

Management of Fusarium wilt of banana by means of biological and chemical control and induced resistance

by

Barbara Nel

Submitted in partial fulfillment of the requirements for the degree of

Magister Scientiae

In the Faculty of Natural and Agricultural Science

University of Pretoria

Pretoria

December 2004

PROMOTER:

Dr. A. Viljoen

CO-PROMOTERS:

Dr. C. Steinberg

Prof. N. Labuschagne



***In loving memory to my father,
who is always in my thoughts and is greatly missed by us all.***

***“Praise the Lord, my soul,
and do not forget how kind He is.
He forgives all my sins
and heals my diseases.
He keeps me from the grave
and blesses me with love and mercy.
He fills my life with good things,
so that I stay young and strong like an eagle.”
Ps. 103:2-5***



DECLARATION

I, the undersigned, declare that the work contained in this thesis is my own original work and that it has not previously, in its entirety or part, submitted for a degree to any other university.

A handwritten signature in black ink, appearing to read 'B Nel', written over a dotted line.

Barbara Nel

TABLE OF CONTENTS

Acknowledgements	I
Preface	II
Chapter 1: Management of Fusarium wilt of banana: A review.	
1. Introduction	2
2. Production of bananas	3
3. Fusarium wilt of banana (Panama disease)	5
3.1 The pathogen	5
3.2 History and distribution of Fusarium wilt of banana	6
3.3 Disease symptoms	7
4. Management of Fusarium wilt of banana	8
4.1 Biological control	9
4.1.1 Fungi	10
<i>Trichoderma</i> and <i>Gliocladium</i> spp.	10
Non-pathogenic <i>Fusarium oxysporum</i>	12
Mycorrhizae	16
4.1.2 Bacteria	17
<i>Pseudomonas</i> and <i>Bacillus</i> spp.	17
Actinomycetes	19
4.1.3 Combinations of microbial agents	20
4.1.4 Hypovirulence	21
4.2 Chemical control	22
4.2.1 Fungicides	23
4.2.2 Surface sterilants	25
4.2.3 Soil fumigation	25
4.2.4 Plant activators	26
4.3 Cultural control	28
4.3.1 Tissue culture bananas	28
4.3.2 Quarantine and sanitation	29
4.3.3 Crop rotation	30
4.3.4 Soil amendments and fertilizers	31

4.3.5 Flood fallowing	32
4.4 Host resistance	33
4.4.1 Natural resistance	33
4.4.2 Plant Breeding	34
4.4.3 Unconventional Improvement	35
4.5 Integrated disease management	36
5. Suppressive soils	38
6. Conclusions	41
7. References	44

Chapter 2:Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana.

Abstract	70
Introduction	71
Materials and methods	73
Results	77
Discussion	79
References	81

Chapter 3:The role of chemical activators in inducing systemically acquired resistance in banana against *Fusarium oxysporum* f.sp. *cubense*.

Abstract	96
Introduction	97
Materials and methods	99
Results	102
Discussion	104
References	109

Chapter 4:Isolation and characterization of non-pathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants.

Abstract	123
----------	-----



Introduction	124
Materials and methods	126
Results	130
Discussion	132
References	135
Chapter 5: Evaluating non-pathogenic <i>Fusarium oxysporum</i> and other potential biological control organisms for suppressing Fusarium wilt of banana.	
Abstract	151
Introduction	152
Materials and methods	155
Results	159
Discussion	160
References	164
Summary	176
Opsomming	179

ACKNOWLEDGEMENTS

First and foremost I would like to thank God, for being my Saviour. You have blessed me with many talents and have shown me how to use them for your honour and glory. My life is in your hands.

I would like to express sincere thanks and appreciation to the following people and institutions:

Altus Viljoen, Christian Steinberg and Prof. Nico Labuschagne, it was an honour to work with such committed and extraordinary promoters. Thank you for your guidance, encouragement and belief in me.

The banana growers in Kiepersol for their assistance during my field trials. Hannes, thank you for your dedication to the trial and the wonderful way you assisted me during the two years. Rodney, Oom Jan and Oom Flip thank you for your support, hospitality and friendship.

The National Research Foundation (NRF), the Banana Growers Association of South Africa (BGASA), the Technology and Human Resources for Industry Programme (THRIP) and the University of Pretoria for financial assistance.

All the suppliers of the fungicides, surface sterilants, chemical activators, and biological agents evaluated in this study. Without whom this study would not have been possible. Also DuRoi Laboratories for proving plants.

Dr. Ben Eisenberg for assistance with statistical analysis of my data and useful discussions.

All the kind people in FABI, for advice and willingness to help. To the banana girls, especially Noëlan, Gerda and Susan, for your friendship, assistance and advice. To the Thursday prayer group, you made me more consistent in my faith by walking with me and encouraging me.

My friends, thank you for your love and for always standing by me. Especially, Gavin, Lieschen, Gerda and Susan I feel privileged to have you in my life. Susan, thank you for the amazing way that you care and for your strength as a friend.

Lastly to my wonderful family, for your love and encouragement. Mom, thank you for being the wonderful strong woman that you are, you are my most dedicated supporter and for praying constantly for me. To my sister Sanet and brother-in-law Hennie, thank you for being so strong for me and for always looking out for me. I love you all.

PREFACE

Fusarium oxysporum f.sp. *cubense* (*Foc*) is responsible for Fusarium wilt, one of the most damaging diseases of banana in the world. Current control strategies involve the use of resistant cultivars and preventing the introduction of the disease into new areas. Even though both the pathogen and disease have been studied for more than 100 years, little progress has been made in the effective management thereof. In fields where *Foc* has been introduced and resistant cultivars are not acceptable to local markets, little can be done to reduce the impact of the disease. This thesis investigates recent developments in plant disease control in order to manage Fusarium wilt of banana. If proven effective, these methods could be combined into an integrated disease management programme.

Since the first report of Fusarium wilt of banana in 1876, various researchers have tried to reduce the impact of the disease or to eliminate *Foc* from infested fields. **Chapter 1** provides the reader with a broad overview of the banana plant, the history of Fusarium wilt, the responsible pathogen and its life cycle. It then reviews management practices investigated in the past and considers alternative management strategies for the future. Finally, it considers an integrated disease management strategy, and discusses the importance of disease suppressive soils.

Considering the importance of disease prevention and the environmental impact of chemicals it was of importance to determine the effectiveness of commercially available fungicides and sterilants. Due to the fact that few fungicides have been tested against Fusarium wilt in the last 40 year, new fungicidal groups were evaluated *in vitro* and *in vivo* in **chapter 2**. Different application methods were used to apply these chemicals in the greenhouse. Secondly, different surface sterilants were evaluated for use as disinfectants to prevent the spread of *Foc*. These sterilants were compared to the ones that are currently being used.

Only a few studies have investigated the use of chemical activators to induce systemic acquired resistance (SAR) in bananas. In **chapter 3**, chemicals that were successful as

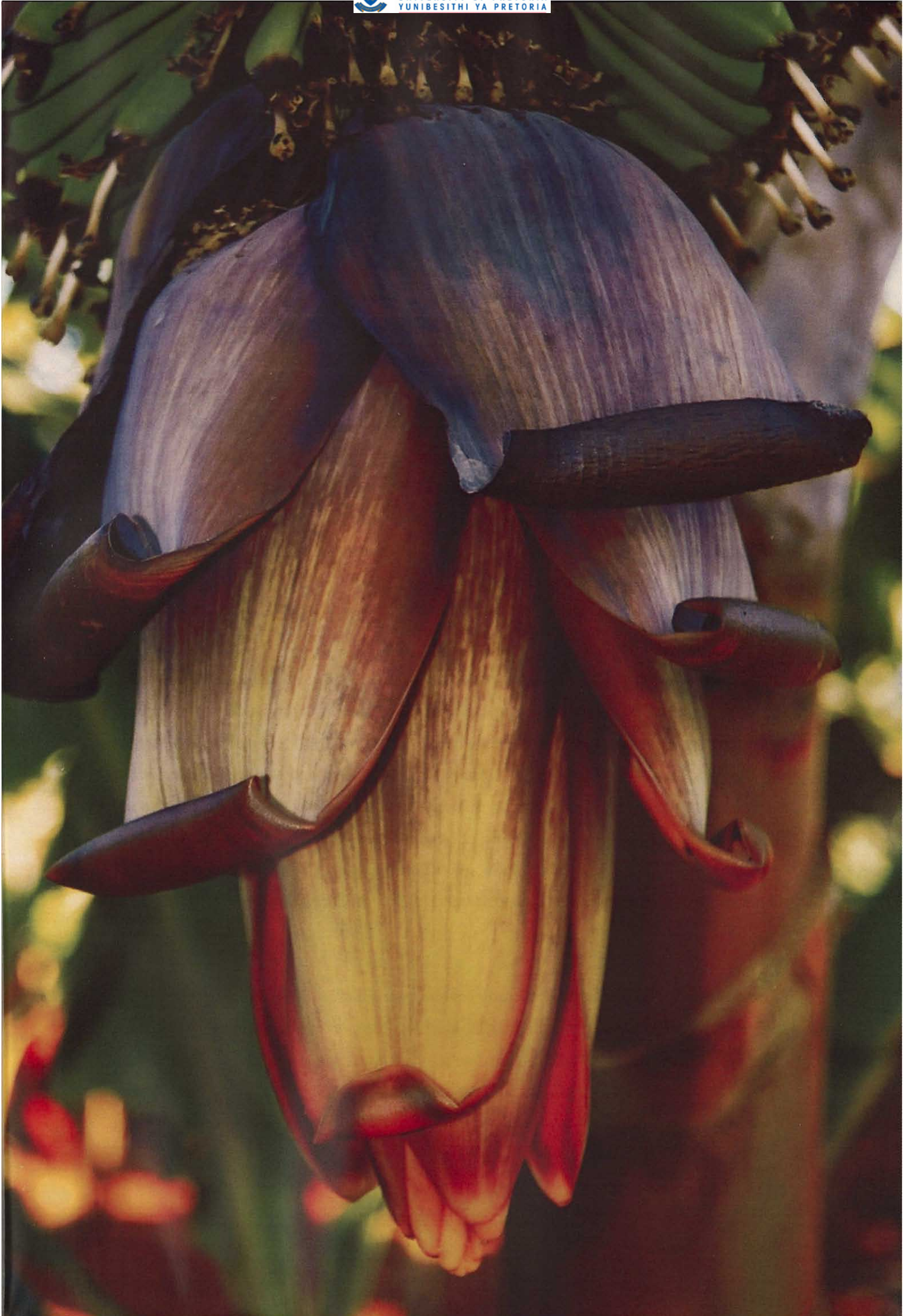
SAR activators on other crops were evaluated in the greenhouse and in the field against a susceptible and tolerant banana cultivar for their ability to activate a resistance response against Fusarium wilt. It further discusses how these chemical activators could be included in an integrated disease management programme.

Biological control is an important management strategy to be considered for Fusarium wilt of banana, as effective biological agents have been identified against other Fusarium wilt diseases. In **chapter 4**, potential non-pathogenic *F. oxysporum* isolates were collected from the root rhizosphere of banana plants in suppressive soils in Kiepersol, South Africa. The strains were identified using morphology and species-specific primers, and characterized by restriction fragment length polymorphism analysis of the intergenic spacer region of the ribosomal RNA operon and pathogenicity testing. The isolates forming similar restriction profiles were grouped together and their genetic relatedness were determined.

Non-pathogenic isolates of *F. oxysporum* from disease suppressive soils have proved effective in reducing Fusarium wilt of agricultural crops. In **chapter 5**, isolates of non-pathogenic *F. oxysporum* and *Trichoderma* spp. from Fusarium wilt suppressive soils in Kiepersol were evaluated for their ability to suppress *Foc* in the greenhouse. These isolates were compared to known biological control agents of Fusarium wilt disease, as well as commercial biological control products.

CHAPTER 1

Management of Fusarium wilt of Banana: A review



1. INTRODUCTION

Fusarium wilt diseases are known to be destructive to many economically important agricultural crops planted around the world (Armstrong and Armstrong, 1981). *Fusarium* wilt is caused by the soil-borne fungus *Fusarium oxysporum* Schlechtend.; Fr., a highly cosmopolitan organism that includes both pathogenic and non-pathogenic strains (Booth, 1971; Armstrong and Armstrong, 1975). Pathogenic members of the fungus can be divided into at least 120 different *formae speciales* (Hawksworth *et al.*, 1995). A *forma specialis* consists of individuals of *F. oxysporum* with similar or identical host ranges, such as those causing disease to banana (*F. oxysporum* f.sp. *cubense* Snyder & Hansen)(*Foc*), date palms (*F. oxysporum* f.sp. *elaeidis* Toovey) and cotton (*F. oxysporum* f.sp. *vasinfectum* Snyder & Hansen). A *forma specialis* can be further divided into subgroups, called races (Armstrong and Armstrong, 1981). Races are determined on the basis of virulence to a set of differential cultivars within the same plant species (Armstrong and Armstrong, 1981).

Fusarium oxysporum is an opportunistic pathogen that takes advantage of weakened or injured hosts. The fungus remains dormant in agricultural soils until stimulated by a susceptible host species (Nelson, 1981). It then germinates, infects the roots and colonises the vascular vessels to cause lethal wilts in plants. The pathogen is primarily spread by the movement of infected plants, plant debris and infected soil, but can also be spread by seeds (Green, 1981). It is active under a wide range of environmental conditions and survives in the soil as chlamydospores (Booth, 1971; Kreutzer, 1972). This makes the elimination of the pathogen from soil by conventional control measures very difficult (Armstrong and Armstrong, 1975).

Management of *Fusarium* wilt diseases depends on the integration of different control strategies, since no single method is fully effective on its own. These strategies concentrate on lowering the amount of inoculum in a field, while enhancing plant vigour and disease tolerance (Erwin, 1981). Preventative measures include restricting the introduction of the disease, early detection of the disease, and effective quarantine and

sanitation methods. The most effective method for control of wilt diseases is probably the use of resistant plants, when they are available (Nelson, 1981). The use of cultural control measures like crop rotation have provided some control over the years against many diseases (Baker, 1981), however, propagules of many of the causal agents of vascular wilt diseases stay viable in the soil for extensive periods. Chemical treatments like soil fumigation and foliar spray treatments have been evaluated against wilt diseases and have provided control in certain instances (Erwin, 1981). Chemical control, however, has economical and environmental implications and can lead to the suppression of other beneficial microorganisms (Erwin, 1981). Biological control and *Fusarium* wilt suppressive soils are receiving increasing attention and can prove to play a major role in integrated control practices of wilt diseases in the future (Baker, 1981).

One of the most important *Fusarium* wilt diseases is *Fusarium* wilt of banana (Panama disease). Panama disease almost destroyed the banana export industry, built on the Gros Michel variety, in Central America during the 1950's (Stover, 1962). Gros Michel was replaced by Cavendish varieties that proved to be resistant to *F. oxysporum* f.sp. *cubense* (*Foc*) in Central America. Apart from the use of resistant varieties, management of *Fusarium* wilt of banana has been difficult. Preventing introduction and limiting the spread of the disease has been effective, but little success was achieved in attempts to eliminate the pathogen from the soil. The objective of this review is to summarize knowledge currently available for the management of *Fusarium* wilt of banana, and to propose future management prospects. Management strategies of other *Fusarium* wilt diseases will also be considered.

2. PRODUCTION OF BANANAS

Bananas belong to the genus *Musa*, and form part of the *Musaceae* family, in the order *Zingiberales* (Simmonds, 1962). The banana plant is a monocotyledonous giant herb that consists of a sympodial rhizome from which both the root system and pseudostem, consisting of tightly clasping leaf sheaths, arise (Karamura and Karamura, 1995; Jones, 2000). Flowers are produced when the apical meristem stops producing leaves and forms

an inflorescence. Once flowering has been completed, the pseudostem dies, and new plants develop from suckers that arise freely from the underground rhizome (Jones, 2000). Since edible bananas are completely or nearly female-sterile, it almost never produces seeds. Plant propagation, therefore, depends on the use of vegetative material such as suckers or rhizome pieces (Simmonds, 1959). *In vitro* propagation of bananas was developed to mass produce uniform and disease-free planting material (Israeli *et al.*, 1995). The commercial production of micropropagated bananas can now be found in many countries, and the *in vitro* techniques can also be applied for the genetic improvement of bananas (Israeli *et al.*, 1995).

Edible bananas originated from two diploid species, *Musa acuminata* Colla and *Musa balbisiana* Colla (Simmonds, 1959). Their origin is estimated to be Southeast Asia for *M. acuminata* (genome AA) and the Indian subcontinent for *M. balbisiana* (genome BB) (Dale, 1999). Most cultivated banana cultivars are triploid hybrids of these two species (Dale, 1999). From Asia, bananas were introduced to the Middle East and Africa. It is assumed that Arab traders introduced bananas into Africa, and from there it spread across the continent (Reynolds, 1927). Many further introductions of bananas from India and Asia followed with the expansion of world trade. The Portuguese and Spanish contributed to the final stages in the worldwide distribution of bananas and plantains, when it was introduced into the Americas (Simmonds, 1959). Today, edible bananas are cultivated in many subtropical and tropical regions of the world, including Asia, Africa, Central and South America, the Caribbean and Oceania (Dale, 1999).

Bananas are the fourth most important staple food crop in the world. The fruit can be produced all year round and provides a stable income to farmers in resource poor areas (Jones, 2000). Bananas are divided into two main groups: dessert bananas and cooking bananas (Anon, 1992; Jones, 2000). Dessert bananas form 43% of the world's production of bananas, and are eaten raw when ripe. The Cavendish subgroup consists of the most popular dessert banana cultivars. Cooking bananas, which account for the remaining 57%, are a staple food that needs to be fried, baked, boiled or roasted before it can be eaten (Anon, 1992). Plantains, which are one of the best-known cooking bananas, are

produced by millions of small farmers throughout the tropics and are an important source of food, fibre and income for the people (Dale, 1999).

Diseases and pests are threatening the worldwide production of bananas (Stöver, 1986). Fungi, bacteria, viruses and nematodes affect different parts of the plant, causing substantial yield losses. Fusarium wilt has also been responsible for considerable economical losses and affects many important cultivars of banana (Jeger *et al.*, 1995). Black leaf streak or black Sigatoka is considered an important disease problem of bananas due to its destructiveness and wide distribution (Ploetz *et al.*, 2003). Bacterial diseases, such as Moko disease and bacterial wilt are also very damaging in many parts of the world (Ploetz *et al.*, 2003). Among the virus diseases, banana bunchy top virus (BBTV) is considered the most destructive, and of the nematodes, *Radopholus similis* (Cobb) is considered most important (Jeger *et al.*, 1995).

3. FUSARIUM WILT OF BANANA (PANAMA DISEASE)

3.1 THE PATHOGEN

The causal agent of Fusarium wilt of bananas, *Foc*, is a soil-inhabiting filamentous fungus that belongs to the section Elegans in the genus *Fusarium* (Stover, 1962). The fungus is characterized by micro- and macroconidia that are produced on branched and unbranched monophialides. Microconidia are one- or two-celled, oval- to kidney-shaped and are produced in false heads. Macroconidia are four- to eight-celled and sickle-shaped with foot-shaped basal cells. Chlamydospores are usually globose and are formed singly or in pairs in hyphae or conidia (Nelson *et al.*, 1983). They are resistant to desiccation and unfavourable environmental conditions, and enable the fungus to survive for more than 30 years in the soil after their hosts have been removed. In the presence of roots, chlamydospores or conidia germinate and penetrate susceptible plants (Armstrong and Armstrong, 1975).

Based on pathogenicity to different banana cultivars, three races of *Foc* have been recognized. Race 1 causes disease in the Gros Michel (AAA) and Silk (AAB) cultivars. Race 2 attacks Bluggoe (ABB), and race 4 infects Cavendish (AAA) cultivars and all the cultivars that are susceptible to race 1 and 2 (Pegg *et al.*, 1995). Race 3 has been omitted as a pathogen of banana, as it only attacks *Heliconia* spp. Substantial variation exists within *Foc* as measured by vegetative compatibility, volatile production, electrophoretic karyotyping and various molecular techniques (Miao, 1990; Bentley *et al.*, 1998; Bentley *et al.*, 1999; O'Donnell *et al.*, 1998). Twenty-one vegetative compatibility groups (VCGs) have been identified from a worldwide collection of *Foc* isolates. In South Africa, only one of these VCG's has been found, namely VCG 0120 (Viljoen, 2002).

3.2 HISTORY AND DISTRIBUTION OF FUSARIUM WILT OF BANANA

Fusarium wilt of banana was first reported from Australia, although the pathogen probably originated in Southeast Asia (Stover, 1962). From Southeast Asia it rapidly spread throughout the world by means of infected rhizomes (Stover, 1962). Fusarium wilt became notorious when it became destructive in the Central American region around the turn of the century, and was given the name Panama disease. During the early 1900's the disease was also recorded in Hawaii, South America, Asia and West Africa, and by 1950, it had spread to most of the banana-producing regions of the world (Jeger *et al.*, 1996).

Devastation was caused to the banana industry in the Central American region by Fusarium wilt during the first half of the 19th century (Stover, 1962). At that time the export industry was totally reliant on the Gros Michel variety, which was highly susceptible to *Foc* race 1. The industry was only saved by the replacement of Gros Michel with Cavendish cultivars. Cavendish cultivars remain resistant to race 1, but are susceptible to race 4, where serious losses are caused in the subtropical regions of Australia, South Africa and the Canary Islands, and tropical regions of the Philippines, Malaysia and Australia (Brandes, 1919; Stover, 1962; Bentley *et al.*, 1998).

For many years, *Foc* has been disseminated locally, nationally and internationally by infected planting material, which may not exhibit symptoms (Moore *et al.*, 1995). Once the pathogen is introduced into a disease-free plantation, the disease can spread from mat-to-mat through root contact (Jeger *et al.*, 1995). The pathogen can also be spread by contaminated irrigation water and soil attached to implements, shoes and vehicles (Stover, 1962). Heavy rainfall can lead to increased spread of the pathogen from plant to plant and from the surface down to the roots. The run-off water may contaminate the irrigation reservoirs and increase the spread of the fungus through the plantation (Simmonds, 1959; Stover, 1962).

3.3 DISEASE SYMPTOMS

Foc infects bananas by penetrating the root tips of the small lateral or feeder roots of the plant (Stover, 1962; Beckman, 1990). Penetration takes place through wounds or injuries that expose the xylem vessels to the pathogen (Sequeira *et al.*, 1958). The fungus then invades the water conducting tissue (xylem) where it produces microconidia that are carried up the plant, plugging the vascular tissue and reducing the movement of water. When blocked by sieve cells, the spores germinate and continue to spread until the entire water conducting system is blocked (Stover *et al.*, 1961; Jeger *et al.*, 1995). Internal symptoms of Fusarium wilt of banana become visible as yellow, red or brownish dots and streaks localized in the vascular strands of the rhizome and pseudostem (Wardlaw, 1961). The discoloration of the rhizome is most severe where the stele joins the cortex (Stover, 1962). In advanced stages of infection, the rhizome discoloration is more prolific and the stains are more intense.

The external symptoms of *Foc* are those typical of vascular wilt diseases. Sometimes the disease symptoms are only visible after the bunch has started to form and the plant is under stress (Brandes, 1919). Infected plants, at first, show premature yellowing of the older leaves. The yellowing of the older leaves will start along the leaf margins and continue to the midrib until the leaves are completely brown and die. The yellowing progresses from the older leaves to the younger leaves. Typical external symptoms of

Panama disease are dead leaves hanging down the pseudostem like a skirt. Splitting of the pseudostem, just above the soil level, may also occur. Eventually the heart leaf dies and the pseudostem will remain standing until it is removed or collapses (Brandes, 1919; Wardlaw, 1961; Stover, 1962).

The environment may have an important influence on *Fusarium* wilt development. In a subtropical country such as South Africa, disease symptoms are best observed and most severe after winter (Viljoen, 2002). Ploetz *et al.* (1990) suggested that *Foc* spreads slower in the Natal province of South Africa than in the Kiepersol area because the winters in Natal are warmer, reducing the stress levels of the plant. Studies done by Beckman (1962) on the susceptible cultivar Gros Michel showed that the defence mechanisms in banana plants were affected by temperature. The spread of the fungus was inhibited at 34°C due to gel and tylose formation by the plant. At temperatures of 21° and 27°C the host responses were delayed and this led to pathogen invasion. The resistant cultivar Lacatan, however, was not affected by any of the different temperatures evaluated. Wardlaw (1961) also stated that the onset of a rainy season, or after less active plant growth periods, the disease incidence is higher.

4. MANAGEMENT OF FUSARIUM WILT OF BANANA

Since the discovery of *Fusarium* wilt of banana, various control methods have been attempted to curb the damage caused by the disease. Yet, no long-term control measures are available other than the planting of resistant cultivars (Moore *et al.*, 1999a). Soil fumigation (Herbert and Marx, 1990), fungicides (Lakshmanan *et al.*, 1987), crop rotation (Hwang, 1985; Su *et al.*, 1986), flood-fallowing (Wardlaw, 1961; Stover, 1962) and organic amendments (Stover, 1962) are some of the control strategies that have been investigated in the past. Studies on biological control and soils that are naturally suppressive to *Fusarium* wilt of banana due to beneficial microorganisms have only recently started (Ploetz *et al.*, 2003). Many effective biological control agents can be found for *Fusarium* wilt diseases of other crops (Marois, 1990; Alabouvette, 1999), which makes biological control a promising alternative for managing *Fusarium* wilt of banana.

Current management practices for Fusarium wilt of banana include the use of disease-free tissue culture plantlets, preventing the introduction of the disease in disease-free areas, and the use of proper sanitation methods. The treatment of vehicles, machinery, tools and footwear with an effective surface disinfectant is important (Deacon, 1984). In fields where *Foc* is already present, the planting of resistant cultivars is essential, if such cultivars are acceptable to the local markets.

4.1 BIOLOGICAL CONTROL

Difficulties encountered with the application of fungicides and consumer acceptance of resistant cultivars make biological control of Fusarium wilt of banana an attractive alternative. Biological control can be achieved by means of a direct or indirect interaction between the control agent and the pathogen (Marios, 1990). Direct biocontrol is achieved when the control agent reduces the pathogen population through antagonistic mechanisms such as parasitism, antibiosis, or competition. Parasitism is when a parasite attacks the mycelium and spores of the fungus, while antibiosis refers to the production of toxic metabolites by an organism that may reduce or prevent germination of fungal propagules, invoke lysis, or inhibit growth after germination (Papavizas and Lumsden, 1980). Competition for nutrients or competition for space usually occurs at the infection site (Marios, 1990). Indirect biocontrol occurs when the control agent interacts with the pathogen through the host. This interaction is also referred to as “induced resistance” or “cross protection”, and is based on the induction of the host’s own defence system (Marois, 1990). However it has been stated that many biocontrol agents employ more than one mechanism to protect plants (Fravel and Engelkes, 1994).

Several microorganisms have been associated with biological control of Fusarium wilt diseases. These include some fungal and bacterial genera (Weller *et al.*, 2002).

4.1.1 FUNGI

Biological control of soil-borne pathogens has been achieved by the application of various fungal species, such as the mycoparasitic species of *Trichoderma* and *Gliocladium*, non-pathogenic isolates of *F. oxysporum* and with arbuscular mycorrhizal (AM) fungi (Papavizas, 1985).

***Trichoderma* and *Gliocladium* spp.**

Trichoderma and *Gliocladium* spp. are widely distributed fungi that occur in nearly all soils around the world. Biological control achieved by means of *Trichoderma* and *Gliocladium* spp. may involve different mechanisms. Mycoparasitism and antibiotic production have been suggested to be the mechanisms for biocontrol for years (Papavizas and Lumsden, 1980; Howell, 1982). However, it later became clear that other mechanisms such as the production of enzymes, competition and induced resistance are also involved (Howell, 2003). The enzymes produced by *Trichoderma* spp. usually break down polysaccharides, chitin and β -glucans, thereby resulting in the destruction of the pathogen's cell wall (Howell, 2003). It is stated that the chitinolytic enzymes produced by *Trichoderma harzianum* Rifai is more active and more effective than enzymes from other sources (Chérif and Benhamou, 1990; Harman *et al.*, 1993; Lorito *et al.*, 1993). They produce cell wall degrading enzymes like chitinases and glucanases, which can destroy other fungi, and then use the cell walls of other fungi as sole carbon and energy sources.

Biological control of soil-borne diseases by *Trichoderma* spp. is well documented (Harman *et al.*, 1980; Papavizas and Lewis, 1983; Hadar *et al.*, 1984; Sivan and Chet 1986), although there has always been little data available on the control of Fusarium wilt diseases. In recent years, however, more information on this topic has become available. *Trichoderma hamatum* (Bonord.) Bainier and especially *T. harzianum* have been effective against diseases caused by *F. oxysporum* (Marois *et al.*, 1981; Sivan and Chet, 1986; Datnoff *et al.*, 1995; Larkin and Fravel, 1998; Thangavelu *et al.*, 2003). Marois *et al.* (1981) showed that a conidial suspension of five fungal antagonists, which included three isolates of *T. harzianum*, provided control against Fusarium crown and root rot of

tomato. Similarly, Sivan (1987) reported the control of Fusarium crown rot of tomato by *T. harzianum* under field conditions. Chérif and Benhamou (1990) demonstrated that the chitinolytic activity of *Trichoderma* spp, isolated from a sample of peat collected in New Brunswick (Canada), was responsible for the inhibition of growth of *F. oxysporum* f.sp. *radicis-lycopersici* Jarvis & Shoemaker. *Trichoderma harzianum* T-22, commercially marketed as a granular formulation (RootShield®) or a water-suspendable drench (PlantShield®), has been shown to reduce Fusarium crown and root rot of tomatoes (Datnoff *et al.*, 1995). Larkin and Fravel (1998) reported reduced disease incidence of Fusarium wilt of tomato by strain T-22 (RootShield®) and *T. hamatum* strain TRI-4. However, RootShield® was only effective at concentrations higher than the recommended dosage and both of these strains were not as effective as non-pathogenic *Fusarium* isolates. Interestingly, Rose and Parker (2003) also found that RootShield® did not provide significant control of Fusarium root and stem rot on cucumber seedlings at the recommended rates of application. Thangavelu *et al.* (2003) reported that *T. harzianum* isolate Th-10, isolated from the rhizosphere of banana plants, was effective in inhibiting mycelial growth of *Foc* race 1. They produced a dried formulation of *T. harzianum* grown on banana leaflets. In two field trials the formulation proved to be effective in the control of *Foc*.

The biological control potential of *Gliocladium* spp. against soil-borne diseases has been reported on many occasions (Howell, 1982; Papavizas, 1985; Lumsden and Locke, 1989; Papavizas and Collins, 1990; Zhang *et al.*, 1996; Larkin and Fravel, 1998). *Gliocladium virens* (= *Trichoderma virens*) Miller, Giddens & Foster, showed to control damping-off caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn in soilless mixes (Lumsden and Locke, 1989). Zhang *et al.* (1996) found that seed treatment with different *G. virens* isolates (G-4 and G-6) resulted in the suppression of Fusarium wilt of cotton. Effective *Gliocladium* spp. have been developed into commercial biocontrol formulations. *Gliocladium virens* GL-21 was first formulated into an alginate pill (GlioGard®) and later a granular fluid (SoilGard®). Another commercial product (Primastop®) was developed from *Gliocladium catenulatum* Gilman & Abbott strain J1446, targeting damping-off, seed rot, root rot, and wilt pathogens (Paulitz, 2001).

Larkin and Fravel (1998) found the biocontrol organism *G. virens* G-21 to be very effective against Fusarium wilt of tomato in the greenhouse when applied at concentrations higher than the recommended dosage. Rose and Parker (2003) evaluated both commercial *Gliocladium* biological formulations against Fusarium root and stem rot of greenhouse cucumber. They found that Primastop® reduced the disease compared to the untreated control plants, while SoilGard® did not provide significant control at the recommended dosage.

Non-pathogenic *Fusarium oxysporum*

Studies on Fusarium wilt diseases of important agricultural crops like banana (Gerlach *et al.*, 1999), basil (Fravel and Larkin, 2002), celery (Schneider, 1984), chickpea (Hervás *et al.*, 1995), cucumber (Mandeel and Baker, 1991), cyclamen (Minuto *et al.*, 1995), flax (Lemanceau and Alabouvette, 1991), muskmelon (Freeman *et al.*, 2002), tomato (Lemanceau and Alabouvette, 1991; Larkin and Fravel, 1998), strawberry (Tezuka and Makino, 1991) and watermelon (Larkin *et al.*, 1996; Freeman *et al.*, 2002) have demonstrated the usefulness of non-pathogenic isolates of *F. oxysporum* for the biological control of Fusarium wilt pathogens. Gerlach *et al.* (1999) observed that non-pathogenic *F. oxysporum* had the potential to provide resistance against *Foc* race 4 for the Cavendish cultivar Williams. They found that several endophytic isolates of *F. oxysporum* derived from symptomless banana roots provided a degree of protection against Fusarium wilt of banana, in the greenhouse. Hervás *et al.* (1995) reported a reduction of Fusarium wilt of chickpea incidence and severity due to the prior inoculation of seeds with non-pathogenic *F. oxysporum* isolates. In addition, Freeman *et al.* (2002) reported that two non-pathogenic mutant strains of *F. oxysporum* f.sp. *melonis* Snyder & Hansen effectively reduced the seedling mortality of both muskmelon and watermelon cultivars. It was also found by Larkin and Fravel (1998) that known non-pathogenic *F. oxysporum* isolates and non-pathogenic isolates of *F. oxysporum* isolated from tomato roots, were both highly effective in providing disease control against Fusarium wilt of tomato.

Biocontrol isolate Fo47, is probably the best-studied non-pathogen of *F. oxysporum* isolate. It was first isolated from the Châteaurenard soil in France that is naturally suppressive to Fusarium wilt of tomato and melon (Alabouvette, 1986). Biological control agent Fo47 has made a huge contribution to the greater understanding of the mechanisms involved in Fusarium wilt suppression. Lemanceau *et al.* (1992) and Duijff *et al.* (1998; 1999) observed that the efficacy of Fo47 was related to its inoculum density. Increasing the ratio of Fo47 to pathogen enhanced the suppression, suggesting competition as a mode of action for control.

Three modes of action have been proposed for suppression of Fusarium wilt diseases by non-pathogenic isolates of *F. oxysporum*. The first mode of action is competition for nutrients. Carbon has been implicated as the major nutrient that the pathogen and the non-pathogen compete for in the soil (Couteaudier and Alabouvette, 1990). Lemanceau *et al.* (1993) confirmed this hypothesis in an *in vitro* study of Fusarium wilt of carnation, when carbon was found to be the major nutrient the pathogen competes for against biocontrol agent Fo47. Larkin and Fravel (1999) later confirmed these results by comparing the ability of a small collection of *F. oxysporum* isolates to utilize substrates present in cell walls and growing actively in close vicinity to tomato roots, they demonstrated that growth habits related to carbon utilization are unique to each isolate of *F. oxysporum*. These traits, however, are not necessarily related to pathogenic or antagonistic ability (Steinberg *et al.*, 1999a; 1999b).

The second mode of action is competition for infection sites at the root surface (as epiphytes) and inside the root (as endophytes) (Schneider, 1984; Fravel *et al.*, 2003). Mandeel and Baker (1991) stated that there are a limited number of infection sites that could be protected by increasing the inoculum density of the non-pathogen. Indeed, control of the disease does not necessarily require a high inoculum density of the non-pathogen, but rather a high ratio of non-pathogen to pathogen. Eparvier and Alabouvette (1994) demonstrated that Fo47 was effective in competing with the pathogen at the apex of the flat root. Fo47 reduced both the colonization rate and the activity of the pathogen in the root tissues. More recently it was shown that, beyond active colonization of the

surface of the tomato root, both pathogenic and non-pathogenic isolates were able to penetrate the epidermal cells and colonize the cortex to some extent. This suggested that a plant-mediated competition between the pathogen and non-pathogen occurred within the roots (Postma and Luttikholt, 1996; Olivain and Alabouvette, 1997; 1998). The way a fungus colonizes the root surface and root tissue is not determined solely by the fungus, as the plant genotype and the biotic and abiotic characteristics of the soil can also play a role (Fravel *et al.*, 2003).

The third mechanism of action is induced resistance. Biles and Martyn (1989) were the first to report that induced resistance was the mechanism involved in the control of Fusarium wilt of watermelon by non-pathogenic isolates of *F. oxysporum*. The split root method proved that there was no interaction between the pathogen and the non-pathogen, and that resistance is due to the non-pathogen that triggers a defence response in the plant (Biles and Martyn, 1989; Mandeel and Baker, 1991; Fuchs *et al.*, 1997; Larkin and Fravel, 1999). The split root method involves the exposure of some roots to non-pathogens, and proving that by means of systemic translation of biochemical processes in the plant, it induces resistance to the pathogen in the other non-exposed roots.

Induced resistance caused by non-pathogenic isolates of *F. oxysporum* against Fusarium wilt diseases had been associated with the increased activities of plant enzymes involved in plant resistance. Using GUS-transformed isolates, Olivain and Alabouvette (1997; 1998) showed that the tomato plant reacted to the fungal invasion by expressing defence reactions such as cell wall thickening and intracellular plugging that were more intense in the case of the non-pathogen. These alterations resulted in the increased activity of chitinase, β -1,3-glucanase, and β -1,4-glucosidase (Fuchs *et al.*, 1997). As a result, these defence reactions always prevented the non-pathogen from reaching the stele, whereas the pathogen grew quickly towards and invaded the vessels. Cachinero *et al.* (2002) acquired similar results when a non-pathogenic isolate of *F. oxysporum* was inoculated into chickpea plants to induce resistance. The inoculation resulted in increased activities of chitinase, β -glucanase and peroxidase in plant roots. Induced resistance responses observed in asparagus due to inoculation with non-pathogenic *F. oxysporum* isolates were

associated with the activation of defence-related enzymes such as peroxidase (POX) and phenylalanine ammonia-lyase (PAL), accumulation of lignin, and enhancement of antifungal activity of root exudates (He *et al.*, 2002).

Suppression of Fusarium wilt due to a non-pathogenic *F. oxysporum* isolate is not always linked to just one biocontrol mechanism, and that all three may be involved. Mandeel and Baker (1991) found that the mechanisms of suppression of Fusarium wilt of cucumber by non-pathogenic agents involved competition in the rhizosphere and infection sites, as well as induction of enhanced resistance in the host. Fuchs *et al.* (1997) demonstrated that the suppression of Fusarium wilt of tomato by Fo47 was mainly by means of induced resistance, and proposed that other mechanisms may also be involved. Larkin and Fravel (1999) used the split-root technique and dose requirements to determine the mechanisms of action used by some non-pathogenic *Fusarium* isolates for the control of Fusarium wilt of tomato. Non-pathogenic *F. oxysporum* and *Fusarium solani* (Mart.) Sacc. isolates (CS-20 and CS-1) were found to act by means of induced resistance, while the biological control isolate Fo47 functioned primarily by competition for nutrients. It was also found by Larkin and Fravel (2002) that non-pathogenic *F. oxysporum* isolate CS-20 significantly reduced Fusarium wilt of tomato under varying environmental conditions. Temperatures ranging from cool to hot conditions and optimal conditions had no effect on the suppression caused by CS-20. In four soils, representing different soil textures and properties, CS-20 still effectively reduced the disease. Different pathogenic races and tomato cultivars were also evaluated, but did not affect the efficacy of biocontrol. These results demonstrated that environmental conditions did not have an effect on the control provided by biocontrol agent CS-20 through induced resistance.

The advantages of using non-pathogens of the same or closely related species to the pathogen, are that they have similar environmental requirements than the pathogen. This means that under conditions where the pathogen is most effective, the biocontrol agent will also be most effective. Postma and Rattink (1992) found that a non-pathogenic *F. oxysporum* isolate (618-12), from a healthy carnation plant suppressed Fusarium wilt of carnation to the same extent as the known biocontrol agent Fo47. This isolate probably

lived in close association and under the same environmental conditions as the pathogen. El Hassni *et al.* (2004) also found that the hypo-aggressive isolate of *F. oxysporum*, isolated from date palm, protected palm seedlings against *F. oxysporum* f.sp. *albedinis* Gordon. They also stated that the protection was not due to antagonism or competition, but resulted from induction of the host plant's defence reactions by the hypo-aggressive isolate.

Mycorrhizae

AM fungi are among the most beneficial root-inhabiting organisms known (Schönbeck, 1979). They are symbiotic fungi, and colonize the roots of their host inter- and intracellularly. AM fungi are beneficial to the host plant by increasing the nutrient-uptake ability of the plant roots, by enhancing water transport in the plant, thus increasing growth and yield, and sometimes by providing a physical barrier against invading pathogens (Schönbeck, 1979). In return, they obtain organic nutrients from the plant for their own growth and reproduction.

In bananas, no long-term studies have been reported on the effect that AM fungi have on Fusarium wilt of banana (Ploetz *et al.*, 2003). A greenhouse study done on micropropagated banana plantlets (Grand Naine) showed that the application of two AM fungi (*Glomus* spp.) enhanced plant development and nutrient uptake. It was further reported that the two *Glomus* spp. reduced internal and external symptoms of Fusarium wilt of banana (Jaizme-Vega *et al.*, 1998). Reports can also be found on the influence that AM fungi had on other Fusarium wilts. In Fusarium wilt of tomatoes and cucumber, less stunting and a reduction of infection was observed when AM fungi were established on the roots (Schönbeck, 1979). Hwang (1992) found that inoculations with AM fungi *G. mosseae* (Nicolaj & Gerd.) Gerd. & Trappe and *G. fasciculatus* (Thax.) Gerd. & Trappe significantly reduced the population density of *F. oxysporum* f.sp. *medicaginis* (Weimer) Snyder & Hansen and *Verticillium albo-atrum* Reinke & Berthier on alfalfa seedlings. The presence of AM fungi led to lower disease severity, increased plant growth, and a reduction in the density of *F. oxysporum* f.sp. *medicaginis* and *V. albo-atrum* in the soil. The symptoms of Fusarium root rot of beans were reduced due to colonization with the

AM fungus *Glomus intraradices* Schenk and Sm. The proposed mode of action was direct competition for infection sites between the pathogen and the symbiont (Filion *et al.*, 2003).

4.1.2 BACTERIA

***Pseudomonas* and *Bacillus* spp.**

Rhizosphere bacteria are present in large numbers on plant root surfaces. As certain strains of these bacteria stimulate plant growth, they are referred to as plant growth-promoting rhizobacteria (PGPR) (Ramamoorthy *et al.*, 2001). PGPR may benefit the host by causing plant growth promotion and/or contributing to disease control (Van Loon *et al.*, 1998). In most cases, PGPR that are effective as biocontrol agents of fungal plant diseases belong to the genera *Pseudomonas* and *Bacillus*. Many soil-borne pathogens have proved to be negatively affected by PGPR, including *F. oxysporum* (Weller, 1988). *Pseudomonas fluorescens* has been found particularly effective in this respect.

One of the most predominant mechanisms involved in the suppression of Fusarium wilt by *Pseudomonas* spp. is competition for iron (Fe) through the production of siderophores (Leong, 1986). Under Fe-limiting conditions, siderophores bind to Fe available in the soil in such a way that it becomes unavailable to the pathogen, forming a ferric-siderophore complex. The Fe deficiency leads to the inhibition of chlamydospore germination and hyphal growth, thereby reducing disease incidence (Van Loon *et al.*, 1998). Lemanceau *et al.* (1992) and Duijff *et al.* (1999) provided evidence of antagonistic activity caused by *Pseudomonas putida* WCS358, which was related to the production of siderophores, more specifically pseudobactin, for the control of Fusarium wilt of carnation and flax, respectively.

In some cases, PGPR bacteria suppress plant diseases by means of inducing natural resistance in plants. Resistance induced by PGPR against soil-borne pathogens is usually associated with ultra-structural cell wall modifications that prevents invasion of the pathogen, followed by biochemical changes like the accumulation of pathogenesis-related

(PR) proteins and/or phytoalexins (Ramamoorthy *et al.*, 2001). Tomato plants treated with *P. fluorescens* strain 63-28 inhibited the growth of *F. oxysporum* f.sp. *radicis-lycopersici*, due to production of phenolic compounds, cell wall thickening and the formation of callose (M’Piga *et al.*, 1997). Van Peer *et al.* (1991) demonstrated that *P. fluorescens* strain WCS417r (rifampicin-resistant mutant) induced resistance against Fusarium wilt of carnation and that this induced response led to the accumulation of phytoalexins in the stem. It was proved that WCS417 induce resistance against Fusarium wilt of radish, but this resistance was not achieved via necrosis or PR protein synthesis (Hoffland *et al.*, 1996). Similar results were found in tomato plants when the resistance induced by WCS417 did not lead to the accumulation of PR proteins in the host plant (Duijff *et al.*, 1998). It was suggested that protection provided by WCS417 in radish and tomato was the same as classical systemic acquired resistance (SAR), but mediated via a partially different pathway (Hoffland *et al.*, 1996; Duijff *et al.*, 1998).

