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**OOMYCETES ASSOCIATED WITH CITRUS AND
EUCALYPTS ROOT ROT IN SOUTH AFRICA**

BONGANI BRUCE O'CLIVE ZWELIBANZI MASEKO

OOMYCETES ASSOCIATED WITH CITRUS AND EUCALYPTS ROOT ROT IN SOUTH AFRICA

This thesis is being submitted in accordance with the requirements for the *MAGISTER SCIENTIAE* degree in the Faculty of Science, Department of Microbiology and Biochemistry at the University of the Orange Free State.



BY

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March 1999

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DECLARATION

I, **Bongani O'clive Zwelibanzi Maseko**, hereby declare that this thesis entitled "**OOMYCETES ASSOCIATED WITH CITRUS AND EUCALYPTS ROOT ROT IN SOUTH AFRICA**" is a result of my own independent work and has hitherto not been submitted for any degree at any other University.

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

Signature of the Candidate

Bongani O'clive Zwelibanzi Maseko

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"Behind every important discovery, is a person who regularly grew weary searching for it.
Behind every fortune, is someone who has laboured long into the night to make it real.
Behind the magnificent work of art is an artist who spent hour after hour, month after
month toiling at tasks that were not so magnificent".

Ralph Marston

Dedicated to my late brother **Makhosonke**, who passed away a few days before graduating
from South African College Teacher of Education (SACTE).

PREFACE

The genera *Phytophthora* and *Pythium* include many species that are mostly plant pathogens, others are saprophytic and a few are human pathogens. *Phytophthora* (Greek: *Phyton*, plant + *phtheiro*, destroyer) comprises a group of plant pathogenic organisms that attack an extremely broad range of agronomically important crops, worldwide. These species range from highly host specific such as *P. infestans* found in potatoes and tomatoes to *P. cinnamomi*, which has more than 1000 hosts.

In South Africa *Phytophthora* spp. are associated with both exotic and indigenous hosts and cause serious economic losses to major agricultural crops, ornamentals, forest and fruit trees. The host list includes avocado, citrus, grapes, commercial *Protea* spp., tomatoes, potatoes, eucalypts, pine and wattle. Their cumulative damage is estimated at billions of Rands annually.

The agricultural importance of the Oomycetes, and of *Phytophthora* in particular, provided a compelling reason to conduct this study. Chapter One of this thesis provides a comprehensive literature review on *Phytophthora* and *Pythium* diseases of citrus and exotic forest tree species planted in South Africa. An attempt was made to consolidate all existing knowledge on *Phytophthora* and *Pythium* and the diseases they cause on these hosts. Disease symptoms and control measures are briefly discussed and special emphasis is placed on the disease situation in South Africa.

The main focus of this research was on *Phytophthora* and *Pythium* spp. associated with root rot of citrus and eucalypts in selected provinces of South Africa. Due to the complexity of the diseases of these exotic hosts, these topics are dealt with separately in this thesis. Part one of this thesis deals with the susceptibility of *E. fraxinoides* and *E. smithii* to *P. cinnamomi*. Both these hosts have a tremendous potential to be planted commercially in cold areas unsuitable for *E. grandis*. Pioneering work conducted by Dr Charlie Clarke of Sappi in 1995 showed that these two species had fast growth rates and excellent wood properties compared to other *Eucalytus* spp. currently planted in high altitude areas. However, *Phytophthora* root rot is one major limiting factor affecting

commercial afforestation using these cold-tolerant eucalypts. In this study, an attempt was made to identify half-sib families of these hosts tolerant to *P. cinnamomi*.

In Chapter Two of this study, *E. fraxinoides* families tolerant to *P. cinnamomi* were screened. A single most virulent isolate of *P. cinnamomi* was used to inoculate young trees in the field. Results obtained following natural mortality and artificial inoculation with *P. cinnamomi* in the field were combined in a Multiple Selection Index (MSI) in order to aid selection of best families.

Chapter Three deals with pot trials of *E. fraxinoides* and *E. smithii* families conducted under greenhouse conditions. Sixty-five *E. fraxinoides* and forty-nine *E. smithii* half-sib families were screened for tolerance to *P. cinnamomi*. A single most virulent isolate was used to inoculate the seedlings. Lesion lengths measured after three weeks were used as criterion to measure disease tolerance or susceptibility among different families.

Root rot is one of the serious diseases affecting fruit yield and production in citrus orchards in South Africa. Several soil-borne pathogens, especially *Phytophthora* spp., and stress factors such as soil compaction and waterlogging have been reported to play a part in the decline of citrus trees. Part two of this thesis, therefore, deals with the role played by *Phytophthora* and *Pythium* spp. in the development of citrus root rot.

Pythium spp. occur in abundance in the rhizosphere of diseased citrus trees in the apparent absence of *Phytophthora* spp. However, the role played by *Pythium* spp. in the development of the citrus root rot complex has never been clearly defined. In Chapter four, a preliminary survey was conducted on selected nurseries and orchards in the Northern and Mpumalanga provinces of South Africa. Diseased plant material and soil samples were collected and assayed for *Phytophthora* and *Pythium* spp.

The pathogenicity of *Phytophthora* and *Pythium* isolates recovered from diseased citrus trees in nurseries and orchards was determined in Chapter Five. Pathogenicity of isolates was determined using rapid screening techniques. These included inoculating *Phytophthora* isolates into citrus fruit, and infecting lupin with different *Pythium* isolates.

Virulent isolates were further inoculated on two commercial citrus rootstocks, Rough Lemon and Troyer Citrange.



CHAPTER ONE
LITERATURE REVIEW

OOMYCETES ASSOCIATED WITH CITRUS AND EUCALYPTS ROOT ROT IN SOUTH AFRICA

GENERAL INTRODUCTION

Oomycetes, also referred to as 'water moulds', are a primitive group of fungi, saprophytic or parasitic on plants and depend on moist conditions for sporulation and spore dispersal (Kendrick, 1992; Erwin & Ribeiro, 1996). This group of microorganisms has been classified in the fungal Kingdom because they share certain morphological features with fungi (Fuller, 1987; Barr, 1992). They have several unique structural, physiological and genetic characteristics that distinguish them from other fungal groups (Zentmyer, 1983; Irwin, Cahill & Drenth, 1995). They have coenocytic colourless mycelia composed mainly of a glucan-cellulose complex rather than of chitin (Bartnicki-Garcia & Wang, 1983), motile biflagellate zoospores produced in zoosporangia, thick-walled oospores, a diploid life cycle (Waterhouse, 1973; Sharma, 1989; Kendrick, 1992) and are unable to synthesize sterols (Hendrix, 1964). Oomycetes have recently been reclassified under a newly described Kingdom, Chromista on the basis of phylogenetic relatedness with brown algae (Gunderson *et al.*, 1987; Dick, 1990a; Förster *et al.*, 1990; Brasier & Hansen, 1992; Sankoff *et al.*, 1992; Paquin *et al.*, 1995; Erwin & Ribeiro, 1996; Agrios, 1997).

The genera *Phytophthora* and *Pythium* both belong to the class Oomycetes, family Pythiaceae (Waterhouse, 1973; Sharma, 1989). Members of this family include some of the most destructive plant pathogens (Kendrick, 1992). They cause serious economic losses of major agricultural crops, ornamentals, forest and fruit trees (Hendrix & Campbell, 1973; Van der Plaats-Niterink, 1981; Erwin & Ribeiro, 1996). A classic example of the destructive nature of the Pythiaceae is potato late blight, caused by *Phytophthora infestans* (Montagne) de Bary, which led to the great Irish famine in 1845 (Gregory, 1983; Bourke, 1991). Another example is that of *P. cinnamomi* Rands that has caused widespread devastation of *Eucalyptus marginata* Donn ex Smith (the Jarrah) (Podger, Doepel & Zentmyer, 1965; Gregory, 1983; Shearer & Tippett, 1989; Podger, James & Mulcahy, 1996) and other flora in Australia (Irwin *et al.*, 1995).

The genus *Phytophthora* (Greek: *phyton*, plant + *phtheiro*, destroyer) includes more than 50 species (Newhook, Waterhouse & Stamps, 1978; Stamps *et al.*, 1990). Most species have been reported as causal agents of a wide range of diseases on a large number of plants (Zentmyer, 1983; Erwin & Ribeiro, 1996). These range from the highly host specific species such as *P. fragariae* Hickman found only on strawberry and raspberry (Cooke, Duncan & Unkles, 1995) to *P. cinnamomi*, which has nearly 1000 hosts (Zentmyer, 1980).

Pythium species are common inhabitants of soil and water (Waterhouse, 1973). They are responsible for serious damage to a wide variety of plant species in many parts of the world (Robertson, 1972; Hendrix & Campbell, 1973) including South Africa (Botha & Coetzer, 1996). The genus *Pythium* is the largest genus of the Pythiaceae (Waterhouse, 1973; Sharma, 1989) with more than 120 recognised species (Dick, 1990b). *Pythium* spp. have been underestimated as plant pathogens since many are non-pathogenic (Hendrix & Campbell, 1973). Many *Pythium* spp. are implicated as primary pathogens causing damping-off on a variety of plants (Waterhouse, 1973; Dick & Ali-Shtayeh, 1986), while some have even been reported as parasites of other *Phytophthora* spp. (Fang & Tsao, 1995) or other fungi (Deacon, 1976). Species such as *P. ultimum* Trow and *P. irregulare* Buisman have a cosmopolitan distribution and wide host ranges (Van der Plaats-Niterink, 1981). *Pythium* spp. such as *P. spinosum* Sawada, have been reported as weak pathogens on a few plants (Van der Plaats-Niterink, 1981). Damping-off of seedlings in nurseries is a common disease symptom associated with *Pythium* spp. (Gibson, 1975; Agrios, 1997).

In this review, citrus and exotic forest tree diseases associated with *Phytophthora* and *Pythium* spp. are discussed. Special reference is paid to the situation in South Africa. Due to the complexity of the diseases of these hosts, they are dealt with separately in this review.



PART ONE

PHYTOPHTHORA AND PYTHIUM SPP. ASSOCIATED WITH CITRUS ROOT ROT IN SOUTH AFRICA

INTRODUCTION

Phytophthora and *Pythium* spp. have been reported in citrus growing countries, as the most destructive soil-borne pathogens associated with citrus. These countries include Australia (Doepel, 1966); China (Ho, 1996); Iran (Fatemi, 1972); Iraq (Hassan, El-Behaldli & Alsaadawi, 1989 a, b); South Africa (Wager, 1942); Taiwan (Ann, 1984); and the United States of America (Martin *et al.*, 1956). Both genera attack seedlings in nurseries leading to damping-off (DeWolfe, Calavan & Sufficool, 1954; Klotz *et al.*, 1966; Whiteside, 1988b), crown and root rot (Graham & Timmer, 1994). In orchards, *Phytophthora* spp. cause foot and fibrous root rot on susceptible rootstocks and scions, resulting in tree and fruit production losses (Timmer & Menge, 1988). This condition is often referred to as "citrus decline" (Tsao, Martin & Davis, 1978). Fatemi (1972) defines citrus decline as any condition that deprives the plant of an adequate root system. Citrus decline is attributed to a number of abiotic and biotic factors (Tsao, Martin & Davis, 1978; Whiteside, 1988a). These include soil compaction (Joubert, 1993; Mkhize, Vanassche & Laker, 1996), waterlogging (Rowe & Beardsell, 1973; Kazlowski, 1984; Calvert & Ford, 1995), nutrient deficiencies (Reese & Koo, 1975), salinity (Blaker & MacDonald 1986; Combrink, 1990), viruses (Ferguson & Garnsey, 1993), nematodes (Van Gundy & Kirkpatrick, 1964; Kaplan, 1988; Noling, 1993) and fungi (especially Pythiaceae fungi and *Fusarium* spp.) (Sherbakoff, 1953; Danderand & Menge, 1993).

In South Africa citrus decline is reported to be caused by the interaction between *Phytophthora nicotianae* van Breda de Haan (Kotzé 1982; 1984; Le Roux *et al.*, 1991), *Pythium* spp. (Wager, 1942; Thompson, Phillips & Nel, 1995), *Fusarium* spp., and citrus nematode (*Tylenchulus semipenetrans*) (Martin, 1960; Labuschagne, Kotzé, & Putterill, 1987; Labuschagne, Van Der Vegte & Kotzé, 1989; Strauss, 1992). Citrus root rot is usually severe when trees are subjected to some stress factors (Labuschagne *et al.*, 1987). This disease complex has also been reported in the USA (Van Gundy & Tsao, 1963; O'Bannon, Leathers & Reynolds, 1967; Duncan, Graham & Timmer, 1993).

***Phytophthora* spp. associated with citrus diseases**

Phytophthora spp. causes the most serious and economically important citrus root diseases in nurseries and orchards (Timmer & Menge, 1988; Menge, 1989). In nurseries, production losses due to *Phytophthora* spp. occur due to damping-off of seedlings in seedbeds and crown rot of young seedlings (Klotz, DeWolfe & Wong, 1958; Carpenter & Furr, 1962; Whiteside, 1988b; Graham & Timmer, 1994). In orchards, *Phytophthora* spp. causes collar and fibrous root rot (Klotz *et al.*, 1958; Ferguson & Timmer, 1987; Timmer & Menge, 1988). Some *Phytophthora* spp., especially *P. citrophthora* (Smith & Smith) Leonian also infect fruit causing brown rot, resulting in pre-harvest and post-harvest fruit losses (Feld, Menge & Pehrson, 1979; Graham & Timmer, 1995).

