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

By

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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.

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Shahasi Yusuf Athman

TABLE OF CONTENTS

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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 disdavantage (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 loose 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., Kashaija, 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.