Certain PGPR bacteria are able to induce resistance by the production of salicylic acid (SA) (Maurhofer *et al.*, 1994). Leeman *et al.* (1996) hypothesized that systemic resistance against Fusarium wilt of radish by *P. fluorescens* strains WCS374 and WCS417 is affected by the availability of Fe. However, they observed, *in vitro*, that the production of SA increased at low Fe availability, suggesting that resistance induced against Fusarium wilt of radish was due to the production of SA. How SA, produced by *Pseudomonas* spp., induces resistance is not yet clear. It was hypothesized by Leeman *et al.* (1996) that siderophore-mediated competition for Fe, reported many times in the literature as the cause of suppression, may not play such a major part as previously thought. To complicate matters further, Pieterse *et al.* (1996) found that WCS417r reduced symptoms caused by the pathogen *F. oxysporum* f.sp. *raphani* Kendr. & Snyder in *Arabidopsis* by means of induced resistance. However, this induced resistance was not dependant on SA accumulation or on the production of PR proteins.

Several studies have investigated the ability of *P. fluorescens* to suppress Fusarium wilt of banana. Banana plantlets dipped in a suspension of *P. fluorescens* showed protection against race 1 and race 4 of *Foc* in the greenhouse (Sivamani and Gnanamanickam,

1988). Root and plant growth were also enhanced by the application of this bacterium. Thangavelu *et al.* (2001) demonstrated that *P. fluorescens* strain Pf10, which was isolated from the rhizosphere of banana roots, was able to detoxify the fusaric acid produced by *Foc* race 1, and reduced wilt incidence by 50%. Rajappan *et al.* (2002) produced a powder formulation of *P. fluorescens* strain Pf-1 that was selected on the basis of *in vitro* results. They found this formulation to be effective against *Foc* in the field, and that it also increased fruit yield.

Bacterial strains other than *P. fluorescence* and *P. putida* have shown some abilities to reduce Fusarium wilt diseases. In Japan, Toyota *et al.* (1994) isolated bacteria from soil suppressive to Fusarium wilt of radish. They found that, out of all the bacteria isolated from the disease suppressive soil, a single isolate of *Pseudomonas cepacia* was able to suppress the disease to the same extent as was found in the disease suppressive soil. Hervas *et al.* (1998) discovered that disease incidence of Fusarium wilt of chickpea was reduced by the application of *Bacillus subtilis*. Hammad and El-Mohandes (1999) isolated exospore-forming bacterial isolates from the rhizosphere of healthy cucumber plants. Of the isolates, *Bacillus mycoides*, showed antagonistic activity and a reduction in the percentage plants affected by Fusarium wilt of cucumber. Bapat and Shah (2000) found that pigeon pea seeds treated with the antagonist *Bacillus brevis* protected the plant against Fusarium wilt of pigeon pea. -

Actinomycetes

Actinomycetes, and especially the genus *Streptomyces*, have shown antagonistic activity towards certain plant fungal pathogens (Crawford *et al.*, 1993; Yuan and Crawford, 1995). Antagonistic activity of actinomycetes to *Foc* has been reported by Meredith (1943a; 1946). In these reports, the incidence of Fusarium wilt was reduced in soil to which cultures of actinomycetes had been added. Harper (1950) and Rombouts (1953) also observed antagonistic activity by bacteria and actinomycetes towards *Foc*. In recent *in vitro* studies, *Streptomyces violaceusniger* strain G10 showed antagonistic activity against *Foc* race 4 (Getha and Vikineswary, 2002). Antifungal metabolites were proposed to be involved. Although the authors suggested that strain G10 should be considered a

potential biological control agent, no studies were conducted in the greenhouse or in the field (Getha and Vikineswary, 2002).

Streptomyces spp. have also been used in the biological control of Fusarium wilt diseases of cotton (Reddi and Rao, 1971), carnation (Lahdenpera and Oy, 1987), asparagus (Smith *et al.*, 1990) and tomato (El-Shanshoury *et al.*, 1996). Larkin *et al.* (1993) found that higher populations of microorganisms, including higher populations of actinomycetes, occurred in soil where a specific watermelon cultivar was planted. They suggested that the specific watermelon cultivar stimulated the microbial activity, which lead to the suppression of Fusarium wilt. Hammad and El-Mohandes (1999) isolated exospore-forming actinomycetes from the rhizosphere of healthy cucumber plants. They found that one actinomycete isolate, identified as *Streptomyces* spp., showed the highest antagonistic activity against the pathogen responsible for Fusarium wilt of cucumber *in vitro*. Further studies also revealed that the *Streptomyces* spp. lowered the disease incidence of Fusarium wilt of cucumber.

4.1.3 COMBINATIONS OF MICROBIAL AGENTS

It is likely that naturally occurring biological control is a result of the combination of more than one microbial antagonist, rather than from high populations of a single antagonist (Ramamoorthy *et al.*, 2001). Most of the studies done on the combinations of biocontrol agents resulted in improved biocontrol (Raupach and Kloepper, 1998). A mixture of bacterial strains could be more effective for the control of Fusarium wilt than the application of individual bacterial strains (Singh *et al.*, 1999). De Boer *et al.* (1999) showed that the combination of two compatible *Pseudomonas* spp. resulted in a better disease suppression of Fusarium wilt of radish, than a single species. Van Loon (1999) observed that, if *Pseudomonas* spp. are compatible *in vitro*, disease suppression by the combination of these species is greater compared to the individual species.

Enhanced disease suppression has also been achieved by the combination of non-pathogenic *F. oxysporum* and *Pseudomonas* spp. (Lemanceau *et al.*, 1992; Leeman *et al.*,

1996; Duijff *et al.*, 1998; Olivain *et al.*, 2004). Lemanceau *et al.* (1992) and Duijff *et al.* (1998; 1999) demonstrated that the combination of non-pathogenic Fo47 and *P. putida* WCS358 suppressed Fusarium wilt of carnation and flax, respectively. In cucumber, *P. putida* and a non-pathogenic *F. oxysporum* were only effective in suppressing Fusarium wilt when they were applied in combination (Park *et al.*, 1988). In the presence of the non-pathogenic *F. oxysporum*, the population densities of fluorescent pseudomonads increased significantly in the rhizosphere of cucumber in soils with a pH of 8.1. It was hypothesized that the high population of non-pathogenic *F. oxysporum* in the soil enhances the root exudations of the cucumber plant, which in turn increases the activity of the pseudomonads and their siderophore production. This led to competition for Fe and a higher suppression of the disease (Park *et al.*, 1988).

There are reports where the combination of isolates did not result in enhanced disease suppression compared to the separate application of antagonists (Sneh *et al.*, 1984; Larkin and Fravel, 1998). Larkin and Fravel (1998) found that no combinations of biological agents gave better control of Fusarium wilt of tomato than non-pathogenic *Fusarium* isolates alone. The efficacy of the isolates may also vary according to the specific conditions and the host it is applied to (Schisler *et al.*, 1997).

4.1.4 HYPOVIRULENCE

The discovery of hypovirulent isolates of plant pathogens presents a potential for biocontrol that might be applicable to Fusarium wilt diseases. Virus-like particles, found in some important fungal pathogens, have in a few cases been associated with reduced growth rate or reduced virulence to the host (Baker, 1987). The term “hypovirulence” was introduced in the 1960s to describe naturally occurring isolates of the chestnut blight fungus *Cryphonectria parasitica* (Murrill) Barr that exhibited reduced virulence (Nuss and Koltin, 1990). Since then the term has widely been used in association with mycoviruses. Apart from reduced virulence, other properties associated with mycoviruses include the alteration of the colony morphology, suppressed conidiation, reduced oxalate accumulation, reduced laccase production, and reduced pigment production

(Anagnostakis, 1982; Nuss and Koltin, 1990). The virus, or virus-like particles, is transmitted by hyphal anastomosis or by germ tubes. This means that the number of anastomosis within vegetative compatibility groups present in a given area and the effect of the environment on these hypovirulent isolates, influence the spread of mycoviruses (Baker, 1987).

Hypovirulence and the presence of double-stranded RNA (dsRNA) has been reported for a number of plant pathogenic fungi, but no information exists on the use of hypovirulence associated with dsRNA, to control *Fusarium* diseases. A few studies have reported the detection of virus-like particles and dsRNA elements in some *Fusarium* spp. (Chosson *et al.*, 1973; Lapierre *et al.*, 1974; Nogawa *et al.*, 1993; Fekete *et al.*, 1995; Woo *et al.*, 1997; Ozlem and Griffin, 1998;). Chosson *et al.* (1973) reported the presence of virus-like particles in *F. oxysporum* f.sp. *lini* (Bolley) Snyder and Hans., the wilt pathogen of flax. Fekete *et al.* (1995) found the presence of dsRNA elements and encapsidated virus-like particles in 55 geographically different isolates of *Fusarium poae* (Peck) Wollenw. Unfortunately, the dsRNA containing isolates showed no changes in sporulation, nor in any morphological characteristics or signs of degeneration. Ozlem and Griffin (1998) reported the presence of dsRNA in six *F. oxysporum* isolates causing seedling disease of soybean. Four segments of dsRNA, with sizes of 4.0, 3.1, 2.7 and 2.2 kb were detected, but no significant differences were found between dsRNA-containing and dsRNA-free hypovirulent isolates in their effects on disease severity. The question arising from such results is the transmissibility of dsRNA among populations of *F. oxysporum* and their putative role in controlling pathogenic forms of the fungus.

4.2 CHEMICAL CONTROL

Chemical control of *Fusarium* wilt diseases has yielded variable degrees of success. While fungicidal applications often depend on the crop and method of application, other forms of chemical treatment promise to have a more general use. Chemical control can be divided into four different categories: fungicides, surface sterilants, fumigants and plant activators.

4.2.1 FUNGICIDES

Some measure of success against Fusarium wilt diseases has been achieved with fungicides belonging to the benzimidazole group, which include fungicides such as benomyl, carbendazim, thiabendazole and thiophanate. Most benzimidazoles are converted to methyl benzimidazole carbamate (MBC, carbendazin) when it comes in contact with the plant surface (Erwin, 1973; Agrios, 1997). Benomyl provided control of Fusarium wilt when it was applied as a drench or a spray on young tomato plants in the greenhouse (Thanassouloupoulos *et al.*, 1970), and as a soil drench on muskmelon (Maraite and Meyer, 1971). Benomyl and thiabendazole also provided control of Fusarium wilt of sweetpotato when the propagated roots, or sprouts, were dipped in the chemical compounds (Nielsen, 1977). Complete control of wilt of chrysanthemum was obtained when benomyl was applied with lime-nitrogen soil amendments (Engelhard and Woltz, 1973). Carbendazim, thiophanate and triophanatemethyl have been used to control Fusarium wilt of cucurbits (Li and Liu, 1990).

Widespread resistance to methyl benzimidazole carbamate has been observed in field pathogens (Baldwin and Rathmell, 1988). Because of this resistance, many new classes of fungicides have been introduced. These include strobilurins, phenylpyrroles, anilinopyrimidines, phenoxyquinolines, oxazolidinediones, spiroketalamines, diarylamines and scytalone dehydrase inhibitors (Gullino *et al.*, 2000). Gullino *et al.* (2002) compared the effectiveness of three strobilurins (azoxystrobin, kresoxym-methyl and trifloxystrobin) with benomyl against Fusarium wilts of carnation, cyclamen, and Paris daisy. In the case of Paris daisy, prochloraz was also included for comparison. Azoxystrobin provided control against Fusarium wilts of the three crops that was similar or better than the control provided by benomyl and prochloraz. In China, an organo-copper fungicide called Homodemycine (HDE) was developed for use against Fusarium wilt of cucurbits (Li and Liu, 1990). It was found that HDE not only effectively controlled wilt disease of cucurbits, but also stimulated the growth of the plant (Li and Liu, 1990).

Various fungicides have been evaluated for their effectiveness against *Foc*. In a study conducted by Meredith (1943b), mercury compounds were found to be the most effective of several chemicals applied to kill *Foc* in air-dried soil. However, no long-term control could be established with the application of mercury compounds in the field (Meredith, 1943b). During 1959 and 1960, Corden and Young (1959; 1960) screened many different fungicides for their fungistatic activity against *Foc*. The chemical R&H-3888 (nitrile) was found to be most effective, followed by EP-161 (methyl isothiocyanate), Vapam (sodium n-methyl dithiocarbamate), allyl alcohol, and Mylone (3,5-dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione). The latter compounds were all approximately equal in their potential for Fusarium wilt control. Nabam was also highly effective when mixed into the soil, while CP 30249 [2-chloro-3-(tolylsulfonyl) propionitrile] was found to be less effective than the other chemicals, due to its failure to move in soil. Application of excess water can, however, increase the activity of CP 30249 in the soil.

In 1959-1960, Phelps performed field experiments in a non-fallowed area in Honduras (Stover, 1962). Laboratory and greenhouse screenings were first conducted and the chemicals Vapan, Mylone, allyl alcohol and formaldehyde performed best when applied as drenches to the soil at that time. A previously flood-fallowed area was then used for the final evaluation of these chemicals. After 9 months, no control by any of the chemicals was found, as the entire banana plantation was diseased. The results demonstrated that the best soil fungicides commercially available at the time were not effective in controlling *Foc* (Stover, 1962). Later, in India, it was reported that rhizome injections of carbendazim provided some short-term protection for Silk plants (Lakshmanan *et al.*, 1987). However, stem injections with carbendazim in South Africa proved to be ineffective (Herbert and Marx, 1990).

Phosphonate fungicides have shown the potential to control fungi other than *Oomycetes* (Heaton and Dullahide 1990; Guest and Grant 1991), and even showed potential control against *Foc* (Davis *et al.*, 1994). Davis *et al.* (1994) observed that phosphonate reduced the growth of *Foc in vitro*. It was also found that the extent of inhibition was affected by the amount of phosphate available to the fungus. *Fusarium oxysporum* f.sp. *dianthi* (Prill.

and Delacr.) Snyder and Hansen and *Fusarium avenaceum* (Fr.) also showed sensitivity towards phosphonate *in vitro*.

4.2.2 SURFACE STERILANTS

The spread of *Fusarium* wilt of banana can effectively be prevented if proper disinfectants or surface sterilants are used. However, in the past disinfecting *Foc*-infested soil on a small scale by drenching with copper sulphate, carbolineum and formaldehyde proved to be unsuccessful and expensive (Brandes, 1919). In Jamaica, Rishbeth and Naylor (1957) conducted studies on disinfectants and found that carbon disulphide was ineffective and formalin gave unreliable results, although it reduced the population of *Foc* in the soil. The application of surface disinfectants, however, is more effective and appropriate for the cleaning of equipment, shoes and machinery.

In Australia, a commercial liquid washing product called 'Farmcleanse' was found to be the most effective disinfectant against *Foc* spores when compared to other fungicides and quaternary ammonium compounds (Moore *et al.*, 1999b). 'Farmcleanse' is a detergent-based degreaser with a quaternary ammonium component that is now used for the disinfection of vehicles, farm equipment and in footbaths. In Australia, 'Farmcleanse' has replaced the previously used chlorine bleach, methylated spirits and copper oxychloride solutions as disinfectant since it is non-corrosive and environmentally friendly (Moore *et al.*, 1999b).

4.2.3 SOIL FUMIGATION

Fumigation has been considered as control strategy to eradicate *Foc* from infested banana soils. Rishbeth and Naylor (1957) attempted to fumigate banana soils in Jamaica without success. Fumigation is said to be effective in containing outbreaks where *Fusarium* wilt is detected at an early stage. In South Africa, the spread of early-detected *Foc* was stopped by treatment of the infested sites with methyl bromide. Unfortunately, *Fusarium* wilt was suppressed for approximately 2 years only, after which the disease spread even more

rapidly than before (Herbert and Marx, 1990). Soil fumigation is considered to be very expensive and can be considered only as a short-term control measure for Fusarium wilt of banana.

4.2.4 PLANT ACTIVATORS

Plants have the ability to activate their own defence mechanisms against attack by foreign pathogens and pests (Kessmann *et al.*, 1994; Ryals *et al.*, 1994). These mechanisms fail when a pathogen is able to avoid triggering or suppresses the resistance reactions, or evades the effects of activated defences. Resistance in a plant may be expressed locally at the site of infection, known as locally acquired resistance (LAR), or may spread systemically to all parts of the plant, known as systemically acquired resistance (SAR) or induced systemic resistance (ISR) (Hammerschmidt *et al.*, 2001). Acquired or induced resistance of plants against pathogens can be achieved by inoculating a plant with incompatible or weak pathogens (Gessler and Kuć, 1982). It has also been found that certain natural and synthetic chemical compounds can activate systemic resistance responses in plants to protect them against pathogen attack (Kessmann *et al.*, 1994).

The activation of plant defence mechanisms is associated with different signal transduction pathways, depending on the initial stimulus. The plant hormones SA, ethylene (ET) and jasmonic acid (JA) are the major players in the network of defence signalling pathways. They have been implicated in acting as secondary signals following pathogen attack (Reymond *et al.*, 2000; Schenk *et al.*, 2000). There is also increasing evidence that interactions exist between the different defence signalling pathways (Genoud and Métraux, 1999; Pieterse *et al.*, 2001). This cross talk between the pathways provides a great regulatory potential for activating multiple resistance mechanisms (Pieterse *et al.*, 2001). Activation of SAR pathways is associated with the transcriptional activation of genes encoding for PR proteins, also termed SAR proteins (Tally *et al.*, 1999). Some of these PR proteins have antifungal or antibacterial activities (Hammerschmidt *et al.*, 2001).

The most thoroughly investigated chemical inducers are those interfering with the SA pathway, such as 2,6-dichloroisonicotinic acid (INA) and benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) commercially known as Bion[®] (Oostendorp *et al.*, 2001). Exogenous application of INA protected cucumber plants against a spectrum of diseases to the same extent as the protection provided after local pre-infection with inducing microorganisms. Also, INA showed no antimicrobial activity *in vitro*, making it compliant with the criteria of a plant activator (Métraux *et al.*, 1991). It has been found that the resistance induced by BTH in monocots appears to be longer lasting than that induced in dicots (Tally *et al.*, 1999). Foliar applications of BTH prior to inoculation provided protection of banana against *Foc* race 4 for approximately 6 weeks after inoculation (Moore *et al.*, 1999b). The exogenous application of BTH also effectively suppressed black Sigatoka leaf disease in banana (Tally *et al.*, 1999). BTH has been included in a number of crop management programmes and has yielded better results when combined with low rates of fungicides or bactericides (Tally *et al.*, 1999).

Other potential resistance-activating chemicals include D,L- β -aminobutyric acid (BABA), which has been reported to activate disease resistance in various crops, especially against the downy mildews (Cohen, 1994a; Tosi *et al.*, 1998; Silué *et al.*, 2002). BABA, as a foliar spray or as a soil drench, also showed widespread action against soil-borne fungi and nematodes (Cohen, 1994b). The mechanism by which this compound induces resistance is still not fully understood. It is not clear if the SA pathway or other pathways are involved in resistance induced by BABA (Cohen 1994a; Siegrist *et al.*, 2000). Another compound, probenazole, was shown to protect rice plants against rice blast and bacterial blight through the activation of host defence mechanisms (Watanabe *et al.*, 1977; Kato *et al.*, 1984). However, *in vitro* results suggested that part of the protection provided by probenazole was due to a direct effect on the pathogen (Watanabe *et al.*, 1977). Cohen *et al.* (1993) published the first report on the local and systemic resistance that jasmonates induced in potato and tomato plants against fungal pathogens. Ethylene or ethylene-releasing compounds have also been reported to activate resistance against some fungal, bacterial and viral diseases (Spanu and Boller, 1989; Ciardi *et al.*, 2000; Knoester *et al.*, 2001). The protein harpin, produced by the pathogenic bacterium

Erwinia amylovora, has been found to induce systemic resistance in plants against various fungi, bacteria and viruses (Brasher, 2000).

4.3 CULTURAL CONTROL

Cultural control should be considered as one of the most important approaches for the management of Fusarium wilt diseases. This approach is environmentally friendly, affordable, and is based on the exclusion of the pathogen, the reduction of pathogen effect, and the enhancement of plant vigour and resistance.

4.3.1 TISSUE CULTURE BANANAS

The primary means whereby *Foc* is introduced into new banana production areas, is by the planting of infected material (Jeger *et al.*, 1995). Since the banana is a vegetatively reproducing plant, the offspring (suckers) could be infected by *Foc* without showing visible symptoms. Planting of infected suckers and corms in Central America was responsible for one of the most important epidemics of plant diseases in agricultural history (Stover, 1962). Today infected suckers are still planted in disease-free areas, spreading the disease to new banana fields around the world.

The use of banana tissue culture plants is a major step towards preventing the spread of Fusarium wilt to disease-free fields (Moore *et al.*, 1999a). Tissue culture plantlets are free of bacterial, fungal and nematode pathogens, they grow more vigorously, have a shorter and more uniform production cycle, and they produce fruit of better quality than suckers (Hwang, 1999). However, if planted in *Foc*-infested fields, they are more vulnerable to Fusarium wilt than plants grown from conventional planting material (Smith *et al.*, 1998). It was suggested that conventional planting material has the advantage of being surrounded by microbial populations with the potential of being antagonists. Conventional planting material is also better adapted to environmental conditions and stress factors than tissue-culture plants that were grown under sterile conditions. Therefore, tissue culture plants need to be exposed to non-deleterious microbes

(including bacteria, fungi, and mycorrhizae) during the period of hardening of before field planting. This can be achieved by using an inoculated potting mixture that will ensure colonization of the root system of banana plants.

4.3.2 QUARANTINE AND SANITATION

Quarantine and sanitation is aimed at the reduction or elimination of the amount of inoculum in a field, plant, or warehouse, to prevent the spread of the pathogen to uninfected areas, and to prevent greater losses due to the spread of the pathogen (Agrios, 1997). The importance of effective quarantine and sanitation methods for the control of *Fusarium* wilt of banana has been emphasised many times (Moore *et al.*, 1999b; Viljoen, 2002).

In South Africa, legislation prevents the movement of banana corms, suckers and soil from one production area to another (Viljoen, 2002). Deacon (1984) recommended several quarantine and sanitation practices to the South African farmers to lower the spread of *Fusarium* wilt. These practices have also been well documented by several other researchers (Jeger *et al.*, 1995; Moore *et al.*, 1999a; 1999b). Once a new outbreak of *Fusarium* wilt of banana is discovered, infected plants should be killed by injecting them and surrounding plants with herbicides such as Roundup® /glyphosate (Moore *et al.*, 1999b). This is done to reduce the amount of inoculum by killing its preferred host plant and to prevent the mat-to-mat spread of the pathogen. The diseased areas should then be 'sealed off' by fencing-in of diseased plants, and by digging a trench around the infected area to prevent the movement of fungal spores in surface run-off water. If the movement of run-off water is not restricted, irrigation sources may eventually become infected. Other recommendations include the use of an effective surface sterilant or disinfectant for the sterilization of farm equipment and other implements that are used during banana production. To avoid the movement of infested soils or plant matter from a diseased block to disease-free blocks, it is suggested that footwear and vehicles should be disinfected, especially between plantations or blocks within a plantation. Regular field

evaluations and early detection of diseased plants could contribute to long-term disease control (Deacon, 1984; Moore *et al.*, 1999b).

4.3.3 CROP ROTATION

By withholding a pathogen's host through crop rotations, it is possible to reduce the pathogen populations in the soil (Baker, 1981). The principle of crop rotation is based on the hypothesis that, with a non-susceptible crop, pathogen levels can be reduced and the cycle of disease build-up in the soil broken. Although crop rotation may not be effective for all pathogens, some instances can be found where crop rotations have contributed substantially to disease management (Martin, 2003).

Crop rotation was evaluated for *Fusarium* wilt management in banana plantations. Sequeira *et al.* (1958) investigated the effect on banana following rotation with velvet beans, sorghum or sugarcane planted for 1 year in *Fusarium* wilt-affected fields. While rotation with sugarcane was found the most effective, the results were not sufficiently convincing. Sequeira (1962a) also found that crop rotation with sugarcane, combined with fallow rotations, reduced the disease incidence significantly. However, in Taiwan, crop rotation with sugarcane or sunflowers for 3 years showed no reduction in *Fusarium* wilt incidence (Hwang, 1985). Crop rotation with paddy-rice has been considered as a control measure against *Fusarium* wilt of banana in Taiwan. Unfortunately, it proved to be only a short-term measure (Hwang, 1985; Su *et al.*, 1986). Meng *et al.* (1999) reported that intercropping with oil palm resulted in a disease incidence of 4.9% after 24 months of inter-cropping, and further trials in this intercropping system is currently in progress. Interestingly, Rishbeth (1955) found that pathogens isolated from sugarcane and oil palm were morphologically similar to *Foc*, but the authors did not inoculate the Gros Michel plants to confirm their pathogenicity. Long-term control of *Fusarium* wilt of banana is unlikely to be achieved through crop rotation, since *Foc* can survive in the soil for long periods without a host (Stover, 1962).

4.3.4 SOIL AMENDMENTS AND FERTILIZERS

Soil amendments, fertilizers and the manipulation of soil pH are the most important crop management strategies (Alabouvette *et al.*, 2004). Proper fertilization can lead to improved plant vigour, which in turn helps to increase the resistance of the plant. It can also have significant effects on the microbial populations in the soil (Alabouvette *et al.*, 2004). Previous studies have shown the influence that Fe availability and soil acidity have on the incidence of diseases caused by *Fusarium* (Scher and Baker, 1982; Domínguez *et al.*, 1995; Peng *et al.*, 1999; Domínguez *et al.*, 2001). The more alkaline the soil is, the less Fe is available to the organisms (Duijff *et al.*, 1995). It is also known that acid soils are more conducive to *Fusarium* wilt diseases and that alkaline soils are more favourable to soil bacteria (Scher and Baker, 1980; Domínguez *et al.*, 2001).

Various soil treatments have been applied in an effort to reduce the incidence of *Fusarium* wilt of banana (Rishbeth and Naylor, 1957; Stover, 1962). In 1923-1927, Knudson performed a series of fertilizer experiments using various fertilizing materials. The results showed that none of the fertilizers evaluated had an influence on the disease incidence (Stover, 1962). Similarly in 1960, Butler found no effect on disease development after extensive experiments with NPK and minor elements in Honduras (Stover, 1962). Meredith (1941) stated that Na nitrate is replacing other nitrogenous fertilizers on many of the larger properties in Jamaica, after the fertilizer has been evaluated in culture. Later in Jamaica, various combinations of inorganic fertilizers, lime, green manure, compost and bagasse were evaluated. It was found that none of the treatments reduced the disease, and the mineral nitrogen fertilizers even enhanced the spread of the disease (Rishbeth and Naylor, 1957). Simmonds (1959) stated that too much sulphate of ammonia is most effective in enhancing *Fusarium* wilt.

Disease severity of *Fusarium* wilt is increased with the application of the ammonium form of nitrogen and decreased with the application of the nitrate forms of nitrogen. The nitrate form of nitrogen tends to make the root zone less acidic. Conversely, the high pH is lost by adding acidifying ammonium nitrogen (Huber and Watson, 1974). Soil

amendments with different sources of Ca or organic matter have proved to be ineffective against Fusarium wilt of banana in South Africa (Ploetz *et al.*, 1990), although it was suggested as control measure by others (Sequeira, 1962b; Sun and Huang, 1985). Peng *et al.* (1999) studied the impact of temperature, soil water content, pH, Ca and Fe on the germination of chlamydospores and disease symptoms in banana plantlets in suppressive and conductive soils. They stated that the suppressiveness of banana wilt could be manipulated with soil amendments. However, these experiments were short-term and the results were not demonstrated in the field.

The decomposition of organic matter is associated with increases of microbial biomass and microbial activities, resulting in enhancement of general suppression. Therefore, addition of organic amendments has been successfully used to increase soil suppressiveness to diseases, including nematode diseases (Lumsden *et al.*, 1983; Cotten and Munkvold, 1998; Serra-Wittling *et al.*, 1996; Widmer *et al.*, 2002) as well as disease suppression in horticultural crops (Hoitink and Boehm, 1999; Tilston *et al.*, 2002; Cotxarrera *et al.*, 2002;). The impact of organic amendments on the infectious activity of soil-borne pathogens is not a general rule. Fusarium wilt or *Pythium* diseases are generally better controlled than *Rhizoctonia solani* diseases (Tuitert *et al.*, 1998; Hoitink and Boehm, 1999). Control of soil-borne plant pathogens was improved using the incorporation of fresh organic matter in combination with a plastic cover and solar heating (Blok *et al.*, 2000). Although modifications of functional diversity as well as genotypic diversity of microbial communities have been reported (Diab *et al.*, 2003; Edel-Hermann *et al.*, 2004), it has been very difficult to reveal the particular stimulation of specific indigenous populations exerting an antagonistic activity towards the pathogens.

4.3.5 FLOOD FALLOWING

Flood fallowing implies the flooding of an agricultural land which is infested with a pathogen with water, and keeping it flooded for several months in an attempt to create nearly anaerobic conditions that will reduce the survival of the pathogen. In this way,

promising results were achieved for the control of Fusarium wilt in Central America in the 1960s (Stover, 1962). The lands were flooded at a depth of 2 to 5 feet, and for a period of about 6 months, which was usually followed by reflooding and replanting. It proved to be a very effective control method, especially during the first cycle after planting, but failed after the second cycle (Wardlaw, 1961).

Complete eradication of *Foc* was not achieved by flood fallowing, and it was speculated that the water used to maintain the flood level was contaminated (Simmonds, 1959). The surviving pathogens were further reduced by the application of fungicides to the soil surface as a drench after flood fallowing and draining. These chemical treatments were excessively expensive. Ferrous sulphate and calcium cyanide were found to be very effective in eradicating *Foc* from banana fields after they were flooded (Wardlaw, 1961). Although soil treatments consisting of fungicides alone were found ineffective, Stover *et al.* (1961) reported significant reductions in the disease development after fungicides such as Vapam, Mylone, allyl alcohol, or formaldehyde were applied to soil after flooding for 3 to 6 months. But even these treatments did not ensure control in some areas at that time. Later, Stover (1990) speculated that flood fallowing might have destroyed the natural suppressiveness of the soil.

4.4 HOST RESISTANCE

Host resistance has proved to be the most effective means to control Fusarium wilt diseases. In most agricultural crops, resistance in plants can be bred for. However, in the absence of seeds, such resistance can only be introduced through unconventional improvement techniques, such as somaclonal variation or genetic transformation.

4.4.1 NATURAL RESISTANCE

Natural resistance to most microorganisms exists in all plants (Agrios, 1997). However, certain microorganisms can overcome the defence mechanisms of a plant and cause disease. Even though it is found that a cultivar or cultivars of a particular agricultural

crop is susceptible to a plant pathogen, it is still possible that resistance to the pathogen might be present in other cultivars, wild types, or closely related plant families. Resistance against a pathogen is based on the plant's ability to rapidly recognize the pathogen and activate its own defence mechanisms.

In banana plants, resistance to races of *Foc* exists in some cultivars, wild species and synthetic diploids developed through breeding programmes (Jeger *et al.*, 1995). The pathogen infects both resistant and tolerant cultivars, and host resistance is only expressed after infection (Beckman, 1987; Beckman, 1990). The success of resistance responses will depend on the rate and extent of host responses. In banana, this response is based on the ability of plants to produce phenolics, deposit lignin, and increase enzymes involved in cell wall strengthening in the tolerant cultivar (Beckman, 1990; De Ascensao and Dubery, 2000).

4.4.2 PLANT BREEDING

The first banana breeding programme at the Fundación Hondureña de Investigación Agrícola (FHIA) was established in Honduras in 1959. The aim of this programme was to develop resistant cultivars against *Foc* race 1 that destroyed the Gros Michel in Central America (Jones, 2000). During the breeding process, fertile diploid pollen from resistant male plants was applied to female flowers of Gros Michel, or other specific triploid clones with female fertility, to obtain resistant tetraploid hybrids (Cheeseman, 1932; Jones, 2000). However, breeding in banana was difficult, as the process is slow and the number of fertile seeds that are obtained is extremely low. During the 1960s, the Cavendish subgroup of bananas replaced Gros Michel cultivar and now dominates the international banana export industry. Since Cavendish cultivars are susceptible to race 4 of *Foc*, an urgent need developed to breed for a resistant Cavendish-type banana. Unfortunately, Cavendish cultivars are sterile, and conventional breeding methods could not be used to improve this subgroup (Ortiz *et al.*, 1995). The FHIA breeding programme has produced several hybrids with resistance to both *Foc* race 1 and 4, such as FHIA-01 (AAAB) (Moore *et al.*, 1995; Jones, 2000) and SH-3640/10 ('High Noon') (Eckstein *et*

al., 1996; De Beer, 1997). The taste of tetraploid bananas produced at FHIA, however, is not acceptable to the Cavendish markets. Attempts are now made to breed for triploid hybrid bananas in Honduras (Rowe and Richardson, 1975) and Guadeloupe (Vakili, 1967; Stover and Buddenhagen, 1986).

Three banana breeding programmes other than the FHIA program also exist. These programs are located in Brazil (EMBRAPA-CNPMPF), Nigeria (IITA), and Guadeloupe (CIRAD-FLHOR) (Jones, 2000). Two non-conventional programmes can be found in Australia (IAEA) and Taiwan (TBRI). Although some of these programmes focus more on resistance to black Sigatoka and other banana diseases, three programmes have shown positive results toward the development of *Foc* resistant cultivars (Ploetz, 1994). The EMBRAPA breeding programme has been aiming at generating Maçã, Prata and other types of variants to replace susceptible cultivars, especially the Maçã (Silk) type (AAB), which is preferred by consumers in Brazil. They used conventional and non-conventional techniques to generate Maçã type variants, which have proved successful. Their programme managed to obtain some Prata, Maçã and Prata Anã tetraploid hybrids, which showed resistance to *Foc* (De Matos *et al.*, 1999).

4.4.3 UNCONVENTIONAL IMPROVEMENT

Natural somatic mutations in banana have been observed since the development of micropropagation techniques for banana using meristem cultures (Hwang *et al.*, 1984). Variants can be grouped into changes in plant stature, leaf morphology, pseudostem colour and bunch characteristics (Hwang and Tang, 2000). It was found that 95% of somaclonal variants are genetically stable, which is an important feature for the improvement of banana cultivars (Hwang and Tang, 2000). At the Taiwan Banana Research Institute (TBRI) in Taiwan, resistant clones have been successfully selected from somaclonal variants since 1983. In the process, a moderately resistant clone GCTCV-215-1 and a highly resistant clone GCTCV-218 have been identified. Both the clones were derived from Giant Cavendish and showed resistance to *Foc* tropical race 4. GCTCV-218, however, proved to have much higher resistance than the previously

selected clones (Hwang, 1999). In addition to somaclonal variation, mutation breeding can be achieved by exposing plant tissue to irradiation and chemical mutagens (Ortiz *et al.*, 1995).

Plant improvement can combine *in vitro* techniques with biotechnological techniques such as protoplast fusion or genetic transformation (Agrios, 1997). Protoplasts under stable conditions can form clusters that develop into callus, which in turn can be used to regenerate plants through protoplast fusion or electroporation. Using this technique, triploid banana plants can be formed by the fusion of a diploid banana cell with a monoploid banana cell (Novak *et al.*, 1989; Megia *et al.*, 1992; Assani *et al.*, 2001). Transformation of bananas has been achieved through particle bombardment (Sági *et al.*, 1995; Becker *et al.*, 2000) and *Agrobacterium tumefaciens*-mediated transformation (Ganapathi *et al.*, 2001; Chakrabarti *et al.*, 2003). Genetic improvement offers the potential to generate a banana plant with resistance to Fusarium wilt and other important banana diseases like black Sigatoka.

4.5 INTEGRATED DISEASE MANAGEMENT

Several aspects of disease control are sometimes combined to achieve a more holistic and sustainable way of disease management. This approach to plant disease control is referred to as integrated disease management (Agrios, 1997). Integrated disease management is usually associated with the control of all the diseases affecting a crop. However, when substantial losses are found due to a specific disease, an integrated disease programme will be developed for that disease. By the combination of more than one control measure it is possible to reduce the initial inoculum, increase the plant resistance, delay the disease development and reduce the effectiveness of initial inoculum at the same time (Agrios, 1997).

Cultural control is sometimes combined with chemical control and host resistance for the control of root diseases (Summer, 1994). In 1987, Baker (1987) proposed that efforts should be made to develop antagonists that are resistant to chemicals so that they can be

applied together (Baker, 1987). Rowe and Farley (1978) achieved control of Fusarium crown and root rot of greenhouse tomatoes by the combination of chemical and biological control. After the activity of the chemical disappeared, the microbial population, which was established in the treated soil, continued to provide protection. A benomyl-resistant biotype of *Trichoderma viride* Pers. (T-1-R9) in combination with a minimal number of benomyl drenches provided control equal to a commercial integrated control procedure against Fusarium wilt of chrysanthemums in the greenhouse (Locke, 1985). These results provided great benefit to the field control of Fusarium wilt of chrysanthemums, where benomyl drenches have been part of their control strategy (Locke, 1985). Minuto *et al.* (1995) reported that the control of Fusarium wilt of cyclamen could be improved by the combined applications of antagonistic *F. oxysporum* isolates with benzimidazole fungicides. Rose and Parker (2003) also observed that in pathogen-infested medium, seed treatment with *Pseudomonas chlororaphis* strain 63-28 and the fungicide thiram significantly reduced plant mortality caused by Fusarium root and stem rot.

Examples of integrated approaches for the control of Fusarium wilt of banana can be found. In 1923-1927, Knudson performed a series of liming trials on infested soils in Guatemala (Stover, 1962). This was combined with crop rotation and the addition of potassium and phosphate fertilizers. However, these combinations had no effect on the disease outbreaks when Gros Michel cultivar was replanted (Stover, 1962). As mentioned earlier, flood fallowing was combined with chemical applications with the aim of reaching a greater degree of control. Unfortunately, no long-term control was ever achieved with these combinations (Stover, 1962). In South Africa, Deacon (1984) recommended the combination of chemical and cultural control by suggesting that every banana sucker should be washed free of soil and inspected for the presence of the disease, followed by a fungicide dip treatment, before planting. He also suggested that weeds should be controlled by means of herbicide application instead of hoe to ensure that the infected soil is not disturbed. In Malaysia, chemical treatments such as Beret and formalin are combined with cultural practices (Meng *et al.*, 1999). However, these attempts failed to control the spread of the pathogen.

Few reports can be found on the integrated management of Fusarium wilt of banana through the use of biocontrol agents. A study done on *P. fluorescens* in combination with wheat bran (saw dust) resulted in a reduced vascular discoloration (Raghuchander *et al.*, 1997). In another study, Saravanan *et al.* (2003) found that the combination of organic amendment (neem cake) with *P. fluorescens* showed the greatest suppression of *Foc* race 1 among other biocontrol combinations evaluated in the greenhouse and in the field. Smith *et al.* (1999) proposed the application of biocontrol agents, isolated from banana roots grown in Fusarium wilt suppressive soil, to tissue-culture plantlets in the nursery. By the application of these biocontrol agents, the banana roots may have a better chance of protection against *Foc*.

5. SUPPRESSIVE SOILS

The occurrence of disease suppressive soils in banana fields requires special consideration in any management strategy for Fusarium wilt. Suppressive soils are soils in which disease severity is reduced despite the presence of the pathogen, susceptible host and favourable environmental conditions (Alabouvette *et al.*, 1993). Fusarium wilt diseases are probably the pathosystems in which suppressive soils have been best studied. The reason for disease suppression is not yet well understood, but may be related to the biological, chemical and physical composition of soils (Louvet *et al.*, 1981; Höper and Alabouvette, 1996).

It has been shown that microorganisms and their interactions are the basis of Fusarium wilt suppressiveness (Alabouvette *et al.*, 1993). In studies of Fusarium wilt suppressive soils, biocidal treatments (steam, methyl bromide, gamma radiation) were used to destroy the microorganisms in the soil, causing the suppressiveness to disappear. The suppressiveness was restored when small amounts of untreated suppressive soils were added to the previously treated suppressive soil (Scher and Baker, 1980; Schneider, 1984; Alabouvette, 1986). Baker (1987) reported that suppressiveness to *formae speciales* of *F. oxysporum* is usually specific, meaning that the suppression is usually linked to a specific organism or organisms. Several groups of microorganisms have been proposed to

contribute to the Fusarium wilt suppressiveness, e.g. *Bacillus*, *Trichoderma* (Sivan and Chet, 1989), *Pseudomonas* (Scher and Baker, 1982; Lemanceau and Alabouvette, 1993), actinomycetes (Peng *et al.*, 1999), and non-pathogenic *F. oxysporum* (Alabouvette, 1986; Larkin *et al.*, 1996; Larkin and Fravel, 1998; 1999). Non-pathogenic *F. oxysporum* has most frequently been linked to soil suppressiveness, along with fluorescent *Pseudomonas* spp. (Scher and Baker, 1982; Alabouvette, 1990; Lemanceau and Alabouvette, 1991; Lemanceau *et al.*, 1992; Lemanceau *et al.*, 1993; Duijff *et al.*, 1998; 1999). Both these microbial groups have showed mechanisms of competition and induced resistance.

Some physical and chemical soil properties can contribute to the suppression provided by microorganisms. It was found that acidic soils are more conducive to Fusarium wilt diseases and alkaline soils are more favourable to growth of soil bacteria (Scher and Baker, 1980; Domínguez *et al.*, 2001). The amount of Fe available in the soil is influenced by the pH of that soil. The more alkaline the soil, the less Fe is available to the organisms. This leads to the hypothesis that the application of *Pseudomonas* can induce soil suppressiveness (Scher and Baker, 1982; Van Loon *et al.*, 1998). Scher and Baker (1982) proved this hypothesis by amending a chelator, ferric ethylenediaminedi-*O*-hydroxyphenylacetic acid (FeEDDHA) and *P. putida*, isolated from a Fusarium-suppressive soil, into a conducive soil, and the soil became suppressive to Fusarium wilt of flax, cucumber and radish. Raaijmakers *et al.* (1995) also demonstrated the role of siderophore-mediated competition for Fe. They suppressed the incidence of Fusarium wilt of radish by applying *P. putida* strain WCS358. Suppression was due to the production of the siderophore pseudobactin 358.

It is believed that the host genotype has the potential to enhance the activity of biocontrol rhizobacteria in the soil that cause disease suppressiveness (Larkin *et al.*, 1993; Liu *et al.*, 1995; Mazzola and Gu, 2002). Larkin *et al.* (1993) demonstrated the development of soil suppressiveness to Fusarium wilt of watermelon was due to the successive planting of a specific watermelon cultivar. They found that the planting of the watermelon cultivar Crimson Sweet (moderately resistant and associated with soil suppressiveness) led to higher populations of non-pathogenic *F. oxysporum*, actinomycetes, fluorescent

pseudomonads, and overall bacteria occurrence than in non-planted soil or soil planted with the cultivar Florida Giant (susceptible to Fusarium wilt). Mazzola and Gu (2002) found that artificially infected cultivars of wheat planted in apple orchard soils, at first had the same level of susceptibility when evaluated in the greenhouse. However, after three growth-cycles, the cultivars varied in their degree of suppression to Rhizoctonia root rot of apple. Mazzola and Gu (2002) also observed that the fluorescent pseudomonad populations found on the wheat cultivars that induced disease suppressiveness, showed greater inhibition *in vitro* against *R. solani* than pseudomonad populations from non-treated soil or soils planted with cultivars that did not induce disease suppressiveness.

Suppressive soils to Fusarium wilt have been recognized in banana plantations since 1923 (Stover, 1962), and are found in several different locations (Stover, 1962; Peng *et al.*, 1999; Domínguez *et al.*, 2001). In Central America the suppression of Fusarium wilt of banana was correlated with the chemical and physical properties of the soil (Smith and Snyder, 1971; 1972). Domínguez *et al.* (1995) reported that Fe availability and soil acidity led to an increased disease incidence of *Foc*. In field studies it was further shown that the water-stability was higher and the pH lower in conducive than in suppressive soils. The suppressive soil had higher clay content, electric conductivity and higher Na solubility (Domínguez *et al.*, 2001). Montmorillonite soils reduced the spread of *Foc*, but this reduction was later correlated to the higher bacterial activity that was stimulated in montmorillonite soils (Stotzky and Martin, 1963; Stotzky and Rem, 1965). Similar results were obtained of the suppression of Fusarium wilt of flax (Höper *et al.*, 1995).