Phytophthora nicotianae and *P. citrophthora* are two of the most common and destructive root pathogens associated with citrus root and collar rot (Ferguson & Timmer, 1987; Timmer & Menge, 1988; Graham & Timmer, 1994). In South Africa *P. nicotianae* is the most common species in nurseries (Wehner, Combrink & Kotzé, 1986) and orchards (Thompson *et al.*, 1995). These authors also reported the absence of *P. citrophthora* in nurseries and orchards. Other *Phytophthora* spp., like *P. citricola* Sawada, *P. cryptogea* Pethybridge & Lafferty have been occasionally isolated from citrus nurseries (Von Maltitz & Von Broembsen, 1985) and orchards (Thompson *et al.*, 1995).

DISEASE SYMPTOMS ASSOCIATED WITH *PHYTOPHTHORA* INFECTIONS

Damping-off

Damping-off is a term used to describe underground, or crown rot of seedlings, due to several soil-borne fungi, including *Phytophthora* spp. (Klotz *et al.*, 1966; Whiteside, 1988b). Damping-off is a problem, known throughout the world to affect newly germinated seedlings of all citrus cultivars (Whiteside, 1988b). Damping-off is usually limited to juvenile seedlings, but can also infect older seedlings in nurseries (Whiteside, 1988b). According to Graham and Timmer (1994) seedlings become resistant soon after the leaves appear and the stem tissue matures. Disease symptoms result when the fungus penetrates the seed coat, or later as the radicle starts to emerge (pre-emergence damping-off). Post-emergence damping-off results when the fungus infects the seedling, just above the ground level, causing it to fall

over and die (Graham & Timmer, 1994). This condition often results in poor, uneven stands of seedlings (Klotz, 1978; Graham & Timmer, 1994).

The majority of commercial citrus nurseries are maintained free of *Phytophthora* spp., through strict sanitation practices (Lee & Roxburgh, 1988). Several disease control strategies are used in controlling damping-off in citrus nurseries. These include monitoring the pathogen status in water, roots and growth media on regular basis (Lee & Roxburgh, 1988). Irrigation water may be filtered or decontaminated to eliminate *Phytophthora* spp. that are commonly found in ponds used for irrigation (Shokes & McCarter, 1979; Le Roux, 1988). Methyl bromide is commonly used to fumigate the growing media (Klotz *et al.*, 1966; Lee & Roxburgh, 1988). Unfortunately, fumigation with methyl bromide kills beneficial mycorrhizal fungi and has detrimental, non target effects. Methyl bromide fumigation been phased out in favour of safer fumigation methods (Erwin & Ribeiro, 1996). Most nurseries in South Africa use composted pine bark as growing media because it is effective in controlling *Phytophthora* disease outbreaks (Lee & Roxburgh, 1988). To prevent possible nursery infestation by humans, copper oxide "Bordeaux powder" is usually sprinkled on the nursery floor (Klotz, 1978).

Foot rot and Gummosis

Foot rot and gummosis are the most destructive diseases of citrus throughout the world (Klotz 1978; Ferguson & Timmer, 1987; Graham & Timmer, 1994). In South Africa the disease was first reported as early as 1891 (Klotz, 1978). Several *Phytophthora* spp. are associated with this disease (Table 1). However, *P. nicotianae* and *P. citrophthora* are the most common causal organisms (Klotz, 1978; Timmer & Menge, 1988). Foot rot results from an infection of the rootstock or scion near ground level (Whiteside, 1971). Infection occurs through wounds or small cracks in the bark, resulting in water soluble gum exudation, hence the term "gummosis" (Timmer & Menge, 1988). Lesions usually spread around the tree trunks, slowly girdling it and resulting in a loss of vigour. Juvenile trees with thin stems can be rapidly girdled and killed (Graham & Timmer, 1994). Commercial scion cultivars are highly susceptible to the disease, sometimes tolerant rootstocks can also be affected (Timmer, 1977). Disease symptoms can be readily seen from above or below the soil surface. The most obvious above surface symptoms are pale green leaves with yellow veins, followed by rapid die-back of the branches with reduced fruit size and yield (Graham & Timmer, 1994). The below surface disease symptoms include necrotic areas that remain attached to the bark, while

invasion of secondary pathogens discolour and eventually kill the wood (Klotz, 1978). This condition is commonly known as dry root rot (Whiteside, 1988a).

In orchards, collar rot problems can best be avoided through improved cultural practices (Klotz, DeWolfe & Miller, 1969; Shea & Broadbent, 1983; Coffey, 1991). For example, new orchards should be established on well-drained sites and only disease-free trees should be planted (Klotz *et al.*, 1968). Properly grafted trees should be planted such that the bud union remains well above the ground (Klotz, 1978), since most commercial scions are susceptible. Burial of the bud union can allow direct contact of bark with infected soil (Klotz, 1978). Use of resistant or tolerant rootstocks such as Trifoliolate orange greatly reduces the impact of the disease (Klotz *et al.*, 1967, 1969).

Chemical control through the use of systemic fungicides (e.g. Metalaxyl and fosetyl-Al) have been useful in controlling foot rot of citrus (Timmer, 1977; Cohen & Coffey, 1978, Farih *et al.*, 1981; McKenzie, 1985; Sandler *et al.*, 1989). Various application methods using phosphonate fungicides have been used successfully in controlling foot rot of citrus (Schutte, Bezuidenhout & Kotzé, 1991; Guest, Pegg & Whiley, 1995). Heat treatment of diseased citrus trees has also been reported effective in controlling foot rot and gummosis (Hough, Mulder & La Grange, 1979).

Fibrous root rot

Infection of the fibrous roots begins when *Phytophthora* zoospores are released from zoosporangia under moist conditions. Zoospores are chemotactically attracted to the root elongation region (Zentmyer, 1961). They encyst and germinate to produce germ tubes that invade and penetrate the host root cortex. Hyphae proliferate within the root tissue leading to decay of fibrous roots (Klotz *et al.*, 1958; Ferguson & Timmer, 1987; Timmer & Menge, 1988).

Phytophthora nicotianae and *P. citrophthora* are the most widespread causal agents associated with fibrous root rot of citrus (Le Roux, 1992; Timmer & Menge, 1988). The first obvious symptom of fibrous root rot is reduced number of rootlets (Klotz, 1978). Fibrous root rot is particularly severe on susceptible rootstocks, however, resistant rootstocks are also infected (Timmer, 1977). The major difference between the susceptible and resistant

rootstocks is that susceptible rootstocks are unable to produce new fibrous roots. They can not keep pace with root death (Graham, 1995a). Infected roots usually have a water-soaked appearance and a discoloured, soft cortex (Klotz, 1978). As the disease progresses, the water and mineral uptake of the trees is hampered and repeated fungal attacks deplete nutrient reserves (Graham & Timmer, 1994). This condition leads to severe yield losses, twig die-back and eventual death of the tree (Ferguson & Timmer, 1987; Timmer & Menge, 1988). In the orchards, fibrous root rot is usually very difficult to control. Several control strategies are currently used in reducing *Phytophthora* diseases on citrus. These include sanitation, cultural and biological control, use of resistant rootstocks and chemical control (Coffey, 1991).

Brown rot of fruit

Brown rot of fruit is a serious disease of citrus resulting in pre-harvest and post-harvest fruit losses (Feld, Menge & Pehrson, 1979; Brown & Eckert, 1988; Brown, 1994). It has been reported in the following countries: South Africa (Doidge & Van der Plank, 1936; Hough, Kellerman & Fourie, 1980), USA (Klotz & DeWolfe, 1961; Feld *et al.*, 1979), Israel (Schiffmann-Nadel & Cohen, 1969; Solel, 1983), India (Rao, 1985) and Australia (Doepel, 1966). The disease is caused by several *Phytophthora* spp., but *P. citrophthora* is the most common causal agent (Feld *et al.*, 1979; Brown, 1994). In Florida, *P. palmivora* has also been reported to cause brown rot (Zitko, Timmer & Sandler, 1991). Symptoms start when the fruit near ground level becomes infected with *Phytophthora* containing soil. Free water on the fruit promotes zoospore production, and these penetrate the intact rind within a relatively short time (Brown & Eckert, 1988).

The disease can easily spread from infected fruit to healthy fruit in pallets during ripening and in packed boxes during storage, resulting in substantial post-harvest fruit losses (Whiteside, 1970, Cohen & Schiffmann-Nadel, 1978a; Brown & Eckert, 1988; Le Roux, 1992; Brown, 1994). Brown rot of fruit is characterised by a light brown discolouration of the rind within few days after infection. The affected area is firm and leathery and remains firm. In humid conditions, white mycelia are formed on the rind surface of the fruit. Infected fruits have a distinct rancid smell, which distinguishes the disease from other fruit rots (Brown & Eckert, 1988; Brown, 1994). The disease is favoured by long duration of fruit wetness, common during long rainy seasons (Brown & Eckert, 1988; Showdon, 1991). Integrated control measures are usually used to reduce brown rot. Pre-harvest application of systemic fungicides

to the canopy is very effective in controlling brown rot (Hough *et al.*, 1980; Rao, 1985). Post-harvest fungicide sprays are not effective in controlling brown rot (Cohen & Schiffmann-Nadel, 1978b) but fruit may be coated with wax containing fungicides (Cohen, 1981; Showdon, 1991).

***Pythium* diseases on citrus**

Pythium spp. are present in citrus soils and are associated with citrus root rot (Havey, 1945; Klotz *et al.*, 1966). However, their role is not clearly understood. *Pythium* spp. could either play a pathogenic or saprophytic role on citrus trees depending on prevailing environmental factors in the rhizosphere, or they could have a protective role against *Phytophthora* spp. (Fang & Tsao, 1995) and other fungi (Deacon, 1976). In California, for example, *P. ultimum* Trow is prevalent in orchards and has been reported to cause root rot of citrus seedlings in greenhouses (Martin *et al.*, 1956; Klotz, 1978). Surveys conducted in South African orchards and nurseries also indicate that *Pythium* spp. are very common in citrus soils (Wehner *et al.*, 1986; Thompson *et al.*, 1995). Decline of citrus trees has been reported in some orchards where *Phytophthora* spp. are absent (Thompson *et al.*, 1995). It is apparent that *Pythium* spp. may be involved, but their role is often underestimated or poorly understood. The nursery disease symptoms caused by *Pythium* spp. are very similar to those of *Phytophthora* spp. According to Whiteside (1988a), *Pythium* spp. are very seldom involved in damping-off. *Pythium aphanidermatum* (Edson) Fitzp. and other *Pythium* spp. may cause damping-off of citrus seedlings (DeWolfe *et al.*, 1954). However, *Phytophthora* spp. are thought to cause more extensive damage to seedlings compared to *Pythium* spp. (Klotz, 1978).

***Pythium* root rot in citrus orchards**

Very little has been published on the role of *Pythium* spp. in citrus root rot. *Pythium* spp. are often reported as components of a root rot complex involving other fungi (Wager, 1942; Fatemi, 1972; Hassan *et al.*, 1989a; Thompson *et al.*, 1995). These include soil-borne fungi such as *Phytophthora* and *Fusarium* spp. (Kotzé, 1984; Labuschagne *et al.*, 1987). However, *Pythium* spp. alone are capable of causing citrus root rot on seedlings (DeWolfe *et al.*, 1954; Klotz, 1978) and on mature trees (Thompson *et al.*, 1995), under favourable conditions in the soil.

SOIL AND ENVIRONMENTAL FACTORS CONTRIBUTING TO ROOT ROT

In most citrus orchards, *Phytophthora* and *Pythium* spp. are widespread and occur naturally (Klotz *et al.*, 1968). However, occurrence and severity of root diseases is determined by pathogen virulence, rootstock susceptibility, and several environmental factors (Whiteside, 1988a).

Soil compaction

Relationship between soil compaction and *Phytophthora* root rot of citrus has been extensively studied (Lutz, Menge & O'Connell, 1986; Joubert, 1993; Mkhize *et al.*, 1996). Soil compaction can be caused by several agricultural practices or may occur naturally in some soil types (Allmaras, Kraft & Miller, 1988). Compacted soil layers also referred to as "hard pans" (Erwin & Ribeiro, 1996) are characterised by non-porosity, high bulk density and soil strength (Allmaras *et al.*, 1988). Over-irrigation of such soils leads to waterlogging which predispose root systems to attack by soil-borne fungi (Joubert, 1993). This often results in adverse changes in the rhizosphere mainly due to poor aeration (Louvet, 1970; Rowe & Beardsell, 1973; Allmaras *et al.*, 1988; Drew, 1992). Soil compaction is one of the major factors contributing to citrus decline in South Africa (Labuschagne, 1994). A number of citrus orchards in Southern Africa were established on fine-textured soil, thus soil compaction is prevalent (Mkhize *et al.*, 1996).

