banana Pests and Diseases in Africa. Proceedings of a research coordination meeting held in Cotonou, Benin, 12-14 November 1991. pp. 384- 387.
- Niere, B.I. (2001). Significance of non-pathogenic isolates of *Fusarium oxysporum* Schlecht.: Fries for the biological control of the burrowing nematode *Radopholus similis* (Cobb) Thorne on tissue cultured banana. PhD thesis, University of Bonn, Bonn. Germany. p118.
- Pereira, J.O., Carneiro-Vieira, M.L. and Azevedo, J.L. (1999). Endophytic fungi from *Musa acuminata* and their reintroduction into axenic plants. World Journal of Microbiology and Biotechnology 15: 37-40.
- Petrini, O. (1991). Fungal endophytes of tree leaves. In: Andrews, J.H and Hirano, S.S. (Eds). Microbial ecology of leaves. Springer-Verlag, New York, USA. pp.179-197.
- Pocasangre, L. (2000). Biological enhancement of tissue culture plantlets with endophytic fungi for the control of the burrowing nematodes *Radopholus similis* and the Panama disease (*Fusarium oxysporum* f. sp. *cubense*). PhD Thesis, University of Bonn, Bonn. Germany. p94.

- Sikora, R.A. and Schuster, R.P. (1999). Novel approaches to nematode IPM. In: Frison, E.A., Gold, C.S., Karamura, E.B. and Sikora, R.A. (Eds). Mobilising IPM for sustainable banana production in Africa. INIBAP, Montpellier, France. pp.127-136.
- Simmonds, N.W. (1987). Classification and breeding of bananas. In: Persley, G.J., and de Langhe E.A., (Eds). Banana and plantain breeding strategies. Workshop Proceedings, Cairns, Australia, 13-17 October 1986. pp. 69-73.
- Speijer, P.R., Gold C.S., Kajumba, C. and Karamura E.B. (1995). Nematode infestation of 'clean' banana planting materials in farmer's fields in Uganda. *Nematologica* 41: 344.
- Speijer, P.R., Kajumba, C. and Ssango, F. (1999a). East African highland banana production as influenced by nematodes and crop management in Uganda. *International Journal of Pest Management* 45: 41-49.
- Speijer, P., Kajumba, C. and W. Tushemereirwe (1999b). Dissemination and adaptation of a banana clean planting material technology in Uganda. *InfoMusa* 8: 11-13.
- Speijer, P.R., Nampala, P.M., Elsen, A., Ekwamu, A. and De Waele, D. (2001). Reinfestation by nematodes and performance of hot-water-treated East African highland cooking bananas as perceived by farmers in Ikulwe, Iganga District, Uganda. *African Plant Protection* 7: 85-89.
- Stanton, J.M. (1999). Assessment of resistance and tolerance of *in-vitro* propagated banana plants to burrowing nematode, *Radopholus similis*. *Australian Journal of Experimental Agriculture* 39: 891-895.