Peng *et al.* (1999) reported that *Foc* chlamydospore germination in suppressive and conducive soils is influenced by changes in certain soil properties (soil pH, Ca and Fe supply, temperature and soil water content). They suggested that soil amendments could be used to manipulate suppressiveness of soils against Fusarium wilt of banana. Ting *et al.* (2002) tried to artificially induce suppressive soil by the application of calcium nitrate [$\text{Ca}(\text{NO}_3)_2$] and *T. harzianum* to the soil, separately and in combination. The results showed that the addition of $\text{Ca}(\text{NO}_3)_2$ was the most effective treatment, followed by the combination of $\text{Ca}(\text{NO}_3)_2$ and *T. harzianum*. It was said that the disease suppression was

due to increasing cell wall lignification, because increased peroxidase and polyphenoloxidase activities were observed. This, however, was not demonstrated in the field. In investigations on the microbial composition of suppressive soils, Peng *et al.* (1999) found that *Foc* suppressive soils had a higher count of bacteria and Actinomycetes than conducive soils, suggesting that the microbial populations in the soil contribute to Fusarium wilt suppressiveness of bananas.

6. CONCLUSIONS

Investigations into management of Fusarium wilt of banana in the past century have often been periodical, and in response to severe outbreaks in new geographical areas. With the enormous damage caused by Fusarium wilt before the 1960's, a concerted effort was made by the United Fruit Company and other research groups to develop management strategies for the disease in Central America (Stover, 1962). However, once Gros Michel was replaced with Cavendish bananas, little emphasis was placed on Fusarium wilt management until new and serious outbreaks occurred on Cavendish bananas in the subtropics and recently in the tropics (Ploetz and Pegg, 2000). Recent research efforts, with a few exceptions, rather focused on the genetic and molecular analysis of *Foc* populations, and the testing of different banana varieties for resistance to Fusarium wilt. The FHIA breeding programme in this period also released a few banana hybrids with good resistance to Fusarium wilt, while several attempts have been made to produce resistance in Cavendish bananas using unconventional breeding strategies such as somaclonal variation. Despite all attempts to find disease resistance in banana, a lack in local acceptance of new varieties prevented this approach from being useful in many banana-growing regions. Management of Fusarium wilt, therefore, remains a research approach that should be investigated far more structured and comprehensively to provide relief to producers around the world.

Prevention of the introduction of Fusarium wilt pathogens in disease-free fields remains the most effective management approach. In this respect, the introduction of *in vitro*-propagated planting material has played a major role. The greater international movement

of plant material and people, as well as the reckless movement of planting material on farms, and between farms and districts, is still contributing to further dissemination of the disease. Early detection and isolation of Fusarium wilt of banana can limit the damage caused by the disease, but offers a constant risk of escape. The real challenge in Fusarium wilt research, other than development of disease resistant plants, remains to be the reduction in the impact of the disease by proper disease management strategies. While certain strategies such as flood fallowing and soil fumigation offered some relief in the past, these were not sustainable, and today, after more than 100 years of Fusarium wilt research on banana, a universally acceptable management strategy has not yet been defined.

As a management tool, chemical control has not proven to be very successful in the past, despite some reports of successful stem injections in India (Lakshmanan *et al.*, 1987), and the fungicidal treatment of banana corms and suckers before planting (Deacon, 1984). The development of fungicides that could be applied as a foliar spray or injected into banana stems, and that could be translocated to the roots, might be a viable option for the control of Fusarium wilt of banana. The application of surface disinfectants plays an important role in practices to prevent the introduction of Fusarium wilt into clean banana fields. However, the greatest potential that chemical products might offer to Fusarium wilt management is probably in terms of chemical activators. Several specific and non-specific chemicals have proved to induce SAR to diseases of pests in many crops. Chemical activators are expected to provide new opportunities for effective and sustainable disease management of several crops in the future. The effect of these products on Fusarium wilt of banana should be further investigated.

Cultural management of Fusarium wilt diseases offers the opportunity to reduce activity of *Foc* in the soil, and enhance the vigour of plants in the field by means of specific fertilization programs. Generally, the severity of most Fusarium wilt diseases was reduced in soils with a pH above 7.0, and with the application of the NO₃ rather than NH₄ form of nitrogen. Other nutrients such as P, K, Ca and Fe have all been proved to affect the incidence of Fusarium wilt diseases, and should be tested for management of

Fusarium wilt of banana. Soil nutrition could be applied in an integrated disease management programme using disease tolerant banana selections, SAR chemicals and biological control agents.

Biological control is probably one disease management strategy that offers the best opportunity for management of Fusarium wilt of banana. Disease suppressive soils have resulted in the identification of numerous microorganisms that have been applied, individually or combined, for control of Fusarium wilt in other crop systems. Suppressive soils have been observed in many banana fields, and while the chemical and physical properties of these soils have been investigated, little work has been done on analysing the microbial composition of such soils. Recent research showed that non-pathogenic isolates of *F. oxysporum* offers the potential for biological control of Fusarium wilt of banana. These non-pathogens can be applied as epiphytic or endophytic microorganisms to roots of bananas in the nursery, and in combination with other potential biological control organisms, chemicals and plant nutrition. The topic of biological control of *Foc* has been poorly investigated, and further research is needed to consider this strategy as an option for control of Fusarium wilt of banana.

The management of Fusarium wilt of banana has become a relevant and important research topic with the outbreaks of the disease in Cavendish bananas in the subtropics and tropics. There are no acceptable varieties available to replace Cavendish bananas as a dessert banana for export and local markets. Since Cavendish bananas are parthenocarpic, they cannot be used as female lines in breeding programmes. The only means to improve Cavendish bananas in the long term is through unconventional techniques such as genetic engineering, a process that might take a decade or more to develop resistant plants. In this period, Fusarium wilt must be managed to reduce losses of banana fields. Furthermore, management strategies should be designed for banana production in developing countries, where new technologies and expensive management strategies are often unaffordable. Since almost 90% of bananas are produced for local consumption, often as a staple food crop, development of management strategies for Fusarium wilt is essential.

7. REFERENCES

- Agrios, G.N. 1997. Plant Pathology. Academic Press, USA, pp. 173-221.
- Alabouvette, C. 1986. Fusarium-wilt suppressive soils from the Châteaurenard region: review of 10-years study. *Agronomie* 6: 273-284.
- Alabouvette, C. 1990. Biological control of Fusarium wilt pathogens in suppressive soils. In: Hornby, D. (Ed.), Biological control of soil-borne plant pathogens. CAB International, Wallingford, UK, pp. 27-43.
- Alabouvette, C. 1999. Fusarium wilt suppressive soils: An example of disease-suppressive soils. *Australasian Plant Pathology* 28: 57-64.
- Alabouvette, C., Backhouse, D., Steinberg, C., Donovan, N.J., Edel-Hermann, V., and Burgess, L.W. 2004. Microbial diversity in soil-effects on crop health. In: Schjonning, P., Elmholt, S., and Christensen, B.T. (Eds), Managing soil quality: Challenges in modern agriculture. CAB International, Wallingford, UK, pp. 121-138.
- Alabouvette, C., Lemanceau, P., and Steinberg, C. 1993. Recent advances in the biological control of Fusarium wilts. *Pesticide Science* 37: 363-373.
- Anagnostakis, S.L. 1982. Biological control of chestnut blight. *Science* 215: 466-471.
- Anon. 1992. Banana and plantain – food for thought. In: INIBAP (Ed), Banana, Plantains and INIBAP. Annual Report 1992, INIBAP, Montpellier, France, pp. 7-11.
- Armstrong, G.M., and Armstrong, J.K. 1975. Reflections on the wilt *Fusaria*. *Annual Review of Phytopathology* 13: 95-103.
- Armstrong, G.M., and Armstrong, J.K. 1981. *Formae speciales* and races of *Fusarium oxysporum* causing wilt disease. In: Nelson, P.E., Toussoun, T.A., and Cook, R.J. (Eds), Fusarium: Disease, Biology and Taxonomy. Pennsylvania State University Press, University Park, USA, pp. 391-399.
- Assani, A., Haicour, R., Wenzel, G., Côte, F., Bakry, F., Foroughi-Wehr, B., Ducreux, G., Aguillar, M.E., and Grapin, A. 2001. Plant regeneration from protoplasts of dessert banana cv. Grande Naine (*Musa* spp., Cavendish sub-group AAA) via somatic embryogenesis. *Plant Cell Reporter* 20: 482-488.

- Baker, K.F. 1981. Biological control. In: Mace, M.E., Bell, A.A., and Beckman, C.H. (Eds), Fungal wilt diseases of plants. Academic Press, New York, USA, pp. 523-561.
- Baker, K.F. 1987. Evolving concepts of biological control of plant pathogens. *Annual Review of Phytopathology* 25: 67-85.
- Baldwin, B.C., and Rathmell, W.G. 1988. Evolution of concepts for chemical control of plant diseases. *Annual Review of Phytopathology* 26: 265-283.
- Bapat, S., and Shah, A.K. 2000. Biological control of fusarial wilt of pigeon pea by *Bacillus brevis*. *Canadian Journal of Microbiology* 46: 125-132.
- Becker, D.K., Dugdale, B., Smith, M.K., Harding, R.M., and Dale, J.L. 2000. Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. Grant Nain via microprojectile bombardment. *Plant Cell Reports* 19: 229-239.
- Beckman, C.H. 1962. The interaction of host, pathogen, and soil temperatures in relation to susceptibility to *Fusarium* wilt of bananas. *Phytopathology* 52: 134-140.
- Beckman, C.H. 1987. The nature of wilt disease of plants. APS Press, St. Paul, Minnesota, USA, 175 pp.
- Beckman, C.H. 1990. Host responses to the pathogen. In: Ploetz, R.C. (Ed.), *Fusarium wilt of banana*. APS Press, St Paul, Minnesota, USA, pp. 93-105.
- Bentley, S., Moore, N.Y., Pegg, K.G., Gerlach, K.S., and Smith, L.J. 1999. Genetic characterisation and detection of *Fusarium* wilt. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of the International Workshop on banana *Fusarium* wilt disease, Genting Highlands Resort, Malaysia, pp. 143-151.
- Bentley, S., Pegg, K.G., Moore, N.Y., Davis, R.D., and Buddenhagen, I.W. 1998. Genetic variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense* analyzed by DNA fingerprinting. *Phytopathology* 12: 1283-1293.
- Biles, C.L., and Martyn, R.D. 1989. Local and systemic resistance induced in watermelons by *formae speciales* of *Fusarium oxysporum*. *Phytopathology* 79: 856-860.

- Blok, W.J., Lamers, J.G., Termorshuizen, A.J., and Bollen, G.J. 2000. Control of soil-borne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* 90: 253-259.
- Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, pp. 237.
- Brandes, E.W. 1919. Banana wilt. *Phytopathology* 9: 339-389.
- Brasher, P. 2000. Protein for replacing pesticides approved. San Diego Union Tribune, April 29, pp. A7.
- Cachinero, J.M., Hervás, A., Jiménez-Díaz, R.M., and Tena, M. 2002. Plant defence reactions against *Fusarium* wilt in chickpea induced by incompatible race 0 of *Fusarium oxysporum* f. sp. *ciceris* and nonhost isolates of *F. oxysporum*. *Plant Pathology* 51: 765-776.
- Chakrabarti, A., Ganapathi, T.R., Mukherjee, P.K. and Bapat, V.A. 2003. MSI-99, a magainin analogue, imparts enhanced disease resistance in transgenic tobacco and banana. *Planta* 216: 587-596.
- Cheeseman, E.E. 1932. Genetic and cytological studies of *Musa* I. Certain hybrids of the Gros Michel banana. *Journal of Genetics* 26: 291-312.
- Chérif, M., and Benhamou, N. 1990. Cytochemical aspects of chitin breakdown during the parasitic action of *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 80: 1406-1414.
- Chosson, J.F., Lapierre, H., Kusiak, C., and Molin, G. 1973. Presence of virus-like particles in fungi of the genus *Fusarium*. *Annual Review of Phytopathology* 5: 324.
- Ciardi, J.A., Tieman, D.M., Lund, S.T., Jones, J.B., Stall, R.E., and Klee, H.J. 2000. Response to *Xanthomonas campestris* pv. *vesicatoria* in tomato involves regulation of ethylene receptor gene expression. *Plant Physiology* 123: 81-92.
- Cohen, Y. 1994a. 3-Aminobutyric acid induces systemic resistance against *Peronospora tabacina*. *Physiology and Molecular Plant Pathology* 44: 273-288.
- Cohen, Y. 1994b. Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-Amino-*n*-Butanoic acids. *Phytopathology* 84: 55-59.

- Cohen, Y., Gisi, U., and Niderman, T. 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* 83: 1054-1062.
- Corden, M.E., and Young, R.A. 1959. The effects of fungicides on *Fusarium oxysporum* f. *cubense* and other soil microorganisms. U.F.-Oregon State College annual reports.
- Corden, M.E., and Young, R.A. 1960. The effects of fungicides on *Fusarium oxysporum* f. *cubense* and the nature of wilt disease resistance. U.F.-Oregon State College annual reports.
- Cotten, T.K., and G.P. Munkvold. 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. *Phytopathology* 88: 550-555.
- Cotxarrera, L., Trillas-Gay, M.I., Steinberg, C. and Alabouvette, C. 2002. Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress Fusarium wilt of tomato. *Soil Biology and Biochemistry* 34: 467-476.
- Couteaudier, Y., and Alabouvette, C. 1990. Quantitative comparison of *Fusarium oxysporum* competitiveness in relation with carbon utilization. *FEMS Microbiology* 74: 261-268.
- Crawford, D.L., Lynch, J.M., Whipps, J.M., and Ousley, M.A. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. *Applied and Environmental Microbiology* 59: 3889-3905.
- Dale, J.L. 1999. Banana biotechnology: already a reality. *AgBiotechNet* Vol 1 November, ABN033.
- Datnoff, L.E., Nemec, S., and Pernezny, K. 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. *Biological Control* 5: 427-431.
- Davis, A.J., Say, M., Snow, A.J., and Grant, B.R. 1994. Sensitivity of *Fusarium oxysporum* f.sp. *cubense* to phosphonate. *Plant Pathology* 43: 200-205.
- Deacon, J.W. 1984. Panama disease of banana in South Africa. *Horticultural Science* 1: 29-31.

- De Ascensao, A.R.D.C.F., and Dubery, I.A. 2000. Panama disease: Cell wall reinforcement in banana roots in response to elicitors from *Fusarium oxysporum* f.sp. *cubense* race four. *Phytopathology* 90: 1173-1180.
- De Beer Z. 1997. Fusarium tolerance and horticultural characteristics of some FHIA hybrids. *Banana Growers Association of South Africa Year Book* 2. Studio Novell, Sandton, pp. 15-17.
- De Boer, M., Van der Sluis, I., Van Loon, L.C., and Bakker, P.A.H.M. 1999. Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of Fusarium wilt of radish. *European Journal of Plant Pathology* 105: 201-210.
- De Matos, A.P., Cordeiro, Z.J.M., De O e Silva, S., Trindade, A.V., and Ferreira, D.M.V. 1999. Research on Fusarium wilt of banana in Brazil: Achievements and current status. In: Molina, A.B., Masdek, N.H.N., and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia*, pp. 95-102.
- Diab, H.G., Hu, S., and Benson, D.M. 2003. Suppression of *Rhizoctonia solani* on impatiens by enhanced microbial activity in composted swine waste-amended potting mixes. *Phytopathology* 93: 1115-1123.
- Domínguez, J., Negrín, M.A., and Rodríguez, C.M. 2001. Aggregate water-stability, particle size and soil solution properties in conducive and suppressive soils to Fusarium wilt of banana from Canary Islands. *Soil Biology and Biochemistry* 33: 449-455.
- Domínguez, J., Rodríguez, C.M., and Hernández-Moreno, J.M. 1995. Iron and Fusarium wilts in banana crops on Andic soils. In: Abadía, J. (Ed), *Iron Nutrition in Soils and Plants*. Kluwer Academic, Dordrecht, The Netherlands, pp. 255-258.
- Duijff, B.J., Erkelens, A., Bakker, P.A.H.M., and Schippers, B. 1995. Influence of pH on suppression of Fusarium wilt of carnation by *Pseudomonas fluorescens* WCS 417r. *Journal of Phytopathology* 143: 217-222.

- Duijff, B.J., Pouhair, D., Olivain, C., Alabouvette, C., and Lemanceau, P. 1998. Implication of systemic induced resistance in the suppression of Fusarium wilt of tomato by *Pseudomonas fluorescens* WSC417r and by nonpathogenic *Fusarium oxysporum* Fo47. *European Journal of Plant Pathology* 104: 903-910.
- Duijff, B.J., Recorbet, G., Bakker, P.A.H.M., Loper, J.E., and Lemanceau, P. 1999. Microbial antagonism at the root level is involved in the suppression of Fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. *Phytopathology* 89: 1073-1079.
- Eckstein, K., Fraser, C., and Temple-Murray, N. 1996. The evaluation of promising new banana cultivars. *Banana Growers Association of South Africa Year Book* 1. Studio Novell, Sandton, pp. 3-6.
- Edel-Hermann, W., Dreumont, C., Perez-Piqueres, A., and Steinberg, C. 2004. Terminal restriction fragment length polymorphism analysis of ribosomal RNA genes to assess changes in fungal community structure in soils. *FEMS Microbiology Ecology* 47: 397-404.
- El Hassni, M., Jaiti, F., Dihazi, A., Ait Barka, E., Daayf, F., and El Hadrami, I. 2004. Enhancement of defence responses against Bayoud disease by treatment of date palm seedlings with hypoaggressive *Fusarium oxysporum* isolate. *Journal of Phytopathology* 152: 182-189.
- El-Shanshoury, A.R. El-Sounoud, S.M.A., Awadalla, O.A., and El-Bandy, N.B. 1996. Effects of *Streptomyces corchorusii*, *Streptomyces mutabilis*, pendimethalin, and metribuzin on the control of bacterial and Fusarium wilt of tomato. *Canadian Journal of Botany* 74: 1016-1022.
- Engelhard, A.W., and Woltz, S.S. 1973. Fusarium wilt of chrysanthemum: Complete control of symptoms with an integrated fungicide-lime-nitrate regime. *Phytopathology* 63: 1256-1259.
- Eparvier, A., and Alabouvette, C. 1994. Use of ELISA and *GUS*-transformed strains to study competition between pathogenic and non-pathogenic *Fusarium oxysporum* for root colonization. *Biocontrol Science and Technology* 4: 35-47.
- Erwin, D.C. 1973. Systemic fungicides: Disease control, translocation, and mode of action. *Annual Review of Phytopathology* 11: 389-422.

- Erwin, D.C. 1981. Chemical control. In: Mace, M.E., Bell, A.A., and Beckman, C.H. (Eds), Fungal wilt diseases of plants. Academic Press, New York, USA, pp. 563-600.
- Fekete, C., Giczey, G., Papp, I., Scabo, L., and Hornok, L. 1995. High-frequency occurrence of virus-like particles with double-stranded RNA genome in *Fusarium poae*. FEMS Microbiology Letters 131: 295-299.
- Filion, M., St-Arnaud, M., and Jabaji-Hare, S.H. 2003. Quantification of *Fusarium solani* f.sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media. Phytopathology 93: 229-235.
- Fravel, D.R., and Engelkes, C.A. 1994. Biological Management. In: Campbell, C.L., and Benson, D.M. (Eds), Epidemiology and Management of Root Diseases. Springer-Verlag, Berlin, Heidelberg, pp. 293-308.
- Fravel, D.R., and Larkin, R.P. 2002. Reduction of Fusarium wilt of hydroponically-grow basil by *Fusarium oxysporum* strain CS-20. Crop Protection 21: 539-543.
- Fravel, D.R., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. New Phytologist 157: 493-502.
- Freeman, S., Zveibil, A., Vintal, H., and Maymon, M. 2002. Isolation of nonpathogenic mutants of *Fusarium oxysporum* f. sp. *melonis* for biological control of Fusarium wilt in Cucurbits. Phytopathology 92: 164-168.
- Fuchs, J.G., Moënné-Loccoz, Y., and Défago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt of tomato. Plant Disease 81: 492-496.
- Ganapathi, T.R., Higgs, N.S., Balint-Kurti, P.J., Arntzen, C.J., May, G.D., and Van Eck, J.M. 2001. *Agrobacterium*-mediated transformation of embryogenic cell suspensions of banana cultivar Rasthali (AAB). Plant Cell Reports 20: 157-162.
- Genoud, T., and Métraux, J.P. 1999. Crosstalk in plant cell signaling: structure and function of the genetic network. Trends in Plant Science 4: 503-507.

- Gerlach, K.S., Bentley, S., Moore, N.Y., Aitken, E.A.B., and Pegg, K.G. 1999. Investigation of nonpathogenic strains of *Fusarium oxysporum* for suppression of Fusarium wilt of banana in Australia. In: Alabouvette, C. (Ed), Second International Fusarium Workshop. INRA, Dijon.
- Gessler, C., and Kuć, J. 1982. Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens. *Phytopathology* 72: 1439-1441.
- Getha, K., and Vikineswary, S. 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. *Journal of Industrial Microbiology and Biotechnology* 28: 303-310.
- Green, R.J. 1981. An Overview. In: Mace, M.E., Bell, A.A., and Beckman, C.H. (Eds), *Fungal wilt diseases of plants*. Academic Press, New York, USA, pp. 1-24.
- Guest, D.I., and Grant 1991. The complex action of phosphonate as antifungal agents. *Biological Reviews* 66: 159-187.
- Gullino, M.L., Leroux, P., and Smith, C.M. 2000. Uses and challenges with novel compounds for plant disease control. *Crop Protection* 19: 1-11.
- Gullino, M.L., Minuto, A., Gilardi, G., and Garibaldi, A. 2002. Efficacy of azoxystrobin and other strobilurins against Fusarium wilts of carnation, cyclamen and Paris daisy. *Crop Protection* 21: 57-61.
- Hadar, Y., Harman, G.E., and Taylor, A.G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. *Phytopathology* 74: 106-110.
- Hammad, A.M.M., and El-Mohandes, M.A.O. 1999. Controlling Fusarium wilt disease of cucumber plants via antagonistic microorganisms in free and immobilized states. *Microbiological Research* 154: 113-117.
- Hammerschmidt, R., Métraux, J.P., and Van Loon, L.C. 2001. Inducing resistance: a summary of papers presented at the First International Symposium of Induced Resistance to Plant Diseases, Corfu, May 2000. *European Journal of Plant Pathology* 107: 1-6.

- Harman, G.E., Chet, I., and Baker, R. 1980. *Trichoderma hamatum* effects on seed and seedling diseases induced in radish and pea by *Pythium* spp. or *Rhizoctonia solani*. *Phytopathology* 70: 1167-1172.
- Harman, G.E., Hayes, C.K., Lorito, M., Broadway, R.M., Di Pietro, A., Peterbauer, C., and Tronsmo, A. 1993. Chitinolytic enzymes of *Trichoderma harzianum*: Purification of chitobiosidase and endochitinase. *Phytopathology* 83: 313-318.
- Harper, J.L. 1950. Studies in the resistance of certain varieties of banana to Panama disease I, II. *Plant and Soil* 2: 374-394.
- Hawksworth, D.L., Kirk, P.M., Sutton, B.C., and Pegler, D.N. 1995. Dictionary of the fungi. CAB International, Wallingford, United Kingdom.
- He, C.Y., Hsiang, T., and Wolyn, D.J. 2002. Induction of systemic disease resistance and pathogen defence responses in *Asparagus officinalis* inoculated with nonpathogenic strains of *Fusarium oxysporum*. *Plant Pathology* 51: 225-230.
- Heaton, J.B., and Dullahide, S.R. 1990. Efficacy of phosphoric acid in other host-pathogens. *Australasian Plant Pathology* 19: 133-134.
- Herbert, J.A., and Marx, D. 1990. Short-term control of Panama disease in South Africa. *Phytophylactica* 22: 339-340.
- Hervás, A., Landa, B., Datnoff, L.E., and Jiménez-Díaz, R.M. 1998. Effects of commercial and indigenous microorganisms on Fusarium wilt development in chickpea. *Biological Control* 13: 166-176.
- Hervás, A., Trapero-Casas, J.L., and Jiménez-Díaz, R.M. 1995. Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f.sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. *Plant Disease* 79: 1110-1116.
- Hoffland, E., Hakulinem, J., and Van Pelt, J.A. 1996. Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. *Phytopathology* 86: 757-762.
- Hoitink, H.A.J., and Boehm, M.J. 1999. Importance of physical and chemical soil properties in the suppressiveness of soils to plant disease. *European Journal of Soil Biology* 32: 41-58.

- Höper, H., and Alabouvette, C. 1996. Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. *European Journal of Soil Biology* 32:41-58.
- Höper, H., Steinberg, C., and Alabouvette, C. 1995. Involvement of clay type and pH in the mechanisms of soil suppressiveness to *Fusarium* wilt of flax. *Soil Biology and Biochemistry* 27: 955-967.
- Howell, C.R. 1982. Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizotonia solani*, and damping-off of cotton seedlings. *Phytopathology* 72: 496-498.
- Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease* 87: 1-11.
- Huber, D.M., and Watson, R.D. 1974. Nitrogen form and plant disease. *Annual Review of Phytopathology* 12: 139-165.
- Hwang, S.C. 1985. Ecology and control of fusarial wilt of banana. *Plant Protection Bulletin. (Taiwan)* 27: 233-245.
- Hwang, S.C. 1999. Recent development on *Fusarium* R&D of banana in Taiwan. In: Molina, A.B., Masdek, N.H.N., and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia*, pp. 39-49.
- Hwang, S.C., and Tang, C.Y. 2000. Unconventional breeding in Taiwan. In: Jones, D.R. (Ed), *Diseases of banana, abacá and enset*. CAB International, Wallingford, UK, pp.449-464.
- Hwang, S.C., Chen, S.L., Lin, J.C., and Lin, H.L. 1984. Cultivation of banana using plantlets from meristem culture. *Horticultural Science* 19: 231-233.
- Hwang, S.F. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of *Verticillium* and *Fusarium* wilts of Alfalfa. *Plant Disease* 76: 239-243.
- Israeli, Y., Lahav, E., and Reuveni, O. 1995. *In vitro* culture of bananas. *Banana diseases*. In: Gowen, S. (Ed), *Bananas and Plantains*. Chapman and Hall, London, pp. 147-178.

- Jaizme-Vega, M.C., Hernández, B.S., and Hernández, J.M.H. 1998. Interaction of arbuscular mycorrhizal fungi and the soil pathogen *Fusarium oxysporum* f.sp. *cubense* on the first stages of micropropagated Grande Naine banana. *Acta Horticulturae* 490: 285-295.
- Jeger, M.J., Eden-Green, S., Thresh, J.M., Johanson, A., Waller, J.M., and Brown, A.E. 1995. Banana diseases. In: Gowen, S. (Ed), *Bananas and Plantains*. Chapman and Hall, London, pp. 337-343.
- Jeger, M.J., Waller, J.M., Johanson, A., and Gowen, S.R. 1996. Monitoring in banana pest management. *Crop Protection* 4: 391-397.
- Jones, D.R. 2000. History of banana breeding. In: Jones, D.R. (Ed), *Diseases of banana, abacá and enset*. CAB International, Wallingford, UK, pp.425-449.
- Karamura, E.B., and Karamura, D.A. 1995. Banana morphology – part II: the aerial shoot. In: Gowen, S. (Ed), *Bananas and Plantains*. Chapman and Hall, London, pp. 190-205.
- Kato, T., Yamaguchi, Y., Harano, T., Yokohama, T., Uyehara, T., *et al.* 1984. Unsaturated hydroxy fatty acids, the self defensive substance in rice plant against rice blast disease. *Chemical Letters* 409-412.
- Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology* 32: 439-459.
- Knoester, M. Linthorst, H.J.M., Bol, J.F., and Van Loon, L.C. 2001. Involvement of ethylene in lesion development and systemic acquired resistance in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Physiological and Molecular Plant Pathology* 59: 45-57.
- Kreutzer, W.A. 1972. *Fusarium* spp. as colonists and potential pathogens in root zones of grassland plants. *Phytopathology* 62: 1066-1070.
- Lahdenpera, M.L., and Oy, K. 1987. The control of *Fusarium* wilt on carnation with a *Streptomyces* preparation. *Acta Horticulturae* 216: 85-92.
- Lakshmanan, P., Selvaraj, P., and Mohan, S. 1987. Efficiency of different methods for the control of Panama disease. *Tropical Pest Management* 33: 373-376.

- Lapierre, H., Kusiak, C., and Molin, G. 1974. Some properties of viral particles of *Fusarium culmorum*. *Annual Review of Phytopathology* 6: 500-501.
- Larkin, R.P., and Fravel, D.R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for the control of Fusarium wilt of tomato. *Plant Disease* 82: 1022-1028.
- Larkin, R.P., and Fravel, D.R. 1999. Mechanisms of action and dose response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. *Phytopathology* 89: 1151-1161.
- Larkin, R.P., and Fravel, D.R. 2002. Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by non-pathogenic *Fusarium* spp. *Phytopathology* 92: 1160-1166.
- Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soil suppressive and conducive to Fusarium wilt of watermelon. *Phytopathology* 83: 1097-1105.
- Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. *Phytopathology* 86: 812-819.
- Leeman, M., Den Ouden, F.M., Van Pelt, J.A., Dirkx, F.P.M., Steijl, H., Bakker, P.A.H.M., and Schippers, B. 1996. Iron availability affects induction of systemic resistance to Fusarium wilt of radish by *Pseudomonas fluorescens*. *Phytopathology* 86: 149-155.
- Lemanceau, P., and Alabouvette, C. 1991. Biological control of Fusarium diseases by fluorescent pseudomonas and nonpathogenic *Fusarium*. *Crop Protection* 10: 279-286.
- Lemanceau, P., and Alabouvette, C. 1993. Suppression of Fusarium wilt by fluorescent pseudomonads – mechanisms and applications. *Biocontrol, Science and Technology* 3: 219-234.
- Lemanceau, P., Bakker, P.A.H.M., de Kogel, W.J., Alabouvette, C., and Schippers, B. 1992. Effect of pseudobactin 358 produced by *Pseudomonas putida* WSC358 on suppression of *Fusarium* wilt of carnations by non-pathogenic *Fusarium oxysporum*. *Applied Environmental Microbiology* 58: 2978-2980.

- Lemanceau, P., Bakker, P.A.H.M., de Kogel, W.J., Alabouvette, C., and Schippers, B. 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and Pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. Applied Environmental Microbiology 59: 74-82.
- Leong, J. 1986. Siderophores: Their biochemistry and possible role in the biocontrol of plant pathogens. Annual Review of Phytopathology 24: 187-209.
- Li, Z., and Liu, C. 1990. A new fungicide – HDE and its application in controlling wilt disease of cucurbits. Pesticide Science 28: 413-418.
- Liu, L., Kloepper, J.W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: duration of protection and effect of host resistance on protection and root colonization. Phytopathology 85: 1064-1068.
- Locke, J.C. 1985. Biological control of Fusarium wilt of greenhouse-grown chrysanthemums. Plant Disease 69: 167-169.
- Lorito, M., Harman, G.E., Hayes, C.K., Broadway, R.M., Tronsmo, A., Woo, S.L., and Di Pietro, A. 1993. Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. Phytopathology 83: 302-307.
- Louvet, J., Alabouvette, C., and Rouxel, F. 1981. Microbiological suppressiveness of some soils to Fusarium wilts. In: Nelson, P.E., Toussoun, T.A., and Cook, R.J. (Eds), Fusarium: Disease, Biology and Taxonomy. Pennsylvania State University Press, University Park, USA, pp. 261-275.
- Lumsden, R.D., and Locke, J.C. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology 79: 361-366.
- Lumsden, R.D., Lewis, J.A., and Millner, P.D. 1983. Effect of composted sewage sludge on several soilborne pathogens and diseases. Phytopathology 73: 1543-1548.
- Mandeel, Q., and Baker, R. 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81: 462-469.

- Maraite, H., and Meyer, J.A. 1971. Systemic fungitoxic action of benomyl against *Fusarium oxysporum* f.sp. *melonis* in vivo. *Netherland Journal of Plant Phytopathology* 77: 1-5.
- Marois, J.J. 1990. Biological control of diseases caused by *Fusarium oxysporum*. In: Ploetz, R.C. (Ed), *Fusarium wilt of banana*. APS Press, St Paul, Minnesota, USA, pp. 77-81.
- Marois, J.J., Mitchell D.J., and Sonada, R.M. 1981. Biological control of *Fusarium* crown and rot of tomato under field conditions. *Phytopathology* 12: 1257-1260.
- Martin, F.N. 2003. Development of alternative strategies for the management of soilborne pathogens currently controlled with methyl bromide. *Annual Review of Phytopathology* 41: 325-350.
- Maurhofer, M., Hase, C., Meuwly, P., Metraux, J.P., and Defago, G. 1994. Induction of systemic resistance of tobacco necrosis virus by root-colonizing *Pseudomonas fluorescens* strain CHAO: influence of the *gaA* gene and of pyoverdine production. *Phytopathology* 84: 139-146.
- Mazzola, M., and Gu, Y.H. 2002. Wheat genotype-specific induction of soil microbial communities suppressive to disease incited by *Rhizoctonia solani* anastomosis group (AG)-5 and AG-8. *Phytopathology* 92: 1300-1307.
- Megia, R., Haicour, R., Rossignol, L., and Sihachakr, D. 1992. Callus formation from cultured protoplasts of banana (*Musa* spp.). *Plant Science* 85: 91-98.
- Meng, L.Y., Leng, T., and Pin, O.K. 1999. *Fusarium* wilt of Cavendish banana and its control in Malaysia. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of the International Workshop on banana *Fusarium* wilt disease, Genting Highlands Resort, Malaysia, pp. 252-259.
- Meredith, C.H. 1941. The effect of sodium nitrate on *Fusarium oxysporum cubense*. *Phytopathology* 31: 564.
- Meredith, C.H. 1943a. The antagonism of '*Actinomyces*' to *Fusarium oxysporum cubense*. *Phytopathology* 33: 403.
- Meredith, C.H. 1943b. Mercury compounds applied to banana plants in the field. *Phytopathology* 33: 835-836.

- Meredith, C.H. 1946. Soil actinomycetes applied to banana plants in the field. *Phytopathology* 36: 983-987.
- Métraux, J.P., Ahl-Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. 1991. Induced systemic resistance in cucumber in response to 2,6-dichloro-isonicotinic acid and pathogens. In: Hennecke, H., and Verma, D.P.S. (Eds), *Advances in molecular genetics of plant-microbe interactions*. Kluwer Academic Publishers, Dordrecht, pp. 432-439.
- Miao, V.P.W. 1990 Using karyotype variability to investigate the origins and relatedness of isolates of *Fusarium oxysporum* f.sp. *cubense*. In: Ploetz, R.C. (Ed), *Fusarium wilt of bananas*. ASP Press, St. Paul, Minnesota, USA, pp. 55-62.
- Minuto, A., Migheli, Q., and Garibaldi, A. 1995. Evaluation of antagonistic strains of *Fusarium* spp. in the biological and integrated control of Fusarium wilt of cyclamen. *Crop Protection* 14: 221-226.
- Moore, N.Y., Bentley, S., Pegg, K.G., and Jones, D.R. 1995. Fusarium wilt of banana. *Musa* disease fact sheet No.5. INIBAP: 1-4.
- Moore, N.Y., Pegg, K.G., Bentley, S., and Smith, L.J. 1999a. Fusarium wilt of banana: Global problems and perspectives. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 11-30.
- Moore, N.Y., Pegg, K.G., Smith, L.J., Langdon, P.W., Bentley, S., and Smith, M.K. 1999b. Fusarium wilt of banana in Australia. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 64-75.
- M'Piga, P., Belanger, R.R., Paulitz, T.C., and Bennhamou, N. 1997. Increased resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiological and Molecular Plant Pathology* 50: 301-320.

- Nelson, P.E. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. In: Mace, M.E., Bell, A.A., and Beckman, C.H. (Eds), Fungal wilt diseases of plants. Academic Press, New York, USA, pp. 51-80.
- Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University Park, USA, 193 pp.
- Nielsen, L.W. 1977. Control of sweetpotato *Fusarium* wilt with benomyl and thiabendazole. Plant Disease Reporter 61: 1-5.
- Nogawa, M., Shimosaka, M., Kageyama, T., and Okazaki, M. 1993. A double-stranded RNA mycovirus from the plant pathogenic fungus, *Fusarium solani* f.sp. *robiniae*. FEMS Microbiology Letters 110: 153-158.
- Novak, F.J., Afza, R., Van Duren, M., Perea-Dallos, M., Conger, B.V., and Xiaolang, T. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) banana (*Musa* spp.). Biotechnology 7: 154-159.
- Nuss, D.L., and Koltin, Y. 1990. Significance of dsRNA genetic elements in plant pathogenic fungi. Annual Review of Phytopathology 28: 37-58.
- O'Donnell, K., Kistler, H.C., Cigelnik, E., and Ploetz, R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National academy of Science of the United States of America 95: 2044-2049.
- Olivain, C., and Alabouvette, C. 1997. Colonization of tomato root by a non-pathogenic strain of *Fusarium oxysporum*. New Phytologist 137: 481-494.
- Olivain, C., and Alabouvette, C. 1998. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f.sp. *lycopersici* discussed in comparison to a non-pathogenic strain. The New Phytologist 141: 497-510.
- Olivain, C., Alabouvette, C., and Steinberg, C. 2004. Production of a mixed inoculum of *Fusarium oxysporum* Fo47 and *Pseudomonas fluorescens* C7 to control *Fusarium* diseases. Biocontrol Science and Technology 14: 227-238.
- Oostendorp, M., Kunz, W., Dietrich, B., and Staub, T. 2001. Induced disease resistance in plants by chemicals. European Journal of Plant Pathology 107: 19-28.

- Ortiz, R., Ferris, R.S.B., and Vuylsteke, D.R. 1995. Banana and plantain breeding. In: Gowen, S. (Ed), *Banana and Plantains*. Chapman and Hall, London, pp. 110-145.
- Ozlem, K., and Griffin, G.J. 1998. Effect of dsRNA-containing and dsRNA-free hypovirulent isolates of *Fusarium oxysporum* on severity of Fusarium seedling disease of soybean in naturally infected soil. *Plant and Soil* 201: 125-135.
- Papavizas, P.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology* 23: 23-54.
- Papavizas, P.C., and Collins, D.J. 1990. Influence of *Gliocladium virens* on germination and infectivity of sclerotia of *Sclerotium rolfsii*. *Phytopathology* 80: 627-630.
- Papavizas, P.C., and Lewis, J.A. 1983. Physiological and biocontrol characteristics of stable mutants of *Trichoderma viride* resistant to MBC fungicides. *Phytopathology* 73: 407-411.
- Papavizas, P.C., and Lumsden, R.D. 1980. Biological control of soilborne fungal propagules. *Annual Review of Phytopathology* 18: 389-413.
- Park, C.S., Paulitz, T.C., and Baker, R. 1988. Biocontrol of Fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. *Phytopathology* 78: 190-194.
- Paulitz, T.C. 2001. Biological control in greenhouse systems. *Annual Review of Phytopathology* 39: 103-133.
- Pegg, K.G., Shivas, R.G., Moore, N.Y., and Bentley, S. 1995. Characterization of a unique population of *Fusarium oxysporum* f.sp. *cubense* causing Fusarium wilt in Cavendish bananas at Carnarvon, Western Australia. *Australian Journal of Agricultural Research* 46: 167-178.
- Peng, H.X., Sivasithamparam, K., and Turner, D.W. 1999. Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biology and Biochemistry* 31: 1363-1374.
- Pieterse, C.M.J., Ton, J., and Van Loon, L.C. 2001. Cross-talk between plant defence signalling pathways: boost or burden? *AgBiotechNet* 3:1-8.

- Pieterse, C.M.J, Van Wees, S.C.M., Hoffland, E., Van Pelt, J.A., and Van Loon, L.C. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *The Plant Cell* 8: 1225-1237.
- Ploetz, R.C. 1994. Panama disease: return of the first banana menace. *International Journal of Pest Management* 40: 326-336.
- Ploetz, R.C., and Pegg, K.G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D.R. (Ed), *Diseases of banana, abacá and enset*. CAB International, Wallingford, UK, pp. 143-172.
- Ploetz, R.C., Herbert, J., Sebasigari, K., Hernandez, J.H., Pegg, K.G., Ventura, J.A., and Mayato, L.S. 1990. Importance of *Fusarium* wilt in different banana-growing regions. In: Ploetz, R.C. (Ed), *Fusarium wilt of bananas*. ASP Press, St. Paul, Minnesota, USA, pp. 9-26.
- Ploetz, R.C., Thomas, J.E., and Slabaugh, W.R. 2003. Diseases of banana and plantain. In: Ploetz, R.C. (Ed), *Diseases of tropical fruit crops*. CAB International, Wallingford, UK, pp 109-112.
- Postma, J., and Luttikholt, A.J.G. 1996. Colonization of carnation stems by a non-pathogenic isolate of *Fusarium oxysporum* and its effect on *Fusarium oxysporum* f. sp. *dianthi*. *Canadian Journal of Botany* 74: 1841-1851.
- Postma, J., and Rattink, H. 1992. Biological control of *Fusarium* wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*. *Canadian Journal of Botany* 70: 1199-1205.
- Raaijmakers, J.M., Leeman, M., and Van Oorschot, M.M.P. 1995. Dose-response relationship in biological control of *Fusarium* wilt of radish by *Pseudomonas* spp. *Phytopathology* 85: 1075-1081.
- Raghuchander, T., Jayashree, K., and Samiyappan, R. 1997. Management of *Fusarium* wilt of banana using antagonistic microorganisms. *Journal of Biological Control* 11: 101-105.
- Rajappan, K., Vidhyasekaran, P., Sethuraman, K., and Baskaran, T.L. 2002. Development of powder and capsule formulations of *Pseudomonas fluorescens* strain Pf-1 for the control of banana wilt. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Disease and Protection)* 109: 80-87.

- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection* 20: 1-11.
- Raupach, G.S., and Kloepper, J.W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology* 88: 1158-1164.
- Reddi, G.S, and Rao, A.S. 1971. Antagonism of soil actinomycetes to some soil-borne plant pathogenic fungi. *Indian Phytopathology* 24: 649-657.
- Reymond, P., Weber, H., Damond, M., and Farmer, E.E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis* *Plant Cell* 12: 707-719.
- Reynolds, P.K. 1927. *The Banana. Its history, cultivation and place among staple foods.* The Riverside Press, Cambridge, p. 181.
- Rishbeth, J. 1955. Fusarium wilt of bananas in Jamaica. I. Some observations on the epidemiology of the disease. *Annual Botany* 19: 293-328.
- Rishbeth, J., and Naylor, A.G. 1957. Fusarium wilt of bananas in Jamaica. III. Attempted control. *Annual Botany* 21: 599-609.
- Rombouts, J.E. 1953. Micro-organisms in the rhizosphere of banana plants in relation to susceptibility of resistance to Panama disease. *Plant and Soil* 4: 276-288.
- Rose, S., and Parker, M. 2003. Efficacy of biological and chemical treatments for control of Fusarium root and stem rot on greenhouse cucumber. *Plant Disease* 87: 1462-1470.
- Rowe, P.R., and Richardson, D.L. 1975. Breeding bananas for disease resistance, fruit quality and yield. Bulletin 2, Tropical Agriculture Research Services (SIATSA), La Lima, Honduras, pp.41.
- Rowe, R.C., and Farley, J.D. 1978. Control of Fusarium crown and root rot of greenhouse tomatoes by inhibiting recolonization of steam-disinfected soil with a captafol drench. *Phytopathology* 68: 1221-1224.
- Ryals, J., Uknes, S., and Ward, E. 1994. Systemic acquired resistance. *Plant Physiology* 104: 1109-1112.