Waterlogging

The negative influence of waterlogging on the citrus rhizosphere is a well-known phenomenon (Stolzy *et al.*, 1959, 1965a, b; Klotz, *et al.*, 1967; Rowe & Beardsell, 1973). Surface water following heavy rains and over-irrigation can contribute to waterlogging, especially in poorly drained soils (Stolzy *et al.*, 1959; Shokes & McCarter, 1979). Waterlogging prevents aeration, nutrients are leached and high evaporation and salinisation are induced (Stolzy *et al.*, 1965a). These stressful conditions harm and predispose the citrus roots to invasion by pathogenic fungi, resulting in root decay (Klotz *et al.*, 1958; Stolzy *et al.*, 1959, 1965a).

Effects of waterlogging on citrus rootstocks

Different citrus rootstock differ in their ability to tolerate waterlogged soils (Rowe & Beardsell, 1973). However, the mechanisms involved in flooding tolerance of certain rootstocks, and role of stress factors on the breakdown of tolerance, have not been clearly

elucidated. Mature trees tolerate waterlogging better than seedlings (Kazlowski, 1984), because the effects of waterlogging are severe during the growth stage (Drew, 1992). Tolerance to waterlogging of fruit trees is largely determined by the rootstock and not the scion (Rowe & Beardsell, 1973; Rowe & Catlin, 1971; Ponnampereuma, 1984). Trifoliolate and rough lemon rootstocks are more tolerant to waterlogging than sour orange and sweet lime rootstocks (Castle, 1987; Castle *et al.*, 1992). Therefore, sound knowledge of rootstock tolerance to waterlogging is essential, especially on poorly drained sites.

Effect of waterlogging on soil-borne diseases

The effect of waterlogging on the development of plant diseases has been the subject of extensive reviews (Griffin, 1970, Cook & Papendick, 1970, 1972; Duniway, 1979, 1983; Drew & Lych, 1980; Stolzy & Sojka, 1984). However, the scope of this section will focus only on diseases, especially those caused by Pythiaceus fungi. Waterlogging increases the severity of plant diseases due to various pathogens, but those caused by Pythiaceus fungi are most frequently encountered (Dunniway, 1979, 1983). These fungi depend entirely on free water to complete their life cycle (Schmitthenner, 1970; Dunniway, 1979). Flooding directly influences the development and occurrence of *Phytophthora* and *Pythium* diseases through the movement of propagules. These propagules are found in abundance in surface water such as streams or lakes, often used for irrigation.

Disease Epidemiology

High moisture and temperature greatly influences formation, survival, and germination of different propagules of Pythiaceus fungi (Cook & Papendick, 1970). Sporangia are structures which produce numerous motile biflagellate zoospores. Zoospores are chemostatically attracted to the elongation zone of the root by exudations (Zentmyer, 1961). They encyst, germinate and infect feeder roots on contact. Fibrous roots are repeatedly infected by the pathogen, and produce more sporangia, which in turn release zoospores. The infection advances in the cortex resulting in the rot of the entire rootlet. Repeated infections continue under favourable conditions, thus fungal populations are maintained (MacKenzie *et al.*, 1983).

Rootstock susceptibility

Commercial citrus trees are usually propagated on rootstocks rather than as seedlings or cuttings, because rootstocks have several advantages (Wutcher, 1979; Castle *et al.*, 1992). Rootstocks affect several horticultural and pathological characteristics of the tree and fruit (Castle *et al.*, 1992). Citrus rootstocks differ greatly in their degree of tolerance to various soil factors, pests, diseases and environmental stress (Wutcher, 1979; Castle *et al.*, 1992; Graham, 1995b). Before 1930, rough lemon was commonly used in South Africa as the rootstock of choice, because of its drought and root rot tolerance (Marloth, 1954; Lee & Roxburgh, 1988). However, blight and increased incidences of tristeza greatly limited the use of rough lemon rootstocks in favour of other rootstocks (Table 3). Rootstock variation strongly influences the location of the areas in which citrus can be grown. For examples, Troyer citrange rootstock is ideal for fine-textured soil, but it is sensitive to saline soils (Wutcher, 1979; Castle *et al.*, 1992).

In South Africa, yield losses caused by citrus decline are estimated to be between R300 million and R600 million annually (Dr N. Labuschagne, University of Pretoria, pers. comm). Continuous evaluation of rootstock performance is, therefore, essential to the citrus industry. Several rootstocks are currently evaluated for tolerance against *Phytophthora nicotianae*, *Fusarium solani* (Mont.) Appel & Wollenw. emend. Snyd & Hans and *Tylenchulus semipenetrans* (Labuschagne, 1994). Resistance of rootstocks to *Phytophthora* is presumed to be genetically inherited and is regarded as a reliable and durable form of biological control (Graham, 1990, 1995a). Disease screening techniques are commonly used to detect differences in disease tolerance of different rootstocks (Carpenter & Fur, 1962; Afek, Szejnberg & Solel, 1990; Agostini *et al.*, 1991).

CONCLUSIONS

Phytophthora spp. are the causal agents of the most destructive diseases of citrus in many citrus growing countries world-wide. In South Africa, *P. nicotianae* is the most common pathogenic species found in nurseries and orchards. Other *Phytophthora* spp. are rare, especially *P. citrophthora*, which is common in other countries. In South Africa, decline of citrus trees is mainly due to the interaction between *P. nicotianae*, *Fusarium solani* and the citrus nematode (*T. semipenetrans*). Most rootstocks currently used in South Africa are

susceptible to waterlogging and soil pathogens. Tree decline is further enhanced by poorly drained soils common in many citrus orchards. All these factors contribute to the predisposition of the root system to fungal invasion.

The role of *Pythium* spp. in the citrus root rot complex is still unclear, despite their abundance in the rhizosphere of healthy and diseased trees. Current disease control measures include integrated disease control strategies such as strict sanitation practices, cultural and biological control, chemical control and use of tolerant rootstocks.



PART TWO

FOREST ROOT DISEASES ASSOCIATED WITH *PHYTOPHTHORA* AND *PYTHIUM* SPP. IN SOUTH AFRICA

Forestry is one of the fastest growing industries in South African and depends mainly on exotic pines, eucalypts and to a limited extent wattle (Anonymous, 1990). Forestry is concentrated in high rainfall areas mainly along the coast (Herbert, 1993). This environment is conducive to *Phytophthora* and *Pythium* spp. infections. This threat is further enhanced by the intensification of forestry through monoculture (Wingfield, Swart & Kemp, 1991).

Members of the genus *Phytophthora*, especially *P. cinnamomi*, are notorious for causing the most destructive root diseases of woody plants, including some of the most important forest species (Zak & Campbell, 1958; Newhook, 1959; Podger & Batini, 1971; Newhook & Podger, 1972; Weste, 1974). In South Africa, *Phytophthora* and *Pythium* spp. have been associated with root diseases of pine (Darvas, Scott & Kotzé, 1978; Linde, Kemp & Wingfield, 1994a), eucalypts (Wingfield & Knox-Davies, 1980; Wingfield, Swart & Von Broembsen, 1989; Linde *et al.*, 1994c) and wattle (Roux, Kemp & Wingfield, 1995). In South Africa diseases of pines, eucalypts and wattle caused by Pythiaceus fungi have been the subject of a number of reviews (Linde, 1993; Linde *et al.*, 1994a; Roux, Kemp & Wingfield, 1995; Roux, 1996) and thus will not be reiterated. The aim of this review will be to summarize recently published information on the diseases caused by these fungi, and control strategies used to reduce their impact. Special emphasis will be placed on *P. cinnamomi* since it is the most important pathogen in the South African forest industry.

PHYTOPHTHORA CINNAMOMI IN SOUTH AFRICA

Phytophthora cinnamomi is a well-known pathogen of diverse hosts throughout the world (Zentmyer, 1980). Wager first recorded it in South Africa on avocado (*Persea americana* Mill.) in 1931. This was nearly a decade after its first isolation from cinnamon trees (*Cinnamomum burmannii* Blume) in Sumatra. Today, *P. cinnamomi* causes serious losses on major agricultural crops, forest trees, ornamentals and indigenous flora (Von Broembsen, 1979). The host list includes avocado (Wager, 1931), grapes (*Vitis vinifera* L.) (Van der Merwe, Joubert & Matthe, 1972), eucalypts and pines (Wingfield & Knox-Davies, 1980), stinkwood (*Ocotea bullata*) (Von Broembsen, Lubbe & Geldenhuys, 1986), silver tree

(*Leucandendron argenteum*) (Van Wyk, 1973) and commercial *Protea* spp. (Von Broembsen & Brits, 1985). *Phytophthora cinnamomi* also poses a potential threat to the indigenous 'fynbos vegetation', endemic to the Western Cape Province of South Africa (Von Broembsen, 1979; Von Broembsen & Kruger, 1985).

Nature and distribution of *P. cinnamomi* in South Africa

Phytophthora cinnamomi is a heterothallic fungus with two mating types, A1 and A2 (Galido & Zentmyer, 1964; Brasier, 1992). During wet soil conditions sexual reproduction occurs when A1 and A2 mating types fuse to form oospores. Oospores are important survival structures, especially during dry periods. Although *P. cinnamomi* has a global distribution, the A2 mating type is more common than the A1 mating type (Zentmyer, 1980, 1988). South Africa is one of the few countries where both mating types have been reported (Zentmyer, 1976). The A2 mating type is frequently associated with agricultural and exotic forest plantations (Von Broembsen, 1984a). The A1 mating type is more prevalent on remote river catchments and "undisturbed" fynbos vegetation (Von Broembsen, 1984b). However, both mating types also occur at almost equal ratios at certain sites of indigenous flora, but with no destructive effects (Von Broembsen, 1979, 1984a; Von Broembsen & Kruger, 1985). These observations led to the hypothesis that *P. cinnamomi* is indigenous and South Africa could be its the centre of origin (Zentmyer, 1988). The high prevalence of the A2 mating type on cultivated lands also justified the hypothesis that *P. cinnamomi* could have been introduced by early Dutch settlers (Von Broembsen, 1989).

The centre of origin of *P. cinnamomi* has been subject of controversial discussions in many countries where both mating types occur (Zentmyer, 1980; Arentz & Simpson, 1986). In Australia particularly, this subject led to a considerable debate in the eastern states (Zentmyer, 1980). The first hypothesis about the origin of *P. cinnamomi* in Victoria suggested that it invaded the region long ago (Pratt & Heather, 1973; Pratt, Heather & Shepherd, 1973). The second is that *P. cinnamomi* was introduced by European settlers (Weste & Taylor, 1971; Marks, Kassaby & Reynolds, 1972; Newhook & Podger, 1972; Marks, Kassaby & Fagg, 1975; Weste, Cooke & Taylor, 1973; Weste & Marks, 1974; Weste, Ruppin & Vithanage, 1976). However, recent evidence suggests that *P. cinnamomi* has been introduced into Australia (Old, Moran & Bell, 1984; Old, Dudzinsky & Bell, 1988).

In South Africa, recent isozyme studies conducted on a large number of isolates indicate that *P. cinnamomi* is an introduced pathogen. This conclusion was based on the low population diversity among the isolates tested (Linde *et al.*, 1997). Similar results have also been reported in Australia (Old *et al.*, 1984, 1988). The centre of origin of *P. cinnamomi* continues to remain unknown but there is strong evidence suggesting that Papua, New Guinea could be the centre of origin (Zentmyer, 1988).

ROOT DISEASES OF PINES, EUCALYPTS AND WATTLE ASSOCIATED WITH PYTHIACEOUS FUNGI

Black Butt on *Acacia mearnsii*

Acacia mearnsii de Wild (Black wattle) is one of the three most important forest species planted commercially in South Africa (Anonymous, 1993). It is planted mainly for tannin production and high quality pulp (Gibson 1975; Rusk, Pennefather & Cronje, 1990; Haigh, 1993). *Acacia mearnsii* is highly susceptible to a root disease complex commonly known as black butt. *Phytophthora* spp. are thought to be the primary causal agents of this disease (Zeijlemaker, 1971; Roux, 1996; Roux & Wingfield, 1997). Black discolouration and gum exudation from the bark near ground level are the most obvious symptoms of this disease (Sherry, 1971; Zeijlemaker, 1971). *Phytophthora nicotianae* has been found to be the most common pathogen associated with this disease (Zeijlemaker & Margot, 1970; Zeijlemaker, 1971). However, recently *P. boehmeriae* Sawada, *P. meadii* McRae and *Pythium irregulare* have been isolated from diseased trees in Mpumalanga and KwaZulu/Natal provinces of South Africa (Roux & Wingfield, 1997). Greenhouse and field pathogenicity tests conducted using these three fungal species indicated that *Phytophthora* spp. were pathogenic to the disease symptoms (Roux & Wingfield, 1997). Pathogenicity tests also indicated that *P. irregulare* plays an insignificant role in the disease, despite its abundance in the soil (Roux, 1996).

Symptoms

Black butt and gummosis refers to a disease complex on *A. mearnsii*, which is characterised by black discolouration of the bark near ground level on old trees. Initial infection takes place when the fungus gains entry through small cracks on the stem. As the disease progresses, the cracks increase in size resulting in gum exudation (Zeijlemaker, 1968, 1971, Wingfield &

Kemp, 1993). The exact cause of black discolouration at the base of infected *A. mearnsii* is unknown, but is thought to be caused by secondary pathogens (Wingfield & Kemp, 1993).

Other pathogens, including *Botryosphaeria dothidea* (Moug.) Ces de Not., have been isolated from advanced cankers (Roux & Wingfield, 1997). Black butt does not totally kill the tree but reduces the yield and bark quality. It damages the most valuable part of the bark and also makes bark striping difficult (Roux *et al.*, 1995). In South Africa, all wattle families in commercial or naturally stands are susceptible to this disease (Roux & Wingfield, 1997). It can be severe on juvenile, fast growing trees in the field (Roux, 1996).

ROOT DISEASES OF PINES AND EUCALYPTS

Exotic pines and eucalypts are widely planted commercially in South Africa. Members of the genera *Phytophthora* and *Pythium*, cause serious damage to these forest species in many parts of the world (Campbell & Hendrix, 1967; Darvas *et al.*, 1978; Davison & Bumbieris, 1973; Marks & Kassaby, 1974; Orosina & Marx, 1975; Sharma, Mohanan & Florence, 1985; Vaartaja & Salisbury 1961; Wardlaw & Palzer, 1985). Disease epidemics caused by *P. cinnamomi*, in particular had led to devastating losses and termination of planting programmes of some pine and eucalypts in South Africa (Linde *et al.*, 1994b).

Phytophthora - related diseases on *Eucalyptus* spp.

Severe root diseases on various *Eucalyptus* spp. associated with *Phytophthora* spp. has been reported in many countries (Podger & Batini 1971; Marks *et al.*, 1972; Newhook & Podger, 1972), including South Africa (Wingfield & Knox-Davies, 1980; Von Broembsen 1984a; Linde, 1993). *Eucalyptus* species belonging to the sub-genus *Monocalyptus*, such as *E. marginata* Sm. are the most susceptible (Weste & Taylor, 1971; Podger, 1972). In South Africa, cold tolerant *Eucalyptus* spp. planted at high altitude areas have been reported to suffer from severe die-back (Wingfield *et al.*, 1989; Linde *et al.*, 1994c). Commercial propagation of *E. fastigata* Dean & Maiden and *E. fraxinoides* Dean & Maiden has been scaled down due to their susceptibility to *P. cinnamomi*. They have been replaced by less susceptible *Eucalyptus* species (Linde *et al.*, 1994b).

Phytophthora cinnamomi and *P. cryptogea* are the most common species associated with *Eucalyptus* die-back (Marks & Kassaby, 1974; Bumbieris, 1976; Pogder, 1978; Hamm & Hansen, 1982). In South Africa, *P. cinnamomi* is more common than *P. cryptogea* and has been reported on several woody plants (Darvas *et al.*, 1978; Donald & Von Broembsen, 1977; Von Broembsen, 1984a; Wager, 1942). However, recently *P. boehmeriae* has been associated with mortality of *E. dunnii* Maiden and *E. macarthurii* Dean & Maiden seedlings (Linde, 1993).

Symptoms

Root rot is the primary disease symptom associated with *Phytophthora* species. The fungus mainly infects fibrous roots and large roots are rarely infected (Podger *et al.*, 1965; Shearer & Tippett, 1989). Secondary disease symptoms resemble those of drought. Diseased trees have wilted leaves, usually preceded by reddening then chlorosis of the leaves. As the disease progresses, the infected trees usually die-back due to poor root systems (Cahill, Grant & Weste, 1985; Weste & Marks, 1987).

Pythium - related diseases on *Eucalyptus* spp.

The importance of *Pythium* spp. as pathogens of mature forest trees has been underestimated (Marks & Kassaby, 1974). Some *Pythium* spp. are capable of causing serious diseases either singly or in combination with *Phytophthora* spp. (Lorio, 1966; Otrrosina & Marx, 1975; Pratt & Heather, 1973). A serious root and collar disease of *E. grandis* W. Hill ex Maiden caused by *Pythium splendens* Braun has been reported in Northern KwaZulu-Natal region of South Africa (Linde *et al.*, 1994a). Pathogenicity tests conducted on two different clones of *E. grandis* indicate a high degree of virulence (Linde *et al.*, 1994a). This report clearly indicates that some *Pythium* spp. pose a serious threat to some *Eucalyptus* clones, previously regarded as resistant. The susceptibility of *E. grandis* clones to root disease associated with *P. splendens* is of greater concern since it is the most propagated species in South Africa. *Pythium* spp. are also thought to contribute to the root disease complex of *E. smithii* (Wingfield & Kemp, 1993).

Phytophthora-related diseases on *Pinus* spp.

Phytophthora spp. are responsible for serious losses in pine plantations and nurseries (Darvas *et al.*, 1978; Heather *et al.*, 1977). Severe losses have been reported on several pine nurseries

in many parts of the country (Donald & Von Broembsen, 1977). *Pinus radiata* D. Don. and *P. clausa* (Chapm) Vasey are both known to be highly susceptible to *P. cinnamomi* both in nurseries and under field conditions (Wingfield & Knox-Davies, 1980). In Australia, *P. radiata* and *P. patula* Schlecht & Chan. have been reported to be susceptible to other *Phytophthora* spp. (Davison & Bumbieris, 1973; Hamm & Hansen, 1982; Heather & Pratt, 1975; Oxenham & Winks, 1963). Very few reports have been published on *Phytophthora* spp. associated with diseased *Pinus* spp. in South Africa (Linde, 1993; Linde *et al.*, 1994b).

Symptoms

According to Gibson (1975), *Pythium* and *Phytophthora* spp. can infect seedlings before or after germination, leading to pre or post-emergence damping-off. Initial disease symptoms include dull yellow needles. As the disease advances, the entire foliage turns yellow and needles drop rapidly leading to the death of the tree. Large patches of dying or dead seedlings are common.

Pythium related diseases on *Pinus* spp.

A number of *Pythium* spp. have been isolated from pine nurseries and plantation throughout South Africa (Linde, 1993). However, very little has been published on the occurrence of these fungi in South African forest soil. A recent survey conducted yielded at least twenty-one *Pythium* spp., nine of which were new records for South Africa (Linde, 1993). *Pythium* spp. have not been considered as important pathogens of forest trees. This is despite numerous reports on the pathogenicity of some *Pythium* spp. (Hendrix & Campbell, 1973; Vaartaja, 1967). Recently, however, *P. irregulare* has been associated with a serious rot disease of *P. patula* seedlings associated with old agricultural land (Linde *et al.*, 1994a).

CURRENT DISEASE MANAGEMENT STRATEGIES

Breeding for resistance and conventional selection

Forest tree improvement programmes through selection and breeding strategies have provided an effective means of reducing the impact of various diseases in South Africa (Wingfield & Kemp, 1993). During recent years, introduction of clonally propagated eucalypts has also proven to be another effective way of controlling the diseases (Wingfield & Kemp, 1993). However, some *Eucalyptus* spp. such as *E. nitens* (Deane & Maiden) Maiden cannot be

propagated using this technique, because of rooting problems and susceptibility to root rot. In such cases conventional breeding strategies are still used. Natural resistance to pathogens with wide host range such as *P. cinnamomi* is often absent (Irwin *et al.*, 1995). However, heritable resistance has been reported in some tree species generally considered susceptible to this pathogen. These include, for example, *P. radiata* (Butcher, Stukely & Crane, 1994).

Improved nursery practices

Cultural practices aimed at avoiding or prevention strategies have greatly reduced the impact of *Phytophthora* and *Pythium* root diseases on many forest nurseries. Some of these strategies include, the use of composted bark (Hoitink, 1980; Hoitink & Fahy, 1986; Huang & Kuhlman, 1991; Spencer & Benson, 1992), filtered or decontaminated irrigation water (Coffey, 1991), the use of disease free nursery stock, and prevention of soil movement.

Biological Control

Biological control of soil-borne pathogens can provide a good alternative method to the use of fungicides (Shea & Broadbent, 1983; Coffey, 1991; Ribeiro & Linderman, 1991). Biocontrol of *Phytophthora* spp. using antagonistic microorganisms has been reported on *Eucalyptus* and *Pinus* spp. (Marx, 1969; Marais & Kotzé, 1976). However, most biocontrol agents have only proven successful *in vitro*. Unfortunately, very little has been done on biocontrol of soil-borne pathogens South Africa.

Chemical Control

Chemical control is only feasible in nurseries since it is impractical to apply under field conditions. Chemicals such as Ridomil[®], and copper oxychloride are commonly used in nurseries to control *Phytophthora*.

CONCLUSIONS

Phytophthora and *Pythium* spp. cause serious diseases of exotic forest trees in South Africa. Cold tolerant *Eucalyptus* spp., mainly planted at high altitude areas, are highly susceptible to *Phytophthora* root rot. Diseases caused by *Phytophthora* spp. on exotic pine, eucalypts and wattle are amongst the most serious diseases in South Africa. A number of previously unreported *Pythium* spp. have been isolated from forest soils but appear to play an

insignificant role in root rot. However, virulence of *P. splendens* on two *E. grandis* clones is of great concern since no root diseases have been previously associated with these commercially valuable clones. In addition, the severe root diseases of *P. patula* seedlings caused by *P. irregulare* associated with previously cultivated land, clearly illustrates the potential some of these *Pythium* spp. have in causing diseases. Current disease management strategies have greatly reduced the impact of *Pythium* and *Phytophthora* diseases in nurseries and plantations. The recently conducted study on population structure of *P. cinnamomi* in South Africa confirmed that it was introduced to South Africa. This newly acquired information could be crucial to breeding programmes aimed at the exploitation of *Eucalyptus*.

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Table 1. *Phytophthora* spp. associated with citrus diseases

Species	Host	Symptom	Country	Reference
<i>P. arecae</i>	Citrus sp.	Leaf blight	USA	Timmer <i>et al.</i> , 1990
<i>P. boehmeriae</i>	<i>C. sinensis</i>	Brown rot	Argentina	Fawcett, 1936
<i>P. cactorum</i>	<i>C. limon</i>	Fruit rot	Brazil & Argentina	Erwin & Ribeiro, 1996
	<i>C. maxima</i>	Stem canker	USA	Wagener & Cave, 1944;
	<i>C. sinensis</i>	Stem canker & fruit rot	Formosa	Ho <i>et al.</i> , 1995
<i>P. capsici</i>	Citrus sp.	Root rot	USA	Wiant & Tucker, 1940
<i>P. cinnamomi</i>	Citrus sp.	Root rot	Brazil	Erwin & Ribeiro, 1996
<i>P. citricola</i>	<i>C. limon</i>	Shoot Blight	South Africa	Von Maltitz & Von Broembsen, 1985; Wager, 1941
	<i>C. paradisi</i>	Fruit rot	South Africa	Ho, Ann & Chang, 1995
	<i>C. sinensis</i>	Fruit rot	Taiwan	Ho <i>et al.</i> , 1995
	<i>C. tankan</i>	Fruit rot	Japan	Ho <i>et al.</i> , 1995
<i>P. citrophthora</i>	Citrus sp.	Gummosis & Root rot Fruit rot	Worldwide	Timmer & Menge, 1988
<i>P. cryptogea</i>	<i>C. limon</i>	Shoot Blight	South Africa	Von Maltitz & Von Broembsen, 1985
<i>P. drechsleri</i>	Citrus sp.	Root rot	USA	Fawcett, 1936,
<i>P. heaveae</i>	Citrus sp.	unknown	Taiwan	Ho <i>et al.</i> , 1995
<i>P. hibernalis</i>	Citrus sp.	Fruit rot	South Africa	Doidge, 1925
<i>P. megasperma</i>	Citrus sp.	Root rot & Stem canker	USA	Erwin & Ribeiro, 1996
<i>P. insolita</i>	Citrus sp.	unknown	Taiwan	Ann, 1984
<i>P. nicotianae</i>	All citrus spp.	Gummosis, Root & Fruit rot	Worldwide	Timmer & Menge, 1988
<i>P. palmivora</i>	Citrus sp.	Root rot, Crown rot, Seedling blight, Shoot dieback, leaf fall, fruit rot	USA, Worldwide	Zitko <i>et al.</i> , 1991 Whiteside, 1988a
<i>P. syringae</i>	Citrus sp. <i>C. arantium</i> <i>C. sinensis</i>	Collar rot	USA South Africa	Erwin & Ribeiro, 1996 Wager, 1942

Table. 2 *Pythium* spp. associated with citrus diseases

<i>Pythium</i> species	Host	Symptom	Country	Reference
<i>P. aphanidermatum</i> (Edson) Fitzp	<i>Citrus</i> sp.	Fruit rot ⁺	South Africa	Wager, 1931
	<i>C. sinensis</i>	Damping-off	USA (California)	DeWolfe <i>et al.</i> , 1954
	<i>C. jambhiri</i>			
* <i>P. irregulare</i> Buisman	<i>Citrus</i> sp.	Fruit rot ⁺	South Africa	Wager, 1941
	<i>C. arantium</i>			
* <i>P. oligandrum</i> Drechsler (= <i>P. cf. artotrogus</i>)	<i>Citrus</i> sp.	N P	South Africa	Wager, 1931; 1941
<i>P. paroecandrum</i> Drechsler	<i>Citrus</i>	ND	South Africa	Thompson <i>et al.</i> , 1995
* <i>P. rostratum</i> Butler	<i>Citrus</i> sp.	N P (Fruit)	USA (California)	Wager, 1942
	<i>C. arantium</i>			
* <i>P. splendens</i> Braun	<i>Citrus</i> sp.	Fruit rot ⁺	South Africa	Wager, 1931
* <i>P. vexans</i> de Bary	<i>Citrus</i> sp.	Fruit rot ⁺	USA (California)	Wager, 1931
	<i>C. arantium</i>			
<i>P. ultimum</i> Trow = (<i>P. debaryanum</i>)	<i>Citrus</i> sp.	Fruit rot ⁺	USA (California)	Wager, 1942
	<i>C. arantium</i>			
<i>P. group G</i>	<i>Citrus</i> sp.	ND	South Africa	Thompson <i>et al.</i> , 1995