- Sági, L., Panis, B., Remy, S., Schoofs, H., De Smet, K., Swennen, R., and Cammue, B.P.A. 1995. Genetic transformation of banana and plantain (*Musa* spp.) via particle bombardment. *Biotechnology* 13: 481-485.
- Saravanan, T., Muthusamy, M., and Marimuthu, T. 2003. Development of integrated approach to manage the fusarial wilt of banana. *Crop Protection* 22: 1117-1123.
- Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., and Manners, J.M. 2000. Coordinate plant defence responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Science of the United States of America* 97: 11655-11660.
- Scher, F.M., and Baker, R. 1980. Mechanism of biological control of a *Fusarium*-suppressive soil. *Phytopathology* 70: 412-417.
- Scher, F.M., and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology* 72: 1567-1573.
- Schisler, D.A., Slininger, P.J., and Bothast, R.J. 1997. Effects of antagonist cell concentration and two-strain mixtures on biological control of *Fusarium* dry rot of potatoes. *Phytopathology* 87: 177-183.
- Schneider, R.W. 1984. Effects of nonpathogenic strains *Fusarium oxysporum* on celery root infection by *F. oxysporum* f.sp. *apii* and a novel use of the Lineweaver-Burke double reciprocal plot technique. *Phytopathology* 74: 646-653.
- Schönbeck, F. 1979. Endomycorrhiza in relation to plant disease. In: Schippers, B., and Gams, W. (Eds), *Soil-borne plant pathogens*. Academic Press, London, pp. 271-280.
- Sequeira, L. 1962a. Control of bacterial wilt of banana by crop rotation and fallowing. *Tropical Agriculture* 38: 211-217.
- Sequeira, L. 1962b. Influence of organic amendments on the survival of *Fusarium oxysporum* f.sp. *cubense* in the soil. *Phytopathology* 52: 976-982.
- Sequeira, L., Steeves, T.A., Steeves, M.W., and Riedhart, J.M. 1958. Role of root injury in Panama disease infections. *Nature (Lond.)* 182: 309-311.

- Serra-Wittling, C., Houot, S., and Alabouvette, C. 1996. Increased soil suppressiveness to *Fusarium* wilt of flax after addition of municipal solid waste compost. *Soil Biology and Biochemistry* 28: 1207-1214.
- Siegrist, J., Orober, M., and Buchenauer, H. 2000. β -Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiological and Molecular Plant Pathology* 56: 95-106.
- Silué, D., Pajot, E., and Cohen, Y. 2002. Induction of resistance to downy mildew (*Peronospora parasitica*) in cauliflower by DL- β -amino-*n*-butanoic acid (BABA). *Plant Pathology* 51: 97-102.
- Simmonds, N.W. 1959. Bananas. Longmans, Green and Co. Ltd, London.
- Simmonds, N.W. 1962. The evolution of Bananas. Longmans, Green and Co. Ltd, London.
- Singh, P.P., Shin, Y.C., Park, C.S., and Chung, Y.R. 1999. Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* 89: 92-99.
- Sivamani, E., and Gnanamanickam, S.S. 1988. Biological control of *Fusarium oxysporum* f.sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. *Plant and Soil* 107: 3-9.
- Sivan, A. 1987. Biological control of *Fusarium* crown rot of tomato by *T. harzianum* under field conditions. *Plant Disease* 71: 587-592.
- Sivan, A., and Chet, I. 1986. Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *Phytopathology* 116: 39-47.
- Sivan, A., and Chet, I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology* 79: 198-203.
- Smith, L.J., Smith, M.K., Hamill, S.D., Hunter, M.N., Pegg, K.G., and Galea, V.J. 1999. Towards improving resistance of micropropagated bananas to *Fusarium* wilt using bacteria and mycorrhizae I. Bioassay development. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia*, pp. 224-233.

- Smith, J., Putnam, A., and Nair, M. 1990. *In vitro* control of *Fusarium* diseases of *Asparagus officinalis* L. with a *Streptomyces* or its polyene antibiotic, faeriefungin. *Journal of Agricultural Food Chemistry* 38: 1729-1733.
- Smith, M.K., Whiley, A.W., Searle, C., Langdon, P.W., Schaffer, B., and Pegg, K.G. 1998. Micropropagated bananas are more susceptible to *Fusarium* wilt than plants grown from conventional material. *Australian Journal of Agricultural Research* 49: 1133-1139.
- Smith, S.N., and Snyder, W.C. 1971. Relationship of inoculum density and soil types to severity of *Fusarium* wilt of sweet potato. *Phytopathology* 61: 1049-1051.
- Smith, S.N., and Snyder, W.C. 1972. Germination of *Fusarium oxysporum* chlamydospores in soils favourable and unfavourable to wilt establishment. *Phytopathology* 62: 273-277.
- Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum* f.sp. *cucumerinum* as affected by fluorescent and lytic bacteria from a *Fusarium*-suppressive soil. *Phytopathology* 74: 1115-1124.
- Spanu, P., and Boller, T. 1989. Ethylene biosynthesis in tomato plants infected by *Phytophthora infestans*. *Journal of Plant Physiology* 134: 533-537.
- Steinberg, C., Whipps, J.M., Wood, D.A., Fenlon, J., and Alabouvette, C. 1999a. Mycelial development of *Fusarium oxysporum* in the vicinity of tomato roots. *Mycological Research* 103: 769-778.
- Steinberg, C., Whipps, J.M., Wood, D.A., Fenlon, J., and Alabouvette, C. 1999b. Effects of nutritional sources on growth of one non-pathogenic strain and four strains of *Fusarium oxysporum* pathogenic on tomato. *Mycological Research* 103: 1210-1216.
- Stotzky, G., and Martin, R.T. 1963. Soil mineralogy in relation to the spread of *Fusarium* wilt of banana in Central America. *Plant and Soil* 18: 317-338.
- Stotzky, G., and Rem, L.T. 1965. Influence of clay minerals on microorganisms I. Montmorillonite and kaolinite on bacteria. *Canadian Journal of Microbiology* 12: 547-563.
- Stover, R.H. 1962. *Fusarial wilt (Panama disease) of bananas and other Musa species*. Commonwealth Mycological Institute, Kew, Surrey, UK, 117 pp.

- Stover, R.H. 1986. Disease management strategies and the survival of the banana industry. *Annual Review of Phytopathology* 24: 83-91.
- Stover, R.H. 1990. Fusarium wilt of banana: some history and current status of the disease. In: Ploetz, R.C. (Ed), *Fusarium wilt of banana*. ASP Press, St. Paul, Minnesota, USA, pp. 1-7.
- Stover, R.H., and Buddenhagen, I.W. 1986. Banana breeding: polyploidy, disease resistance and productivity. *Fruits* 41: 175-191.
- Stover, R.H., Hildreth, R.C., and Thornton, N.C. 1961. Studies on Fusarium wilt of bananas. VII. Field control. *Canadian Journal of Botany* 39: 197-206.
- Su, H., Hwang, S., and Ko, W. 1986. Fusarial wilt of Cavendish bananas in Taiwan. *Plant Disease* 70: 814-818.
- Summer, D.R. 1994. Cultural Management. In: Campbell, C.L., and Benson, D.M. (Eds), *Epidemiology and Management of Root Diseases*. Springer-Verlag, Berlin, Heidelberg, pp. 309-333.
- Sun, S.K., and Huang, J.W. 1985. Formulated soil amendments for controlling Fusarium wilt and other soilborne disease. *Plant Disease* 69: 917-920.
- Tally, A., Oostendorp, M., Lawton, K., Staub, T., and Bassi, B. 1999. Commercial development of elicitors of induced resistance to pathogens. In: Agrawal, A.A., Tuzun, S., and Bent, E. (Eds), *Induced plant defences against pathogens and herbivores*. APS Press, St Paul, Minnesota, USA, pp. 375-369.
- Tezuka, N., and Makino, T. 1991. Biological control of Fusarium wilt of strawberry by nonpathogenic *Fusarium oxysporum* isolated from strawberry. *Annals of Phytopathology Society of Japan* 57: 506-511.
- Thanassouloupoulos, C.C., Giannopolitis, C.N., and Kitsos, G.T. 1970. Control of Fusarium wilt of tomato and waterlemon with benomyl. *Plant Disease Reporter* 54: 561-564.
- Thangavelu, R., Palaniswami, A., and Velazhahan, R. 2003. Mass production of *Trichoderma harzianum* for managing Fusarium wilt of banana. *Agriculture Ecosystem and Environment* 103: 259-263.

- Thangavelu, R., Palaniswami, A., Ramakrishnan, G., Doraiswamy, S., Muthukrishnan, S., and Velazhahan, R. 2001. Involvement of fusaric acid detoxification by *Pseudomonas fluorescens* strain Pf10 in the biological control of Fusarium wilt of Banana caused by *Fusarium oxysporum* f.sp. *cubense*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Disease and Protection) 108: 433-445.
- Tilston, E.L., Pitt, D., and Groenhof, A.C. 2002. Composted recycled organic matter suppresses soil-borne diseases of field crops. New Phytologist 154: 731-740.
- Ting, A.S.Y., Sariah, M., Jugah, K., and Anuar, A.R. 2002. Effect of artificially induced suppressive soil of Fusarium wilt. InfoMusa 12: 33-34.
- Tosi, L., Luigetti, R., and Zazzerini, A. 1998. Induced resistance against *Plasmopara helianthi* in sunflower plants by DL-beta-amino-n-butyric acid. Journal of Phytopathology 146: 295-299.
- Toyota, K., Yamamoto, K., and Kimura, M. 1994. Isolation and characterization of bacteria responsible for the suppression of *Fusarium oxysporum* f.sp. *raphani* on the host rhizoplane. Soil Science and Nutrition 40: 381-390.
- Tuitert, G., Szczech, M., and Bollen, G.H. 1998. Suppression of *Rhizoctonia solani* in potting mixtures amended with compost made from organic household waste. Phytopathology 88: 764-773.
- Vakili, N.G. 1967. The experimental formation of polyploidy and its effect in the genus *Musa*. American Journal of Botany 54: 24-36.
- Van Loon, L.C. 1999. Improved biological control of Fusarium wilt by combinations of antagonistic micro-organisms in the rhizosphere of crop plants. UBI.3227.
- Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J. 1998. Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology 36: 453-483.
- Van Peer, R., Niemann, G.J., and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81: 728-734.
- Viljoen, A. 2002. The status of Fusarium wilt (Panama disease) of banana in South Africa. South African Journal of Science 98: 341-344.

- Wardlaw, C.W. 1961. Banana diseases, including plantains and abaca. Longmans, Green and Co. Ltd, London, 648 pp.
- Watanabe, T., Igarashi, H., Matsumoto, K., Seki, S., Mase, R., and Sekizawa, Y. 1977. The characteristics of propanazole (Oryzemat) in the control of rice blast. *Journal of Pesticides Science* 2: 291-296.
- Weller, D.M. 1988, Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* 26: 379-407.
- Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B. and Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40: 309-348.
- Widmer, T. L., Mitkowski, N.A., and Abawi, G.S. 2002. Soil organic matter and management of plant-parasitic nematodes. *Journal of Nematology* 34: 289-295.
- Woo, S.L., Lorito, M., Muccifora, S., Scala, F., Gori, P., and Noviello, C. 1997. Presence of double-stranded RNA and virus-like particles in isolates of *Fusarium oxysporum* f.sp. *phaseoli*. *Phytopathology* 87: S104.
- Yuan, W.M., and Crawford, D.L. 1995. Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Applied and Environmental Microbiology* 61: 3119-3128.
- Zhang, J., Howell, C.R., and Starr, J.L. 1996. Suppression of *Fusarium* colonization of cotton roots and Fusarium wilt by seed treatments with *Gliocladium virens* and *Bacillus subtilis*. *Biocontrol Science Technology* 6: 175-187.

CHAPTER 2

Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana



ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), is the most important disease of bananas in South Africa. There is no effective control measure for this disease other than the use of resistant cultivars. Unfortunately, the locally grown Cavendish cultivars are all highly susceptible to *Foc* “subtropical” race 4, the only variant of the pathogen that occurs in South Africa. Currently, the only option available to manage the disease is to prevent the introduction of the fungus into new fields. This can be achieved by using effective sterilants to disinfect contaminated tools, machinery and clothes. The first objective of this study was to evaluate newly available fungicides, representing the main fungicidal groups, *in vitro* and *in vivo* for their efficacy against *Foc*. The second objective was to evaluate surface sterilants that can be used for sterilization purposes. Of the fungicides tested, prochloraz and propiconazole significantly inhibited mycelial growth *in vitro* at concentrations of 1 µg.g⁻¹ and 5 µg.g⁻¹, respectively. The *in vivo* studies of the fungicides showed that benomyl and the DMI fungicides lowered the disease severity of *Foc* when applied as a dip treatment. Results also proved that certain quaternary ammonium compounds are effective as sterilants against *Foc* and should replace the ineffective sterilants that are currently being used. These newly identified sterilants are environmental friendly, non-toxic and non-corrosive sterilants. Further field evaluations of the fungicides are required to determine if the effect observed in the greenhouse can be integrated into field management of Fusarium wilt.

INTRODUCTION

Fusarium wilt of banana is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder and Hansen (*Foc*) (Stover, 1962). The fungus infects the banana roots, colonising the vascular system of the rhizome and pseudostem, and inducing characteristic wilting symptoms before the plant eventually dies (Wardlaw, 1961; Stover, 1962). Evidence suggests that *Foc* originated in Southeast Asia (Ploetz and Pegg, 1997) and from there disseminated rapidly throughout the world with infected rhizomes (Stover, 1962). During the first half of the 20th century, Fusarium wilt caused major destruction in the Central American region, and since Panama was one of the most severely affected countries, the disease adopted the name Panama disease (Jeger *et al.*, 1996). Due to the destruction Fusarium wilt caused in Central America, the international export trade was forced to convert from the susceptible Gros Michel to the resistant Cavendish cultivars (Ploetz *et al.*, 2003). Unfortunately, Cavendish cultivars in subtropical countries of the world are susceptible to race 4 of *Foc* (Ploetz and Pegg, 2000).

Fusarium wilt of banana is controlled effectively by applying disease-preventive practices such as planting in fields not infested with *Foc* using disease-free propagation material. Once infested with the pathogen, the only way of continuing banana production is by means of planting cultivars with resistance to the disease (Deacon, 1984; Ploetz and Pegg, 2000; Viljoen, 2002; Ploetz *et al.*, 2003). In South Africa, cultivars with resistance to *Foc* have been identified, but these cultivars are not acceptable to the local market (Viljoen, 2002). While research programmes around the world now aim at producing Cavendish bananas with resistance against pests and diseases, efforts to develop an integrated management programme for Fusarium wilt should not be neglected. Such a programme could combine disease tolerance with strategies such as chemical, biological and cultural control.

Before Gros Michel was replaced with Cavendish bananas in Central America in the 1960's, several studies investigated the potential application of chemical and cultural

control strategies in *Fusarium* wilt management (Stover, 1962). Various laboratory and greenhouse evaluations of fungicides were performed, and some of these compounds were found to effectively inhibit *Foc* growth during *in vitro* studies and in the greenhouse (Meredith, 1943; Corden and Young, 1959; 1960; Stover, 1962). However, the fungicides that performed best in these studies were unable to control *Fusarium* wilt effectively in the field (Meredith, 1943; Stover, 1962). After 1960, few reports can be found on the use of fungicides for the control of *Foc*. In South Africa, Deacon (1984) recommended that every banana sucker should be washed free of soil and inspected for the presence of the disease, followed by a dip treatment with a fungicide mix before planting, in order to limit the spread of the disease. In India, stem injections with carbendazim decreased the disease incidence of cultivar Rastali (i.e. Silk), but it proved to be ineffective in South Africa (Lakshmanan *et al.*, 1987; Herbert and Marx, 1990). Even though it is clear that chemical control of *Fusarium* wilt of banana has received little attention in the past 40 years, fungicides remain essential for effective control of many plant diseases. The significant discovery of new fungicides has often led to a series of follow-up products, which should be considered when designing an integrated disease management programme (Gullino *et al.*, 2000).

Fusarium wilt of banana has been disseminated from one country or growing region to another by means of infected rhizomes or suckers and adhering soil. These rhizomes and suckers often did not exhibit symptoms (Moore *et al.*, 1995). Within production areas or between fields, the pathogen is spread to non-infested areas in contaminated irrigation water, and with farm equipment, shoes and vehicles (Ploetz, 1994). The use of effective disinfectants or surface sterilants is, therefore, essential in preventing the spread of *Foc*. Disinfecting *Foc*-infested soil in small areas by drenching with copper sulfate, carbolineum and formaldehyde proved to be unsuccessful and expensive (Brandes, 1919). In Jamaica, Rishbeth and Naylor (1957) found carbon disulphide and formalin ineffective and unreliable when evaluated as soil disinfectants. Even soil fumigation with methyl bromide proved to be effective only as a short-term control measure in South Africa, due to the successful re-invasion of the pathogen after a period of approximately 2 years (Herbert and Marx, 1990). The application of surface disinfectants, therefore, is more

effective and appropriate for the cleaning of equipment, shoes and machinery. In Australia, a commercial product 'Farmcleanse' was found to be the most effective disinfectant of *Foc* spores when compared to other fungicides and quaternary ammonium compounds (Moore *et al.*, 1999). 'Farmcleanse' is a detergent-based degreaser with a quaternary ammonium additive that has replaced chlorine bleach, methylated spirits and copper oxychloride solutions as disinfectant of vehicles, farm equipment and in footbaths, since it is non-corrosive and more environmentally friendly (Moore *et al.*, 1999). In South Africa, copper oxychloride was almost exclusively used for the disinfection of vehicles, farm equipment and shoes prior to this study (Viljoen, personal communication).

The aim of this study was, firstly, to screen a range of fungicides, including new chemical formulations, for their ability to inhibit mycelial growth of *Foc in vitro*. Secondly, the aim was to determine the efficacy of some of these fungicides against banana wilt in the greenhouse, and lastly, to evaluate the efficacy of various surface sterilants in suppressing *Foc* colony development.

MATERIALS AND METHODS

Isolates used and inoculum preparation

All isolates of *Foc* used in this study represent subtropical race 4 (VCG 0120), and were isolated from diseased banana plants in South Africa. Their virulence to banana plantlets and their cultural characteristics were established earlier by Groenewald *et al.* (unpublished data). For the *in vitro* evaluation of fungicides, a slow (CAV 031) and a fast growing (CAV 086) isolate were selected. For the *in vivo* studies, two highly virulent isolates (CAV 045 and CAV 092) were selected. These isolates were all grown for 7 days at 25°C on half-strength potato dextrose agar (PDA) (19 g of Difco PDA powder, 19 g of Biolab agar, 1000 ml H₂O) under cool-white and near-ultraviolet fluorescent lights before being used for fungicide evaluation. Mycelial plugs (0.5 cm in diameter) from the margins of these cultures were aseptically transferred to the centres of fungicide-amended PDA for the *in vitro* studies, and inoculated into autoclaved millet seeds in 500-ml

Erlenmeyer flasks for the *in vivo* studies (Strauss and Labuschagne, 1995). Inoculated flasks were incubated at 25°C for 2 weeks during which the flasks were shaken twice weekly to ensure equal distribution of the inoculum. Infested millet seeds were then added to steam-pasteurised soil at a rate of 15 ml seeds per 500 ml soil. The inoculated soil was thoroughly mixed to obtain a final concentration of 3% seed inoculum.

For evaluation of sterilants, a pathogenic isolate of *Foc* (CAV 027) was grown for 7 days on half-strength PDA at 25°C under cool-white and near-ultraviolet fluorescent lights. To prepare a conidial spore suspension, mycelia were transferred into 250-ml Erlenmeyer flasks containing Armstrong *Fusarium* medium (Booth, 1977). The flasks were placed on a rotary shaker operating at 170 rpm at 25°C for 5 days, after which the conidial suspension was passed through cheesecloth to separate the mycelium from the spores. The spore concentration in the remaining liquid medium was determined using a hemacytometer, and diluted with sterile distilled water to a final concentrations of 10^5 spores.ml⁻¹.

Selection of fungicides and surface sterilants

Nine fungicides, representative of seven different chemical groups, were selected for *in vitro* evaluation against *Foc* (Table 1). These fungicides included several new commercial products that had not previously been tested for activity against *Foc*. All the fungicides were tested for efficacy against both *Foc* isolates mentioned earlier, except for phosetyl-Al that was only evaluated against CAV 086. Five fungicides evaluated *in vivo* were selected on the basis of their performance in the *in vitro* evaluation. Before fungicide trials were conducted, phytotoxicity experiments were set up to determine the optimal concentration at which chemicals should be applied to the banana plantlets. Chemicals were tested for phytotoxicity at concentrations of 5, 10, 25 and 50 ppm of the active ingredient (a.i.). Nine commercially available surface sterilants were further selected for evaluation against *Foc* at four different spore concentrations (Table 2).

Evaluation of fungicides

In vitro evaluation: Fungicides were suspended in sterile distilled water and added to autoclaved PDA to achieve final concentrations of 1, 5, 10, 50, 100 ppm (a.i.). The fungicide-amended PDA was dispensed aseptically into 9-cm-diameter plastic Petri dishes. Mycelial plugs of single-spore isolates of *Foc* were then transferred to the centres of the fungicide-amended media. The dishes were incubated for 7 days at 25°C under cool-white and near-ultraviolet fluorescent lights, after which colony diameter was measured (two perpendicular measurements per colony). The mycelial growth on the fungicide-amended PDA was compared with the growth of the pathogen on unamended PDA plates serving as controls. Each treatment was replicated five times. The *in vitro* study was conducted twice.

In vivo evaluation: Pathogen-free tissue culture banana plantlets (of the cultivar Chinese Cavendish) were obtained from Du Roi Laboratories in Letsitele, South Africa. After the roots of the plants were washed to remove excess sterile soil, the 5-cm plantlets were placed in 250-ml plastic cups filled with tap water to allow substantial root development. Strips of sponge were used to support the plants that were stabilized through holes in the lids of the cups. Plants received a weekly nutrient solution consisting of 0.45g Agrasol® 'O 3:2:8 (Fleuron, P.O. Box 31245, Braamfontein, 2017), 0.3 g calcium nitrate monohydrate and 0.15 g Micromix® (Fleuron) L⁻¹ tap water, with a pH of 7.0. After 4 weeks, the approximately 10-cm plants were used for the *in vivo* evaluations of fungicides.

Plants with roots of more or less uniform length (15.0±0.5cm) were selected for greenhouse evaluation of fungicides against *Fusarium* wilt. All roots were slightly bruised by manually squeezing the rootball by hand to ensure infection by the pathogen. Three application methods were tested for each fungicide, namely root dipping at planting, soil drenching immediately after planting, and soil drenching 1 week after planting. For the root dipping, roots of the banana plantlets were submerged for 10 min in a 25 ppm (a.i.) concentration of the five selected fungicides and replanted in 12.5-cm-diameter pots containing steam-pasteurised soil infested with a 3% millet seed inoculum

of *Foc*. Soil drench applications were made after plants had been replanted by drenching the roots of plantlets with 100 ml of a 25 ppm (a.i.) concentration of each fungicide. Similarly, plants receiving soil drench treatments 1 week after planting were also treated with 100 ml of a 25 ppm (a.i.) concentration of each fungicide. Plants treated with 100 ml distilled water and planted in soil infested and uninfested with *Foc* served as controls. The plants were transferred to phytotrons with a photoperiod of 12 hours and at a day/night temperature regime of 28/20°C for 7 weeks. Six replicate plants were used for each treatment, and the trial was repeated. Disease development was estimated by cutting the pseudostem open with a scalpel and by assigning a disease severity rating to each plant according to Inibab's Technical Guidelines number 6 (Carlier *et al.*, 2002) (Table 3).

Evaluation of surface sterilants

In vitro tests were performed to evaluate various compounds for use in the sterilization of contaminated equipment, shoes, and vehicles. *Foc* microconidia at concentrations of 10^5 spores.ml⁻¹ were mixed with each of the sterilants to achieve final concentrations of 0.01, 0.1, 1 and 10 times the recommended dosages. After an exposure time of 5 min, a 1-ml sample of each sterilant-suspension containing *Foc* conidia was spread evenly over PDA plates and incubated at 25°C under cool-white and near-ultraviolet fluorescent lights. Sterile distilled water containing *Foc* spores were used as controls. The developing *Foc* colonies were counted after 4 days. The experiment was performed using spore dilutions of 10^4 , 10^3 , 10^2 and 10^1 spores.ml⁻¹. In addition, time exposure experiments were also conducted at a concentration of 0.1 of the recommended dosage. The three sterilants that showed the lowest *Foc* colony development were tested at different time intervals of 30 sec, 5 min, 15 min and 30 min. The entire experiment was repeated with five replicates of each treatment. The number of developing *Foc* colonies was used to estimate the efficacy of the sterilants.

Statistical analysis

Data from repetitive *in vitro* fungicide experiments were pooled together and subjected to analysis of variance (ANOVA). Mean differences were separated according to Duncan's

Multiple Range ($P \leq 0.05$). Data obtained for the greenhouse trials were discrete and required a non-parametric analysis. Data were analysed based on Wilcoxon scores (Sokal and Rohlf, 1981). Differences between treatments were significant at $P \leq 0.05$ (SAS Institute Inc., SAS/STAT Users Guide, Version 8, Cary, NC: SAS Institute Inc., 1999). Data from the surface sterilant evaluations were pooled together and analysed based on the General Linear Model Procedure (GLM) as given in SAS (SAS Institute Inc., SAS/STAT Users Guide, Version 8, Cary, NC: SAS Institute Inc., 1999). Differences between treatments were significant at $P \leq 0.05$.

RESULTS

Evaluation of fungicides

In vitro evaluation: All fungicides, except benomyl and captab at a concentration of 1 ppm (a.i.), significantly reduced the mycelial growth of *Foc* isolate CAV 086 in culture (Fig. 1A). The demethylation-inhibiting (DMI) fungicides (prochloraz, propiconazole and the combination of cyproconazole and propiconazole), however, were most effective. Of the nine fungicides evaluated, prochloraz was the only one to inhibited mycelial growth of *Foc* completely at all concentrations tested. The two DMI-triazole fungicides, the combination of cyproconazole and propiconazole as well as propiconazole, at a concentration of 5 ppm (a.i.), also completely inhibited growth of the pathogen *in vitro* (data not presented). Benomyl was also effective but to a lesser degree. Conversely, media amended with copper oxychloride, captab, azoxystrobin and phosetyl-Al showed poor inhibition of *Foc* growth, even at 100 ppm (a.i). Although the phosetyl-Al did not show total inhibition of growth, it reduced the density of the mycelia considerably.

Very similar results were obtained with *Foc* isolate CAV 031, than with the fasted growing CAV 086. Prochloraz was again the most effective fungicide, showing total inhibition of fungal growth at all the concentration tested (Fig. 1B). Prochloraz was followed by cyproconazole/propiconazole and benomyl, which restricted growth at concentrations of 10 ppm (a.i.) and higher. Propiconazole completely inhibited fungal

growth at concentrations of 100 ppm (a.i.). The fungicides captab, kresoxim-methyl, azoxystrobin and copper oxychloride were less effective.

In vivo evaluation: Banana plants treated with all the chemicals at concentrations of 50 ppm (a.i.) developed severe symptoms of phytotoxicity. When treated at concentrations of 5, 10 and 25 ppm (a.i.), none of the plants developed signs of phytotoxicity. The fungicide benomyl and the DMI fungicides, prochloraz, propiconazole and cyproconazole/propiconazole significantly reduced the incidence of *Foc* with the root dip and soil drench treatments 1 week after planting, if we compare the 95% confidence intervals of the fungicide treatments with the pathogen-infested control plants (Fig. 2). Cyproconazole/propiconazole, applied as root dip treatment, was the most effective in reducing the disease severity, resulting in a disease reduction of 80.6% (Table 4). Prochloraz and propiconazole were also highly effective as root dip treatments, with disease reductions of 77.8% and 75.0%, respectively. Benomyl was found the most effective applied as a soil drench treatment 1 week after planting and resulted in a disease reduction of 72.2% (Table 4). Phosetyl-Al, applied to the banana plantlets either as root dip or soil drench treatments, had no significant reduction in the disease severity. The external symptoms on the plants were not as informative as the internal symptoms, however slight differences could be observed (Fig. 4). No internal discoloration was observed in the uninfested control plants, while the infested control plants showed a high level of disease severity based on internal discoloration. The experiment was repeated twice. Data obtained from both trials resulted in similar results.

Evaluation of surface sterilants

The most conclusive results were obtained when the sterilants were applied to a spore concentration of 10^2 spores.ml⁻¹. Therefore, data from only this concentration is presented in Fig. 3). All the sterilants showed a reduction in the number of *Foc* colonies that developed when applied at a concentration of 10 times more than the recommended dosage (Fig. 3). A dose-dependent response could be observed, with the efficacy of the sterilants increasing when higher concentrations of the sterilants were used. Of all the sterilants tested, Sporekill, Jik and Prazin proved to be the most effective at all

concentrations, and completely suppressed colony development, with the exception at a concentration of 0.01 of the recommended dosage. Sporekill, Prazin and Jik were effective at exposure times of 5, 15 and 30 min. Sporekill was the only sterilant that showed no *Foc* colony development after an exposure time of 30 sec, (data not presented). Prazin and Jik were not completely effective at an exposure time of 30 sec.

DISCUSSION

Demethylation-inhibiting (DMI) fungicides, which include the imidazoles (prochloraz) and the triazoles (propiconazole and cyproconazole/propiconazole), are one of the most important groups of fungicides (Baldwin and Rathmell, 1988). They are also known as the “azole group”. It is this group of fungicides that showed the greatest effectiveness in inhibiting *Foc* growth *in vitro* and suppression of the disease in the greenhouse. DMI fungicides are a chemically diverse group, and they act by inhibiting the demethylation step in the biosynthesis of sterol, which is needed in fungal cell walls. They most likely bind to cytochrome P-450 involved in sterol demethylations (Uesugi, 1998). In this study, prochloraz and cyproconazole/ propiconazole proved to be the most effective of all the fungicides evaluated.

Similar results have been reported on the use of prochloraz against other *Fusarium* spp. (Strauss and Labuschagne, 1993; Song *et al.*, 2004). Strauss and Labuschagne (1993) found prochloraz to be the most effective fungicide against *Fusarium solani* (Mart.) Sacc. *in vitro* and *in vivo*. They applied the fungicide as a drench treatment to the roots of citrus seedlings 7 days after inoculation with *F. solani*. Song *et al.* (2004) found prochloraz and carbendazim to be the most effective fungicides in inhibiting mycelial growth of *F. oxysporum* causing Fusarium wilt of tomato. They also observed in a hydroponic cultivation system that prochloraz had a preventative effect of 69.9% and a curative effect of 34.4%.

Benomyl is a systemic fungicide that acts as a multiplication inhibitor during fungal mitosis (Uesugi, 1998). In this study, benomyl inhibited the mycelial growth of both *Foc*

isolates effectively at concentration greater than 10 ppm (a.i.). In the greenhouse trials benomyl was most effective, especially as a soil drench treatment 1 week after planting and as a root dip treatment. Similarly, Allen *et al.* (2004) performed *in vitro* fungicide evaluations against *Fusarium circinatum* Nirenberg & O'Donnell, *F. oxysporum*, *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg and *F. solani*. They found that benomyl at all concentrations, completely inhibited fungal growth of all four *Fusarium* species. However, prochloraz and cyproconazole/ propiconazole were not included in their study. Ram *et al.* (2004) reported corm dip treatments with different fungicides for the control of corm rot caused by *F. oxysporum* f.sp. *gladioli*. They reported that benomyl was the most effective treatment, followed by carbendazim and captab. They also observed that dip treatments of 60 min were much more effective than 30 min.

Chemical control can be combined with biological control products in an integrated disease management programme. Elmer and McGovern (2004) combined commercial biological products comprising beneficial microbes with fungicides and found that the combination provided a higher degree of suppression of *Fusarium* wilt of cyclamen. Similarly, Minuto *et al.* (1995) found an enhanced degree of control against *Fusarium* wilt of cyclamen after the combination of the benzimidazole fungicide carbendazim and antagonistic *Fusarium* spp. Fungicides have the potential to be part of an integrated disease management programme for *Fusarium* wilt of banana. However, fungicides could only be applied to banana plants by means of soil drenches or root dipping, since stem injections have proved to be ineffective in the past (Herbert and Marx, 1990). It is also important to determine what impact these fungicides will have on the natural soil microflora and suppressiveness of the soil, and what combination of biological control agents can be applied with these fungicides.

It was observed in this study that more effective surface sterilants than the ones previously used against *Foc* contamination are available on the market. The quaternary ammonium compounds such as Sporekill and Prazin are generally considered as fast-acting disinfectants and are active at low concentrations (Tanner, 1989). Sporekill

prevented colony development of *Foc* at an exposure time of only 30 sec. This makes Sporekill a highly effective compound for the sterilization of *Foc* contaminated equipment, vehicles and shoes in the field. Jik and Prazin also provided complete inhibition of conidial germination as reflected in reduced colony formation at low concentrations, and can be used for sterilization purposes. These three compounds were more effective against *Foc* than copper oxychloride and Farmcleanse, which have been used in water baths and for disinfection of machinery, field equipment and workers' shoes in South Africa and Australia, respectively. Prochloraz was also included in the sterilization experiment to determine its effectiveness on *Foc* colony development *in vitro*. Prochloraz, being a fungicide rather than a sterilant was, however, far less effective for this purpose.

In South Africa surface sterilants are already part of the integrated disease management programme of banana. However, the disinfectants that were used in the past proved to be ineffective and, based on the findings of the current study, farmers have been informed that the previously used sterilants should be replaced with Sporekill or Prazin. Sporekill and Prazin are relatively inexpensive, environmentally friendly, non-toxic and non-corrosive sterilants. Since there is currently no effective control measures available for the control of Fusarium wilt of banana, prevention is of critical importance. Many farmers have not yet adopted any preventative strategies and can, therefore, contribute to the spread of Fusarium wilt of banana.

REFERENCES

- Allen, T.W., Enebak, S.A., and Carey, W.A. 2004. Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. *Crop Protection* 23: 979-982.
- Baldwin, B.C., and Rathmell, W.G. 1988. Evolution of concepts for chemical control of plant disease. *Annual Review of Phytopathology* 26: 265-283.
- Booth, C. 1977. *Fusarium*. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, UK, p. 10.
- Brandes, E.W. 1919. Banana wilt. *Phytopathology* 9: 339-389.

- Carlier, J., De Waele, D., and Escalant, J.V. 2002. Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. INIBAP Technical Guidelines 6, Montpellier France.
- Corden, M.E., and Young, R.A. 1959. The effects of fungicides on *Fusarium oxysporum* f.sp. *cubense* and other soil microorganisms. U.F.-Oregon State College annual reports.
- Corden, M.E., and Young, R.A. 1960. The effects of fungicides on *Fusarium oxysporum* f.sp. *cubense* and the nature of wilt disease resistance. U.F.-Oregon State College annual reports.
- Deacon, J.W. 1984. Panama disease of banana in South Africa. Horticultural Science 1: 29-31.
- Elmer, W.H., and McGovern, R.J. 2004. Efficacy of integrating biologicals with fungicides for the suppression of Fusarium wilt of cyclamen. Crop Protection 23: 909-914.
- Gullino, M.L., Leroux, P., and Smith, C.M. 2000. Uses and challenges with novel compounds for plant disease control. Crop Protection 19: 1-11.
- Herbert, J.A., and Marx, D. 1990. Short-term control of Panama disease in South Africa. Phytophylactica 22: 339-340.
- Jeger, M.J., Waller, J.M., Johanson, A., and Gowen, S.R. 1996. Monitoring in banana pest management. Crop Protection 4: 391-397.
- Lakshmanan, P., Selvaraj, P., and Mohan, S. 1987. Efficiency of different methods for the control of Panama disease. Tropical Pest Management 33: 373-376.
- Meredith, C.H. 1943. Mercury compounds applied to banana plants in the field. Phytopathology 33: 835-836.
- Minuto, A., Migheli, Q., and Garibaldi, A. 1995. Evaluation of antagonistic strains of *Fusarium* spp. in the biological and integrated control of Fusarium wilt of cyclamen. Crop Protection 14: 221-226.
- Moore, N.Y., Bentley, S., Pegg, K.G., and Jones, D.R. 1995. Fusarium wilt of banana. *Musa* disease fact sheet No.5. INIBAP: 1-4.

- Moore, N.Y., Pegg, K.G., Smith, L.J., Langdon, P.W., Bentley, S., and Smith, M.K. 1999. Fusarium wilt of banana in Australia. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 64-75.
- Ploetz, R.C. 1994. Panama disease: return of the first banana menace. *International Journal of Pest Management* 40: 326-336.
- Ploetz, R.C., and Pegg, K.G. 1997. Fusarium wilt of banana and Wallace's line: Was the disease originally restricted to his Indo-Malayan region? *Australian Plant Pathology* 26: 239-249.
- Ploetz, R.C., and Pegg, K.G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D.R. (Ed), Diseases of banana, abacá and enset. CAB International, Wallingford, UK, pp. 143-172.
- Ploetz, R.C., Thomas, J.E., and Slabaugh, W.R. 2003. Diseases of banana and plantain. In: Ploetz, R.C. (Ed), Diseases of tropical fruit crops. CAB International, Wallingford, UK, pp 109-112.
- Ram, R., Manuja, S., Dhyani, D., and Mukherjee. 2004. Evaluations of fortified fungicide solutions in managing corm rot disease of gladiolus caused by *Fusarium oxysporum*. *Crop Protection* 23: 783-788.
- Rishbeth, J., and Naylor, A.G. 1957. Fusarium wilt of bananas in Jamaica. III. Attempted control. *Annual Botany* 21: 599-609.
- Sokal, R.R., and Rohlf, F.J. 1981. Biometry: The principles and practice of statistics in biological research. W.H. Freeman and Company, San Francisco.
- Song, W., Zhou, L., Yang, C., Cao, X., Zhang, L., and Liu, X. 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. *Crop Protection* 23: 243-247.
- Stover, R.H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Commonwealth Mycological Institute, Kew, Surrey, UK, 117 pp.
- Strauss, J., and Labuschagne, N. 1993. *In vitro* and *in vivo* effect of fungicides on *Fusarium solani* associated with citrus roots. *Applied Plant Science* 7: 56-59.

- Strauss, J., and Labuschagne, N. 1995. Pathogenicity of *Fusarium solani* isolates on citrus roots and evaluation of different inoculum types. *Toegepaste Plantwetenskap* 9: 48-52.
- Tanner, R.S. 1989. Comparative testing and evaluation of hard-surface disinfectants. *Journal of Industrial Microbiology* 4: 145-154.
- Uesugi, Y. 1998. Fungicide Classes: Chemistry, uses and mode of action. In: Hutson, D., and Miyamoto, J. (Eds), *Fungicide activity, chemical and biological approaches to plant protection*. John Willey and Sons, Chichester, England, pp. 23-56.
- Viljoen, A. 2002. The status of *Fusarium* wilt (Panama disease) of banana in South Africa. *South African Journal of Science* 98: 341-344.
- Wardlaw, C.W. 1961. *Banana diseases, including plantains and abaca*. Longmans, Green and Co. Ltd, London, 648 pp.

TABLE 1. Fungicides evaluated *in vitro* and *in vivo* against *Fusarium oxysporum* f.sp. *cubense*.

Chemical group	Active ingredient	Formulation	
		Type ^a	% Active ingredient
Benzimidazole	Benomyl	WP	500g/kg
Copper compounds	Copper oxychloride	WP	850g/kg
DMI-imidazole	Prochloraz	EC	450g/l
DMI –triazole	Cyproconazole/ propiconazole	EC	330g/l
DMI-triazole	Propiconazole	EC	250g/l
Phosphorous acid	Phosetyl-Al		200g/l
Phthlalimide	Captab	WP	500g/kg
Strobilurins	Azoxystrobin	Gran	500g/kg
Strobilurins	Kresoxim-methyl	EC	500g/kg

^a WP, wettable powder; EC, emulsifiable concentrate; Gran, granule.

TABLE 2. Surface sterilants evaluated *in vitro* against *Fusarium oxysporum* f.sp. *cubense*.

Trade name	Active ingredient
Chloride	Ca-hypochloride
Clean Green	Non-ammonium compound
Cupravit	Copper oxychloride
Desogerm	Biquanidine and quaternary ammonium compound
Farmcleanse	Quaternary ammonium compound
Jik	Sodium hypochloride
Prazin agri	Polymeric biquanidine hydrochloride and quaternary ammonium compound
Omega	Prochloraz
Sporekill	Poly dimethyl ammonium chloride

TABLE 3. Disease severity rating scale used to record internal symptoms caused by *Fusarium oxysporum* f.sp. *cubense* in banana plants (Carlier *et al.*, 2002).

Disease severity	Disease symptoms
0	Corm completely clean, no vascular discoloration
1	Isolated points of discoloration in vascular tissue.
2	Discoloration of up to 1/3 of vascular tissue.
3	Discoloration of between 1/3 and 2/3 of vascular tissue.
4	Discoloration greater than 2/3 of vascular tissue.
5	Total discoloration of vascular tissue.

TABLE 4. Effect of fungicide treatments on severity of *Fusarium* wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* on the banana cultivar Chinese Cavendish in the greenhouse.

Treatment	Disease severity ^a (%)	Disease reduction (%)
Uninfested control ^b	0.0	-
Infested control control ^c	75.0	-
Phosetyl-Al		
Root dip ^d	75.0	-
Soil drench after planting ^e	58.3	22.2
Soil drench 1 week after planting ^f	62.5	16.7
Benomyl		
Root dip	25.0	66.7
Soil drench after planting	58.3	22.2
Soil drench 1 week after planting	20.8	72.2
Cyproconazole/propiconazole		
Root dip	14.6	80.6
Soil drench after planting	50.0	33.3
Soil drench 1 week after planting	33.3	55.6
Propiconazole		
Root dip	18.8	75.0
Soil drench after planting	41.7	44.4
Soil drench 1 week after planting	35.4	52.8
Prochloraz		
Root dip	16.7	77.8
Soil drench after planting	45.8	38.9
Soil drench 1 week after planting	37.5	50.0

^a Mean of six plants per treatment. Disease severity assessed by visually rating individual plants according to Inibab's Technical Guidelines number 6 (Carlier *et al.*, 2002).

^b Plants treated with distilled water and planted in uninfested soil.

^c Plants treated with distilled water and planted in soil infested with *Foc* (CAV 045 and CAV 092) pathogenic isolates.

^d Roots of plants were dipped in a 25 ppm (a.i.) suspension of each fungicide for 10 min.

^e Plants were drenched with 100 ml of a 25 ppm (a.i.) suspension of each fungicide immediately after planting.

^f Plants were drenched with 100 ml of a 25 ppm (a.i.) suspension of each fungicide one week after planting.

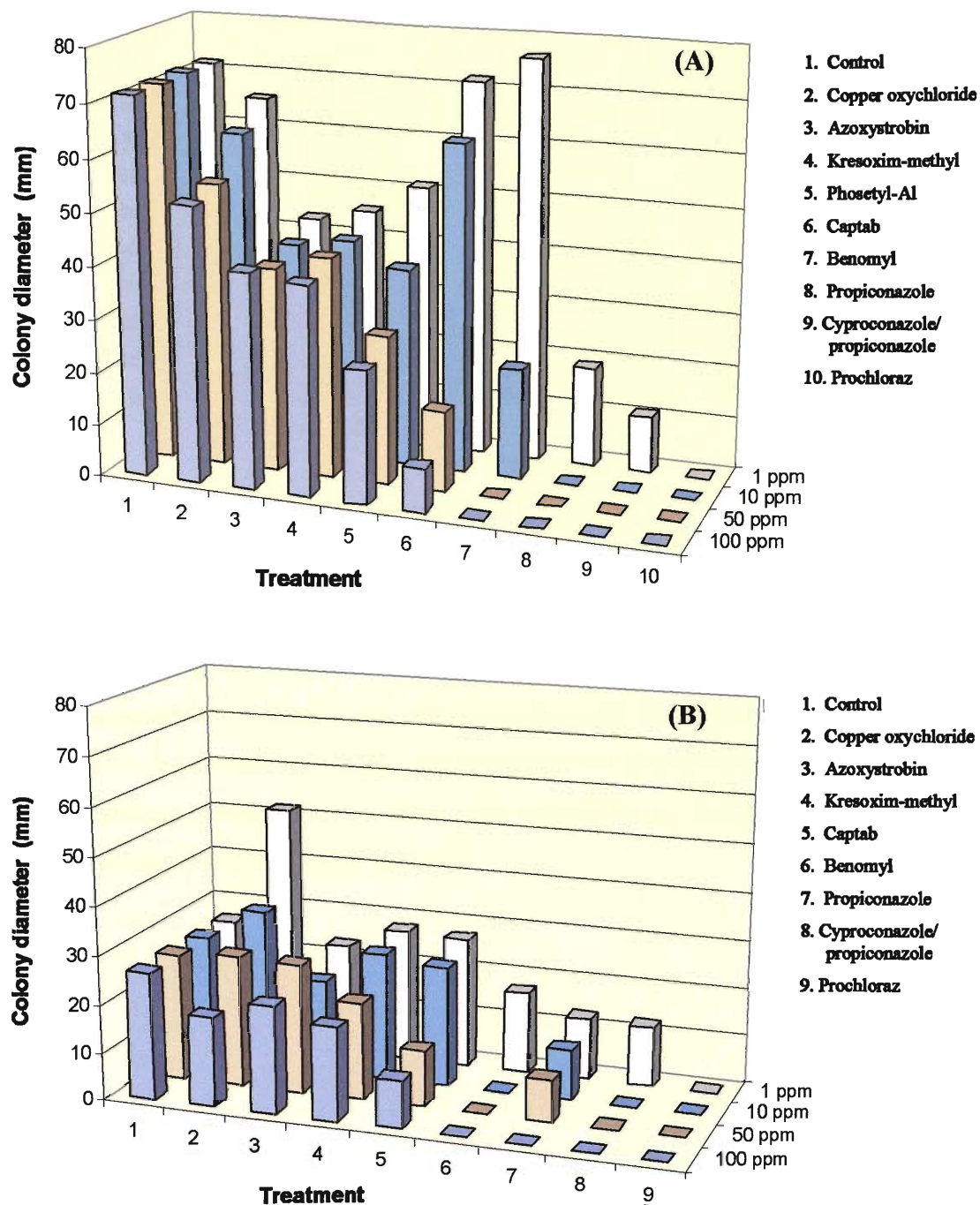


FIGURE 1. Effect of fungicides on the mycelial growth of *Fusarium oxysporum* f.sp. *cubense* isolates CAV 086 (A) and CAV 031 (B) on potato dextrose agar. Mean differences were separated according to Duncan's Multiple Range test ($P \leq 0.05$). (A) LSD=2.952; (B) LSD=2.195.