* Weakly pathogenic, ⁺ Artificially inoculated, ND not determined NP, non-pathogenic, = synonym

Table 3. Some pathological characteristics of commercial citrus rootstocks used in South Africa

Common Name	Species or Hybrid	Major use	Tolerance to root pathogens & stress factors					References
			Biotic		Abiotic			
			1	2	3	4	5	
Trifoliolate orange	<i>Poncitrus trifoliata</i>	Rubidoux & Australian trifoliolate selections are mainly used as rootstock	R*	S	R*	T	T	Wutcher, 1979; Syvertsen <i>et al.</i> , 1983; Castle, 1987; Rabe & Von Broembsen, 1991
Troyer citrange	<i>C. sinensis</i> x <i>P. Trifoliata</i>	Rootstock	R*	I	R	T	T	Wutcher, 1979; Syvertsen <i>et al.</i> , 1983; Castle, 1987; Rabe & Von Broembsen, 1991
Carrizo citrange	<i>C. sinensis</i> x <i>P. trifoliata</i>	Rootstock	R*	I	S	T	T	Wutcher, 1979; Syvertsen <i>et al.</i> , 1983; Castle, 1987; Rabe & Von Broembsen, 1991
Cleopatra mandarin	<i>C. reticulata</i> Blanco	Rootstock	I	R*	S	S	I	Castle, 1987; Castle <i>et al.</i> , 1992
Express mandarin	<i>C. reticulata</i> Blanco	Rootstock for navels and Valencia	I	R	S	S	I	Castle <i>et al.</i> , 1992; Joubert, 1992
X 639 hybrid	<i>P. trifoliata</i> x <i>C. reticulata</i> , cv. Cleopatra mandarin	Rootstock for Eureka lemons	R	X	R	X	I	Rabe & Von Broembsen, 1991
Sour orange	<i>C. aurantium</i> L.	One a popular rootstock but used less frequently	IR	S	S	T	X	Castle, 1987; Castle <i>et al.</i> , 1992
Rough lemon	<i>C. jambhiri</i> Lush.	Largely used as rootstock before 1930	S	S	S	S*	S	Marloth, 1954; Van Broembsen, 1983
Sweet orange	<i>C. sinensis</i> (L.) Osbeck	Used as scion but once a popular rootstock in RSA	S*	S	S	T	S	Castle <i>et al.</i> , 1992; Rabe & Von Broembsen, 1991
Alemow	<i>C. macrophylla</i> Wester	Rootstock	IR	IR	S	S	X	Wutcher, 1979; Castle <i>et al.</i> , 1992
Swingle citrumela	<i>P. trifoliata</i> x <i>C. paradisi</i>	Not extensively used in RSA as rootstocks	R	IR	R	I	X	Castle, 1987; Castle <i>et al.</i> , 1992
Volkamericana	Lemon hybrid	Rootstock	Ss	S	S	S	S	Castle, 1987; Castle <i>et al.</i> , 1992; Rabe & Von Broembsen, 1991

1= *Phytophthora*; 2= *Fusarium* blight; 3 = Nematodes; 4 = Flooding; 5= Soil compaction; R =Resistant; S = Susceptible; I = Intermediate; X = unknown; IR = Intermediately resistant; * = Highly

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U.O.V.S. BIBLIOTEK

CHAPTER TWO

SCREENING AND SELECTION OF *EUCALYPTUS FRAXINOIDES* FAMILIES FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI*

Abstract

Phytophthora cinnamomi is a destructive soil-borne pathogen hindering commercial afforestation of some cold tolerant eucalypts in high altitude areas of South Africa. *Eucalyptus fraxinoides* trials were established at two sites in Mpumalanga and KwaZulu/Natal Provinces of South Africa where *P. cinnamomi* is known to occur. After 28 months, the growth rate and mortality within families was recorded and families ranked accordingly. Field mortality of 52% and 30%, respectively, was recorded at the two sites. Attempts were also made to select disease tolerant *E. fraxinoides* families using stem inoculations of individual trees in field trials. A single most virulent strain of *P. cinnamomi* was used to inoculate three-year old trees in the field. Mortality and lesion length following inoculations at the two sites were compared. In addition, lesion lengths and the diameter of the trees were combined in a multiple trait selection index. Seven apparently disease tolerant families of *E. fraxinoides* were identified. Stem inoculation of young trees was found to be a more reliable method to determine disease tolerance of families than recording mortality in a plantation.

INTRODUCTION

Phytophthora cinnamomi Rands is well known as a destructive root pathogen of a diverse range of hosts (Zentmyer, 1980). Included in this wide host range are important forest tree species (Podger & Batini, 1971; Newhook & Podger, 1972). *Eucalyptus fraxinoides* Dean & Maiden is a commercially desirable species for cultivation in high altitude areas that are unsuitable for *E. grandis* Hill ex Maid. (Herbert, 1993). *Eucalyptus fraxinoides* grows vigorously on cold sites and has excellent pulping properties, including high cellulose yield and low lignin content (Clarke, 1995).

In South Africa, cold-tolerant *Eucalyptus* spp. such as *E. fastigata* Deane & Maiden, *E. smithii* Donn. ex Smith and *E. fraxinoides* are susceptible to root rot caused by *P. cinnamomi* (Wingfield & Knox-Davies, 1980; Herbert, 1993). Susceptibility of *E. fastigata* and *E. smithii* to *P. cinnamomi* has led to the gradual replacement with less susceptible hosts such as *E. macarthurii* (Herbert, 1993; Linde, Kemp & Wingfield, 1994). However, in areas severely affected by *P. cinnamomi* die-back, some individuals of the susceptible tree species survive even after long periods of exposure to the pathogen (Weste & Kennedy, 1997). This suggests the existence of some form of disease tolerance and has led to various breeding projects aimed at selecting disease tolerant trees. Recently, genetically based resistance of *Pinus radiata* D. Don and *E. marginata* Sm., both susceptible to *P. cinnamomi*, as been reported in Australia (Butcher, Stukely & Chester, 1984; Cahill, Bennett & McComb 1992; Stukely & Crane, 1994).

In this study, families of *E. fraxinoides* were assessed for disease tolerance using mortality following natural infection and stem inoculations in the field. Lesion development and tree diameter were combined in a multiple trait selection index to assist in the evaluation of the best families for growth and disease tolerance.

MATERIALS AND METHODS

Plant material and disease evaluation

Seeds were collected from healthy *E. fraxinoides* trees, following die-back caused by *P. cinnamomi*, in a former commercial plantation at Lothair. Additional seeds were also selected from indigenous Australian stands where outbreaks of *P. cinnamomi* had

occurred. Two trial sites of *E. fraxinoides* were established at Lothair, Mpumalanga and Petrusvlei, KwaZulu-Natal. Families were planted in a design consisting of three replications of sixteen trees in row plots (1m x 3m spacing). Forty-two families were planted at Lothair (26° 28' South, 30° 41' East, Altitude: 1500m, Rain fall: 900 mm) and 36 families were planted at Petrusvlei (29° 14' South, 30° 25' East, Altitude: 1300m, Rainfall: 1174 mm). After 28 months, the mortality within the families was recorded and families were ranked accordingly.

Isolations from soil and diseased trees

Soil samples were collected from the rhizosphere of healthy and dying trees. A citrus leaf baiting technique was used to isolate *P. cinnamomi* from the soil (Grimm & Alexander, 1973). Leaf disks were then plated on selective media PARP and PARPH to isolate the pathogen (Tsao & Ocana, 1969). Cultures were incubated at 15-20°C, in the dark for five days. Isolations were also made from diseased plant tissue using the same selective media.

Inoculation trials

A single most virulent strain (CP 470, A2 mating-type) of *P. cinnamomi* (Linde, Kemp & Wingfield, 1999) was used to inoculate three-year old trees at Lothair and Petrusvlei. Three-year old *E. fraxinoides* trees were inoculated using a 9mm cork borer to remove the bark from each tree 1 m above the ground. A mycelium-covered agar plug was then inserted into the wound and sealed with masking tape to reduce desiccation. Lesion lengths were measured after six weeks and reisolations from the diseased tissue conducted. Lesion development in the inner bark was measured (Tippett *et al.*, 1983).

Statistical analysis

A randomised block design was used for field trials and two-way analysis of variance was conducted. Mean lesion length were used to rank families. Results from trials were compared using Spearman's ranking correlation (Cadogan & Sutton, 1994). The ranking of families according to lesion length was compared with ranking according to natural mortality in the field.

Multiple Trait Selection Index

Lesion lengths and diameters of three-year-old trees were combined in a multiple trait selection index. The selection index was used to identify the best individual trees for growth and disease tolerance in each family (Cotterill & Dean, 1990). The index was constructed using family and individual tree information as follows.

$$I = b_1P_{dbh} + b_4F_{dbh} - b_3F_{con} - b_2P_{con} \quad (1)$$

Where I is the index value,

P_{dbh} and P_{con} are the individual tree values for diameter at breast height and lesion lengths, respectively, and F_{dbh} and F_{con} are the family means for diameter at breast height and lesion length, respectively.

The regression components, b_1 to b_4 were determined from:

$$[b] = [P] - I [A][w] \quad (2)$$

Where $[P]$ and $[A]$ are matrices of the phenotypic and genetic variances and covariance's, respectively, and $[b]$ and $[w]$ are vectors of index coefficients and economic weights, respectively.

RESULTS

Survival in the field

Eucalyptus fraxinoides was found to grow vigorously at both sites but persistently suffered high mortality that begun shortly after establishment but gradually stabilised through the rotation (Fig. 1). At 28 months, tree growth rate and mortality were recorded and families were ranked according to their survival at the two sites. A higher level of mortality was found at Lothair (52%) than at Petrusvlei (30%). No correlation was found between the Lothair and Petrusvlei trials (Fig. 2). Three South African families (4, 15 & 5) were found to have excellent survival rates at both sites. In general, South African families were more tolerant to *P. cinnamomi* than Australian families. Variation in tolerance to this fungus

was found within the different families at both sites. Poor recovery of *P. cinnamomi* was observed at both sites, instead several *Pythium* spp. were recovered from the soil.

Field inoculation trials

Brown necrotic lesions, extending above and below the point of inoculation, were observed six weeks after inoculation (Fig. 1). *Phytophthora cinnamomi* was readily recovered from inoculated trees at both sites. Considerable variation in lesion length among the different families was found at both sites. The average lesion length ranged from 82mm – 332mm and 154mm – 448mm at Petrusvlei and Lothair, respectively (Fig. 3 & 4). Significant differences ($P \leq 0.05$) in lesion lengths were also found on trees of various families. Although it was possible to separate the extremes for susceptibility, intermediate levels could not be distinguished.

Families were ranked according to their tolerance to infection after artificial inoculation in the two trials. The rankings of the families in the two trials correlated significantly ($P \leq 0.05$; $r = 0.5$) (Fig. 5). This indicates that the best families were rated amongst the top ten in both trials. A significant ranking correlation was observed only at Lothair (= 0.4), between survival and tolerance to infection after inoculation (Fig. 6). No correlation was found between natural mortality and artificial inoculation at the Petrusvlei trial site (Fig. 7).

Multiple Trait Selection Index

Lesion lengths and the tree diameter results were combined in the multiple trait selection. This test provided the best means of selecting trees that were not only tolerant to *P. cinnamomi* but that also had superior growth qualities. Seven *P. cinnamomi* tolerant families were identified, namely 10, 13, 15, 18, 19, 21 and 27. Results of artificial inoculation provided the best measure of disease tolerance of surviving trees since *P. cinnamomi* was unlikely to be evenly distributed throughout a naturally infested site.

DISCUSSION

Results obtained from this study show a wide variation in tolerance and susceptibility to *P. cinnamomi* among *E. fraxinoides* families. Of all the families screened using stem

inoculation with a virulent strain of *P. cinnamomi* at Lothair and Petrusvlei, seven tolerant families were identified. These tolerant families have the potential to be used for commercial propagation at suitable sites.

The same families of *E. fraxinoides* were planted at both Petrusvlei and Lothair. The observed differences in mortality, following natural infection by *P. cinnamomi*, at the two sites was unexpected. This could possibly be due to higher pathogen density at Lothair compared at Petrusvlei. Also, the pathogen's virulence may vary under different environments (Carson & Carson, 1989). Alternatively, trees could have escaped infection and thus resulted in the lower mortality recorded at Petrusvlei. According to Namkoong, Kang & Brouard, (1988), tree species usually express disease tolerance traits differently at different sites, and genotype rankings for the trait may also change with site.

Failure to recover *Phytophthora* spp. from both soil and plant material at infected sites may be attributed to seasonal population fluctuation of the pathogen (Lutz & Menge, 1986). *Phytophthora cinnamomi* is usually dormant during dry periods and only becomes active during rainy seasons (Zentmyer, 1980). Linde (1993) reported poor recovery of *Phytophthora* but recovered a number of *Pythium* spp. from Lothair. Poor recovery of *Phytophthora* spp. appears to be common in *Eucalyptus* and *Pinus* spp. soils (Erwin & Ribeiro, 1996).

The range of susceptibility and tolerance observed among *E. fraxinoides* families in this study suggests that several genes control these traits. According to Vanderplank (1982), polygenic inheritance in general is synonymous with variation. This variable, polygenic phenomenon appears to be common in forest trees (Namkoong *et al.*, 1988).

Breeding for disease tolerance in plants relies heavily on detailed knowledge of the genetics of host resistance and pathogen virulence (Wolfe & McDermott, 1994). A study on the population structure of *P. cinnamomi* in South Africa has recently been completed (Linde *et al.*, 1997). In this study, a low genetic diversity amongst the South African isolates was reported (Linde *et al.*, 1997), despite the occurrence of both mating types (Von Broembsen, 1984a, b; Zentmyer, 1988). This is indicative of an introduced pathogen and low levels of sexual reproduction (Old, Moran & Bell, 1984; Old, Dudzinsky & Bell,

1988; Linde *et al.*, 1997). In the present study, an isolate selected for its high level of virulence (Linde *et al.*, 1997) was used for inoculation, which should ensure the reliability of the screening strategy used.

The differences in genetic expression of *E. fraxinoides* at Lothair and Petrusvlei may be largely caused by variation within families. This variation in susceptibility/tolerance to *P. cinnamomi* was also found among individual trees. However, inoculation of young *E. fraxinoides* trees in the field provided the best method of evaluating tolerance to *P. cinnamomi*.

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Figure 1: *Eucalyptus fraxinodes* trials at Lothair and Petrusvlei. **A.** Two-year old trees at Lothair, two rows of trees consisting of tolerant family along side a susceptible family with dead trees. **B.** Tolerant families at Petrusvlei after two years. **C.** Discoloured lesion after inoculation with an isolate of *Phytophthora cinnamomi*, arrow indicates inoculation point.



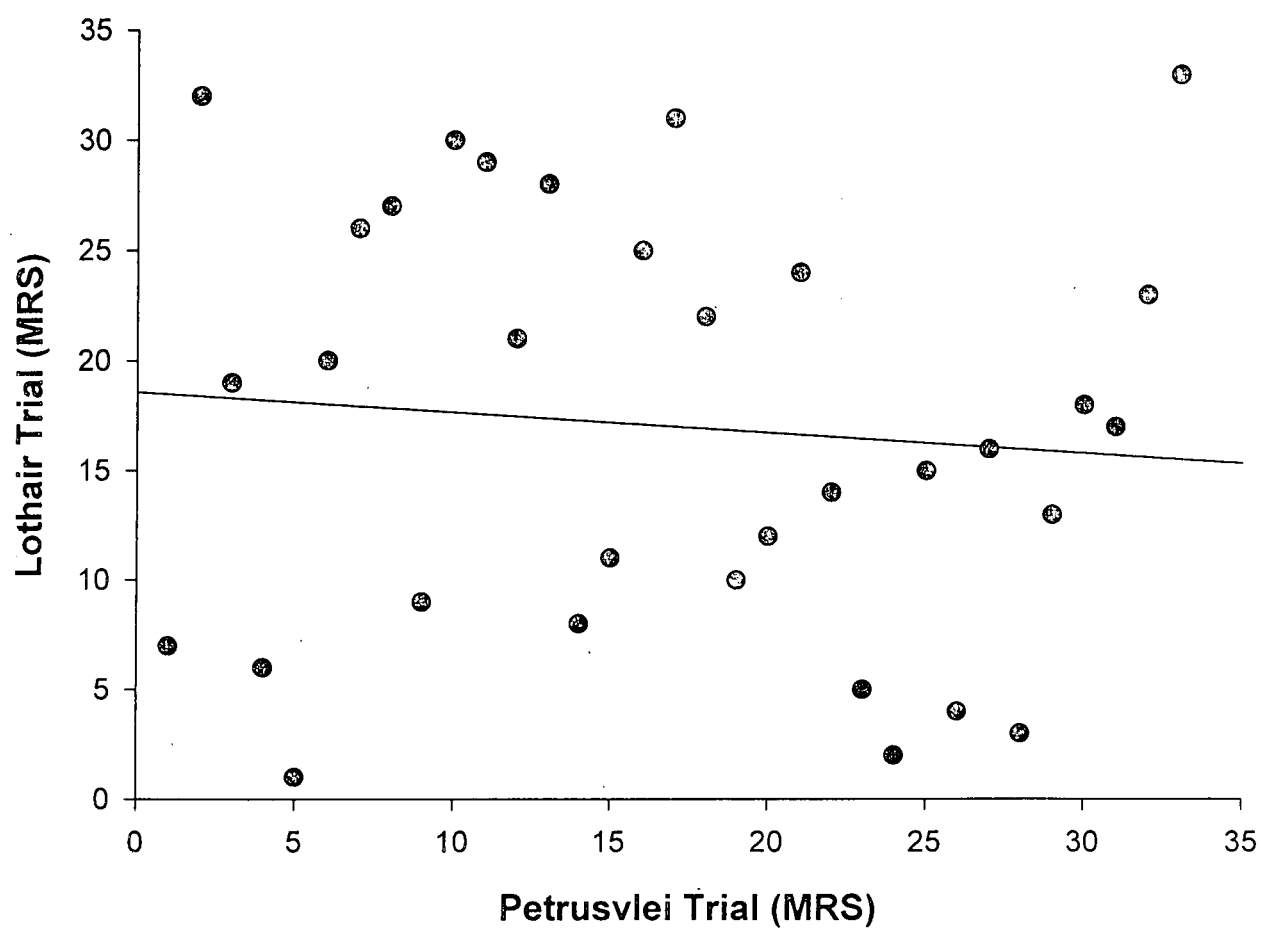


Figure 2: Comparison of the ranking (mean rank scores) between the *Eucalyptus fraxinoides* families at two trail sites following natural mortality after 2 years in *Phytophthora cinnamomi* infected soil.

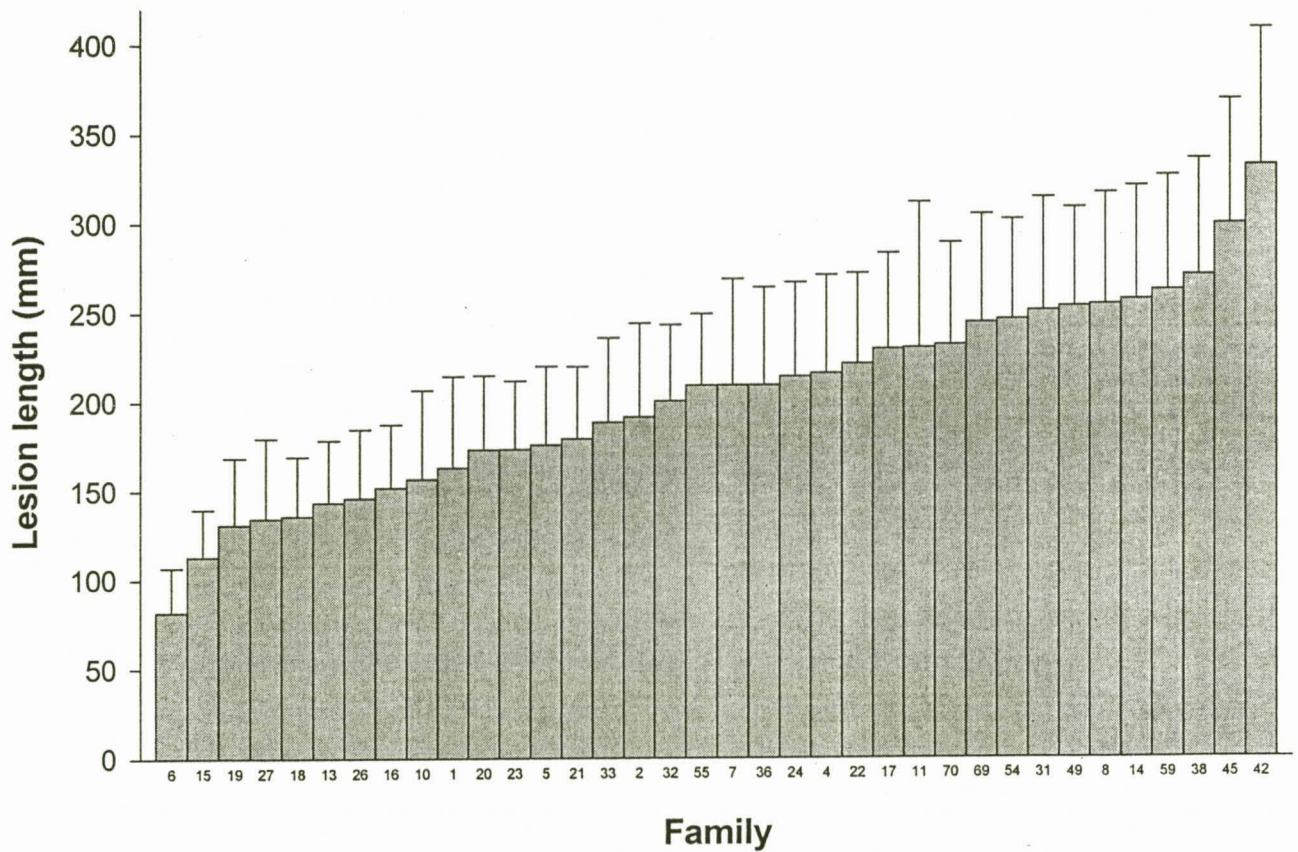


Figure 3: Mean stem lesion length (mm) on 3-year old *Eucalyptus fraxinoides* families six weeks after inoculation with an isolate of *Phytophthora cinnamomi* at Petrusvlei (\bar{x} = 16 trees)

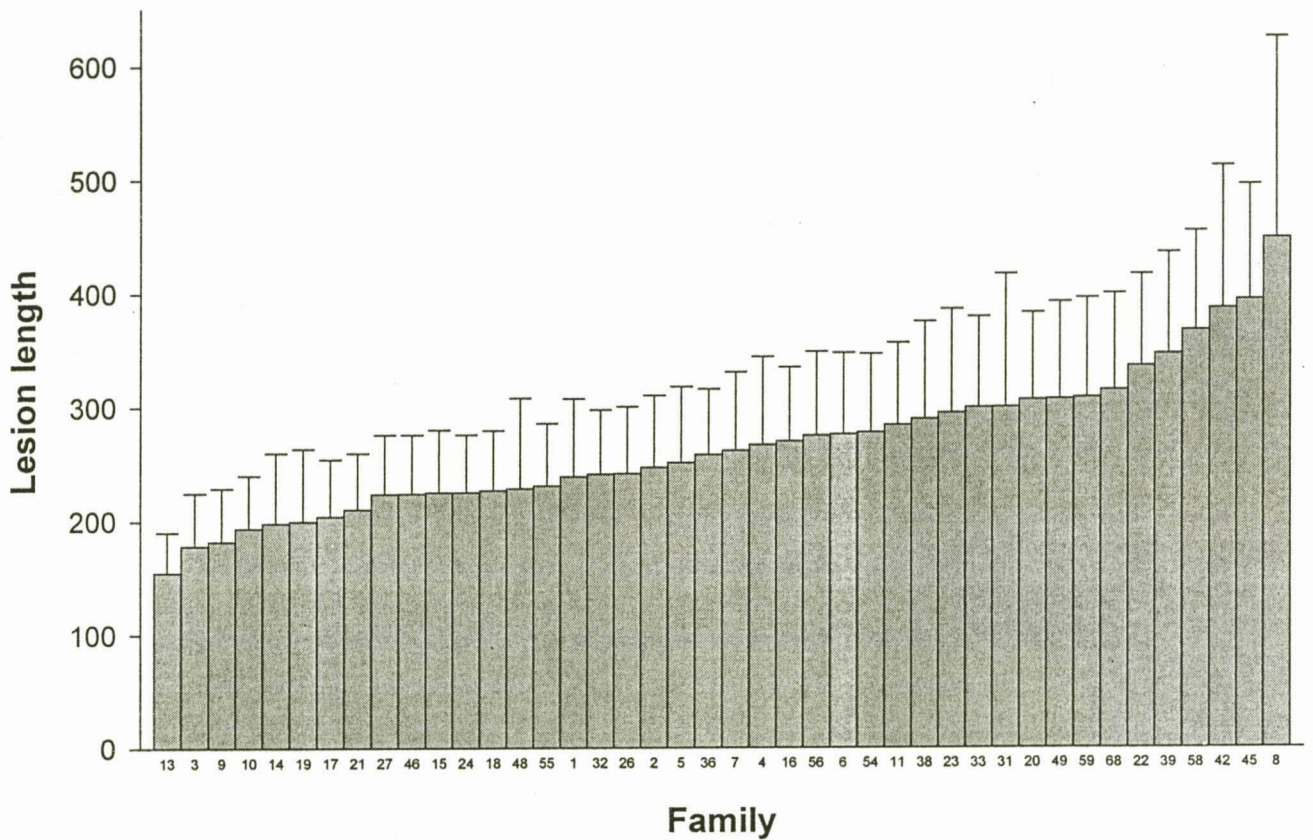


Figure 4: Mean stem lesion length (mm) on 3-year old *Eucalyptus fraxinoides* families six weeks after inoculation with an isolate of *Phytophthora cinnamomi* at Lothair ($\bar{x} = 16$ trees)