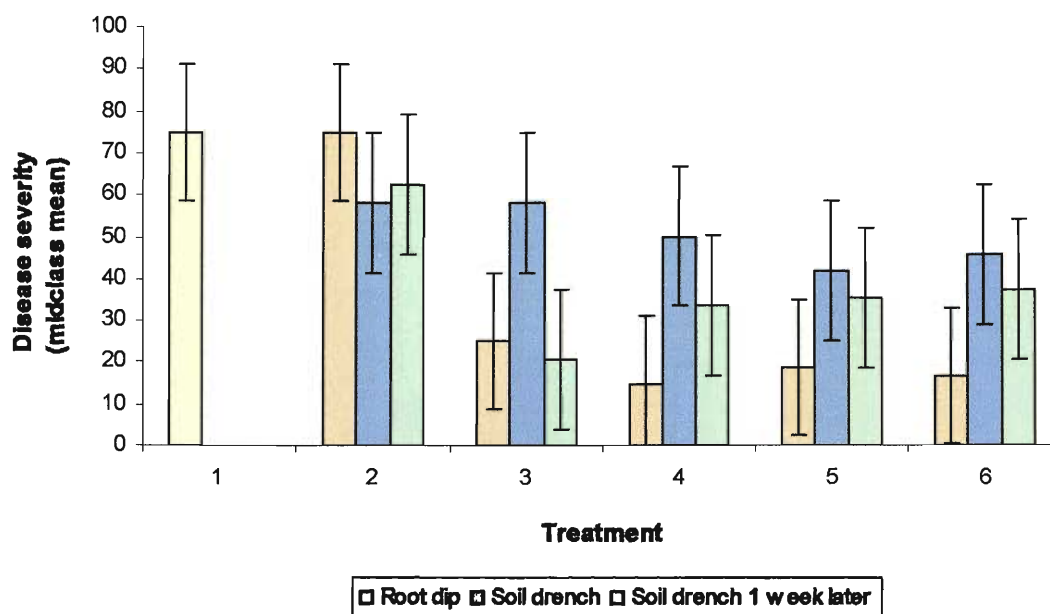


FIGURE 2. Effect of different fungicides in controlling *Fusarium* wilt development caused by *Fusarium oxysporum* f.sp. *cubense* in the banana cultivar Chinese Cavendish in the greenhouse. Treatments are as followed: (1) Control, (2) phosetyl- Al, (3) benomyl, (4) cyproconazole/propiconazole, (5) propiconazole and (6) prochloraz. Midclass mean was calculated taking in account the disease severity in all six plants per treatment. Range bars represent 95% confidence intervals.

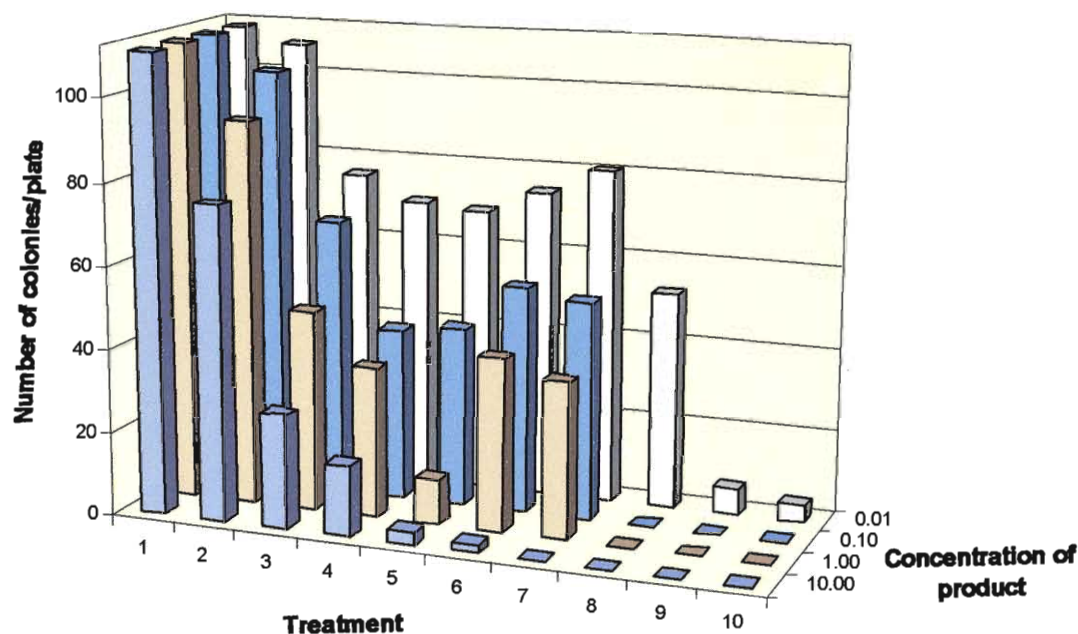


FIGURE 3. Effect of surface sterilants at different concentrations of the product, at the spore concentration of 10^2 spore.ml⁻¹ of *Fusarium oxysporum* f.sp. *cubeense* conidia. Treatments were as followed: (1) Control, (2) Chloride, (3) Omega, (4) Cupravit (copper oxychloride), (5) Farmcleanse, (6) Clean green, (7) Desogerm, (8) Prazin Agri, (9) Jik, and (10) Sporekill. Mean value determined by the General Linear Model Procedure (GLM) ($P \leq 0.05$). LSD=2.795.

FIGURE 4. Effect of fungicides evaluated on the external disease development of *Fusarium* wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) in the greenhouse with three application methods. (A) From left to right, untreated *Foc* infested control (x2), water treated uninfested control. Plants in photos (B)-(F) from left to right represent the three application methods: root dip before planting, soil drench at planting, and soil drench 1 week after planting with the fungicides cyproconazole/ propiconazole (B), benomyl (C), phosetyl-Al (D), prochloraz (E), and propiconazole (F).





CHAPTER 3

The role of chemical activators in inducing systemically acquired resistance in banana against *Fusarium oxysporum* f.sp. *cubense*



ABSTRACT

Certain chemical compounds have the ability to activate natural resistance responses in plants. These chemicals are known as chemical inducers or chemical activators. The activation of resistance responses in banana plants to protect them against Fusarium wilt has not been broadly evaluated before. The aim of this study was, therefore, to evaluate chemical compounds that induce systemic acquired resistance against diseases and pests of other crops, for their ability to induce resistance against Fusarium wilt of banana. Two Cavendish banana cultivars were evaluated in the greenhouse and in the field, namely the susceptible Williams and the tolerant DRS1 cultivar. Six different chemical activators were applied to tissue culture plantlets 2 weeks before and 2 weeks after inoculation with the pathogen in the greenhouse. Field applications of four different chemical activators were applied on a monthly basis. Of the chemicals evaluated in the greenhouse, only BTH reduced disease incidence of the Williams cultivar significantly, while SNP and Hrp-protein showed promising results on the Williams and DRS1 cultivars in the field, respectively. These chemicals have the potential to form part of an integrated disease management programme for Fusarium wilt of banana.

INTRODUCTION

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) can lead to the enhanced protection of plants against diseases and/or pests (Hammerschmidt *et al.*, 2001). SAR refers to the activation of systemic resistance responses in plants by certain natural and synthetic chemical compounds (Kessmann *et al.*, 1994), while ISR involves the biological induction of systemic resistance by prior inoculation of plants with incompatible or weak pathogens (Gessler and Kuć, 1982). During systemic resistance, protection is not restricted to the treated parts of the plant, but involves protection of the untreated plant parts as well (Sticher *et al.*, 1997). This follows activation of SAR signal transduction pathway(s) after induction of so-called SAR genes by chemical or biological elicitors. SAR genes include those that encode for pathogenesis-related (PR) proteins, some with antifungal or antibacterial activities (Hammerschmidt *et al.*, 2001). Induced resistance is associated with modifications of the cell wall, production of phytoalexins, or activation of programmed cell death, also known as the hypersensitive reaction (HR) (Métraux, 2001).

Several signalling compounds have been implicated in the regulation of the resistance response. These include the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), several proteins like harpin, reactive oxygen intermediates (ROIs) and nitric oxide (NO) (Wasternach and Parthier, 1997; Delledonne *et al.*, 1998; McDowell and Woffenden, 2003). The signalling compounds are activated by exogenous application of chemical activators. In many cases, it has been reported that infection of plants with virulent or avirulent pathogens may also activate the enhanced production of signalling compounds in the plant (Reymond *et al.*, 2000; Schenk *et al.*, 2000). The SA-, JA-, and ET-dependent defence pathways play a dominant role in SAR and ISR responses (Pieterse *et al.*, 2001). SA has been implicated as the dominant signalling molecule for the SAR reaction, although it now appears that other systemic signals can also be involved in the induction of SAR (Métraux, 2001). The ISR response requires the operation of signalling pathways responding to the plant hormones JA and ET (Feys and Parker, 2000). There is increasing evidence that interactions exist between the different

defence signalling pathways (Genoud and Métraux, 1999; Pieterse *et al.*, 2001). This cross talk between the pathways provides a great regulatory potential for activating multiple resistance mechanisms (Pieterse *et al.*, 2001).

Various natural or synthetic substances have the ability to induce systemic resistance in plants by exogenous applications. The most studied synthetic compounds are those interfering with the SA pathway, such as 2,6-dichloroisonicotinic acid (INA) and benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH). BTH was the first chemical activator to be commercially marketed as BION[®], ACTIGARD[™] and BOOST[®] (Métraux *et al.*, 1991; Görlach *et al.*, 1996; Kunz *et al.*, 1997).

Synthetic compounds also induce SAR through pathways other than the SA pathway. Cohen *et al.* (1993) first reported that JA and jasmonic methyl ester (JME) induce local and systemic resistance in plants using the JA pathway. Lawton *et al.* (1994) found that the ET-releasing agent ethephon induced an SAR-gene expression via a SA-dependent pathway. Another potential chemical inducer is the non-protein D,L- β -aminobutyric acid (BABA), which has been reported to activate disease resistance in various crops, especially against downy mildews (Cohen, 1994a; Tosi *et al.*, 1998; Siegrist *et al.*, 2000; Silué *et al.*, 2002). The involvement of NO in regulation of plant defence responses to pathogen infection has been demonstrated by Delledonne *et al.* (1998) and Durner *et al.* (1998). Durner *et al.* (1998) found that the treatments with NO donors such as sodium nitroprusside (SNP), triggered the expression of defence-related genes encoding PR1 protein and phenylalanine ammonia lyase (PAL). Even extracts from plants and microorganisms can induce resistance in plants. The protein harpin, produced by the pathogenic bacterium *Erwinia amylovora*, has been found to induce systemic resistance in plants against various fungi, bacteria and viruses (Brasher, 2000; Moffat, 2001).

One of the most destructive diseases of agricultural crops is Fusarium wilt of banana (Panama disease), caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder and Hansen (*Foc*) (Stover, 1962). *Foc* colonizes the water conducting xylem vessels of the banana plant eventually resulting in the blockage of the

vessels. Management strategies for Fusarium wilt of banana rely on preventing the introduction of the disease into a clean area and the use of resistant cultivars (Deacon, 1984; Ploetz and Pegg, 2000; Viljoen, 2002; Ploetz *et al.*, 2003). Host resistance is expressed only after infection, and a successful resistance response, therefore, depends on the rate and extent of recognition of the pathogen (Beckman, 1987; Beckman, 1990). In banana, this resistance response is based on the ability of plants to produce phenolics, deposit lignin, and increase enzymes involved in cell wall strengthening (Beckman, 1990; De Ascensao and Dubery, 2000). Cavendish cultivars with resistance against *Foc* race 4 are not available (Viljoen, 2002). The activation of plant defence mechanisms by means of chemical activators, therefore, offers great prospects for the control of *Foc* that cannot be obtained by conventional methods. The objective of this study was to evaluate a variety of chemical plant activators for reducing Fusarium wilt in susceptible and tolerant Cavendish bananas. All chemicals used in this study have previously been proven to induce systemic resistance in plants other than banana.

MATERIALS AND METHODS

Plant material

Pathogen-free tissue culture banana plantlets were obtained from Du Roi Laboratories in Letsitele, South Africa. Two Cavendish cultivars, the susceptible Williams and the tolerant DRS1, were selected for both greenhouse and field evaluations. For the greenhouse studies, 5-cm plantlets were placed in 250-ml plastic cups filled with tap water, and fertilized weekly with a nutrient solution (Chapter 2). After 4 weeks, sufficient root development had taken place and the approximately 10-cm plants were considered ready for greenhouse evaluations. For the field experiment, plantlets were planted into 2 L plastic bags in composted pine bark medium, fertilized with a slow releasing fertilizer and allowed to develop to 20-cm plants before transplanting in the field (January 2002 and 2003). A standard fertilization programme was applied throughout the duration of the field trial.

Fungal isolates and inoculum preparation

Pathogenic *Foc* isolates (CAV 045 and CAV 092) were used for the greenhouse inoculation. The isolates were grown on half-strength potato dextrose agar (PDA) under cool-white and near-ultraviolet fluorescent lights for 7 days. To prepare a conidial spore suspension, mycelia were transferred into 250-ml Erlenmeyer flasks containing Armstrong Fusarium medium, prepared according to the method of Booth (1977). The flasks containing the fungal inoculum were incubated on a rotary shaker operating at 170 rpm at 25°C for 5 days, after which the spore suspension was passed through cheesecloth to separate the mycelia from the spores. The remaining spore concentration in the liquid medium was determined using a hemacytometer, and diluted with sterile distilled water to a final concentration of 5×10^6 spores.ml⁻¹. The roots of banana plantlets in each of the plastic cups were inoculated with 5 ml of the spore suspension, to give a final concentration of 10^5 spores.ml⁻¹. For field infection, the banana plants were planted in a field known to be heavily infested with *Foc* race 4 (VCG 0120).

Chemicals

Six chemical activators were selected for the greenhouse trials (Table 1). Before final concentrations were decided upon, all the chemical compounds were evaluated for phytotoxicity to banana plantlets. Aqueous solutions of the chemicals were either sprayed onto the foliage of the plants or mixed with the water in the plastic cups. Concentrations of 0.25, 0.50, 1.00 and 5.00 g/L of product were screened for BTH, Hrp-protein and BABA, while concentrations of 0.25, 0.50, 1.00 and 5.00 ml/L of the product were screened for ethephon, IAA or menadione sodium bisulphite (MSB), and methyl jasmonic acid (MeJA). Plants were evaluated for the development of chlorotic areas on leaves 2 days after treatment. From the results obtained in the greenhouse trials, four chemical activators were selected for the field trials (Table 1). During the second year of evaluation, it was decided to replace ethephon with sodium nitroprusside (SNP).

Greenhouse trials

A foliar treatment with aqueous solutions of the six chemicals was carried out at concentrations decided upon after completion of the phytotoxicity tests (Table 1). The

products were sprayed to run-off using a hand sprayer. The plants were left on the bench until droplets dried (usually 2-3 hours). Two weeks after the spray treatments the plants were inoculated with *Foc*. The roots of half of the plants were slightly bruised by manually squeezing the rootball, and the rest left intact before inoculation. The plants were transferred to phytotrons with a photoperiod of 12 hours and at a day/night temperature regime of 28/20°C for 7 weeks. A second foliar treatment was applied 2 weeks after inoculation. Control treatments included banana plantlets sprayed with distilled water and inoculated with *Foc* or non-inoculated plants sprayed with distilled water. Eight replicate plants were used for each treatment, and the entire experiment was repeated twice. Plants were removed from the plastic cups and evaluated for the presence of the disease 4-5 weeks after inoculation. Disease development was estimated by cutting the pseudostem open with a scalpel and by assigning a disease severity rating to each plant according to Inibab's Technical Guidelines number 6 (Carlier *et al.*, 2002).

Field trials

Two annual field trials were conducted at the FABI field trial site in the Kiepersol area, South Africa, over a period of 2 years. In the first year, treatments included BTH, ethephon, Hrp-protein and IAA. In the second year, ethephon was replaced with SNP and IAA with MSB. The compounds were each dissolved in water and sprayed on both the abaxial and adaxial leaf surfaces of the banana plants with a 1000-ml spray bottle, 1 month and a week before planting. Control plants were left untreated throughout the trial. The trial consisted of 10 plants per treatment, repeated five times in a completely randomised block design. After planting, all treatments were applied monthly with a backpack sprayer. Rows of susceptible plants surrounded each block of treated plants to prevent drift occurring between the various treatments. The standard disease rating scale (Carlier *et al.*, 2002) for Fusarium wilt of banana was used to determine disease severity. Plants were evaluated for the presence of disease after 6 months for five consecutive months.

Statistical analysis

Data obtained for the greenhouse trials were discrete and required a non-parametric analysis. Data were analysed based on Wilcoxon scores (Sokal and Rohlf, 1981). Differences between treatments were significant at $P \leq 0.05$. The disease severity of the field trial was calculated using the formula of Sherwood and Hagedorn (1958) and data were further analysed based on the GLM Procedure as given in SAS (1999). Differences between field trial treatments were significant at $P \leq 0.05$.

RESULTS

Phytotoxicity

When drenched with BTH, MeJA and IAA, at concentrations of 0.5 g/L and higher and ethephon at a concentration of 0.25 g/L and higher, banana plantlets developed necrotic areas on the leaves. Spray treatments showed less damage to the leaves, although BTH and MeJA at concentrations of 1.0 g/L, and ethephon at a concentration of 0.5 ml/L and higher induced some phytotoxicity symptoms. BABA and Hrp-protein applied as drench or spray treatments were found non-toxic at all the concentration evaluated. Consequently, concentrations presented in Table 1 were chosen for greenhouse and field evaluation.

Greenhouse trials

Effect of chemical activators: Disease severity of Fusarium wilt of banana plantlets was reduced by most chemical activators tested in this study (Fig. 1). The measure of reduction, however, depended on the banana cultivar used and whether the roots had been bruised or not. BABA, Hrp-protein and BTH showed a reduction in disease severity when applied to the susceptible Williams cultivar (Fig. 1A). BTH was the only treatment that differed significantly from the control treatment (Table 2), and reduced disease severity by 34 and 77% in Williams plants where roots were slightly bruised and left intact, respectively ($P \leq 0.05$) (Tables 2 and 3). Interestingly, MeJA, ethephon and IAA resulted in disease incidence slightly higher than the control plants with severe internal and external wilt symptoms noticeable (Fig. 1A). Significant reduction could be observed with all the

chemical activators evaluated on the Williams cultivar where the roots were left intact, according to the less conservative T-test (Table 3). On the tolerant DRS1 cultivar, only ethephon resulted in an increased disease severity, while treatment with Hrp-protein rendered a similar disease severity than the control treatment (Fig. 1B). No significant difference in disease severity was found on the DRS1 cultivar, whether the roots were bruised or not (Tables 2 and 3).

Effect of banana cultivar: Williams and DRS1 differed substantially in their ability to tolerate *Foc* (Fig. 1). In the untreated inoculated controls, the Williams plants developed almost twice the amount of disease than DRS1. Significant differences in disease severity due to the application of chemical activator occurred only on the Williams cultivar (Tables 2 and 3).

Effect of root bruising: Root bruising by manually squeezing the rootball had a huge effect on disease development, both in the susceptible Williams and tolerant DRS1 cultivars. What was most noticeable was that all treatments significantly reduced disease severity in Williams plants with at least 50% when roots were left intact. In the DRS1 plants BTH reduced disease severity substantially and high disease reductions were also observed with the other chemicals when the roots were left intact (Table 3).

Field Trials

Field evaluation of chemical activators was repeated over 2 years. As the results were similar in both years, only data of the second trial is presented because of damage caused to the initial trial by a hippopotamus. Since neither ethephon nor IAA reduced disease incidence in the first trial, these two compounds were replaced with SNP and MSB in the second trial.

Effect of chemical activators: Under field conditions, disease reduction was observed on Williams with Hrp-protein and SNP, and on DRS1 with Hrp-protein only (Figs. 2-4). Hrp-protein and SNP showed a significant reduction in disease severity on the Williams cultivar ($P \leq 0.05$) (Table 4). However, the average disease severity obtained with these two chemicals was still more than 65%. Surprisingly, BTH enhanced disease

development on both Williams and DRS1 cultivars in the field (Table 4). Ethepon was excluded from the second field trial, as it substantially increased disease development and reduced plant growth.

Effect of banana cultivar: Disease development in Williams bananas was more than double that recorded in DRS1. Even though the Hrp-protein reduced disease incidence by 23% in DRS1, this reduction was not significantly different from the control treatment (Table 4). The other three chemicals namely BTH, MSB and SNP increased disease severity when compared to the control treatment (Fig. 2).

DISCUSSION

A limited amount of information is available on the exogenous application of chemical activators to induce SAR against Fusarium wilt in banana. This study, therefore, represents the first comprehensive report on the activity of several chemical activators in reducing the severity of the disease, both under greenhouse and field conditions. Earlier research efforts involved preliminary greenhouse testing of BTH (Moore *et al.*, 1999) and MSB (Borges *et al.*, 2004), but neither compounds have been evaluated in the field. Viljoen (unpublished data) investigated the effect of several chemical activators that stimulate the SA pathway in field trials, but did not include activators that induce systemic resistance via other pathways.

In the current study BTH provided significant protection to the susceptible Cavendish banana cultivar in the greenhouse, but not in the field. This is consistent with an earlier study by Moore *et al.* (1999), where it was shown that pre-treatment of banana plants with BTH provided protection against *Foc* race 4 for about 6 weeks in controlled environment cabinets. In the field however, it proved to be ineffective and plants appeared heavily diseased exhibiting severe yellowing. BTH is a synthetic equivalent of SA, and functions as an elicitor that switches on the SA signalling pathway, thereby activating an entire cascade of resistance reactions in plants (Görlach *et al.*, 1996; Tally *et al.*, 1999).

One possible explanation for the difference in *Fusarium* wilt development in the greenhouse and field, is that winter temperatures in the subtropics might alter the plant's physiology in such a way that SAR in Cavendish bananas are not properly induced. If we look at the effect BTH had on other crops, Tally *et al.* (1999) reported that yellowing and stunting occurred on burley type tobacco plants due to the application of BTH. This type of phytotoxicity effect seemed to be correlated, at least somewhat, with cool, cloudy conditions. In South Africa, banana plants are exposed to cold winter temperatures, often combined with cloudy conditions. It was further stated by Kessmann *et al.* (1994) that crop-tolerance problems can be associated with the exogenous application of SA, and that there is a narrow safety margin separating the rates at which the compound is effective and the rate at which it becomes phytotoxic. Görlach *et al.* (1996) found that a single application of BTH protected wheat against powdery mildew in the field for an entire season, combining long-lasting protection with an ecologically desirable, low-application rate. Benhamou and Bélanger (1998) found that the exogenous application of BTH to tomato plants increased protection against *Fusarium oxysporum* f.sp. *radicis-lycopersici* Jarvis & Shoemaker. In the current study, however, field applications were applied monthly, even during the cool winter months that might have influenced systemic protection of banana plants against *Fusarium* wilt. The hypothesis that rate of applications and climatic conditions influence the effectiveness of this chemical activator on banana plants, requires further investigation. This can be done by doing proper physiological and biochemical studies under the different climatic conditions, and by applying different rates of BTH in the subtropics.

A second possible explanation why BTH was effective in the greenhouse but not in the field could be the difference in plant age. BTH induced resistance against powdery mildew (*Oidium neolycopersici* L. Kiss) in tobacco but not in tomato, while in tomato resistance was induced against grey mould (*Botrytis cinerea* Pers.) but not in tobacco (Achuo *et al.*, 2004). However, when Chivasa *et al.* (1997) and Murphy *et al.* (2000) applied SA to tobacco plants, it induced resistance against *B. cinerea*. The difference between the two studies was that Chivasa *et al.* (1997) and Murphy *et al.* (2000) used much younger plants (only 3 weeks old) than Achuo *et al.* (2004).

In the field, SNP and Hrp-protein were found to be most effective on the Williams and DRS1 cultivars, respectively. In fact, Hrp-protein was the only product protecting DRS1 in the field. SNP was ineffective in protecting DRS1, while Hrp-protein was also able to significantly reduce disease incidence in the Williams cultivar. Hrp-protein has previously been reported to protect tomato plants against bacterial wilt (Moffat, 2001). Whether this compound is effective in protecting plants under diverse (stressful) environmental conditions is not known. Hrp-protein provided substantial protection of Williams plants in the greenhouse as well, whether roots were bruised or left intact, and on the DRS1 plants when roots were left intact. This makes it a good candidate to be considered in an integrated disease management programme for Fusarium wilt of banana. As a late inclusion in the field trial, SNP was not evaluated under greenhouse conditions, but its protection of Williams plants in the field is noteworthy, even though it did not protect DRS1. The mechanism by which SNP induce resistance is still not clear, as plants may have many ways to transduce a NO signal (Durner *et al.*, 1998). Durner *et al.* (1998) found that treatments with NO donors (similar to SNP), triggered the expression of defence-related genes in tobacco plants and tobacco suspension cells. There is also evidence that NO interacts with ROIs and SA in plants to induce the HR and defence gene expression (Hausladen and Stamler, 1998; McDowell and Dangl, 2000).

BABA has been reported to have an effect against soil-borne fungi (Cohen, 1994b; Oka *et al.*, 1999). However, it did not significantly lower the incidence of Fusarium wilt in the greenhouse. It is not yet clear if resistance induced by BABA involves the SA pathway or another pathway (Silué *et al.*, 2002), but Cohen (1994b) observed that a relatively high concentration of the amino acid was required for protection against *Phytophthora infestans* (Mont.) de Bary in tomato. Zhang and Reddy (2001) also found that BABA at high concentrations was the only chemical activator that significantly reduced late leaf spot disease in peanut in the greenhouse. The other chemicals they evaluated included SA, sodium salicylate, INA and BTH. Field trials were also conducted and revealed that none of the chemical activators, including BABA, provided a significant reduction in disease.

The ET-releasing agent ethephon did not induce resistance in tobacco plants against TMV (Brederode *et al.*, 1991), but did induce a SAR-gene expression via a SA-dependent pathway in *Arabidopsis* (Lawton *et al.*, 1994). Certain fungi and bacteria can produce ET as a pathogenicity factor, while the application of ET or ET-releasing compounds have in certain cases been found to aggravate diseases (Knoester *et al.*, 2001). However, exogenous application of ET or ET-releasing compounds have also been associated with the activation of resistance against some fungal, bacterial and viral diseases (Feys and Parker, 2000; Knoester *et al.*, 2001). ET has thus been associated with both disease resistance as well as symptom development (Pieterse *et al.*, 2001). In certain plant species, wounding increases endogenous levels of JA, and exogenous application of JA stimulates the expression of defensive compounds (Reinbothe *et al.*, 1994; Staswick and Lehman, 1999). However, JA levels are sometimes constitutively high in young tissue without inducing defence pathways (Harms *et al.*, 1995). In these cases, JA might be inactivated or sequestered in some way that does not stimulate a response. Neither ethephon nor MeJA had a significant effect on Fusarium wilt of banana development either in the greenhouse or in the field in the current study, and some even enhanced disease development. This enhancement was usually associated with the bruising of plant roots before inoculation in the greenhouse. Nor did IAA and MSB, earlier reported to induce resistance responses in banana (Borges *et al.*, 2004), effectively reduced Fusarium wilt in this study. MSB is associated with the increase of free indole-acetic acid (IAA) levels in the plant (Rama-Rao *et al.*, 1985).

One of the most important findings of this study was the effect that root bruising had on the activity of SAR chemicals. Wounds provide easy entry points for Fusarium wilt pathogens (Sequeira *et al.*, 1958; Beckman, 1987; 1990) from where they rapidly colonise and block the vascular tissue in plants. This leaves little opportunity for plants to respond to attack by the pathogen. However, in instances where the plant has sufficient opportunity to recognize and respond to a pathogen, protection would be more efficient (Beckman, 1990). This is clearly demonstrated by the highly significant reduction in Fusarium wilt incidence in banana plants when roots were left intact.

In the current study it was observed in the greenhouse that, although DRS1 had a lower disease severity due to its semi-tolerant character, the chemical inducers had a similar effect on both cultivars. In the field, however, the chemical inducers had a slightly different effect on the two cultivars. Resistance responses may be cultivar specific, but there are other factors that must also be considered. For example, resistance may be growth stage specific (seedling vs. mature plant) or tissue specific (leaves vs. roots) (Lyon and Newton, 1999). Kuć (2001) indicated that some chemical agents could be more effective against some disease than others. Different compounds and pathways may mediate different biochemical resistance, and may antagonise the activation or effectiveness of another. In other cases, however, it was found that two pathways may also result in an enhanced resistance response (Van Wees *et al.*, 2000).

Not many chemical activators have been tested for inducing resistance in crops under field conditions. In cases where they have been tested, the level of control was found to be less than that found in the laboratory (Schönbeck *et al.*, 1982; Lyon and Newton, 1999). Often these compounds do not show a clear dose-response curve. Some have excessive response with low levels of the product and they may or may not show increased response with increased doses. Also, more studies have been conducted on dicotyledonous plants, and less is known about SAR in monocotyledonous plants (Görlach *et al.*, 1996; Oostendorp *et al.*, 2001). In general, resistance induced in monocots by BTH appears to be much longer lasting than that in dicots (Oostendorp *et al.*, 2001). Tally *et al.* (1999) stated that in monocot plants fewer applications are required and that defence responses can remain active for many weeks. A substantial amount of research is, therefore, needed to optimise SAR application for the protection of bananas against Fusarium wilt in the field.

Tolerance in banana, combined with SAR chemical activators, might offer a major contribution towards developing an integrated disease management programme for Fusarium wilt. This is clearly demonstrated by the effect of the Hrp-protein that reduced disease incidence of a susceptible cultivar Williams to that of a tolerant one, DRS1, from 84% to 24.5%. Reports can also be found where the use of BTH has yielded better results when combined with low rates of fungicides or bactericides in a number of crop

management programmes (Tally *et al.*, 1999). It is, therefore, possible to combine some of the chemical activators with a fungicide and achieve some form of protection. Since this is one of only a few reports that can be found on the use of chemical activators on banana, we are still far from discarding chemical activators as potential important control agents of Fusarium wilt of banana.

REFERENCES

- Achuo, E.A., Audenaert, K., Meziane, H., and Höfte, M. 2004. The salicylic acid-dependent defence pathway is effective against different pathogens in tomato and tobacco. *Plant Pathology* 53: 65-72.
- Beckman, C.H. 1987. The nature of wilt disease of plants. APS Press, St. Paul, Minnesota, USA, 175 pp.
- Beckman, C.H. 1990. Host responses to the pathogen. In: Ploetz, R.C. (Ed.), *Fusarium wilt of banana*. APS Press, St Paul, Minnesota, USA, pp. 93-105.
- Benhamou, N. and Bélanger, R.R. 1998. Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* in tomato. *Plant Physiology* 118: 1203-1212.
- Booth, C. 1977. *Fusarium*. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, UK, p. 10.
- Borges, A.A., Borges-Pérez, A., and Fernández-Falcón, M. 2004. Induced resistance to Fusarial wilt of banana by menadione sodium bisulphite treatments. *Crop Protection* 23: 1245-1247.
- Brasher, P. 2000. Protein for replacing pesticides approved. San Diego Union Tribune, April 29, p. A7.
- Brederode, F.T., Linthorst, H.J.M., and Bol, J.F. 1991. Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. *Plant Molecular Biology* 17: 1117-1125.
- Carlier, J., De Waele, D., and Escalant, J.V. 2002. Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. INIBAP Technical Guidelines 6, Montpellier France.

- Chivasa, S., Murphy, A.M., Naylor, M., and Carr, J.P. 1997. Salicylic acid interferes with tobacco mosaic virus replication via a novel salicylhydroxamic acid-sensitive mechanism. *The Plant Cell* 9: 547-557.
- Cohen, Y. 1994a. 3-Aminobutyric acid induces systemic resistance against *Peronospora tabacina*. *Physiology and Molecular Plant Pathology* 44: 273-288.
- Cohen, Y. 1994b. Local and systemic control of *Phytophthora infestans* in tomato plants by DL-3-Amino-*n*-Butanoic acids. *Phytopathology* 84: 55-59.
- Cohen, Y., Gisi, U., and Niderman, T. 1993. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* 83: 1054-1062.
- De Ascensao, A.R.D.C.F., and Dubery, I.A. 2000. Panama disease: Cell wall reinforcement in banana roots in response to elicitors from *Fusarium oxysporum* f.sp. *cubense* race four. *Phytopathology* 90: 1173-1180.
- Deacon, J.W. 1984. Panama disease of banana in South Africa. *Horticultural Science* 1: 29-31.
- Delledonne, M., Xia, Y., Dixon, R., and Lamb, C. 1998. Nitric oxide functions as a signal in plant disease resistance. *Nature* 394: 585-588.
- Durner, J., Wendehenne, D., and Klessig, D.F. 1998. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proceedings of the National Academy of Science, USA* 95: 10328-10333.
- Feys, B.J., and Parker, J.E. 2000. Interplay of signaling pathways in plant disease resistance. *Trends in Genetics* 16: 449-455.
- Genoud, T., and Métraux, J.P. 1999. Crosstalk in plant cell signaling: structure and function of the genetic network. *Trends in Plant Science* 4: 503-507.
- Gessler, C., and Kuć, J. 1982. Induction of resistance to *Fusarium* wilt in cucumber by root and foliar pathogens. *Phytopathology* 72: 1439-1441.
- Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 8: 629-643.

- Hammerschmidt, R., Métraux, J.P., and Van Loon, L.C. 2001. Inducing resistance: a summary of papers presented at the First International Symposium of Induced Resistance to Plant Diseases, Corfu, May 2000. *European Journal of Plant Pathology* 107: 1-6.
- Harms, K., Atzorn, R., Brash, A., Kühn, H., Wasternak, C., Willmitzer, L., and Peña-Cortés, H. 1995. Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid JA levels in transgenic potato plants but not to a corresponding activation of JA-responding genes. *Plant Cell* 7: 1645-1654.
- Hausladen, A., and Stamler, J.S. 1998. Nitric oxide in plant immunity. *Proceedings of the National Academy of Science, USA* 95: 10345-10347.
- Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology* 32: 439-459.
- Knoester, M., Linthorst, H.J.M., Bol, J.F., and Van Loon, L.C. 2001. Involvement of ethylene in lesion development and systemic acquired resistance in tobacco during the hypersensitive reaction to tobacco mosaic virus. *Physiological and Molecular Plant Pathology* 59: 45-57.
- Kuč, J. 2001. Concepts and direction of induced systemic resistance in plants and its application. *European Journal of Plant Pathology* 107: 7-12.
- Kunz, W., Schurter, R., and Maetzke, T. 1997. The chemistry of benzothiadiazole plant activators. *Pesticide Science* 50: 275-282.
- Lawton, K., Potter, S.L., Uknes, S., and Ryals, J. 1994. Acquired resistance signal transduction in *Arabidopsis* is ethylene independent. *Plant Cell* 6: 581-588.
- Lyon, G.D., and Newton, A.C. 1999. Implementation of elicitor mediated induced resistance in agriculture. In: Agrawal, A.A., Tuzun, S., and Bent, E. (Eds), *Induced plant defences against pathogens and herbivores*. APS Press, St Paul, Minnesota, USA, pp. 299-318.
- McDowell, J.M., and Dangl, J.L. 2000. Signal transduction in the plant immune response. *Trends in Biochemistry Science* 25: 79-82.
- McDowell, J.M., and Woffenden, B.J. 2003. Plant disease resistance genes: recent insights and potential applications. *Trends in Biotechnology* 21: 178-183.

- Métraux, J.P. 2001. Systemic acquired resistance and salicylic acid: current state of knowledge. *European Journal of Plant Pathology* 107: 13-18.
- Métraux, J.P., Ahl-Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. 1991. Induced systemic resistance in cucumber in response to 2,6-dichloro-isonicotinic acid and pathogens. In: Hnennecke, H., and Verma, D.P.S. (Eds), *Advances in molecular genetics of plant-microbe interactions*. Kluwer Academic Publishers, Dordrecht, pp. 432-439.
- Moffat, A.S. 2001. Finding new ways to fight plant diseases. *Science* 292: 2270-2273.
- Moore, N.Y., Pegg, K.G., Smith, L.J., Langdon, P.W., Bentley, S., and Smith, M.K. 1999. Fusarium wilt of banana in Australia. In: Molina, A.B., Masdek, N.H.N., and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 64-75.
- Murphy, A.M., Holcombe, L.J., and Carr, J.P. 2000. Characteristics of salicylic acid-induced delay in disease caused by a necrotrophic fungal pathogen in tobacco. *Physiological and Molecular Plant Pathology* 57: 47-54.
- Oka, T., Cohen, Y., and Spiegel, Y. 1999. Local and systemic resistance to the root-knot nematode in tomato by DL- β -Amino-*n*-Butyric Acid. *Phytopathology* 89: 1138-1143.
- Oostendorp, M., Kunz, W., Dietrich, B., and Staub, T. 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* 107: 19-28.
- Pieterse, C.M.J., Ton, J., and Van Loon, L.C. 2001. Cross-talk between plant defence signalling pathways: boost or burden? *AgBiotechNet* 3:1-8.
- Ploetz, R.C., and Pegg, K.G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D. (Ed), *Diseases of banana, Abaca and Enset*. CAB International, Wallingford, UK, pp.143-159.
- Ploetz, R.C., Thomas, J.E., and Slabaugh, W.R. 2003. Diseases of banana and plantain. In: Ploetz, R.C. (Ed), *Diseases of tropical fruit crops*. CAB International, Wallingford, UK, pp. 109-112.

- Rama-Rao, A.V., Ravichandran, K., David, S.B., and Ranade, S. 1985. Menadione sodium bisulphate: a promising plant growth regulator. *Plant Growth Regulation* 3: 111-118.
- Reinbothe, S., Mollenhauer, B., and Reinbothe, C. 1994. JIPs and RIPs: The regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. *The Plant Cell* 6: 1197-1209.
- Reymond, P., Weber, H., Damond, M., and Farmer, E.E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* 12: 707-719.
- Schönbeck, F., Dehne, H.W., and Balder, H. 1982. On the efficiency of induced resistance under practical conditions I. Powdery mildew on grapevine, cucumber and wheat. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Disease and Protection)* 89: 177-184.
- Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., and Manners, J.M. 2000. Coordinate plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Science, USA* 97: 11655-11660.
- Sequeira, L., Steeves, T.A., Steeves, M.W., and Riedhart, J.M. 1958. Role of root injury in Panama disease infections. *Nature (Lond.)* 182: 309-311.
- Sherwood, R.T., and Hagedorn, D.J. 1958. Determining common root rot potential of pea fields. *Wisconsin Agricultural Experimental Station Research Bulletin* 531: 12.
- Siegrist, J., Orober, M., and Buchenauer, H. 2000. β -Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. *Physiological and Molecular Plant Pathology* 56: 95-106.
- Silué, D., Pajot, E., and Cohen, Y. 2002. Induction of resistance to downy mildew (*Peronospora parasitica*) in cauliflower by DL- β -amino-*n*-butanoic acid (BABA). *Plant Pathology* 51: 97-102.
- Sokal, R.R., and Rohlf, F.J. 1981. *Biometry: The principles and practice of statistics in biological research*. W.H. Freeman and Company, San Francisco.

- Staswick, P.E., and Lehman, C.C. 1999. Jasmonic acid-signaled responses in plants. In: Agrawal, A.A., Tuzun, S., and Bent, E. (Eds), Induced plant defenses against pathogens and herbivores. APS Press, St Paul, Minnesota, USA, pp. 117-136.
- Sticher, L., Mauch-Mani, B., and Métraux, J.P. 1997. Systemic acquired resistance. Annual Review of Phytopathology 35: 235-270.
- Stover, R.H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Commonwealth Mycological Institute, Kew, Surrey, UK, 117 pp.
- Tally, A., Oostendorp, M., Lawton, K., Staub, T., and Bassi, B. 1999. Commercial development of elicitors of induced resistance to pathogens. In: Agrawal, A.A., Tuzun, S., and Bent, E. (Eds), Induced plant defences against pathogens and herbivores. APS Press, St Paul, Minnesota, USA, pp. 375-369.
- Tosi, L., Luigetti, R., and Zazzerini, A. 1998. Induced resistance against *Plasmopara helianthi* in sunflower plants by DL-beta-amino-n-butyric acid. Journal of Phytopathology 146: 295-299.
- Van Wees, S.C.M., De Swart, E.A.M., Van Pelt, J.A., Van Loon, L.C., and Pieterse, C.M.J. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proceedings of the National Academy of Science, USA 97: 8711-8716.
- Viljoen, A. 2002. The status of Fusarium wilt (Panama disease) of banana in South Africa. South African Journal of Science 98: 341-344.
- Wasternach, C., and Parthier, B. 1997. Jasmonate-signalled plant gene expression. Trends in Plant Science 2: 302-307.
- Zhang, S., and Reddy, M.S. 2001. Lack of induced systemic resistance in peanut to late leaf spot disease by plant growth-promoting rhizobacteria and chemical elicitors. Plant Disease 85: 879-884.

TABLE 1. Chemical activators evaluated for suppression of Fusarium wilt in the banana cultivars Williams and DRS1 in the greenhouse and field.

Chemical activator	Active ingredient	Concentration of product	
		Greenhouse	Field
Bion	Benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH)	0.10 g/L	0.10 g/L
BABA	DL-3-amino-n-butanoic acid (BABA)	1.00 g/L	
Ethapon	Ethepon	0.25 ml/L	
Methyl jasmonic acid (MeJA)	Methyl Jasmonic Acid (MeJA)	0.50 ml/L	
Messenger	Harpin protein (Hrp)	0.70 g/L	0.70 g/L
Param	Indole acetic acid (IAA)	1.00 ml/L	1.00 ml/L
Menadione Sodium Bisulfite (MSB)	Menadione Sodium Bisulfite (MSB)	1.00 ml/L	1.00 ml/L
Sodium nitroprusside (SNP)	Sodium nitroprusside (SNP)		10.00 ml/L

TABLE 2. Effect of chemical activators on severity of Fusarium wilt of banana (cultivars Williams and DRS1) in the greenhouse. Roots were slightly bruised before inoculation with *Fusarium oxysporum* f.sp. *cubense*. Significance determined as $P\leq0.05$.

Treatment	Disease severity (%)		Disease reduction (%)		P value ^a	
	Williams	DRS1	Williams	DRS1	Williams	DRS1
Control	78.13	43.75				
Ethepon	84.38	53.13	-	-	-	-
MeJA	87.50	37.50	-	14.29	-	0.3511
IAA	81.25	34.38	-	21.42	-	0.1630
BABA	65.63	34.38	16.00	21.42	0.2481	0.1630
Hrp-protein	59.38	43.75	24.00	-	0.0845	1.0000
BTH	51.56	37.50	34.00	14.29	0.0153	0.3511

^aComparison of control with treatments by means of t-test (LSD).

TABLE 3. Effect of chemical activators on severity of Fusarium wilt of banana (cultivars Williams and DRS1) in the greenhouse. Roots were left intact before inoculation with *Fusarium oxysporum* f.sp. *cubense*. Significance determined as $P\leq0.05$.