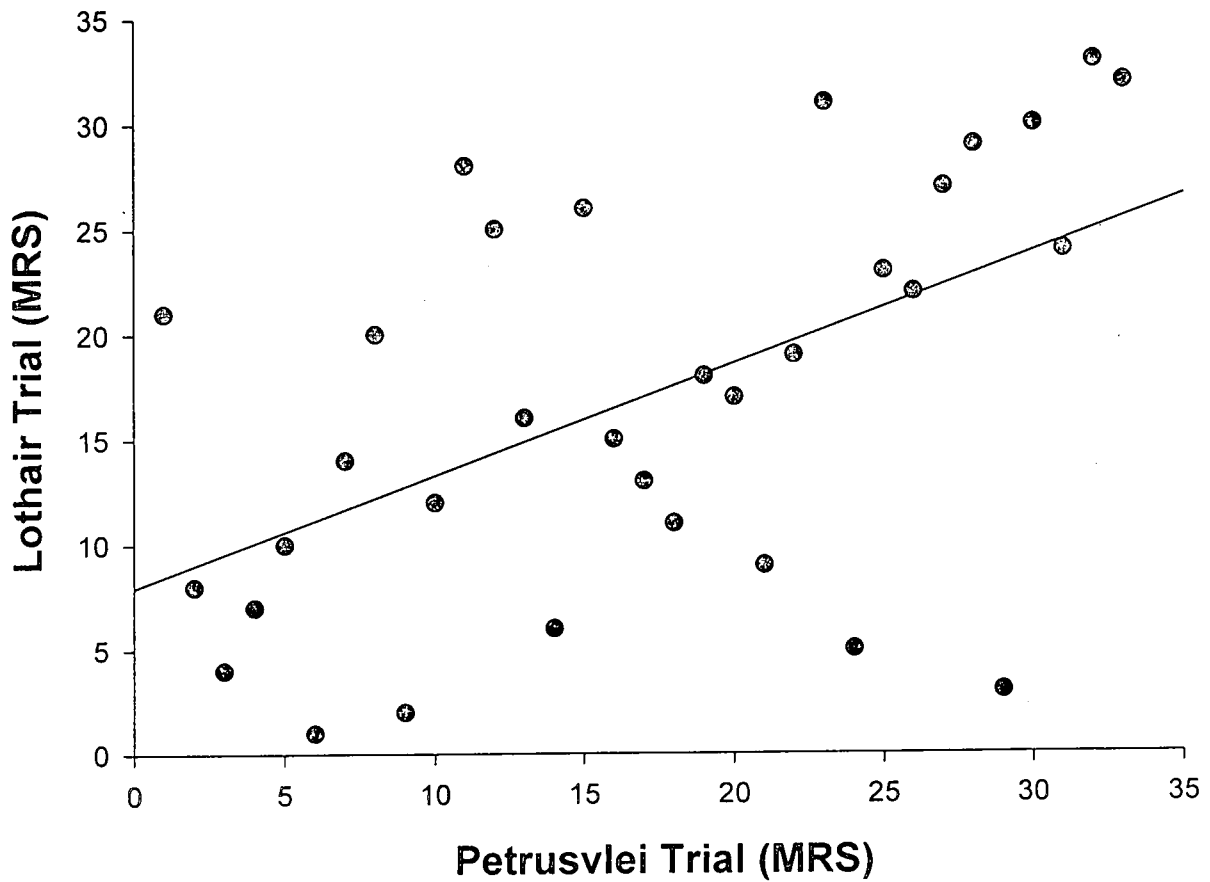


Figure 5: Comparison of the ranking (mean rank scores) of *Eucalyptus fraxinoides* families following inoculation with an isolate of *Phytophthora cinnamomi* at Petrusvlei and Lothair trials ($\bar{x} = 16$ trees)

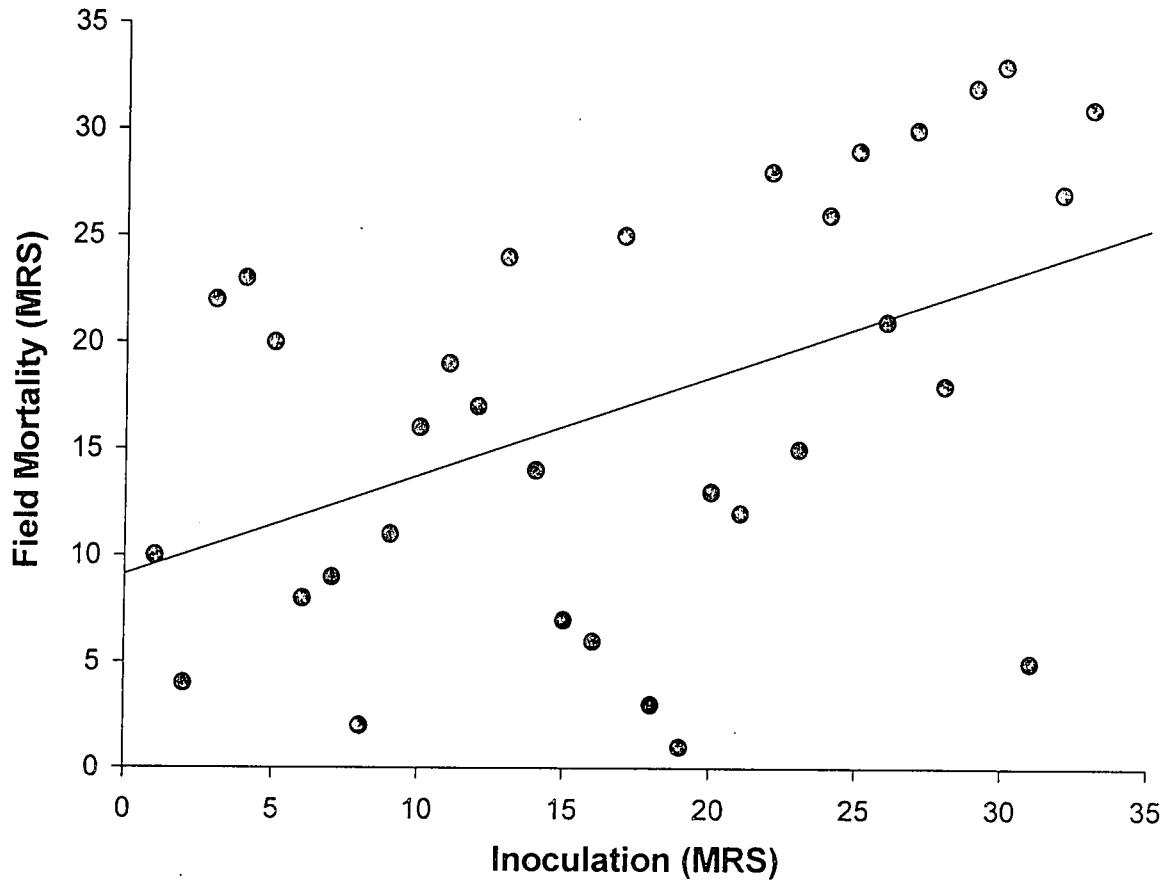


Figure 6: Mean rank scores (MRS) for natural mortality of *Eucalyptus fraxinoides* families versus inoculation with an isolate of *Phytophthora cinnamomi* at Lothair trial ($\bar{x} = 16$ trees)

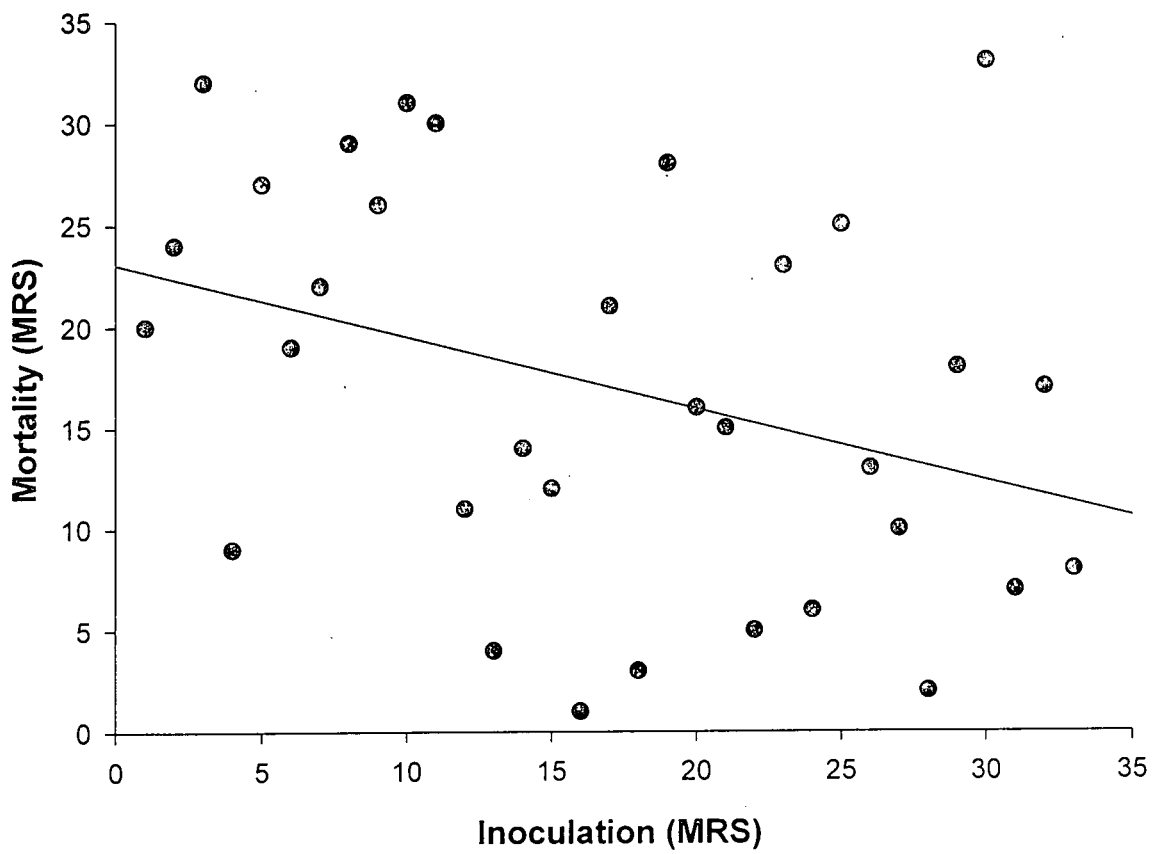


Figure 7: Mean rank scores (MRS) for natural mortality of *Eucalyptus fraxinoides* families versus inoculations with an isolate of *Phytophthora cinnamomi* at Petrusvlei trial ($\bar{x} = 16$ trees)



CHAPTER THREE

SCREENING OF COLD TOLERANT EUCALYPT SEEDLINGS FOR TOLERANCE TO *PHYTOPHTHORA CINNAMOMI*

Abstract

Cold tolerant *Eucalyptus* spp. in South Africa are known to be highly susceptible to *Phytophthora cinnamomi*. This fungus causes root rot and leads to large-scale losses to the South African forestry industry. Sixty-five *Eucalyptus fraxinoides* and forty-nine *E. smithii* families were, therefore, evaluated for tolerance to the fungus, using stem inoculations in pot trials. The lengths of resulting necrotic lesions, measured after three weeks, were used as the criterion to evaluate disease tolerance. Families were ranked according to the average lesion length of between eight to ten tree plots in three replications. Disease tolerance varied between and among both *E. fraxinoides* and *E. smithii* families. The ranking of the families differed between replicates as is reflected by the high coefficient of variance for each experiment. This method was, thus, found unreliable for screening of the seedlings of both *E. fraxinoides* and *E. smithii* for tolerance to *P. cinnamomi*.

INTRODUCTION

Forestry is one of the fastest growing industries in South Africa and depends mainly on exotic pine and eucalypts (Van der Zel, 1993). *Eucalyptus grandis* W. Hill ex Maid. and its hybrids are the most widely planted species in the country (Schönau, Stubbings & Norris, 1993). *Eucalyptus grandis* tends to grow poorly in areas with low rainfall and cold winter temperatures (Herbert, 1993). Other *Eucalyptus* spp., such as *E. nitens* Maid., *E. dunnii* Maid., *E. saligna* Sm., and *E. macarthurii* Dean & Maiden are, therefore, usually planted in these areas. Unfortunately, these species often do not yield high quality pulp when compared to *E. grandis* (Clarke, 1995).

The increasing demand for high quality pulp and paper has forced the forestry industry to consider introducing alternative *Eucalyptus* spp. which do not compromise wood quality. *Eucalyptus fraxinoides* Dean & Maiden and *E. smithii* Donn. ex Smith are two ideal species for commercial propagation in these colder areas. Both species are cold tolerant, grow vigorously and produce high quality pulp and paper (Clarke, 1995). However, they are susceptible to *Phytophthora cinnamomi* Rands root rot disease (Wingfield & Knox-Davies, 1980). Recently conducted field trials on *E. fraxinoides* and *E. smithii* demonstrated that some individual trees survived on sites known to be affected by *P. cinnamomi* (Clarke, 1995). In addition, disease tolerance has been demonstrated in the field using artificial inoculations of three-year old *E. fraxinoides* trees (Wolfaardt *et al.*, 1997).