Treatment	Disease severity (%)		Disease reduction (%)		P value ^a	
	Williams	DRS1	Williams	DRS1	Williams	DRS1
Control	48.44	15.63				
Ethepon	21.88	9.38	54.83	39.99	0.0153	0.3511
MeJA	15.63	7.81	67.73	50.03	0.0029	0.2443
IAA	17.19	6.25	64.51	60.01	0.0045	0.1630
BABA	12.50	10.94	74.19	30.01	0.0012	0.4839
Hrp-protein	23.44	4.69	51.61	70.00	0.0222	0.1043
BTH	10.94	3.13	77.42	79.97	0.0007	0.0639

^a Comparison of control with treatments by means of t-test (LSD).

TABLE 4. Effect of chemical activators on severity of *Fusarium* wilt of banana (cultivars Williams and DRS1) caused by *Fusarium oxysporum* f.sp. *cubense* in the field. Significance determined as $P\leq0.05$.

Treatment	Disease severity (%)		Disease reduction (%)		<i>P</i> value ^a	
	Williams	DRS1	Williams	DRS1	Williams	DRS1
Control	84.00	32.00				
BTH	85.50	39.00	-	-	-	-
MSB	81.00	40.00	3.57	-	0.6865	-
Hrp-protein	69.00	24.50	17.86	23.44	0.0487	0.3156
SNP	66.00	43.00	21.43	-	0.0192	-

^a Comparison of control with treatments by means of t-test (LSD).

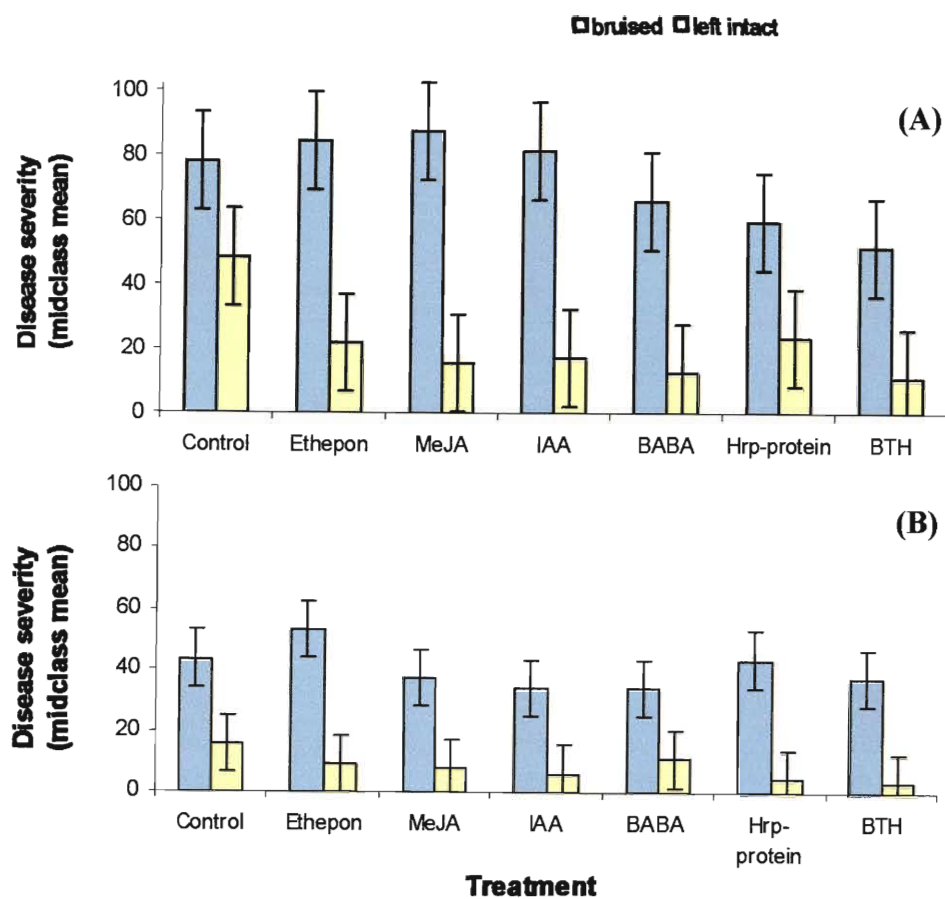


FIGURE 1. Effect of chemical activators on severity of *Fusarium* wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* on the cultivars (A) Williams and (B) DRS1 in the greenhouse. Midclass mean was calculated taking in account the disease severity in all the plants per treatment. Range bars indicate the 95% confidence interval for each treatment.

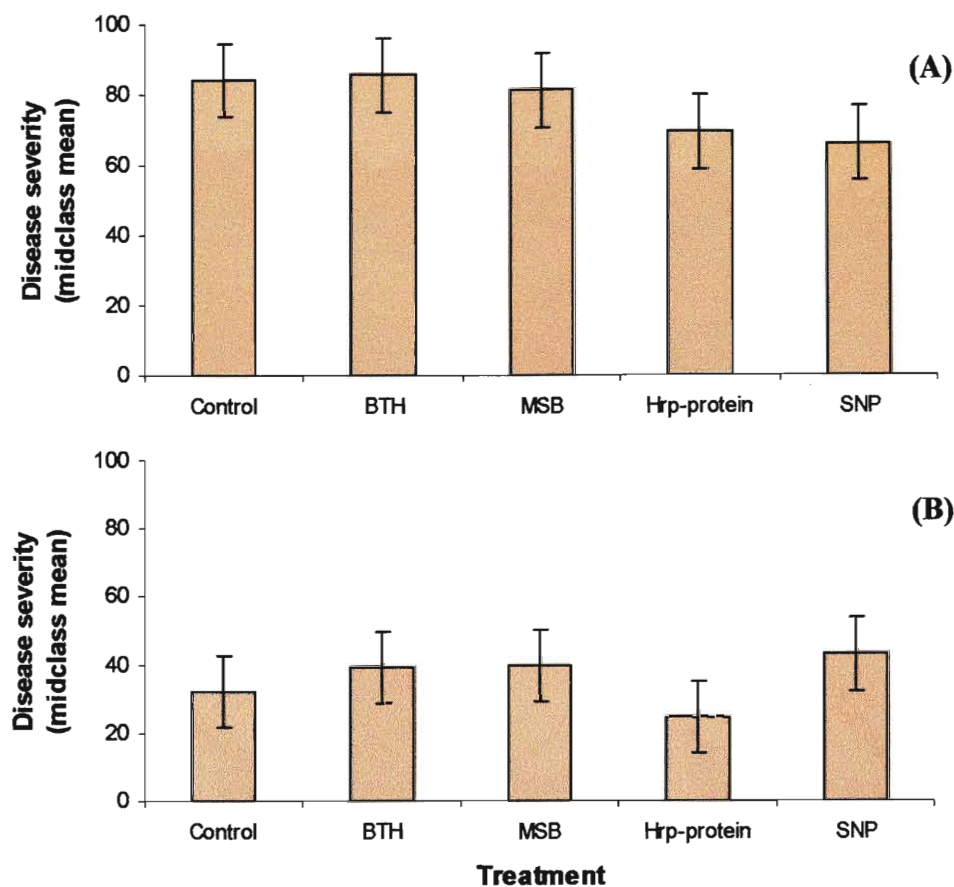


FIGURE 2. Effect of chemical activators on severity of *Fusarium* wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* on the cultivars (A) Williams and (B) DRS1 in the field. Mid-class mean was calculated taking in account the disease severity of the five repetitive blocks per treatment. Range bars indicate the 95% confidence interval for each treatment.

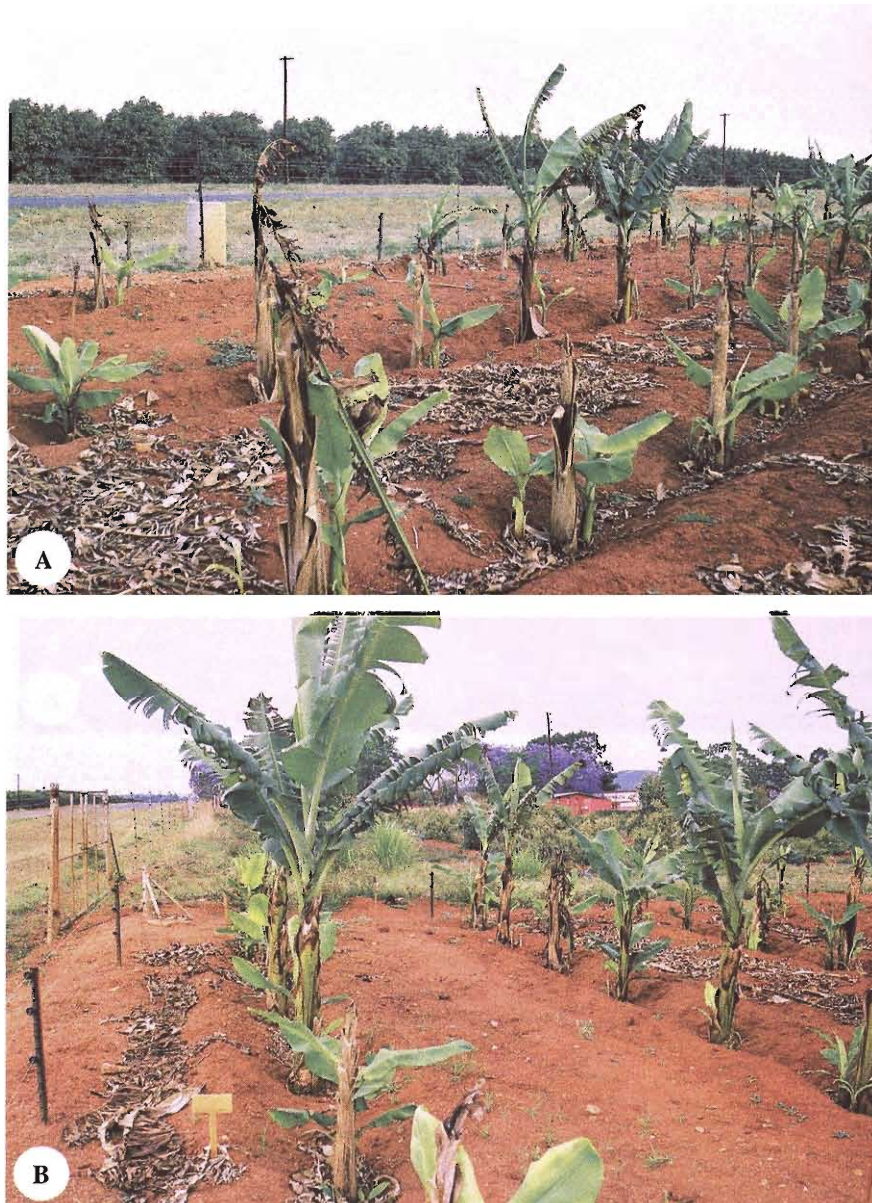


FIGURE 3. Effect of chemical activators SNP on severity of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* on the Williams in the field. (A) Williams control and (B) SNP treatment.

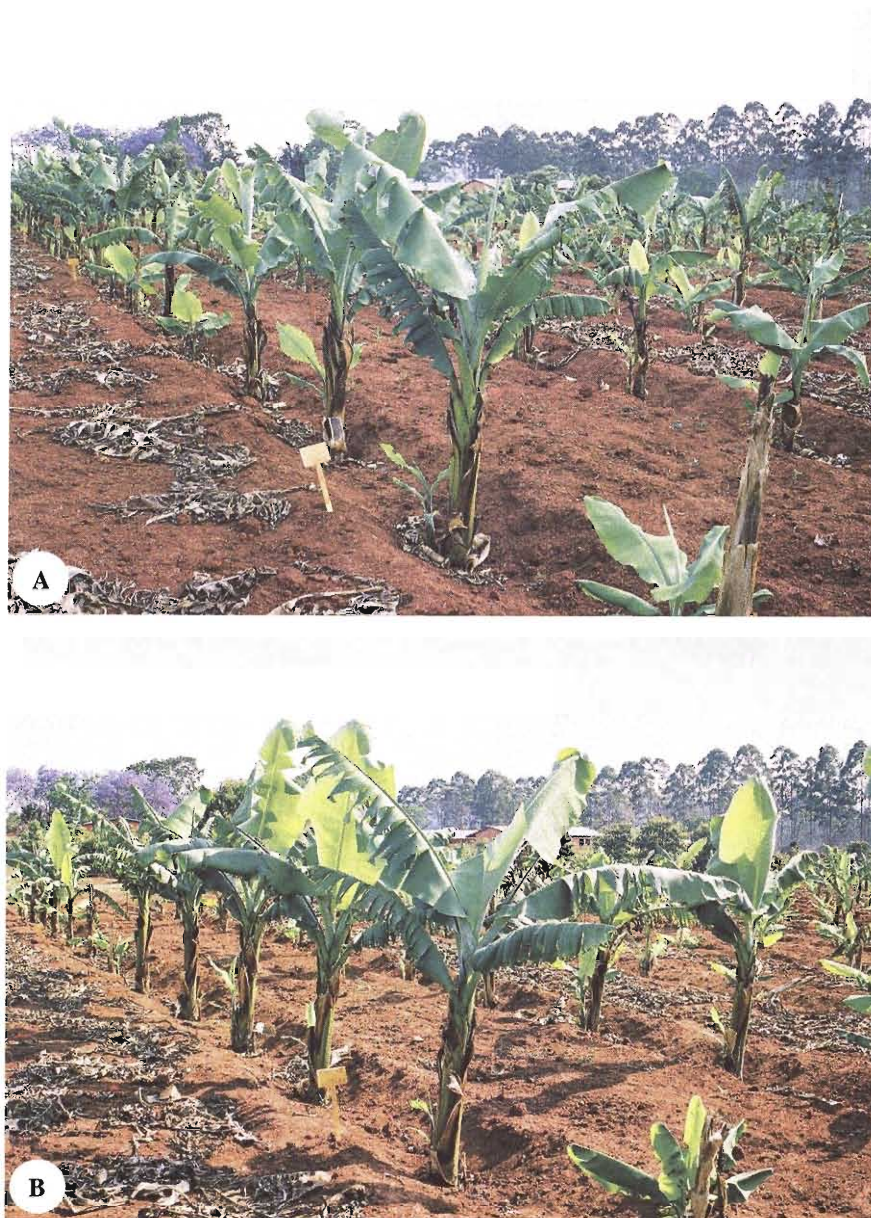


FIGURE 4. Effect of chemical activator Hrp-protein on severity of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* on the DRS1 cultivar in the field. (A) DRS1 control and (B) Hrp-protein treatment.

CHAPTER 4

Isolation and characterization of non-pathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA



ABSTRACT

Fusarium oxysporum is a common and ubiquitous soil-borne fungus responsible for wilt diseases of many agricultural crops. Some isolates of the fungus, however, are non-pathogenic, and have been implicated in the biological control of *Fusarium* wilt diseases. These non-pathogenic isolates are often present in soils where pathogenic members of the species do not cause disease, despite the occurrence of susceptible crops and favourable environmental conditions. One of the most serious diseases of agricultural crops is *Fusarium* wilt (Panama disease) of banana, caused by *F. oxysporum* f.sp. *cubense* (*Foc*). The objectives of this study were to isolate and identify non-pathogenic *F. oxysporum* isolates from reported suppressive soils, and to determine the diversity of these isolates. Three *Fusarium* wilt suppressive soil sites were identified in Kiepersol, one of the main banana-producing regions in South Africa. From the rhizosphere of banana plants, more than 100 *Fusarium* strains were isolated using *Fusarium*-selective media. The *Fusarium* isolates were identified to species level using morphological characteristics and species-specific primers for *F. oxysporum*. Pathogenicity testing was done to confirm that these isolates are non-pathogenic towards banana plantlets. A PCR-based restriction fragment length polymorphism analysis of the intergenic spacer region of the ribosomal RNA operon was used to characterize the non-pathogenic isolates of *F. oxysporum*. These isolates were compared to isolates of *Foc* from South Africa and the known biological control isolate of *F. oxysporum*, Fo47. Isolates found to be non-pathogenic towards banana plantlets grouped in 12 different genotypes and could be distinguished from *Foc*. Interestingly, Fo47 was included in one of the genotype groups from suppressive soils in South Africa. From the results it is concluded that the population structure of non-pathogenic *F. oxysporum* isolates found in suppressive soils of banana is highly diverse and should be investigated as potential biological control agents.

INTRODUCTION

Fusarium oxysporum Schlechtend. f.sp. *cubense* (E.F. Smith) Snyder & Hans. (*Foc*) is a soil-borne fungus responsible for Fusarium wilt of banana (Stover, 1962). Fusarium wilt, commonly referred to as Panama disease, is one of the most serious and destructive diseases of banana (Ploetz and Pegg, 2000). The disease was discovered in Australia in 1876 and, by 1950, had spread rapidly to most of the banana-producing countries of the world (Ploetz *et al.*, 1990). Fusarium wilt was first noticed in South Africa in 1946 (Ploetz *et al.*, 1990) and, today, five of the six banana-producing areas are affected by the disease (Viljoen, personal communication). No control measure for Fusarium wilt has been found effective other than the use of resistant cultivars. Unfortunately, all Cavendish cultivars grown locally are highly susceptible to *Foc* “subtropical” race 4, the only variant of the fungus that occurs in South Africa. Since only Cavendish banana cultivars are acceptable to the local market, and improvement of Cavendish bananas is difficult and time-consuming, it is important that strategies other than disease resistance be considered for the management of Fusarium wilt in the country (Viljoen, 2002).

Biological control of Fusarium wilt diseases has become an increasingly popular disease management consideration in recent years, given the environmentally friendly nature and the discovery of novel mechanisms of plant protection associated with certain microorganisms (Weller *et al.*, 2002; Fravel *et al.*, 2003). Fusarium wilt suppressive soils have been reported in many regions of the world, and suppression has generally been shown to be due to biological factors (Scher and Baker, 1980; Alabouvette *et al.*, 1993). This had led to studies of antagonistic microorganisms in the soil, their identification, and the mechanisms involved in their disease suppression. Most of these studies have found that non-pathogenic strains of *F. oxysporum* are associated with the natural suppressiveness of soil to Fusarium wilt diseases (Smith and Snyder, 1971; Alabouvette, 1990; Postma and Rattink, 1992; Larkin *et al.*, 1996). Non-pathogenic isolates of *F. oxysporum* were also found to effectively colonize the plant rhizosphere and roots without inducing any symptoms (Elias *et al.*, 1991; Olivain and Alabouvette, 1999).

Identification of *Fusarium* species from the soil is often challenging, since it relies on minor differences in morphology and because different cultural conditions can cause the same species to vary (Doohan, 1998). The differentiation of *F. oxysporum* from several other species of *Fusarium* that belongs to the section Elegans and Liseola, especially, can sometimes be difficult (Fravel *et al.*, 2003). Molecular tools have, therefore, been developed to support morphological identifications. Edel *et al.* (1997a) developed a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method targeting a fragment of the ribosomal (r)DNA that includes the internal transcribed spacer (ITS) region for the identification of *Fusarium* species. Edel *et al.* (2000) also developed a rDNA-targeted oligonucleotide probe and PCR assay specific for *F. oxysporum*. Mishra *et al.* (2002) developed a PCR-based assay for rapid identification of some *Fusarium* species. This technique is based on the ITS region of the rDNA.

The ITS region can be used for the differentiation of species, although, its variation at intraspecific level within *F. oxysporum* was found to be low (Edel *et al.*, 1995). The intergenic spacer (IGS) region, which separates rDNA repeat units, appears to evolve more rapidly and is more variable than the ITS region (Hillis and Dixon, 1991). Edel *et al.* (1995) evaluated three different methods to determine the diversity within non-pathogenic isolates of *F. oxysporum*. They found that PCR-RFLP analysis of the IGS region was a rapid technique to determine the genetic relatedness among isolates of non-pathogenic *F. oxysporum*. Appel and Gordon (1995) also demonstrated that the diversity between pathogenic and non-pathogenic *F. oxysporum* isolates could be determined by RFLP analysis of the IGS region of rDNA.

The objectives of this study were to isolate and identify non-pathogenic *F. oxysporum* strains from the rhizosphere of healthy banana roots in Fusarium wilt suppressive soils, to use IGS sequence analysis to determine the genetic differences among these isolates, to determine the genetic relatedness of the non-pathogenic isolates, and to compare them with pathogenic isolates of *F. oxysporum*.

MATERIALS AND METHODS

Isolates used

Three sites with *Fusarium* wilt suppressive soil properties were identified in Kiepersol (Mpumalanga Province), one of the main banana-producing regions in South Africa. The areas were geographically isolated from each other since they occurred on three different farms in Kiepersol. It was observed in all three sites that development of *Fusarium* wilt of banana was slow or was absent, even though the plants in these sites were surrounded by diseased plants in severely affected banana fields. These sites will be referred to as sites 1, 2 and 3. To isolate *F. oxysporum* strains from the rhizosphere of banana roots, five healthy banana plants were selected at each site, and five root pieces collected from each plant. Rhizosphere samples were collected twice from all three areas, once in October 2002 and once in March 2003, in order to counter any seasonal effect on the strains that were collected. The soil around the banana plants was tilled and roots were sampled about 15-30 cm deep. The roots were placed in 10-ml glass bottles containing sterile distilled water. Water and root suspensions were vigorously shaken to remove the adhering soil, after which the roots were removed from the glass bottles. Bottles were transported from the field to the laboratory in a cooler bag containing ice packs. In the laboratory, dilution series were made of each suspension and plated onto Komada medium (Komada, 1975) for the isolation of *F. oxysporum*. Single-spore isolates were selected from each root sample collected. Each single-spore isolate was grown on filter paper overlaid on potato dextrose agar (PDA) (39 g of Difco PDA powder, 1000 ml H₂O). The colonised filter paper was removed from the agar plate, dried, and stored at 4°C at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

For comparative purposes, the non-pathogenic *F. oxysporum* isolate Fo47, from a wilt-suppressive soil of the Châteaurenard region of France, was obtained from Dr. C. Steinberg (INRA, Dijon, France). The efficacy of Fo47 in reducing the severity of *Fusarium* wilt diseases of other crops has been well demonstrated (Lemanceau and Alabouvette, 1991; Alabouvette *et al.*, 1993; Fuchs *et al.*, 1997). The *Foc* isolates (CAV

045 and CAV 129) used in this study were obtained from infected banana plant material in the KwaZulu-Natal area of South Africa.

Identification of *Fusarium oxysporum* from rhizosphere soil

Cultural and morphological identification: Isolates of *F. oxysporum* were identified according to their cultural and morphological characteristics as described by Nelson *et al.* (1983). The single-spore cultures were grown on PDA medium to determine their growth rate and colony pigmentation. Cultures were incubated at 25° and 30°C for 7 to 10 days in the dark, after which the colony diameter was measured and colony colour determined. Single-spore isolates were also placed on carnation leaf agar (CLA) (20 g of Biolab agar, 1000 ml H₂O, one or two 5 mm sterilized carnation leaves per Petri dish) and incubated for 14 days under cool-white and near near-ultraviolet fluorescent lights to investigate the presence and shape of the macroconidia, microconidia and chlamydospores. Morphological characteristics were studied using light microscopy.

Molecular species identification: For DNA extractions, all isolates of *F. oxysporum* were grown on half strength PDA medium for 7 days at 25°C under cool-white and near near-ultraviolet fluorescent lights. Mycelia were then scraped directly from agar plates and used for DNA isolation. Total genomic DNA was isolated using the method described by Raeder and Broda (1985), with minor modifications. Cultures were homogenized with a pestle in 300 µl DNA extraction buffer in an eppendorf tube, freeze-dried in liquid nitrogen and boiled in water for 5 min. After adding 700 µl phenol-chloroform (1:1), samples were vortexed and centrifuged for 7 min at 14 000 rpm. The upper aqueous layer was transferred to a new tube and the phenol-chloroform step repeated until the white interface disappeared. The rest of the procedure was performed similar to that described by Raeder and Broda (1985), with the only exception that tubes were centrifuged for 10 min after the precipitation step. DNA was dried under vacuum, after which the resulting pellet was resuspended in 100-200 µl SABAX water. RnaseA (10 µg/µl) was added to the DNA samples, and incubated at 37°C for three to four hours to digest any residual protein or RNA. The DNA was visualized on a 1% agarose gel (wt/v) (Boehringer Mannheim, Germany) stained with ethidium bromide and viewed under an ultra-violet light. DNA

concentrations were estimated by comparing the intensity of ethidium bromide fluorescence of the DNA sample to a known concentration of lambda DNA marker (marker III) (Roche Diagnostics, South Africa).

Isolated DNA (50-90 ng) was used as template for the PCR reaction. Two primers, designed specific to the ITS region of the rDNA operon of *F. oxysporum* (Mishra *et al.*, 2002), were used for the molecular identification of *F. oxysporum* isolates from the banana root rhizosphere. The primer pair of FOF1 (5'- ACA TAC CAC TTG TTG CCT CG -3') and FOR1 (5'- CGC CAA TCA ATT TGA GGA ACG -3') was synthesized by Inqaba biotec (Pretoria, South Africa). Amplification conditions were similar to those described by Mishra *et al.* (2002). Reactions were carried out in a 20 µl reaction volume containing PCR buffer (10 mM Tris-HCL, 1.5 mM MgCl₂, 50 mM KCL, pH 8.3) (Roche Diagnostics, South Africa), 0.2 mM each dNTP (Roche Diagnostics, South Africa), 0.3 µM of each primers FOF1 and FOR1, and 1 U Taq DNA polymerase (Roche Diagnostics, South Africa). SABAX water was used to achieve the final volume of 20 µl.

DNA amplifications were performed in an Eppendorf Mastercycler gradient PCR machine (Eppendorf Scientific, Germany). The following conditions were used: an initial denaturation temperature of 94°C for 60 s, followed by 25 cycles of template denaturation for 60 s at 94°C, primer annealing for 30 s at 58°C and chain elongation for 60 s at 72°C, with a final extension of 7 min at 72°C. Negative and positive control were included in each reaction, containing SABAX water and no template, and DNA of a known *F. oxysporum* isolate, respectively. The amplified products were verified using 2% agarose gel electrophoresis in 1X Tris Acetic acid EDTA (TAE, pH 8.0) buffer and stained with ethidium bromide and visualised under ultra-violet light. A 100 bp molecular weight marker XIV (Roche Diagnostics, South Africa) was used to determine the size of the PCR products.

Pathogenicity testing: A subsample of 60 isolates that were identified as *F. oxysporum*, and the biological control agent Fo47, were evaluated for their ability to cause disease in small banana plantlets. Isolates were grown for 7 days on half-strength PDA plates at 25°C under cool-white and near UV fluorescent lights, after which mycelia of these

cultures were transferred to Armstrong *Fusarium* sporulation media (Booth, 1977) in 500-ml Erlenmeyer flasks. The flasks were placed on a rotary shaker operating at 170 rpm at 25°C for 5 days, after which the different suspensions were passed through cheesecloth to separate the mycelia from the spores. The spore concentration in the liquid medium was determined using a haemocytometer, and diluted with sterile distilled water to a concentration of 5×10^6 spores.ml⁻¹.

Pathogen-free tissue culture banana plantlets (Williams's cultivar) obtained from Du Roi laboratories, Letsitele, South Africa were used for the pathogenicity testing. The 5-cm plants were planted in a aquaculture system (Chapter 2). After a 4-week period of adjustment, in which the plants received nutrition (Chapter 2), three plants were inoculated with each of the *F. oxysporum* isolates. Each 250-ml plastic cup was inoculated with 5 ml of a spore concentration of 5×10^6 spores.ml⁻¹, which gave a final inoculum concentration of 10^5 spores.ml⁻¹. The roots of the plants were slightly bruised by manually squeezing the rootball to ensure infection by the pathogen. The pathogenic *Foc* isolates CAV 045 and CAV 129 were included as positive controls. Healthy banana plants inoculated with sterile distilled water were used as negative controls. Plants were placed in phytotrons with a photoperiod of 12 hours and at a day/night temperature regime of 28/20°C. After 4 weeks the plants were removed from the cups and evaluated for the presence of the disease. The standard disease rating scale (Carlier *et al.*, 2002) for *Fusarium* wilt of banana was used to record the severity of the disease.

Genotypic characterization of non-pathogenic *Fusarium oxysporum* isolates

PCR amplification of the IGS region: To determine genetic relatedness among non-pathogenic isolates from the banana root rhizosphere, isolates identified as *F. oxysporum* were analysed by means of PCR-RFLPs. The IGS region of the rDNA of each isolate was amplified using the oligonucleotide primers PNFo (5'- CCC GCC TGG CTG CGT CCG ACT C -3') and PN22 (5'- CAA GCA TAT GAC TAC TGG C -3'). The forward primer PNFo, which anneals to the nucleotides 636-657 in the IGS sequence, was designed according to the IGS sequence of *F. oxysporum* f.sp. *melonis* Fom24 (Edel *et al.*, 1995), while the reverse primer PN22 was taken from a conserved region at the 5' end of the

Saccharomyces cerevisiae 18S rRNA gene. Primers were synthesized by Inqaba biotec. The PCR conditions were similar to those described by Edel *et al.* (1995). Amplifications were performed in volumes of 50 µl containing the following: DNA template (50-90 ng), PCR buffer (10 mM Tris-HCL, 1.5 mM MgCl₂, 50 mM KCL, pH 8.3), 0.25 mM each dNTP, 0.2 µM of each primers PNFo and PN22, and 2 U Taq DNA polymerase. SABAX water was used to achieve the final volume of 50 µl.

PCR reactions were performed in an Eppendorf Mastercycler gradient PCR machine. Condition included 30 cycles of denaturation at 95°C for 90 s, followed by primer annealing at 50°C for 60 s and extension at 72°C for 90 s. A sample containing SABAX water and no DNA template was included as a negative control, while DNA of a known *F. oxysporum* isolate was included as positive control. The PCR products were run on a 0.8% agarose gel stained with ethidium bromide and visualized under ultra-violet light. A lambda DNA marker (marker III) (Roche Diagnostics, South Africa) was used to determine the size of the PCR products.

DNA restriction digests and electrophoresis: Restriction enzymes were selected similar to the enzymes used by Edel *et al.* (1995). Restriction enzymes *Hae*III, *Hinf*I, *Msp*I, *Rsa*I, *Scr*FI (Roche Diagnostics, South Africa) and *Hha*I (Promega) are all 4-base cutting enzymes. The restriction enzyme (2 Units) was added directly to 10 µl unpurified PCR amplification products, 1X restriction buffer and SABAX water to achieve an end reaction volume of 20 µl, which was incubated at 37°C for 3-4 hours. Digested fragments were run on an ethidium bromide stained gel consisting of 3-4% agarose at 60 V for 2 hours. Fragments were run against a 100 bp molecular marker for size estimation. Gels were visualized under ultra-violet light.

RESULTS

Identification of *Fusarium oxysporum* from rhizosphere soil

Cultural and morphological identification: Isolates of *F. oxysporum* were selected on Komada medium (Komada, 1975), and their identity verified by means of morphological

characteristics as described by Nelson *et al.* (1983). *Fusarium oxysporum* produced abundant oval to kidney-shaped microconidia in false heads and abundant macroconidia, slightly sickle-shaped, thin-walled and delicate, with an attenuated apical cell and a foot-shaped basal cell (Fig. 1). Short monophialides could be seen, and chlamydospores were present and formed singly or in pairs (Fig. 1). Culture growth was rapid on PDA, producing white aerial mycelium, some tinged with purple. The under-surface in some cultures was dark purple, while in others it varied from light purple to pink and even peach. *Fusarium solani* (Mart.) Sacc. and *Fusarium semitectum* Berk. & Ravenel, as well as a number of unknown *Fusarium* spp. were also isolated from the soil. These, however, were not included in the PCR-RFLP analysis.

Molecular identification: DNA isolation resulted in high concentrations of DNA (50-90 ng/μl) for all isolates used in this study. The primer set FOF1 and FOR1 permitted the amplification of a single DNA fragment of approximately 340 bp in size (Fig. 2). All isolates identified morphologically as *F. oxysporum* amplified with this primer set, while isolates identified as other *Fusarium* spp. did not amplify any fragments.

Pathogenicity testing: Only one of the 60 isolates of *F. oxysporum* (CAV 278) from the rhizosphere soil and the two pathogenic *Foc* isolates incited Fusarium wilt of banana plantlets. None of the other isolates, including the biocontrol agent Fo47, was pathogenic, and did not cause wilt symptoms on any of the inoculated banana plants. These isolates were, therefore, considered as non-pathogens of banana.

Genotypic characterization of non-pathogenic *Fusarium oxysporum* isolates

The primer set PNFo and PN22 permitted the amplification of a single DNA fragment of the IGS region of approximately 1700 bp for each of the 60 isolates of *F. oxysporum* from the rhizosphere soil, as well as for the *Foc* isolates included for comparison (Fig. 3). No size variation was observed between any of the PCR products. When digested with the six restriction enzymes, unique patterns were found for each of the individual enzymes. For each enzyme, patterns that displayed fragments with similar sizes were grouped together and a letter was awarded to each specific pattern (A-H) (Figs. 4 and 5).

The number of restriction patterns produced by each enzyme was as follows: *HaeIII* produced five, *HhaI* three, *HinfI* four, *MspI* four, *RsaI* eight and *ScrfI* only two (Fig. 4). Restriction fragments less than 50 bp were not taken into consideration since it was not possible to determine their correct size by means of electrophoresis.

Twelve IGS genotypes could be distinguished by a six-letter code designated to each isolate (Table 1). The biggest IGS genotype that was formed, DBBACB (group 6), included 18 non-pathogenic *F. oxysporum* isolates. The two pathogenic *Foc* isolates grouped with one of the *F. oxysporum* isolates in IGS genotype ABAACA (group 2), while the well-known biological control isolate Fo47 grouped with three non-pathogenic *F. oxysporum* isolates within the IGS genotype DBBADB (group 7). Several of the genotypes were found at all three sites (Table 1). All IGS genotypes found in this study were present in site 1, while only eight and six of the genotypes were present in sites 2 and 3, respectively (Table 2). The latter sites, however, also yielded the least number of isolates. The isolates sampled in October 2002 group very similar to the isolates sampled in March 2003 (Table 1), with the exception of three genotypes. These exceptions, however only contained a few isolates in the IGS genotypic group.

DISCUSSION

A diverse population of non-pathogenic or saprophytic isolates of *F. oxysporum* dominated the rhizosphere of healthy banana plants in Fusarium wilt suppressive soils in three different fields in South Africa. In one site, an isolate of *Foc* was obtained from healthy roots, confirming that the Fusarium wilt pathogen was indeed present in these soils. This finding substantiates the apparent suppressive nature of these soils. Previous studies on other Fusarium wilt diseases have found that populations of saprophytic *Fusarium* spp. are more diverse and reach higher levels in suppressive soils than in conducive soils (Wensley and McKeen, 1963; Louvet *et al.*, 1981). Nash and Snyder (1965) and Smith and Snyder (1971; 1972) observed that saprophytic *F. oxysporum* established abundantly in suppressive soils in a variety of clonal types, while pathogenic *F. oxysporum* seemed to establish with difficulty in suppressive soils. Due to their higher

numbers in suppressive soils, saprophytic *Fusarium* clones have the ability to utilize substrates better and compete more effectively against the pathogen for the ecological sites in suppressive soils than in conducive soils (Louvet *et al.*, 1981; Gordon *et al.*, 1989). The primary objective of this study was to determine the diversity in non-pathogenic *F. oxysporum* isolates in the banana root rhizosphere for potential use as biological control agents, as effective biocontrol isolates of non-pathogenic *F. oxysporum* have been obtained from healthy plants before (Ogawa and Komada, 1984; Postma and Rattink, 1992). However, a future study on the relationship and interaction between saprophytic and parasitic micro-organisms in suppressive soils will lead to a better understanding of natural resistance and disease control.

Since the identification of *Fusarium* spp. based on cultural and morphological features can sometimes be confusing and incorrect, it was important to characterise the isolates obtained from the banana root rhizosphere as true non-pathogenic *F. oxysporum* isolates. Techniques involving molecular identification methods and virulence determination were therefore used. The ITS region investigated in this study has previously been successfully used to display variation within fungal genera and in the differentiation of other species (Chen, 1992; Carbone and Kohn, 1993). The PCR amplifications of the ITS region as developed by Mishra *et al.* (2002) was, therefore, valuable in permitting the amplification of a small fragment of the isolates morphologically identified as *F. oxysporum*. Such precise identification is a prerequisite for forthcoming studies.

The great diversity of IGS genotypes found within non-pathogenic isolates of *F. oxysporum* on banana was highly significant. These polymorphisms observed among isolates of *F. oxysporum* are most likely due to sequence differences, as have been demonstrated by Appel and Gordon (1995; 1996), Edel *et al.* (1997b; 2001) and Steinberg *et al.* (1997), who also observed substantial differences among non-pathogenic isolates of *F. oxysporum*. Their data further provided evidence that isolates morphologically identifiable as *F. oxysporum* are genetically diverse. Intraspecific variation in the IGS region may reflect a slow rate of concerted evolution in a species characterized by infrequent sexual reproduction, or a predominantly clonal mode of

reproduction by selective mutation (Appel and Gordon, 1996). Vegetative compatibility group (VCGs) studies can be used to help determine the clonality among non-pathogenic population of *F. oxysporum*. It was shown that one IGS type might include one or several VCGs, most of them often being a single member in the case of non-pathogenic populations, but one VCG never includes isolates from different IGS types. It has also been observed that one VCG may be present among the pathogenic isolates while a number of VCGs can be found among isolates from soil (Steinberg *et al.*, 1997). Similarly, few DNA polymorphisms have been noticed among isolates of the same VCG in *Foc* (Kistler *et al.*, 1991). Bentley *et al.* (1999) observed that PCR-RFLP analysis of fourteen South African *Foc* isolates representing VCG 0120 all belonged to the same IGS type.

It was possible to determine the genetic relatedness of non-pathogenic *F. oxysporum* isolates to the pathogenic isolates of *Foc*. The single isolate identified as *F. oxysporum* (CAV 278) virulent to banana plantlets shared the same IGS genotype (IGS group 2) as the two pathogenic isolates of *Foc*. This was a significant finding, considering that the isolates were obtained from two very distant banana-growing regions in South Africa. While it is speculated that *Foc* was introduced into the Kiepersol area from KwaZulu-Natal (Viljoen, 2002), the same RFLP fingerprinting pattern again indicated the highly conserved nature of this clonal pathogen that was introduced into a different area several decades ago. This isolate also produced a characteristic aroma known to be associated with some isolates of the Fusarium wilt pathogen of banana (Stover, 1962). Only three pathogenic isolates were used in this study, but the fact that the *Foc* pathogen does not have a great diversity in South Africa (Visser, 2003), while the natural populations of *F. oxysporum* in banana soils are highly diverse, raises even more questions related to the reproductive mode and evolution of this important fungal species.

Similar genotypes of the non-pathogenic *F. oxysporum* isolates were obtained from all three the collection sites in Kiepersol. The fact that more non-pathogens were obtained from site 1 than from site 2 and 3 could be due to a number of factors. These include physical and chemical soil composition, temperature, sample procedure, and most likely

the cropping history of the various sites. In an IGS RFLP analysis study performed by Edel *et al.* (1997b), the *F. oxysporum* population structure of uncultivated soil was compared to populations isolated from the roots of four plant species. It was found that a considerable diversity within the populations of *F. oxysporum* exists, and that the genotypic population structure of uncultivated soil differed from the populations associated with the roots of wheat and tomato. Certain IGS genotypes were detected more commonly on tomato and certain IGS types were more commonly found on wheat, suggesting that the roots of wheat and tomato plants had a selective effect on the population structure of *F. oxysporum* (Edel *et al.*, 1997b). In the current study, it is possible that isolates from site 1, 2 and 3 are genetically similar because banana root exudates may favour their selectiveness. Stover (1961) proposed that the survival of *Foc* in the soil might depend upon favourable root exudates, as root exudates of the susceptible banana cultivar Gros Michel differed considerably from the exudates of the resistant cultivar Lacatan. He also found that different clones of *F. oxysporum* reacted differently to the various exudates.

One of the most fascinating findings of the current study was that the known biological control agent Fo47, isolated in France, grouped with three of the non-pathogenic isolates of *F. oxysporum* from two collection sites in Kiepersol. It would be difficult to explain how this is possible, and more discriminating molecular analysis are required to substantiate this phenomenon. It would also be most interesting to compare the ability of the indigenous isolates to suppress Fusarium wilt of banana to that of Fo47.

REFERENCES

- Alabouvette, C. 1990. Biological control of Fusarium wilt pathogens in suppressive soils. In: Hornby, D. (Ed), Biological control of soil-borne plant pathogens. CAB International, Wallingford, UK, pp. 27-43.
- Alabouvette, C., Lemanceau, P., and Steinberg, C. 1993. Recent advances in the biological control of Fusarium wilts. Pesticide Science 37: 363-373.

- Apple, D.J., and Gordon, T.R. 1995. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. *Experimental Mycology* 19: 120–128.
- Appel, D.J., and Gordon, T.R. 1996. Relationships among pathogenic and non-pathogenic isolates of *Fusarium oxysporum* based on the partial sequence of the intergenic spacer region of the ribosomal DNA. *Molecular Plant-Microbe Interactions* 9: 125-138.
- Bentley, S., Moore, N.Y., Pegg, K.G., Gerlach, K.S., and Smith, L.J. 1999. Genetic characterisation and detection of Fusarium wilt. In: Molina, A.B., Masdek, N.H.N. and Liew, K.W. (Eds), *Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia*, pp. 143-151.
- Booth, C. 1977. *Fusarium*. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, UK, p. 10.
- Carbone, I., and Kohn, L.M. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the *Sclerotiniaceae*. *Mycologica* 85: 415-427.
- Carlier, J., De Waele, D., and Escalant, J.V. 2002. Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. INIBAP Technical Guidelines 6, Montpellier France.
- Chen, W. 1992. Restriction fragment length polymorphisms in enzymatically amplified ribosomal DNAs of the three heterothallic *Pythium* species. *Phytopathology* 82: 1467-1472.
- Doohan, F.M. 1998. The use of species-specific PCR-based assays to analyse *Fusarium* ear blight of wheat. *Plant Pathology* 47: 197-205.
- Edel, V., Steinberg, C., Avelange, I., Laguerre, G., and Alabouvette, C. 1995. Comparison of three molecular methods for the characterization of *Fusarium oxysporum* strains. *Phytopathology* 85: 579–585.
- Edel, V., Steinberg, C., Gautheron, N., and Alabouvette, C. 1997a. Evaluation of restriction analysis of polymerase chain reaction (PCR)-amplified ribosomal DNA for the identification of *Fusarium* species. *Mycological Research* 101: 179-187.

- Edel, V., Steinberg, C., Gautheron, N., and C. Alabouvette. 1997b. Populations of nonpathogenic *Fusarium oxysporum* associated with roots of four plant species compared to soilborne populations. *Phytopathology* 87: 693-697.
- Edel, V., Steinberg, C., Gautheron, N., and Alabouvette, C. 2000. Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. *Mycological Research* 104: 518-526.
- Edel, V., Steinberg, C., Gautheron, N., Recorbet, G., and Alabouvette, C. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *FEMS Microbiology Ecology* 36: 61-71.
- Elias, K.S., Schneider, R.W., and Lear, M.M. 1991. Analysis of vegetative compatibility groups in non-pathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. *Canadian Journal of Botany* 69: 2089-2094.
- Fravel, D.R., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157: 493-502.
- Fuchs, J.G., Moëgne-Loccoz, Y., and Défago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to *Fusarium* wilt of tomato. *Plant Disease* 81: 492-496.
- Gordon, T.R., Okamoto, D., and Jacobson, D.J. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f.sp. *melonis* and other species of *Fusarium*. *Phytopathology* 79: 1095-1100.
- Hillis, D.M., and Dixon, M.T. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *Quarterly Review of Biology* 66: 411-453.
- Kistler, H.C., Momol, E.A., and Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. *Phytopathology* 81: 331-336.
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8: 115-125.
- Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1996. Suppression of *Fusarium* wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. *Phytopathology* 86: 812-819.

- Lemanceau, P., and Alabouvette, C. 1991. Biological control of *Fusarium* diseases by fluorescent pseudomonas and nonpathogenic *Fusarium*. *Crop Protection* 10: 279-286.
- Louvet, J., Alabouvette, C., and Rouxel, F. 1981. Microbiological suppressiveness of some soils to *Fusarium* wilts. In: Nelson, P.E., Toussoun, T.A. and Cook, R.J. (Eds), *Fusarium: Disease, Biology and Taxonomy*. Pennsylvania State University Press, University Park, USA, pp. 261-275.
- Mishra, K.P., Fox, R.T.V., and Culham, A. 2002. Development of a PCR-based assay for rapid and reliable identification of pathogenic *Fusaria*. *FEMS Microbiology Letters* 10793: 1-4.
- Nash, S.M., and Snyder, W.C. 1965. Quantitative and qualitative comparison of *Fusarium* populations in cultivated fields and noncultivated parent soils. *Canadian Journal of Botany* 43: 939-945.
- Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* species: An illustrated manual for identification. Pennsylvania State University Press, University Park, USA, 193 pp.
- Ogawa, K., and Komada, H. 1984. Biological control of *Fusarium* wilt of sweet potato by non-pathogenic *Fusarium oxysporum*. *Annals of the Phytopathological Society of Japan* 50: 1-9.
- Olivain, C., and Alabouvette, C. 1999. Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. *New Phytologist* 141: 497-510.
- Ploetz, R.C., and Pegg, K.G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D. (Ed), *Diseases of banana, Abaca and Enset*. CAB International, Wallingford, UK, pp.143-159.
- Ploetz, R.C., Herbert, J., Sebasigari, K., Hernandez, J.H., Pegg, K.G., Ventura, J.A., and Mayato, L.S. 1990. Importance of *Fusarium* wilt in different banana-growing regions. In: Ploetz, R.C. (Ed), *Fusarium wilt of bananas*. ASP Press, St. Paul, Minnesota, USA, pp. 9-26.

- Postma, J., and Rattink, H. 1992. Biological control of Fusarium wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*. Canadian Journal of Botany 70: 1199-1205.
- Raeder, U., and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17-20.
- Scher, M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70: 412-417.
- Smith, S.N., and Snyder, W.C. 1971. Relationship of inoculum density and soil types to severity of Fusarium wilt of sweet potato. Phytopathology 61: 1049-1051.
- Smith, S.N., and Snyder, W.C. 1972. Germination of *Fusarium oxysporum* chlamydospores in soils favourable and unfavourable to wilt establishment. Phytopathology 62: 273-277.
- Steinberg, C., Edel, V., Gautheron, N., Abadie, C., Valleys, T., and Alabouvette, C. 1997. Phenotypic characterization of natural populations of *Fusarium oxysporum* in relation to genotypic characterization. FEMS Microbiology Ecology 24: 73-85.
- Stover, R.H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Commonwealth Mycological Institute, Kew, Surrey, UK, 117 pp.
- Stover, R.H., Hildreth, R.C., and Thornton, N.C. 1961. Studies on Fusarium wilt of bananas. VII. Field control. Canadian Journal of Botany 39: 197-206.
- Viljoen, A. 2002. The status of Fusarium wilt (Panama disease) of banana in South Africa. South African Journal of Science 98: 341-344.
- Visser, M. 2003. Molecular biological studies of the Fusarium wilt pathogen of banana in South Africa. PhD thesis, University of Pretoria, South Africa, 155 pp.
- Weller, D.M., Raaijmakers, J.M., McSpadden-Gardener, B.B., and Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 40: 309-348.
- Wensley, R.H., and McKeen, C.D. 1963. Populations of *Fusarium oxysporum* f.sp. *melonis* and their relation to the wilt potential of two soils. Canadian Journal of Microbiology 9: 237-249.

TABLE 1. Intergenic spacer region genotype groups obtained with restriction fragment length polymorphism analysis of *Fusarium oxysporum* isolates collected from the rhizosphere soil of banana plants in Fusarium wilt suppressive soils, and their pathogenicity status.



Group	Isolate	Collection site	Date isolated	Pathogenicity	IGS genotype ^a					
					<i>Hae</i> III	<i>Hha</i> I	<i>Hinf</i> I	<i>Msp</i> I	<i>Rsa</i> I	<i>Scr</i> FI
1	CAV 251	Kiepersol, site 1, SA	03/03	Non-pathogen	A	B	B	A	A	B
	CAV 257	Kiepersol, site 1, SA	03/03	Non-pathogen	A	B	B	A	A	B
	CAV 259	Kiepersol, site 1, SA	03/03	Non-pathogen	A	B	B	A	A	B
	CAV 272	Kiepersol, site 2, SA	03/03	Non-pathogen	A	B	B	A	A	B
	CAV 275	Kiepersol, site 2, SA	03/03	Non-pathogen	A	B	B	A	A	B
	CAV 276	Kiepersol, site 2, SA	03/03	Non-pathogen	A	B	B	A	A	B
2	CAV 045 ^b	Port Edward, SA		Pathogen	A	B	A	A	C	A
	CAV 129 ^b	Port Edward, SA		Pathogen	A	B	A	A	C	A
	CAV 278	Kiepersol, site 1, SA	03/03	Pathogen	A	B	A	A	C	A
3	CAV 221	Kiepersol, site 1, SA	10/02	Non-pathogen	A	B	A	A	G	A
	CAV 227	Kiepersol, site 1, SA	10/02	Non-pathogen	A	B	A	A	G	A
4	CAV 239	Kiepersol, site 1, SA	10/02	Non-pathogen	B	B	B	A	C	B
	CAV 279	Kiepersol, site 1, SA	03/03	Non-pathogen	B	B	B	A	C	B
	CAV 212	Kiepersol, site 2, SA	10/02	Non-pathogen	B	B	B	A	C	B
	CAV 280	Kiepersol, site 2, SA	03/03	Non-pathogen	B	B	B	A	C	B
	CAV 281	Kiepersol, site 2, SA	03/03	Non-pathogen	B	B	B	A	C	B
	CAV 282	Kiepersol, site 3, SA	03/03	Non-pathogen	B	B	B	A	C	B
5	CAV 236	Kiepersol, site 1, SA	10/02	Non-pathogen	C	A	C	C	E	B
	CAV 261	Kiepersol, site 1, SA	03/03	Non-pathogen	C	A	C	C	E	B
	CAV 271	Kiepersol, site 2, SA	03/03	Non-pathogen	C	A	C	C	E	B
	CAV 249	Kiepersol, site 3, SA	10/02	Non-pathogen	C	A	C	C	E	B
6	CAV 224	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 225	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 226	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 229	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 230	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 242	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 243	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 252	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	A	C	B
	CAV 256	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	A	C	B
	CAV 258	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	A	C	B
	CAV 264	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	A	C	B
	CAV 210	Kiepersol, site 2, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 217	Kiepersol, site 2, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 220	Kiepersol, site 2, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 269	Kiepersol, site 2,	03/03	Non-pathogen	D	B	B	A	C	B
	CAV 201	Kiepersol, site 3, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 205	Kiepersol, site 3, SA	10/02	Non-pathogen	D	B	B	A	C	B
	CAV 247	Kiepersol, site 3, SA	10/02	Non-pathogen	D	B	B	A	C	B
7	Fo47 ^c	France		Non-pathogen	D	B	B	A	D	B
	CAV 254	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	A	D	B
	CAV 211	Kiepersol, site 2, SA	10/02	Non-pathogen	D	B	B	A	D	B
	CAV 277	Kiepersol site, 2, SA	03/03	Non-pathogen	D	B	B	A	D	B
8	CAV 240	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	A	H	B
	CAV 202	Kiepersol, site 3, SA	10/02	Non-pathogen	D	B	B	A	H	B
9	CAV 262	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	B	B	B

Group	Isolate	Collection site	Date isolated	Pathogenicity	IGS genotype ^a					
					<i>Hae</i> III	<i>Hha</i> I	<i>Hinf</i> I	<i>Msp</i> I	<i>Rsa</i> I	<i>Scr</i> FI
	CAV 263	Kiepersol, site 1, SA	03/03	Non-pathogen	D	B	B	B	B	B
	CAV 274	Kiepersol, site 2, SA	03/03	Non-pathogen	D	B	B	B	B	B
10	CAV 231	Kiepersol, site 1, SA	10/02	Non-pathogen	D	B	B	D	C	B
11	CAV 241	Kiepersol, site 1, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 255	Kiepersol, site 1, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 265	Kiepersol, site 1, SA	03/03	Non-pathogen	D	C	D	A	F	B
	CAV 219	Kiepersol, site 2, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 270	Kiepersol, site 2, SA	03/03	Non-pathogen	D	C	D	A	F	B
	CAV 273	Kiepersol, site 2, SA	03/03	Non-pathogen	D	C	D	A	F	B
	CAV 200	Kiepersol, site 3, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 244	Kiepersol, site 3, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 248	Kiepersol, site 3, SA	10/02	Non-pathogen	D	C	D	A	F	B
	CAV 253	Kiepersol, site 3, SA	03/03	Non-pathogen	D	C	D	A	F	B
12	CAV 233	Kiepersol, site 1, SA	10/02	Non-pathogen	E	B	B	A	C	B
	CAV 209	Kiepersol, site 2, SA	10/02	Non-pathogen	E	B	B	A	C	B
	CAV 245	Kiepersol, site 3, SA	10/02	Non-pathogen	E	B	B	A	C	B
	CAV 246	Kiepersol, site 3, SA	10/02	Non-pathogen	E	B	B	A	C	B

^a RFLP analysis of the PCR amplified intergenic spacer region of the rDNA cut with the specified enzyme. Each letter represents the same PCR-RFLP pattern.

^b Pathogenic isolates of *Fusarium oxysporum* f.sp. *cubense* from KwaZulu-Natal, South African.

^c Known non-pathogenic *Fusarium oxysporum* isolates Fo47 from suppressive soils in France.

TABLE 2. The number of *Fusarium oxysporum* isolates obtained from Fusarium wilt suppressive soils in banana fields in Kiepersol, South Africa. The isolates were grouped according to their PCR-restriction fragment length polymorphisms of the intergenic spacer region.

IGS group	Number of isolates from		
	Site 1	Site 2	Site 3
ABBAAB	3	3	0
ABAACA	1	0	0
ABAAGA	2	0	0
BBBACB	2	3	1
CACCEB	2	1	1
DBBACB	11	4	3
DBBADB	1	2	0
DBBAHB	1	0	1
DBBBBB	2	1	0
DBBDCB	1	0	0
DCDAFB	3	3	4
EBBACB	1	1	2
Total	30	18	12

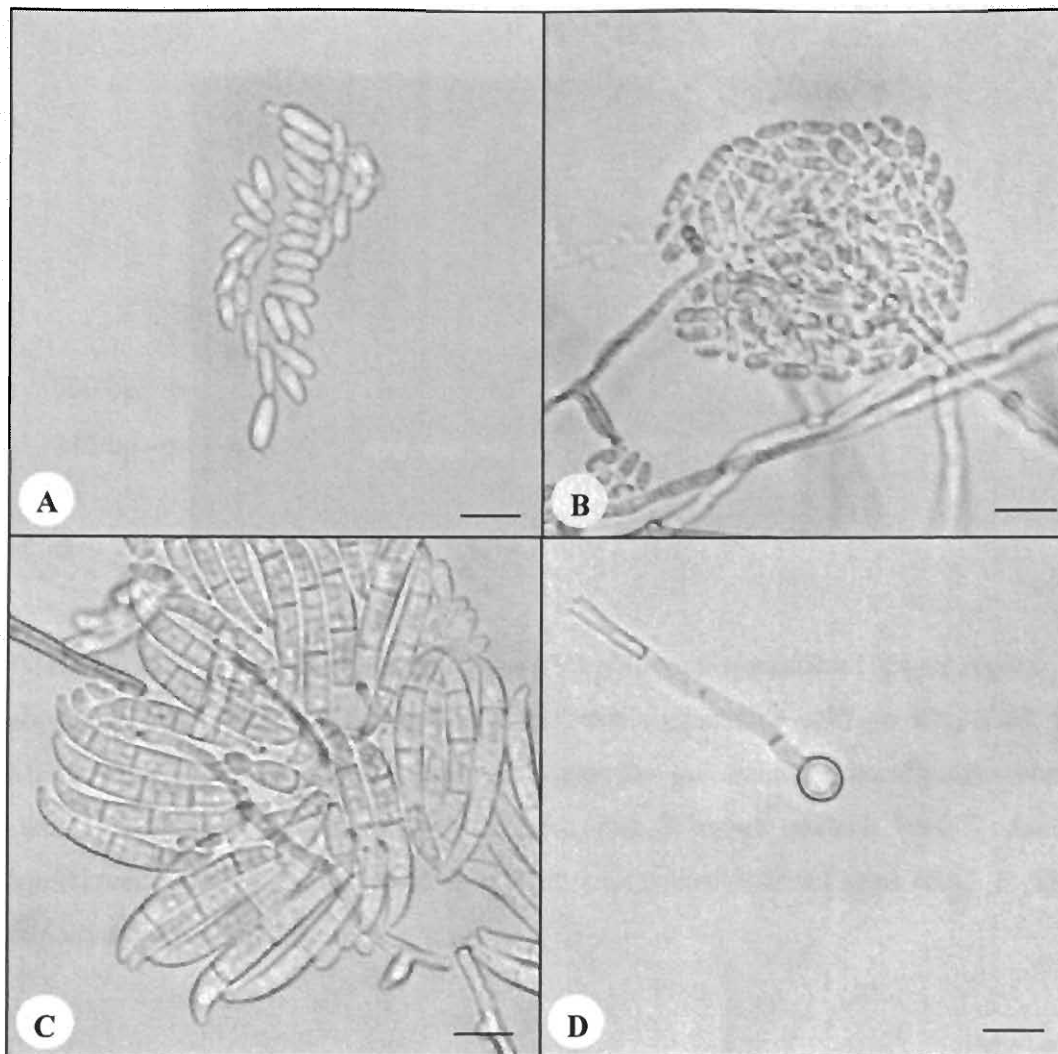


FIGURE 1. Morphological characteristics of *Fusarium oxysporum* isolated from suppressive soils in Kiepersol, South Africa. (A) Oval to kidney-shaped microconidia, (B) microconidia produced in false heads on short monophialides, (C) macroconidia are slightly sickle-shaped, thin-walled and delicate, with an attenuated apical cell and a foot-shaped basal cell, and (D) chlamydospores formed singly or in pairs. Scale bar = 10 µm.

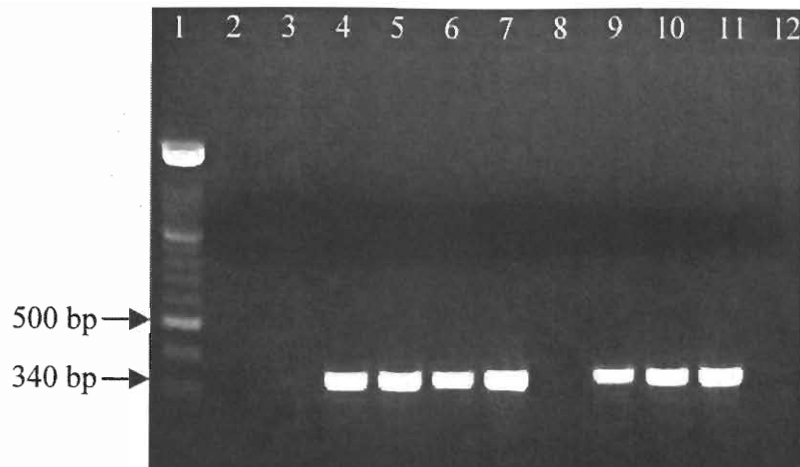


FIGURE 2. PCR amplification products of the internal transcribed spacer region of the ribosomal DNA of the *Fusarium* isolates from suppressive soils in Kiepersol, South Africa. PCR products visualized on a 2% agarose gel stained with ethidium bromide. Lane 1, 100 bp molecular weight marker; lane 2, water control; lane 3, *Fusarium semitectum*; lanes 4-7 and 9-11, *Fusarium oxysporum* isolates; and lanes 8 and 12, *Fusarium solani* isolates.

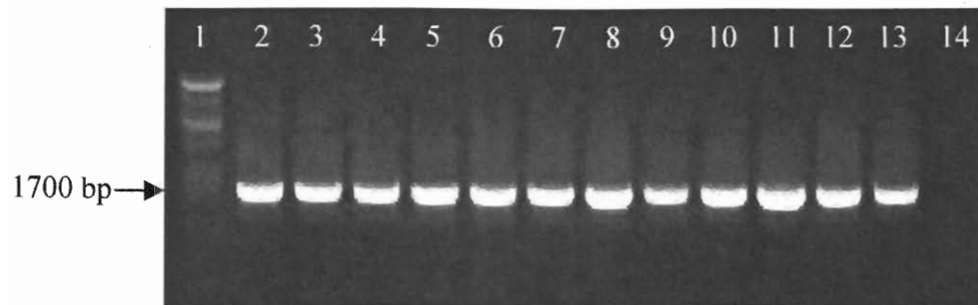
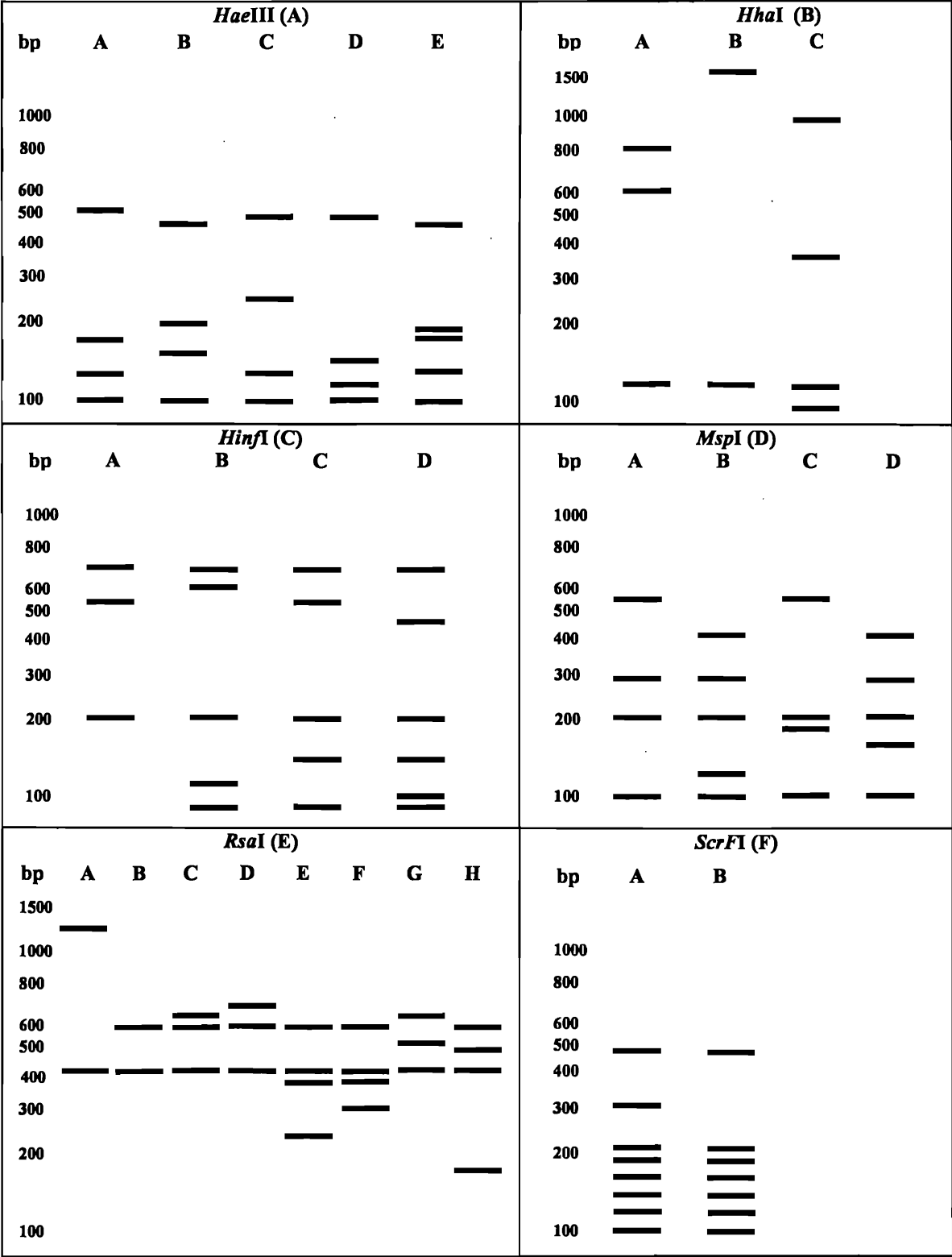


FIGURE 3. PCR amplification products of the intergenic spacer region of the ribosomal DNA of the *Fusarium oxysporum* isolates. PCR products visualized on a 0.8% agarose gel stained with ethidium bromide. Lane 1, λ molecular weight marker; lanes 2-13, *Fusarium oxysporum* isolates; and lane 14, water control.

FIGURE 4. Restriction fragment length polymorphism patterns obtained for *Fusarium oxysporum* isolates from the root rhizosphere of banana plants. Each of the illustrations represent the RFLP pattern produced when the intergenic spacer region of the ribosomal DNA was digested with the restriction enzymes *Hae*III (A), *Hha*I (B), *Hinf*I (C), *Msp*I (D), *Rsa*I (E), and *Scr*FI (F).



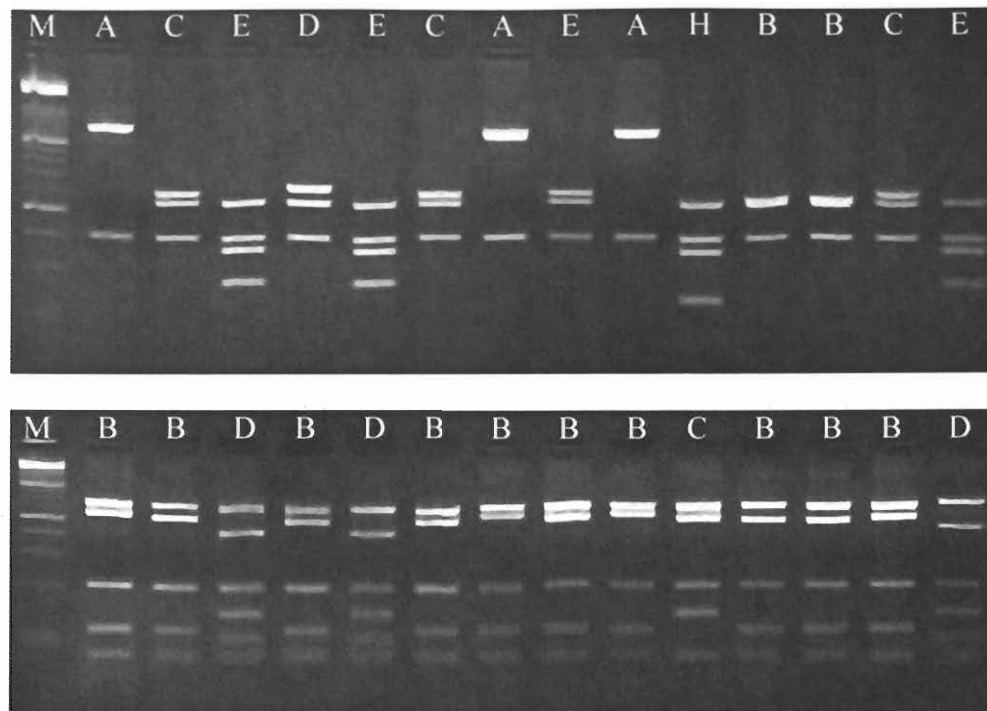
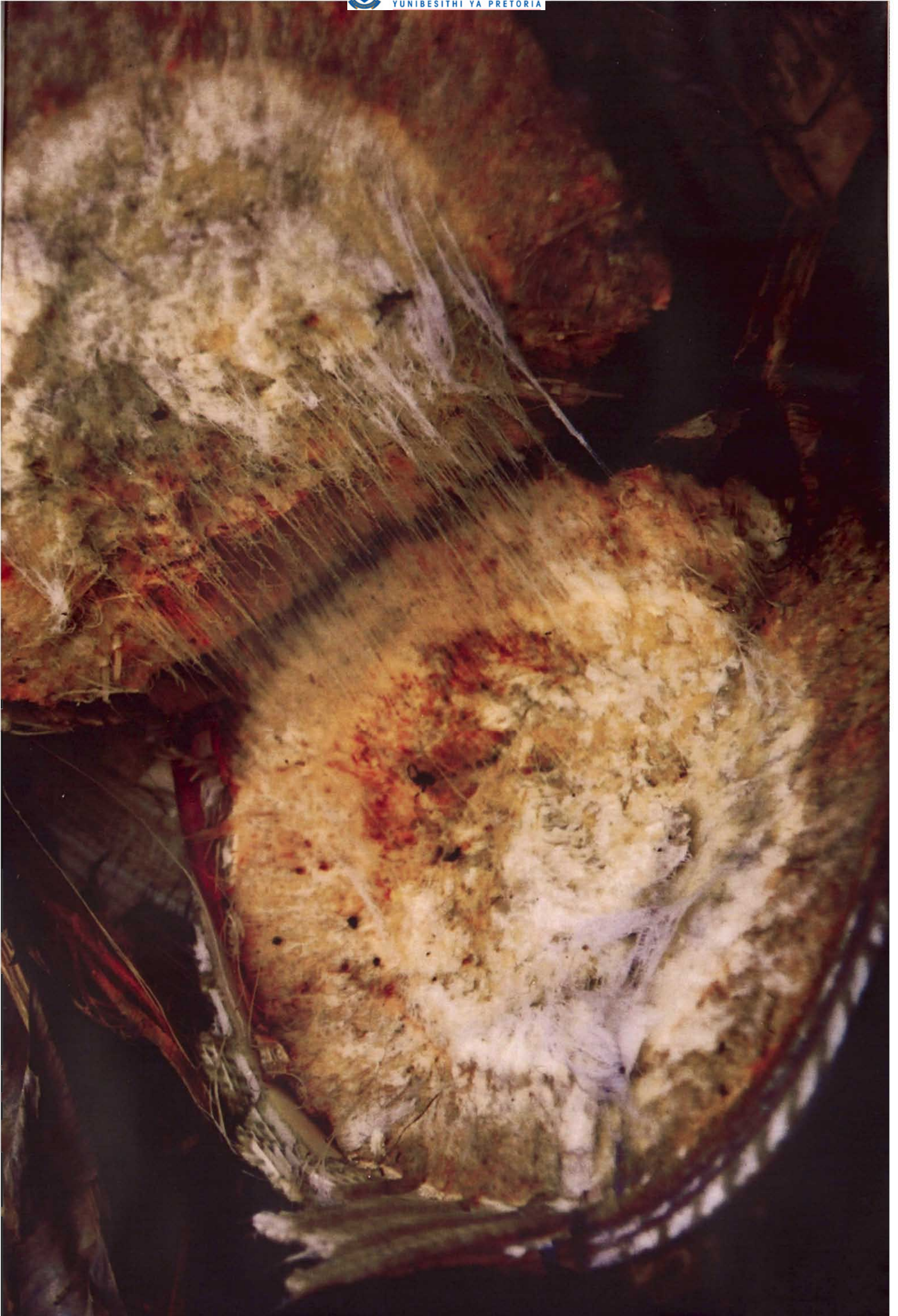


FIGURE 5. Restriction fragments of amplified IGS products of various *Fusarium oxysporum* isolates digested with *Rsa*I (top) and *Hinf*I (bottom). Isolates of *F. oxysporum* with similar restriction patterns were grouped together and assigned the same letter. Lane M represents the 100 bp molecular weight marker. Restriction fragments visualized on a 3-4% agarose gel stained with ethidium bromide.



ABSTRACT

Biological control offers an alternative management strategy for several Fusarium wilt diseases. While many biological control agents have been evaluated for application against Fusarium wilt diseases, little work has been done to investigate biological control of Fusarium wilt of banana. The aim of this study was to evaluate the ability of non-pathogenic *F. oxysporum* and *Trichoderma* isolates from suppressive soils in South Africa to suppress Fusarium wilt of banana in the greenhouse. Several biological control agents and commercial biological control products were included in the study. The isolates were first screened *in vitro* by placing the potential biocontrol agent and pathogen on opposite sides of potato dextrose agar plates. During the greenhouse evaluations, the fungal and bacterial isolates were established on the banana roots before they were replanted to pathogen-infested soil, while the commercial biocontrol agents were applied to banana roots as described by the supplier. Banana plantlets were evaluated for disease development after 7 weeks. None of the non-pathogenic isolates suppressed *Foc in vitro*, while slight suppression was observed with the two *Trichoderma* isolates. Results of the greenhouse evaluations revealed that the well-known biological control agent Fo47 did not suppress *Foc* significantly. Two *Pseudomonas fluorescens* strains, one that is the well-known WCS 417 and two of the non-pathogenic *F. oxysporum* isolates were found to be highly effective in reducing disease severity. These isolates should be further evaluated for potential application in the field, independently and in combination.

INTRODUCTION

Fusarium wilt is regarded as one of the most devastating diseases of banana, affecting plantations in almost all banana-growing countries of the world (Ploetz *et al.*, 1990). The disease is caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (E.F. Smith) Snyder & Hansen (*Foc*) (Stover, 1962). *Foc* survives as chlamydospores that, when they come into contact with banana roots, will germinate to infect the lateral or feeder roots of banana (Beckman, 1990). After infection, the pathogen will colonize and block the plant's vascular system, a process that would lead to wilting and, eventually, plant mortality (Ploetz and Pegg, 2000). Once established, *Foc* survives in banana fields for several decades, thereby rendering future production of bananas in that particular field almost impossible (Stover, 1962).

Control methods effective against Fusarium wilt of banana are limited. Control strategies, therefore, focus mostly on preventing the pathogen from introduction into disease-free areas, and the development of disease resistant varieties (Deacon, 1984; Ploetz and Pegg, 2000; Viljoen, 2002; Ploetz *et al.*, 2003). Disease prevention is mostly based on the use of pathogen-free plantlets produced in tissue culture (Moore *et al.*, 1999a), while the movement of people, vehicles and equipment must be well controlled (Moore *et al.*, 1999b). The *Foc* race 1-resistant Cavendish varieties have replaced the highly susceptible Gros Michel banana in Central America. However, in countries where Cavendish bananas are susceptible to "subtropical" race 4 of *Foc*, resistant hybrids or varieties are not always acceptable to the local commercial markets (Viljoen, 2002). New and environmentally friendly control strategies, therefore, should be considered.

Biological control offers a potential alternative to the use of resistant banana varieties against *Foc*. Several reports have previously demonstrated the successful use of biological control agents against Fusarium wilt diseases (Lemanceau and Alabouvette, 1991; Alabouvette *et al.*, 1993; Larkin and Fravel, 1998; Weller *et al.*, 2002). Most of these biocontrol agents have been isolated from soils naturally suppressive to Fusarium wilts (Smith and Snyder, 1971; Alabouvette, 1986; Larkin *et al.*, 1993; Larkin *et al.*,

1996). In such soils, the disease incidence remains low, despite the presence of a susceptible host and the pathogen (Alabouvette *et al.*, 1993). The microbial organisms that contribute to soil suppressiveness include non-pathogenic *F. oxysporum*, *Bacillus* spp., *Trichoderma* spp., *Pseudomonas* spp. and actinomycetes (Alabouvette, 1986; Larkin *et al.*, 1996; Larkin and Fravel, 1998; 1999). However, non-pathogenic *F. oxysporum* and fluorescent *Pseudomonas* spp. have most frequently been linked to soil suppressiveness (Scher and Baker, 1980; Scher and Baker, 1982; Alabouvette, 1990; Postma and Rattink, 1992; Liu *et al.*, 1995; Hoffland *et al.* 1996; Larkin *et al.*, 1996; Van Loon *et al.*, 1999).

One of the best known and most effective non-pathogenic strains of *F. oxysporum* and *Pseudomonas fluorescens* are Fo47 and WCS417, respectively. Fo47 was isolated from a soil naturally suppressive to Fusarium wilt of tomato and melon at Châteaurenard, France (Alabouvette, 1986), and has effectively reduced the incidence of Fusarium wilt of flax (Duijff *et al.*, 1999), carnation (Lemanceau *et al.*, 1992; 1993) and tomato (Fuchs *et al.*, 1997; Duijff *et al.*, 1998). WCS417 was isolated from the rhizosphere of wheat grown in a field suppressive to take-all disease of wheat (Lamers *et al.*, 1988). Studies on Fusarium wilt disease of carnation (Van Peer *et al.*, 1991), tomato (Duijff *et al.*, 1998) and radish (Leeman *et al.*, 1995; Hoffland *et al.*, 1996) have demonstrated its effectiveness as biological control agent.

Disease suppression by a biological control agent or agents can be achieved by direct antagonism against the pathogen, or from an indirect action through induced resistance modes or mechanisms of the host plant. A number of chief modes of action have been proposed that antagonistic microorganisms use to control a disease, however it should be taken into account that a single antagonistic microorganisms may express several modes of action (Alabouvette and Lemanceau, 1998). Three modes of action have been proposed for suppression of Fusarium wilt diseases by non-pathogenic *F. oxysporum*. These include competition for nutrients (Couteaudier and Alabouvette, 1990), competition for infection sites at the root surface (Schneider, 1984; Mandeel and Baker, 1991) and the induction of systemic resistance (Biles and Martyn, 1989). Similar mechanisms have been shown by fluorescent *Pseudomonas* spp., which suppress disease

by competing for iron and nutrients and, through antibiosis, or by the production of antifungal compounds (Scher and Baker, 1980; Scher and Baker, 1982; Sneh *et al.*, 1984). Induced systemic resistance has also been reported to be associated with *Pseudomonas* spp. (Van Peer *et al.*, 1991; Leeman *et al.*, 1995; Hoffland *et al.*, 1996; Duijff *et al.*, 1998). The enzymes chitinases and β -1,3-glucanases, produced by *Trichoderma* spp., play an important role in the mycoparasitism against *Fusarium* spp. (Chérif and Benhamou, 1990; Harman *et al.*, 1993; Lorito *et al.*, 1993). It might also result in induced resistance against the pathogen (Howell, 2003).

Biological control of Fusarium wilt of banana by non-pathogenic *F. oxysporum* (Gerlach *et al.*, 1999), *Trichoderma* sp. (Thangavela *et al.*, 2003), *P. fluorescens* (Sivamani and Gnanamanickam, 1988; Raghuchander *et al.*, 1997; Thangavelu *et al.*, 2001; Rajappan *et al.*, 2002) and arbuscular mycorrhizal (AM) fungi (Jaizme-Vega *et al.*, 1998) have been documented. Gerlach *et al.* (1999) observed that endophytic non-pathogenic *F. oxysporum* isolated from healthy banana plants provided a level of protection against Fusarium wilt of banana. In India, *Trichoderma harzianum* Rifai strain TH-10, isolated from the rhizosphere of banana plants, protected banana against *Foc* race 1 (Thangavelu *et al.*, 2003). Several studies also investigated the ability of *P. fluorescens* to suppress the activity of *Foc* race 1 and race 4 in the greenhouse, and some positive results were obtained (Sivamani and Gnanamanickam, 1988; Thangavelu *et al.*, 2001; Rajappan *et al.*, 2002). Jaizme-Vega *et al.* (1998) observed that the application of AM fungi to micropropagated banana plantlets (Grand Naine) reduced the internal and external symptoms of *Foc* race 4. However, no long-term studies have been reported on the effect that AM fungi have on Fusarium wilt of banana in the field (Ploetz *et al.*, 2003).

The objective of this study was to evaluate the potential of non-pathogenic *F. oxysporum* isolates from the rhizosphere soil of banana roots, to suppress Fusarium wilt of banana. Several known biological control agents of Fusarium wilt diseases, as well as commercial biological control products were also evaluated for their effectiveness against Fusarium wilt of banana.

MATERIALS AND METHODS

Selection of potential biological control organisms

Several newly isolated fungal strains, biological control organisms with known or potential activity against soil-borne fungal pathogens, as well as some commercial biocontrol agents were evaluated for control of *Fusarium* wilt of banana (Table 1). These included non-pathogenic isolates of *F. oxysporum* and *Trichoderma* spp. isolated from the rhizosphere of banana roots, *F. oxysporum* isolate Fo47 (obtained from Dr. C. Steinberg, INRA, Dijon, France), *P. fluorescens* strain WSC417 provided by Prof. L.C. Van Loon (University of Utrecht, The Netherlands), an isolate of *P. fluorescens* (*Psf*) from Prof. Mike Wallace (Dept. Microbiology, University of Natal, Pietermaritzburg, South Africa), and a mixture of two mycorrhizae isolates from Dr. R. Sinclair (Amphigro, Pretoria, South Africa). Commercial formulations of *Trichoderma* spp. (TS1) and the bacterial strain *Bacillus subtilis* (B-rus) were provided by Prof. P. Steyn (Stimuplant, Pretoria, South Africa), while a formulation containing a *Bacillus* sp., a *Pseudomonas* sp., and a *Gliocladium* sp., known as Patostop[®], was supplied by Dr. van Wyk (Soygro Ltd, Potchefstroom, South Africa).

Non-pathogenic isolates of *F. oxysporum*, and isolates of *Trichoderma* spp. were isolated from the rhizosphere of banana roots in *Fusarium* wilt suppressive soils in the field. Soil and root samples were collected from three areas in Kiepersol (Mpumalanga Province), one of the main banana-producing regions in South Africa. Segments of the root system with adhering rhizosphere soil from healthy banana plants were used for rhizosphere and rhizoplane organism dilution plating on general and selective media (Chapter 4). *Fusarium* spp. were recovered using Komada's selective medium (Komada, 1975), while potato dextrose agar (PDA) was used for the isolation of the *Trichoderma* spp. All isolates of *F. oxysporum* were evaluated for their pathogenicity to banana plantlets and then assigned to restriction fragment length polymorphism (RFLP) fingerprinting groups, using the polymerase chain reaction (PCR) (Chapter 4). Representative non-pathogenic *F. oxysporum* isolates from the PCR-RFLP groups were selected for evaluation as potential biological control agents.

***In vitro* evaluation for biological control activity**

Bacterial and fungal isolates were evaluated for their ability to inhibit growth of the virulent *Foc* isolate CAV 045 *in vitro*. The *Foc* isolate was first grown on PDA at 25°C under cool-white and near-ultraviolet fluorescent lights, before a mycelial plug was removed from the culture and placed in the centre of another PDA plate that served as the test plate (mycelial side facing down). The respective bacterial isolates were then spot-inoculated at four points around the edges of the test plates containing the pathogen. The activity of the bacterial cultures were assessed after 6 days, using a scale from 1-4, where 1=bacterial colony completely overgrown by *Foc*, 2=overgrown but bacterial colony visible, 3=grown to the edge of the bacterial colony and 4=visible inhibition zone.

For testing of fungal activity against *Foc*, mycelial plugs of both the fungal isolates and *Foc* isolate were placed on opposite sides of PDA plates. They were placed 4 cm apart from each other, with the mycelia in direct contact with the agar. Fungal activity was assessed after 10 days, using a scale from 1-5, where 1=test fungus completely overgrown by *Foc*, 2=test fungus growing over *Foc*, 3=mycelia inter-growing, 4=mycelia grown up to each other and stopped, and 5=visible inhibition zone. Control plates consisted of 2 *Foc* mycelial plugs. Five replicate plates were used for each bacterial and fungal isolate evaluated.

***In vivo* evaluation for Fusarium wilt suppression**

Plant material: Pathogen-free tissue culture banana plantlets (of the Cavendish cultivar Chinese Cavendish) were obtained from Du Roi Laboratories in Letsitele, South Africa. After the roots of the plants were washed to remove excess sterile soil, the 5-cm plantlets were placed in 250-ml plastic cups filled with tap water, and fertilized weekly with a nutrient solution (Chapter 2). After 4 weeks, sufficient root development had taken place, and the approximately 10-cm plants were replanted in 250-ml cup filled with steam pasteurized soil.

Inoculations: The *F. oxysporum* isolates, which included the biocontrol agent Fo47 and non-pathogenic isolates from the banana root rhizosphere, were grown for 7 days at 25°C

on half-strength PDA under cool-white and near-ultraviolet fluorescent lights, after which mycelia from these cultures were transferred to 500-ml Erlenmeyer flasks containing 250 ml of Armstrong Fusarium medium (Booth, 1977). The flasks containing the fungal inoculum were then placed on a rotary shaker operating at 170 rpm at 25°C for 5 days, after which the spore suspension was passed through cheesecloth to separate the mycelium from the spores. The spore concentration in the liquid medium of each flask was determined using a hemacytometer, and diluted to final concentrations of 10^7 spores.ml⁻¹. The two field strains of *Trichoderma* (T22 and T5), which were selected based on minor inhibition observed *in vitro* against *Foc*, were grown on half strength PDA plates for 4 days. Sterile distilled water was poured onto the fungal cultures, and conidia were collected after carefully abrading the agar. The conidial suspensions of the two strains were combined (TB2), filtered through cheesecloth, counted using a hemacytometer, and diluted to a final concentration of 10^6 spores.ml⁻¹. Banana plantlets were inoculated by applying 40-50 ml of each fungal spore suspension to the steam pasteurized soil as a drench, using six plants per treatment. After 7 days, all the plants were replanted in larger 12.5-cm-diameter pots.

Pseudomonas fluorescens strain WCS417 and the isolate of *P. fluorescens* (*Psf*) obtained from the University of Natal were both grown for 48 h at 25°C on King's B medium agar plates (King *et al.*, 1954). Bacteria were scraped from medium and suspended in sterile distilled water. The bacterial densities of the suspensions were measured by direct observation on a hemacytometer and adjusted by dilution. Roots of each banana plantlet were treated by drenching with 40-50 ml of a 10^8 cells ml⁻¹ suspension 3 days before they were replanted in 12.5-cm-diameter pots.

The commercial strains of *Trichoderma* (TS1) and *B. subtilis* (B-rus) were both dissolved in distilled water at a concentration of 1g/L. The *Trichoderma* strain was applied to banana roots as a drench treatment of 40-50 ml per plant 7 days before they were replanted. Plants were placed in the commercial *Bacillus subtilis* (B-rus) suspension for 24 hours, after which plants were planted in 250-ml cups filled with steam pasteurized soil. After 3 days, the plants were replanted in the 12.5-cm-diameter pots. Plants treated

with Patostop® were drenched with 40-50 ml of the suspension 3 days before being replanted. Lastly, the two mycorrhizae (MHZ) isolates were added to the roots of the banana plantlets, as a bead formulation (isolate type 1 contained 10 spores per bead and isolate type 2 contained 20 ± 5 spores per bead), during planting in 250-ml cups and allowed to colonize the banana roots for 7 days. Before the inoculated plants were replanted into the larger pots, 1-cm-long pieces of two randomly selected roots were plated onto PDA and microscopically examined to confirm the presence of the respective biocontrol products.

Greenhouse trial: A virulent isolate of *Foc* race 4 (CAV 045) was grown for 7 days on half-strength PDA under cool-white and near-ultraviolet fluorescent lights. Mycelial plugs (0.5 cm in diameter) from the margins of this culture were used to inoculate sterilized millet seeds in 500-ml Erlenmeyer flasks (Chapter 2). Infested millet seeds were added to steam sterilized soil at the rate of 15 ml per 500 ml of soil. The inoculated soil was mixed and dispensed in 12.5-cm-diameter plastic pots, to obtain a 3% inoculum concentration in the soil. Plants previously treated with potential biological control products were then transplanted to pots containing soil infested with the *Fusarium* wilt pathogen. Before replanting, the roots of the plants were slightly bruised by manually squeezing the rootball by hand to ensure infection by the pathogen. Control treatments included banana plantlets which were planted in soils either infested or uninfested with *Foc*. Plants were transferred to phytotrons with a photoperiod of 12 hours and at a day/night temperature regime of 28/20°C for 7 weeks. Six replicate plants were used for each treatment, and the entire experiment was repeated twice. Disease development was estimated by cutting the pseudostem open with a scalpel and by assigning a disease severity rating to each plant according to Inibab's Technical Guidelines number 6 (Carlier *et al.*, 2002).

Statistical analysis

Data obtained from the *in vivo* experiment were discrete and, therefore, required a non-parametric analysis. Data were analyzed based on Wilcoxon scores (Sokal and Rohlf, 1981). Differences between treatments were significant at $P \leq 0.05$ (SAS, 1999).

RESULTS

***In vitro* evaluation for biological control activity**

None of the bacterial isolates showed any visible inhibition of *Foc* after 6 days of incubation. Of the 22 *Trichoderma* isolates selected for *in vitro* evaluation, only strains T22 and T5 showed minor inhibition of *Foc*. These two strains therefore, were selected for *in vivo* evaluation as potential biocontrol organisms. No inhibition zones were observed between the non-pathogenic and pathogenic isolates, but the isolates did not overgrow each other.

***In vivo* evaluation for Fusarium wilt suppression in the greenhouse**

Of the twenty-four non-pathogenic isolates of *F. oxysporum* evaluated for potential biological control activity, fourteen isolates significantly ($P \leq 0.05$) reduced Fusarium wilt incidence (Table 2). Only four non-pathogenic *F. oxysporum* isolates, however, had a 95% confidence limit significantly different from the untreated inoculated control treatment (Fig. 1). Isolate CAV 255 was the most effective non-pathogenic isolate in terms of suppressing disease incidence of *Foc*, with a disease reduction of 87.4% (Table 2). The second most effective isolate was CAV 241, with a disease reduction of 75.0%. The biological control agent Fo47 did not reduce Fusarium wilt severity significantly in banana.

Significantly ($P \leq 0.05$) less disease severity was also observed with the two *P. fluorescens* strains WCS417 and *Psf*, and these two strains rendered disease reduction of 87.4% and 83.4%, respectively (Table 2)(Fig. 2). In the case of the commercial formulation B-rus and Patostop[®] was significant ($P \leq 0.05$) reductions in disease severity of Fusarium wilt of banana were observed. Patostop were slightly more effective than B-rus with a disease reduction of 66.6%, compared to B-rus with a disease reduction of 62.4% (Table 2). All the bacterial strains evaluated, except B-rus, had 95% confidence limits significantly different from the untreated inoculated control treatment (Fig. 1).

The combination of the two field isolates of *Trichoderma* (TB2) reduced disease severity significantly ($P \leq 0.05$), and was more effective than the commercial formulation of

Trichoderma spp. (TS1), which did not show a significant disease reduction. The simultaneous application of the two mycorrhizae isolates (MHZ) to banana roots rendered a significant degree of control and a disease reduction of 70.8% was observed (Table 2). Only the MHZ treatment had a 95% confidence limit significantly different from the untreated inoculated control treatment (Fig. 1). No disease development occurred in the untreated uninoculated control plants (Fig. 2).

DISCUSSION

Several organisms, both newly isolated from the rhizosphere of banana roots and existing commercial formulations, provided a reduced incidence of Fusarium wilt of Cavendish bananas in greenhouse trials. These organisms included non-pathogenic members of *F. oxysporum*, isolates of *P. fluorescens*, and to a lesser extent combinations of either *Trichoderma* spp. or AM fungi. The most promising results were obtained with two isolates of *F. oxysporum* from banana roots in the Kiepersol area, and the two isolates of *P. fluorescens*.

In this study, more than half of the non-pathogenic *F. oxysporum* isolates from the banana root rhizosphere rendered a significant reduction in Fusarium wilt development. This is consistent with the work of Gerlach *et al.* (1999) who found that certain isolates of non-pathogenic *F. oxysporum*, isolated from symptomless banana roots, could provide protection against Fusarium wilt of banana. Interestingly, the most effective non-pathogenic *F. oxysporum* isolates in this study (CAV 255 and CAV 241) produced identical IGS-RFLP patterns (DCDAFB) (Chapter 4), suggesting that they might be genetically related. In fact, four of the six best performing non-pathogenic *F. oxysporum* isolates against Fusarium wilt, belong to the same IGS genotype. Fo47, the biological control agent effective against several other Fusarium wilt diseases (Lemanceau *et al.*, 1992; 1993; Fuchs *et al.*, 1997; Duijff *et al.*, 1999) did not protect banana plants from Fusarium wilt in the current study. However, work done by Larkin and Fravel (1998; 1999) indicated that non-pathogenic isolates, including Fo47, can differ in their efficacy, as well as in their mechanism(s) and dose requirements to suppress a disease.

WCS417, a strain of *P. fluorescens* with known biological control activity against Fusarium wilt diseases of several crops (Van Peer *et al.*, 1991; Leeman *et al.*, 1995; Hoffland *et al.*, 1996; Duijff *et al.*, 1998) proved to be effective against Fusarium wilt of banana in this study for the first time. It was also found that the strain of *P. fluorescens* from South Africa effectively reduced disease severity, almost to the same extent as the strain WCS417. Sivamani and Gnanamanickam, (1988) reported that banana plantlets treated with a native strain of *P. fluorescens* from India, showed a degree of protection against race 1 and race 4 of *Foc* in the greenhouse. Thangavelu *et al.* (2001) demonstrated that *P. fluorescens* strain Pf10, which was isolated from the rhizosphere of banana roots, reduced wilt incidence by 50%. Since *P. fluorescens* has now been found to suppress Fusarium wilt of banana in studies by several investigators, it should be considered as a potentially strong candidate to include as biological control agent in integrated disease management programmes. It must, however, be pointed out that no study has confirmed the effectiveness of *P. fluorescens* under field conditions, and the mode of action of the bacterium has not yet been established.

A moderate reduction in Fusarium wilt incidence was achieved by application of the commercial product of *B. subtilis* (B-rus), *Trichoderma*, and the mycorrhizae. Although *Bacillus* spp. have not previously been evaluated for biocontrol against Fusarium wilt of banana, it was found to reduce the incidence of Fusarium wilt of chickpea (Hervás *et al.*, 1998) and cucumber (Hammad and El-Mohandes, 1999). Thangavelu *et al.* (2003) further reported that *Trichoderma* strains isolated from the rhizosphere soil of banana proved to be effective against *Foc* race 1.

The application of biological control organisms in combinations has often yielded better results than the organisms applied individually. For instance, the combination of non-pathogenic *F. oxysporum* and *Pseudomonas* spp. have enhanced disease suppression of Fusarium wilt of carnation (Lemanceau *et al.*, 1992), cucumber (Park *et al.*, 1988) and flax (Duijff *et al.*, 1998). In the current study, the commercial biocontrol formulation Patostop (*Bacillus* sp., *Pseudomonas* sp. and *Gliocladium* sp.) proved to be effective against Fusarium wilt of banana, but did not provide better control than the *Pseudomonas*

spp. alone. It must be mentioned that the *Pseudomonas* strain used in Patostop is not similar to the other *Pseudomonas* strains used in this study. De Boer *et al.* (1999) showed that the combination of two compatible *Pseudomonas* spp. resulted in a better disease suppression of Fusarium wilt of radish than a single strain. Although biological control agents were combined in this study, the combinations only consisted of either *Trichoderma* or mycorrhizae. It would be interesting to determine whether the combinations of different types of organisms found to be effective in this study could provide better control of Fusarium wilt than the individual organisms.

In a study on Fusarium wilt of watermelon, Larkin *et al.* (1996) investigated the role of microorganisms in soil suppressiveness. He found that some bacteria, actinomycetes, and other fungal isolates, which may play a role in soil suppression, were not very effective when tested individually in the field. He stated that these isolates may not be effective individually, but may enhance the overall suppressive effects in combination with other groups of antagonistic organisms or by providing a background of general suppression in which the specific suppression by *F. oxysporum* operates. Certain strains of fluorescent *Pseudomonas* spp. can enhance the suppression provided by antagonistic *F. oxysporum* in some soil systems (Park *et al.*, 1988; Lemanceau and Alabouvette, 1991; Lemanceau *et al.*, 1992; Duijff *et al.*, 1999). If an isolate is found to be ineffective individually, it is possible that the isolate will be more effective in combination with other microorganism or that the isolate may help to increase the overall suppression provided by other organisms in the soil.

If the mode of action of an antagonist is known, it is possible to base a screening procedure on *in vitro* tests, specifically focusing on that particular mode of action (Alabouvette *et al.*, 1993). Apart from Fo47 and WCS417, whose modes of action have mainly been linked to competition and induced resistance (Van Peer *et al.*, 1991; Lemanceau *et al.*, 1993; Eparvier and Alabouvette, 1994; Hoffland *et al.*, 1996; Fuchs *et al.*, 1997; Duijff *et al.*, 1998; 1999), the modes of action of potential biological control agents used in this study are still unknown. *In vitro* testing against *Foc* provided insufficient evidence that antagonism or antibiosis is involved, with the possible

exception of the two isolates of *Trichoderma*. However, if antagonism is not found in culture, it does not mean that antagonism would not occur in the soil, and *vice versa*. (Scher and Baker, 1980).

It was interesting to note that several non-pathogenic *F. oxysporum* isolates from the rhizosphere of banana roots were more effective in reducing Fusarium wilt incidence of banana than the known biological control agent Fo47. It was also found that the combination of the two *Trichoderma* spp. isolated from healthy banana roots, showed more effectiveness than the commercial *Trichoderma* formulation. In his analysis of similar findings, Cook (1993) suggested that organisms isolated from the environment where the pathogen occurs naturally may be more effective than biological control agents isolated from other hosts and under different environmental conditions. He reasoned that this is possible because, in the natural environment, the pathogen and non-pathogen have been co-existing for years. Cook's (1993) hypothesis was tested by Larkin and Fravel (1998) when they evaluated numerous bacteria and fungi against Fusarium wilt of tomato. They found, however, that several tomato root-colonizing organisms reduced the disease, but that none of them were more effective or as good as the known biocontrol agents. Considering the findings of this and other previous studies, it appears that biological control of soil-borne diseases is not as simplistic as is often presented. Specificity between a potential biocontrol agent and the pathogen, as well as the interaction between biocontrol agent and plants must be carefully investigated before such organisms can be presented as commercial products.

It is clear that biological control can add to the control of Fusarium wilt of banana, especially in an integrated management programme. However, a lot of additional research is still needed before this complex phenomena is sufficiently understood. Until now, one of the reasons why biological control has not, as yet, been integrated in all control systems, is the lack of consistency in the performance of the individual biological control agent (Weller, 1988; Fravel *et al.*, 2003). For research on biological control of Fusarium wilt of banana, finding a potential biological control agent, or a combination of biological control organisms, and understanding their modes of action, are the first steps only.

Future steps will include optimization of the activity of these microorganisms in the field, the effect of application time and intervals on sustainable plant protection, the survival of the biological control agent in the soil and on roots within the existing ecological status of the soil, its interaction with other soil microflora, both pathogenic and non-pathogenic to banana, and the influence that these microorganisms have on the host plant. Only then would one be in a position to fully exploit the potential application of biological organisms in the control of Fusarium wilt of banana.

REFERENCES

- Alabouvette, C. 1986. Fusarium-wilt suppressive soils from the Châteaurenard region: review of 10-years study. *Agronomie* 6: 273-284.
- Alabouvette, C. 1990. Biological control of Fusarium wilt pathogens in suppressive soils. In: Hornby, D. (Ed), *Biological control of soil-borne plant pathogens*. CAB International, Wallingford, UK, pp. 27-43.
- Alabouvette, C., and Lemanceau, P. 1998. Joint action of microbials for disease control. In: Hall, F.R., and Menn, J.J. (Eds.), *Methods in biotechnology*, vol. 5: *Biopesticides: Use and delivery*. Humana Press Inc., Totowa, NJ, pp. 117-135.
- Alabouvette, C., Lemanceau, P., and Steinberg, C. 1993. Recent advances in the biological control of Fusarium wilts. *Pesticide Science* 37: 363-373.
- Beckman, C.H. 1990. Host responses to the pathogen. In: Ploetz, R.C. (Ed.), *Fusarium wilt of banana*. APS Press, St Paul, Minnesota, USA, pp. 93-105.
- Biles, C.L., and Martyn, R.D. 1989. Local and systemic resistance induced in watermelons by *formae speciales* of *Fusarium oxysporum*. *Phytopathology* 79: 856-860.
- Booth, C. 1977. *Fusarium*. Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, UK, p. 10.
- Carlier, J., de Waele, D., and Escalant, J.V. 2002. Global evaluation of *Musa* germplasm for resistance to Fusarium wilt, *Mycosphaerella* leaf spot diseases and nematodes. INIBAP Technical Guidelines 6, Montpellier France.

- Chérif, M., and Benhamou, N. 1990. Cytochemical aspects of chitin breakdown during the parasitic action of *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 80: 1406-1414.
- Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31: 53-80.
- Couteaudier, Y., and Alabouvette, C. 1990. Quantitative comparison of *Fusarium oxysporum* competitiveness in relation with carbon utilization. *FEMS Microbiology* 74: 261-268.
- Deacon, J.W. 1984. Panama disease of banana in South Africa. *Horticultural Science* 1: 29-31.
- De Boer, M., Van der Sluis, I., Van Loon, L.C., and Bakker, P.A.H.M. 1999. Combining fluorescent *Pseudomonas* spp. strains to enhance suppression of Fusarium wilt of radish. *European Journal of Plant Pathology* 105: 201-210.
- Duijff, B.J., Pouhair, D., Olivain, C., Alabouvette, C., and Lemanceau, P. 1998. Implication of systemic induced resistance in the suppression of Fusarium wilt of tomato by *Pseudomonas fluorescens* WSC417r and by nonpathogenic *Fusarium oxysporum* Fo47. *European Journal of Plant Pathology* 104: 903-910.
- Duijff, B.J., Recorbet, G., Bakker, P.A.H., Loper, J.E., and Lemanceau, P. 1999. Microbial antagonism at the root level is involved in the suppression of Fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. *Phytopathology* 89: 1073-1079.
- Eparvier, A., and Alabouvette, C. 1994. Use of ELISA and *GUS*-transformed strains to study competition between pathogenic and non-pathogenic *Fusarium oxysporum* for root colonization. *Biocontrol Science and Technology* 4: 35-47.
- Fravel, D.R., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157: 493-502.
- Fuchs, J.G., Moëgne-Loccoz, Y., and Défago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt of tomato. *Plant Disease* 81: 492-496.

- Gerlach, K.S., Bentley, S., Moore, N.Y., Aitken, E.A.B., and Pegg, K.G. 1999. Investigation of nonpathogenic strains of *Fusarium oxysporum* for suppression of Fusarium wilt of banana in Australia. In: Alabouvette, C. (Ed), Second International Fusarium Workshop. Dijon, France: INRA-CMSE, p. 28.
- Hammad, A.M.M., and El-Mohandes, M.A.O. 1999. Controlling Fusarium wilt disease of cucumber plants via antagonistic microorganisms in free and immobilized states. Microbiological Research 154: 113-117.
- Harman, G.E., Hayes, C.K., Lorito, M., Broadway, R.M., Di Pietro, A., Peterbauer, C., and Tronsmo, A. 1993. Chitinolytic enzymes of *Trichoderma harzianum*: Purification of chitobiosidase and endochitinase. Phytopathology 83: 313-3128.
- Hervás, A., Landa, B., Datnoff, L.E., and Jiménez-Díaz, R.M. 1998. Effects of commercial and indigenous microorganisms on Fusarium wilt development in chickpea. Biological Control 13: 166-176.
- Hoffland, E., Hakulinem, J., and Van Pelt, J.A. 1996. Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. Phytopathology 86: 757-762.
- Howell, C.R. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease 87: 1-11.
- Jaizme-Vega, M.C., Hernández, B.S., and Hernández, J.M.H. 1998. Interaction of arbuscular mycorrhizal fungi and the soil pathogen *Fusarium oxysporum* f.sp. *cubense* on the first stages of micropropagated Grande Naine banana. Acta Horticulturae 490: 285-295.
- King, E.O., Ward, M.K., and Raney, D.E. 1954. Two simple media for the demonstration of phycocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 44: 301-307.
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research 8: 115-125.

- Lamers, J.G., Schippers, B., and Geels, F.P. 1988. Soil-borne diseases of wheat in the Netherlands and results of seed bacterization with pseudomonads against *Gaeumannomyces graminis* var. *tritici*. In: Jorna, M.L., and Sloodmaker, L.A.J. (Eds), Cereal Breeding Related to Integrated Cereal Production, Pudoc, Wageningen, pp.134-139.
- Larkin, R.P., and Fravel, D.R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for the control of Fusarium wilt of tomato. Plant disease 82: 1022-1028.
- Larkin, R.P., and Fravel, D.R. 1999. Mechanisms of action and dose response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89: 1151-1161.
- Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soil suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83: 1097-1105.
- Larkin, R.P., Hopkins, D.L., and Martin, F.N. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. Phytopathology 86: 812-819.
- Leeman, M., Van Pelt, J.A., Den Ouden, F.M., Heinsbroek, M., Bakker, P.A.H.M., and Schippers, B. 1995. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85: 1021-1027.
- Lemanceau, P., and Alabouvette, C. 1991. Biological control of Fusarium diseases by fluorescent *Pseudomonas* and nonpathogenic *Fusarium*. Crop Protection 10: 279-286.
- Lemanceau, P., Bakker, P.A.H.M., De Kogel, W.J., Alabouvette, C., and Schippers, B. 1992. Effect of pseudobactin 358 produced by *Pseudomonas putida* WSC358 on suppression of *Fusarium* wilt of carnations by non-pathogenic *Fusarium oxysporum*. Applied Environmental Microbiology 58: 2978-2980.

- Lemanceau, P., Bakker, P.A.H.M., De Kogel, W.J., Alabouvette, C., and Schippers, B. 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and Pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. Applied Environmental Microbiology 59: 74-82.
- Liu, L., Kloepper, J.W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85: 695-698.
- Lorito, M., Harman, G.E., Hayes, C.K., Broadway, R.M., Tronsmo, A., Woo, S.L., and Di Pietro, A. 1993. Chitinolytic enzymes produced by *Trichoderma harzianum*: Antifungal activity of purified endochitinase and chitobiosidase. Phytopathology 83: 302-307.
- Mandeel, Q., and Baker, R. 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. Phytopathology 81: 462-469.
- Moore, N.Y., Pegg, K.G., Bentley, S., and Smith, L.J. 1999a. Fusarium wilt of banana: Global problems and perspectives. In: Molina, A.B., Masdek, N.H.N., and Liew, K.W. (Eds), Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 11-30.
- Moore, N.Y., Pegg, K.G., Smith, L.J., Langdon, P.W., Bentley, S., and Smith, M.K. 1999b. Fusarium wilt of banana in Australia. In: Molina, A.B., Masdek, N.H.N., and Liew, K.W. (Eds), Banana Fusarium wilt management: Towards sustainable cultivation. Proceedings of the International Workshop on banana Fusarium wilt disease, Genting Highlands Resort, Malaysia, pp. 64-75.
- Park, C.S., Paulitz, T.C., and Baker, R. 1988. Biocontrol of Fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. Phytopathology 78: 190-194.
- Ploetz, R.C., and Pegg, K.G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D.R. (Ed), Diseases of banana, abacá and enset. CAB International, Wallingford, UK, pp. 143-172.

- Ploetz, R.C., Herbert, J., Sebasigari, K., Hernandez, J.H., Pegg, K.G., Ventura, J.A., and Mayato, L.S. 1990. Importance of *Fusarium* wilt in different banana-growing regions. In: Ploetz, R.C. (Ed), *Fusarium wilt of bananas*. ASP Press, St. Paul, Minnesota, USA, pp. 9-26.
- Ploetz, R.C., Thomas, J.E., and Slabaugh, W.R. 2003. Diseases of banana and plantain. In: Ploetz, R.C. (Ed), *Diseases of tropical fruit crops*. CAB International, Wallingford, UK, pp 109-112.
- Postma, J., and Rattink, H. 1992. Biological control of *Fusarium* wilt of carnation with a nonpathogenic isolate of *Fusarium oxysporum*. *Canadian Journal of Botany* 70: 1199-1205.
- Raghuchander, T., Jayashree, K., and Samiyappan, R. 1997. Management of *Fusarium* wilt of banana using antagonistic microorganisms. *Journal of Biological Control* 11: 101-105.
- Rajappan, K., Vidhyasekaran, P., Sethuraman, K., and Baskaran, T.L. 2002. Development of powder and capsule formulations of *Pseudomonas fluorescens* strain Pf-1 for the control of banana wilt. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Disease and Protection)* 109: 80-87.
- Scher, F.M., and Baker, R. 1980. Mechanism of biological control of a *Fusarium*-suppressive soil. *Phytopathology* 70: 412-417.
- Scher, F.M., and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. *Phytopathology* 72: 1567-1573.
- Schneider, R.W. 1984. Effects of nonpathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f.sp. *apii* and a novel use of the Lineweaver-Burke double reciprocal plot technique. *Phytopathology* 74: 646-653.
- Sivamani, E., and Gnanamanickam, S.S. 1988. Biological control of *Fusarium oxysporum* f.sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. *Plant and Soil* 107: 3-9.
- Smith, S.N., and Snyder, W.C. 1971. Relationship of inoculum density and soil types to severity of *Fusarium* wilt of sweet potato. *Phytopathology* 61: 1049-1051.

- Sneh, B., Dupler, M., Elad, Y., and Baker, R. 1984. Chlamydospore germination of *Fusarium oxysporum* f.sp. *cucumerinum* as affected by fluorescent and lytic bacteria form a Fusarium-suppressive soil. *Phytopathology* 74: 1115-1125.
- Sokal, R.R., and Rohlf, F.J. 1981. *Biometry: The principles and practice of statistics in biological research*. W.H. Freeman and Company, San Francisco.
- Stover, R.H. 1962. Fusarial wilt (Panama disease) of bananas and other *Musa* species. Commonwealth Mycological Institute, Kew, Surrey, UK, 117 pp.
- Thangavelu, R., Palaniswami, A., Ramakrishnan, G., Doraiswamy, S., Muthukrishnan, S., and Velazhahan, R. 2001. Involvement of fusaric acid detoxification by *Pseudomonas fluorescens* strain Pf10 in the biological control of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz (Journal of Plant Disease and Protection)* 108: 433-445.
- Thangavelu, R., Palaniswami, A., and Velazhahan, R. 2003. Mass production of *Trichoderma harzianum* for managing Fusarium wilt of banana. *Agriculture Ecosystem and Environment* 103: 259-263.
- Van Loon, L.C., Bakker, P.A.H.M., and Pieterse, C.M.J. 1999. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology* 36: 453-483.
- Van Peer, R., Niemann, G.J., and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* 81: 728-734.
- Viljoen, A. 2002. The status of Fusarium wilt (Panama disease) of banana in South Africa. *South African Journal of Science* 98: 341-344.
- Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* 26: 379-407.
- Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B., and Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40: 309-348.

TABLE 1. Description and sources of biological control isolates and products evaluated against *Fusarium* wilt of banana in the greenhouse trials.

Treatments ^a	Organism	Source
MHZ	2 strains	Amphigro (Dr. R. Sinclair)
B-rus	<i>Bacillus subtilis</i>	Stimuplant (Prof. P. Steyn)
Patostop	<i>Gliocladium</i> sp. <i>Bacillus</i> sp. <i>Pseudomonas</i> sp.	Soygro Ltd, Potchefstroom (Dr. v Wyk)
WCS417	<i>P. fluorescens</i> ^b	Suppress soil of wheat (Prof. L.C. v. Loon)
<i>Psf</i>	<i>P. fluorescens</i>	Dept. Microbiology, University of Natal, Pietermaritzberg
TS1	<i>Trichoderma</i> spp.	Stimuplant (Prof. P. Steyn)
TB2	<i>Trichoderma</i> spp.	South Africa, suppressive soil
Fo47	<i>F. oxysporum</i> ^c	France, <i>Fusarium</i> -suppressive soil
CAV 202	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 209	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 210	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 211	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 212	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 231	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 236	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 239	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 240	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 241	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 244	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 245	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 247	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 249	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 251	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 255	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 261	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 263	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 270	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 271	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 274	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 275	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 277	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 282	<i>F. oxysporum</i>	South Africa, suppressive soil
CAV 045 ^d	<i>F. o. cubense</i>	Port Edwards, South Africa

^a Isolate code or trade name of potential biocontrol product.

^b *Pseudomonas fluorescens*

^c *Fusarium oxysporum*

^d The pathogenic isolate of *Fusarium oxysporum* f.sp. *cubense* used to infect banana plantlets.

TABLE 2. Effect of treatments on the disease development of Fusarium wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* as evaluated in the greenhouse.

Treatment ^a	Disease severity (%)	Disease reduction (%)	<i>P</i> value ^b
CAV045	50.0		
Fo47	41.7	16.6	0.4449
CAV 240	50.0	-	1.0000
CAV 261	45.8	8.4	0.7023
CAV 212	41.7	16.6	0.4449
CAV 277	41.7	16.6	0.4449
CAV 251	39.6	20.8	0.3399
CAV 209	33.3	33.4	0.1276
CAV 231	33.3	33.4	0.1276
CAV 236	31.3	37.4	0.0868
CAV 245	31.3	37.4	0.0868
CAV 263	29.2	41.6	0.0573
CAV 271	27.1	45.8	0.0367
CAV 274	27.1	45.8	0.0367
CAV 211	25.0	50.0	0.0229
CAV 202	22.9	54.2	0.0138
CAV 210	22.9	54.2	0.0138
CAV 249	22.9	54.2	0.0138
CAV 275	22.9	54.2	0.0138
CAV 239	20.8	58.4	0.0081
CAV 244	20.8	58.4	0.0081
CAV 270	20.8	58.4	0.0081
CAV 247	16.7	66.6	0.0026
CAV 282	14.6	70.8	0.0014
CAV 241	12.5	75.0	0.0007
CAV 255	6.3	87.4	<.0001
B-rus	18.8	62.4	0.0046
Patostop	16.7	66.6	0.0026
<i>Ps</i> f	8.3	83.4	0.0002
WCS417	6.3	87.4	<.0001
TS1	33.3	33.4	0.1276
TB2	18.8	62.4	0.0046
MHZ	14.6	70.8	0.0014

^aIsolate code or trade name of commercial biocontrol product.

^bComparison of control with treatments by means of t-tests (LSD)

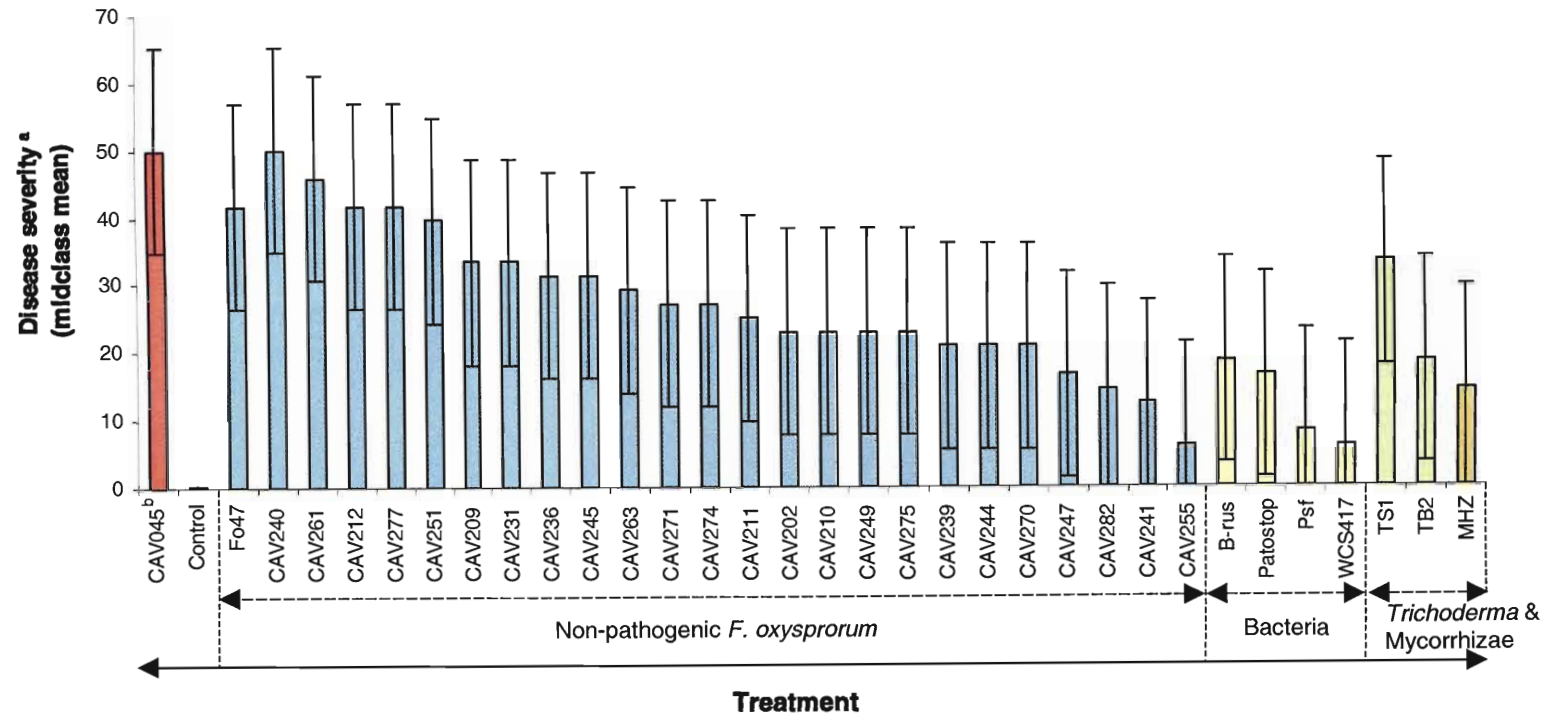
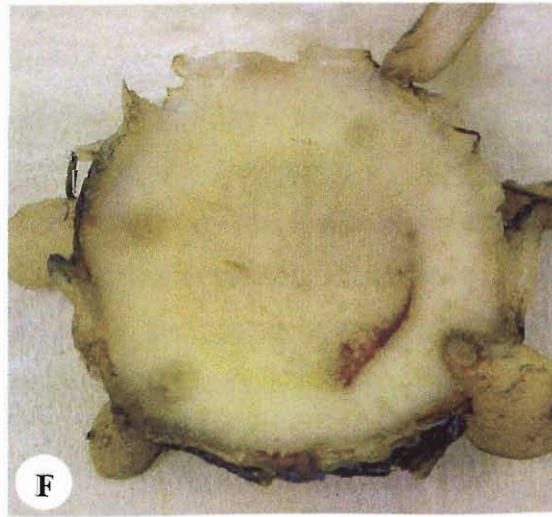
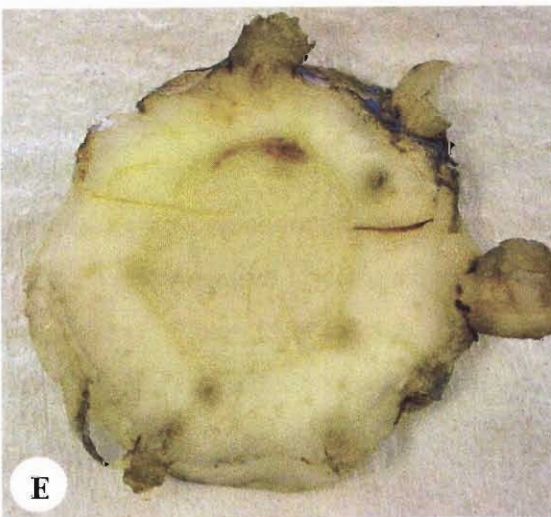
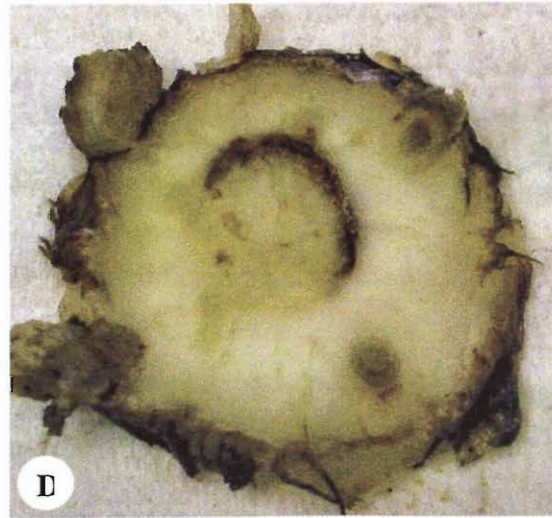
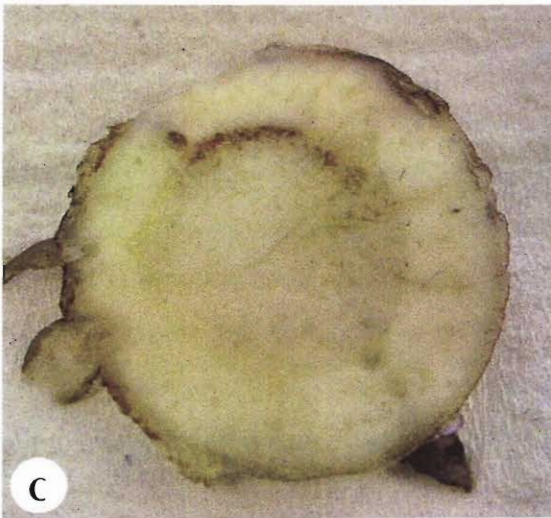
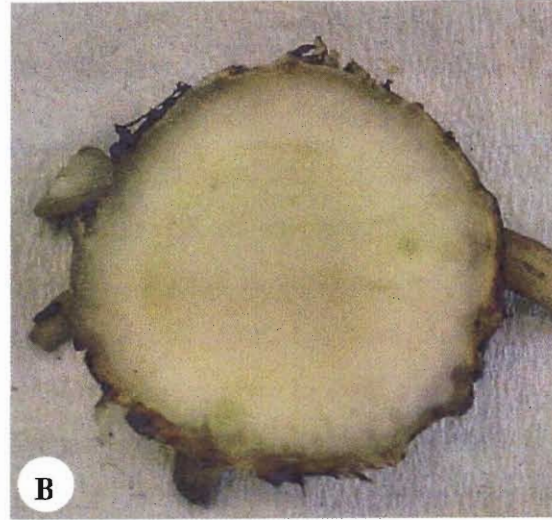
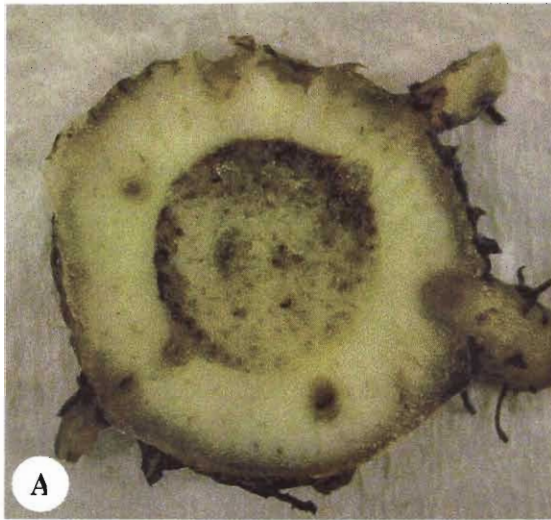


FIGURE 1. Development of Fusarium wilt in banana caused by the pathogen *Fusarium oxysporum* f.sp. *cubense*, as affected by treatments with various potential biological control agents and commercial products. Midclass mean value was calculated for the disease severity of six replicate plants per treatment. Range bars indicate a the 95% confidence interval for each treatment.

^a Disease severity determine according to the disease rating scale of INIBAP.

^b Untreated control infected with *Foc* pathogenic strain.

FIGURE 2. Internal symptoms of Fusarium wilt on banana plantlets in the greenhouse following treatments with the pathogenic isolate of *Fusarium oxysporum* f.sp. *cubense* (CAV 045) only (A), without the pathogen and with biological control agents. (C) Treatments with a non-pathogenic isolate of *F. oxysporum* from suppressive soil and *Foc*, (D) with the biological control isolate Fo47 and *Foc*, (E) with *Pseudomonas fluorescens* strain WCS 417, and (F) with *P. fluorescens* (*Pfs*) strain and *Foc*.



SUMMARY

Management of Fusarium wilt of banana, one of the most important diseases of agricultural crops, is complicated and involves the consideration of factors such as the biology, epidemiology and population structure of the pathogen, and genetic resources and production practices of the crop. The development of an integrated disease management programme, therefore, is of great importance in countries where the Fusarium wilt pathogen, *Fusarium oxysporum* f.sp. *cubense* (*Foc*), has been introduced into banana fields, and where resistant cultivars are not acceptable to local markets. To achieve this, it is important to investigate new management strategies and to review methods that have been less successful in the past. These management practices need to be practical and affordable. Since certain cultural practices have proven to be effective, management practices that could compliment them should be considered. This thesis has attempted to investigate such practices in order to develop an integrated disease management programme for Fusarium wilt of banana.

One of the most important findings of this study, was that the surface sterilant previously used to prevent the introduction of the Fusarium wilt into uninfected areas in South Africa, are not effective. The sterilants Sporekill and Prazin proved to be highly effective, and are now recommended to replace the sterilants previously used. Several fungicides reduced mycelial growth of *Foc in vitro*, with the DMI fungicides and Benomyl found to be the most effective. The same fungicides reduced the disease severity of Fusarium wilt in the greenhouse significantly, especially when they were applied as root dip treatments. None of the fungicides found effective against *Foc* have been evaluated in the field against *Foc* before. The next step, therefore, would be to evaluate root dip treatments combined with drench treatment in the field. Although it is expected that these fungicides might have a negative effect on the microbial populations in the soil, this has yet to be investigated. Fungicides may even weaken or stress the pathogen, making it more vulnerable for the action of an effective biocontrol agent or agents.

Chemical activators are probably one of the most attractive strategies to combat *Fusarium* wilt of banana, since it stimulate the plants' own defence system. Banana plantlets were found to be quite sensitive to the amount and method whereby chemical activators were applied. The activator benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester induced resistance against *Foc* on the susceptible Williams cultivar in the greenhouse, but not in the field. In field studies, environmental conditions were much more variable than in the greenhouse, which made it difficult to evaluate the effectiveness of chemical activators. Sodium nitroprusside and a product containing the harpin protein showed promising results on the Williams and DRS1 cultivars, respectively. These activators need to be considered as part of an integrated disease management programme. Since they are not directly applied to the soil, they will not have a negative effect on the microbial populations in the soil.

Several *Fusarium* isolates had been collected from banana fields with disease suppressive soils in Kiepersol, South Africa. Most of these isolates were *F. oxysporum*, and with the exception of one isolate, proved to be non-pathogenic to banana plants. A PCR-based restriction fragment length polymorphism (RFLP) analysis of the intergenic spacer region of the ribosomal RNA operon grouped the non-pathogenic *F. oxyporum* isolates into 12 distinct genotypes. A great diversity could be seen among the non-pathogenic isolates compared to the pathogenic *Foc* isolates. The known-biological control agent Fo47 grouped with three of the South African isolates, while the one pathogenic isolate grouped with the pathogenic *Foc* from diseased Cavendish bananas in South Africa. By using PCR-RFLPs, we were able to rapidly characterize the structure of non-pathogenic isolates of *F. oxysporum* in disease suppressive soils in Kiepersol. This could assist us in our search for potential biological control agents for *Fusarium* wilt of banana.

Representative isolates from the 12 genotype groups were selected for evaluation of *Fusarium* wilt suppressive properties in banana. These non-pathogenic *F. oxyporum* isolates appeared to be good biological control candidates and was compared to known biological control agents and commercial biological control products. Fourteen of the non-pathogenic isolates, the combination of two *Trichoderma* strains form suppressive

soils in South Africa, and two *Pseudomonas fluorescens* isolates were found to significantly reduce Fusarium wilt development in the greenhouse. The commercial products Patostop, B-rus and a mixture of arbuscular mycorrhizae were also found to suppress the disease severity of *Foc* significantly. The well-know biological control agent Fo47 proved to be not effective. Results concluded that two of the non-pathogenic *F. oxysporum* isolates and the two *P. fluorescens* isolates, one of which was the well-known WCS 417, were the most effective of all the agents evaluated. Since combinations of biocontrol agents may provide even more consistent and effective control than a single agent, future research will include the combination of biocontrol agents found effective in this study. It would also be of great value to determine the mode of action of these isolates, so that isolates with different modes of action could be combined to enhance the suppression effect. Biological control can be a very useful component of an integrated disease management programme, since the effective agent or agents can easily be established on tissue culture banana plantlets before they are planted in the field.

OPSOMMING

Een van seker die mees belangrikste grondgedraagte siektes in landbou, is *Fusarium* verwelksiekte van piesangs. In Suid-Afrika, is die siekte verantwoordelik vir ernstige verliese in die piesang produksie. Aangesien daar geen weerstandbiedende kultivars beskikbaar is wat deur die mark aanvaar word nie, is dit van kardinale belang dat 'n geïntegreerde siekte beheer program vir Suid-Afrika ontwikkel word. Voordat so 'n program saamgestel kan word, is dit belangrik dat verskeie faktore aangaande die patogeen en piesang produksiepraktyke in ag geneem moet word. Beheermaatreëls moet prakties en bekostigbaar wees, en moet die reeds bestaande praktyke kan bevoordeel. Studies wat in hierdie tesis aangebied word, oorweeg beheermaatreëls wat gekombineer kan word met die huidige praktyke, nadat vorige praktyke ook in ag geneem is. Daar word gesoek na nuwe meer doeltreffende en ekonomiese metodes om siektes te beheer. Metodes wat doeltreffend aangewend kan word om die voorkoms van die siekte te verminder.

In vitro en *in vivo* studies het getoon dat die DMI swamdoders en Benomil die groei van die patogeen en die ontwikkeling van *Fusarium* verwelksiekte die meeste onderdruk. Die beste resultate is in die glashuis gevind nadat die wortels van plante in die middels gewee is. Positiewe resultate is ook verkry met die grondtoediening van Benomil 'n week nadat plante geplant is in *Foc* geïnfecteerde grond. Die chemiese beheer van *Fusarium* verwelksiekte kan verder ondersoek word deur die effek van die grondtoedienings en wortelbehandelings in die veld te ondersoek. Daar word egter verwag dat die swamdoders moontlik 'n negatiewe uitwerking op die mikrobiese aktiwiteit in die grond kan veroorsaak. Die gebruik van effektiewe ontsmettingmiddels is uiters belangrik vir die voorkomende beheer van *Fusarium* verwelksiekte op piesangs. Die ontsmettingmiddel, koper oxichloried, wat tot onlangs in Suid Afrika gebruik was, is ondoeltreffend gevind vir ontsmettingsdoeleindes. Prazin en Sporekill, twee omgewingsvriendelike middels, is baie effektief gevind en word dus aanbeveel vir die ontsetting van voertuie, skoene en veld toerusing.

Chemiese plant aktiveerders stimuleer plante om hulleself te beskerm deur middel van weerstandsmeganismes. Piesang plante het sensitiwiteit getoon toonoor die konsentrasie en die toedieningsmetode van hierdie chemiese aktiveerders. In die glashuisproewe het die aktiveerder benzo-(1,2,3)thiadiazole-7-carbothioic suur S-metiel ester weerstand gestimuleer in die Williams kultivar. As gevolg van veranderende toestande in die veld was dit moeiliker om die chemiese aktiveerders se werking te evalueer. Nogtans het die middels natrium nitroprussied en 'n produk wat die proteïen harpin bevat die voorkoms van siekte op die Williams en DRS1 plante verlaag. Chemiese aktiveerders behoort sterk oorweeg te word as deel van 'n geïntegreerde beheer program, aangesien chemiese aktiveerders nie direk tot die grond aangewend word nie, en geen negatiewe uitwerking op die natuurlike mikrobiële populasies in die grond uitoefen nie.

Verskeie *Fusarium* isolate is geïsoleer vanuit siekte onderdrukkende gronde in die Kiepersol area van Suid-Afrika. Die meeste van die isolate is geïdentifiseer as *F. oxysporum*. 'n PKR-gebaseerde restriksie fragment lengte polimorfisme (RFLP) ontleding van die "intergenic spacer region" van die ribosomale DNS operon het die nie-patogeniese *F. oxysporum* isolate in 12 verskillende genotipes opgedeel. 'n Groot diversiteit was sigbaar onder die nie-patogeniese isolate in vergelyking met die patogeniese *Foc* isolate. Die bekende beheer agent, Fo47 het gegroepeer saam met drie van die Suid Afrikaanse nie-patogene. Hierdie tegniek het ons instaat gestel om die nie-patogeniese populasie van onderdrukkende gronde in Kiepersol vinnig te karakteriseer en potensiele biologiese agente te identifiseer.

Verteenwoordigende isolate van die 12 genotipiese groepe wat geïdentifiseer is, is geselekteer vir verdere evaluasie. Dit is gevind dat die isolate goeie kandidate vir moontlike bio-beheer agente maak. Die onderdrukkingsvermoë van die nie-patogene is vergelyk met die van bekende bio-beheer agente en komersiële produkte wat beskikbaar is. Veertien van die nie-patogene, die kombinasie van twee *Trichoderma* spp., en twee *Pseudomonas fluorescens* isolate het die siekte ontwikkeling van *Fusarium* verwelking merkwaardig onderdruk in die glashuis. Die komersiële produkte Patostop[®], B-rus en die kombinasie van twee mycorrhizae isolate is ook gevind om die voorkoms van siekte te

verlaag. Die wel-bekende biobeheer agent Fo47 is oneffektief gevind teen *Fusarium* verwelksiekte van piesangs. Resultate van die studie het bewys dat twee van die nie-patogeniese *F. oxysporum* isolate en twee *P. fluorescens* isolate, waarvan een die wel-bekende WCS 417 is, uiters effektiewe beheer agente teen *Foc* is. Toekomstige studies sal fokus op die kombinasie van die bio-beheer agente wat die meeste potensiaal getoon het in die studie, asook hulle meganismes van werking. Biologiese beheer is van groot waarde vir 'n geïntegreerde beheer program. Dit kan maklik met bestaande beheer maatreëls gekombineer word en potensiële biologiese beheer agente kan vooraf op weefselkultuur plante in die kwekery gevestig word.