Some *Eucalyptus* spp. such as *E. nitens* and *E. smithii* cannot be propagated by cuttings because of their inability to root (Van Wyk, 1993). Commercial propagation of *E. fraxinoides* and *E. smithii* is usually achieved through open-pollinated crosses and breeding based on half-sibling families. Incorporating disease tolerance in such a commercial tree improvement programme is a prerequisite for successful introduction of these exotic tree species (Bingham, Hoff & McDonald, 1971). Stem inoculations have been used successfully to screen half-sibling families of *Pinus radiata* D. Don. (Butcher, Stukely & Chester, 1984) and *Eucalyptus marginata* Don. ex Sm. (Stukely & Crane,

1994) for tolerance to *P. cinnamomi*. This technique could, therefore, be useful to screen and select disease tolerant families of *E. fraxinoides* and *E. smithii*.

The aim of this study was to screen seedlings from half-sibling families of *E. fraxinoides* and *E. smithii* for tolerance to *P. cinnamomi* using stem inoculations in greenhouse experiments. This technique can be cost effective and has several advantages. It can be conducted and completed in a short period of time, more individuals can be screened than in field trials and it is also not necessary to wait for trees to mature before they are evaluated.

MATERIALS AND METHODS

Plant Material

The seedlings used in this study were the progeny of open pollinated crosses of parent trees in breeding orchards that were previously affected by *P. cinnamomi* die-back. Thus, parents were trees that had survived die-back and had superior form and growth rate. Additional seedlings were selected from seed collected in Australia and planted in South Africa. Seedlings were grown in composted bark medium to suppress soil-borne pathogens. The seedlings were kept in a shade house until they had developed stems of between 5-10 mm in diameter. During this period they were watered daily and pruned to discourage formation of multiple stems. A week before inoculation, seedlings were moved to a greenhouse and allowed to acclimatise to this environment.

Greenhouse trials

From April 1996 until February 1997, *E. fraxinoides* and *E. smithii* seedlings were inoculated using a single virulent strain of *P. cinnamomi* (CP 470) (Linde, Kemp & Wingfield, 1999). A five-day-old culture of *P. cinnamomi* grown on Potato Dextrose Agar (PDA) was used. A 4 mm diameter cork borer was used to remove bark from the seedlings, at 10 cm above the soil level. A mycelium-covered agar plug taken from the actively growing margin of a colony was then inserted into a wound on the stem.

Parafilm was used to seal the wounds and thus restrict desiccation. The inoculated seedlings were kept in a greenhouse and lesion lengths were measured three weeks after inoculation (Fig. 1). Inoculated seedlings were selected at random with the aim of recovering *P. cinnamomi* from lesions. The diseased plant material was surface-disinfected with 70% ethanol for 30 seconds. Small pieces of the bark were then cut using a sterile blade and plated onto a selective agar medium (PARP ad PARPH) for *Phytophthora* spp. (Tsao & Ocana, 1969).

Experimental design and statistical analysis

Seventy *Eucalyptus fraxinoides* and fifty-one *E. smithii* families were evaluated for tolerance to *P. cinnamomi*. A randomised block experimental design, with three blocks was used. Each replicate consisted of 8 to 10 seedlings that were inoculated at three different times of the year (April 1996 to September 1996). Shorter lesion lengths served to indicate disease tolerance. A one-way analysis of variance was done and tested for significant differences between family means with Tukey's test at 95% confidence (Winer, 1971).

RESULTS

Eucalyptus smithii trials

Distinctive necrotic brown lesions developed, below and above the points of inoculation, on all inoculated stems, five days after inoculation (Fig. 1A). The lesions continued to extend at different rates on different seedlings throughout the monitoring period. In some families, lesions did not spread rapidly, rather, were contained. However, on susceptible families lesions continued to expand for three weeks, after which the lesions were measured. In severely infected families (9, 15, 26 and 38) seedlings exhibited wilting symptoms. *Phytophthora cinnamomi* was successfully recovered from the infected tissue when plated onto the selective medium.

Families differed in tolerance to *P. cinnamomi* in the three replicate trials (Fig. 2). No correlation was found between the ranking of the families in any of the three replications conducted. The coefficient of variance for the experiment was high (360%). In each of the replicate trials conducted, families reacted differently and showed varying degrees of tolerance to *P. cinnamomi* (Fig. 2). In the first replication, families were ranked according to the lesion length following inoculation with *P. cinnamomi*. Families were arranged from the least susceptible to most susceptible. Although some families showed the same level of susceptibility or tolerance to the pathogen in all replications, the experiment was not repeatable and inconsistent results were obtained. For example, in all replications, families 9, 15, 26 and 38 ranked amongst the most susceptible while families 25, 39 and 47 showed low to moderate disease tolerance. The variation in lesion lengths within each family appeared to increase with age. This is evident when comparing the variation in size of the standard error bars for all replications. Few families (17, 52, 53, 54 and 55) showed improved *P. cinnamomi* tolerance in the second and third replication.

Eucalytus fraxinoides trials

Brown necrotic lesions similar to those observed on *E. smithii* trials were seen after inoculation (Fig. 1B). No single family was found to be immune to *P. cinnamomi* infection. In general, *E. fraxinoides* families yielded larger lesions than *E. smithii* families when inoculated with *P. cinnamomi* (Fig. 3). No lesions developed on the control seedlings, while *P. cinnamomi* was successfully recovered from diseased stem tissue of inoculated seedlings.

Significant differences ($P \leq 0.05$) in susceptibility were found between families. Results were also more reproducible than those obtained in the *E. smithii* experiments (Figs. 2 & 3). Some families were consistent in their level of susceptibility or tolerance to *P. cinnamomi* in all three replications. For example, families 29, 59 and 68 were found to be the most tolerant, while families 67 and 61 were highly susceptible (Fig. 2). These tolerant families have the potential to be used in further breeding programmes.

DISCUSSION

The technique used to screen *E. smithii* and *E. fraxinoides* families for tolerance to *P. cinnamomi* proved to be unreliable in this study. No consistency was found between the three replicate trials conducted for each of the *E. smithii* or the *E. fraxinoides* families screened. Thus some families were relatively disease tolerant in one trial and less or more so in other trials.

Some obvious trends in susceptibility and tolerance were evident for *E. smithii* and *E. fraxinoides* families. For example, families 25, 39 and 47 of *E. smithii* were found to be moderately tolerant to *P. cinnamomi*. Significant differences in susceptibility or tolerance in families 48, 52, 54 and 55 were also found in all *E. fraxinoides* replicate trials. Although no correlation was found in all trials, *E. fraxinoides* results were more reproducible than those of *E. smithii*.

The lack of correlation and low levels of repeatability obtained in both the *E. smithii* and *E. fraxinoides* trials was due to genetic variation within families. It has been shown that there is a large genetic variation among different half-sibs of the same family (Stukely & Crane, 1994). In contrast, to our results a similar technique was used successfully to screen *P. radiata* (Butcher *et al.*, 1984) and *E. marginata* families for tolerance to *P. cinnamomi* (Stukely & Crane, 1994). It is, therefore, apparent that other factors may have contributed to the failure of the experiments conducted.

In this study, the age of trees differed at the time of inoculation. It is evident from the results obtained that there is variation in susceptibility or tolerance to *P. cinnamomi* during the early stages of growth of *E. smithii* and *E. fraxinoides*. This difference in the age of the trees at different inoculation times could have contributed to the lack of correlation between the replicate trials of both hosts. In general, younger trees tend to be more susceptible to pathogens than older trees (Carson & Carson, 1989).

Results obtained from the greenhouse trials following artificial inoculation with *P. cinnamomi* were found to be less accurate than those obtained later under field conditions (Wolfaardt *et al.*, 1997). According to Namkoong *et al.*, (1988) artificial inoculations in greenhouse trials are often less accurate than natural infections in the field since greenhouse tests produce overly severe symptoms. Therefore, changing the method of disease assessment and scoring could possibly improve replication between field and greenhouse trials for both species.

The methods of inoculation, screening and evaluation are crucial when ranking families in greenhouse trials. A large number of individuals are usually needed when conducting such trials so as to reduce experimental error (Wright, 1976). A low number of trees per block and in replications could have contributed to the lack of correlation between results obtained from the replicate trials. Therefore, increased replication could reduce error.

The technique used to screen *E. fraxinoides* and *E. smithii* families for *P. cinnamomi* tolerance was unreliable. Inconsistent results were found in all replicate trials conducted. A large genetic variation was found amongst individuals with families. This may have led to the poor expression of the desired *P. cinnamomi* tolerance trait within half-sib families screened. Incorporating disease tolerance in *E. smithii* and *E. fraxinoides* families is a challenging exercise, due to the genetic complexity of these hosts.

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Figure 1: Results obtained following artificial inoculation of *Eucalyptus smithii* and *Eucalyptus fraxinoides* families with *Phytophthora cinnamomi* after three weeks. Stems of *Eucalyptus smithii* (A) and *Eucalyptus fraxinoides* (B) with and without the bark removed. The arrows show distinct brown lesions, above and below the inoculation points after three weeks.



A

B

Figure 2: Three replicate trials of *Eucalyptus smithii* families showing comparative lesion lengths following inoculation with *Phytophthora cinnamomi*. Bars represent the mean (\pm SE) lesion length (mm). Families are ranked from the least susceptible to the most susceptible in the first replication only.

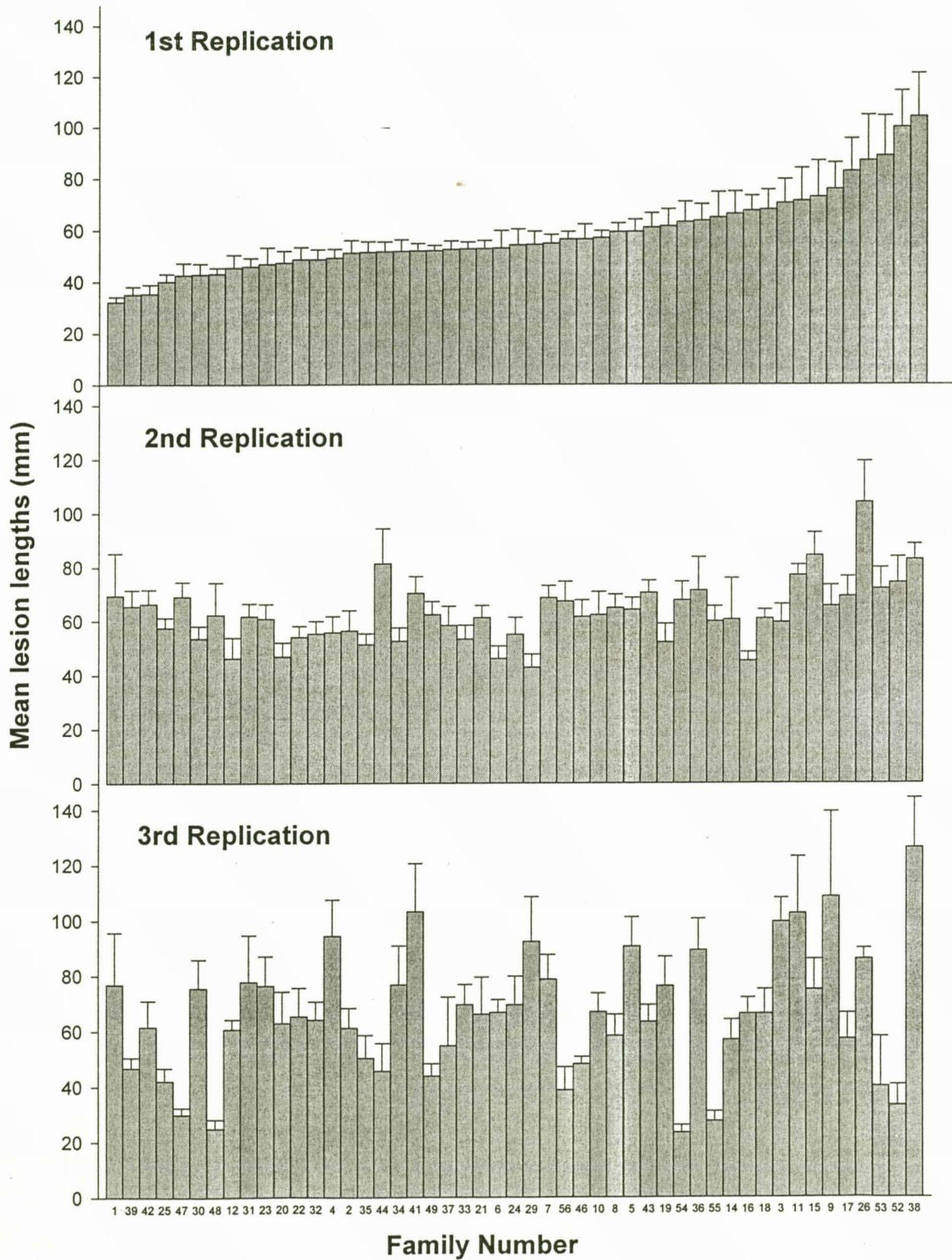
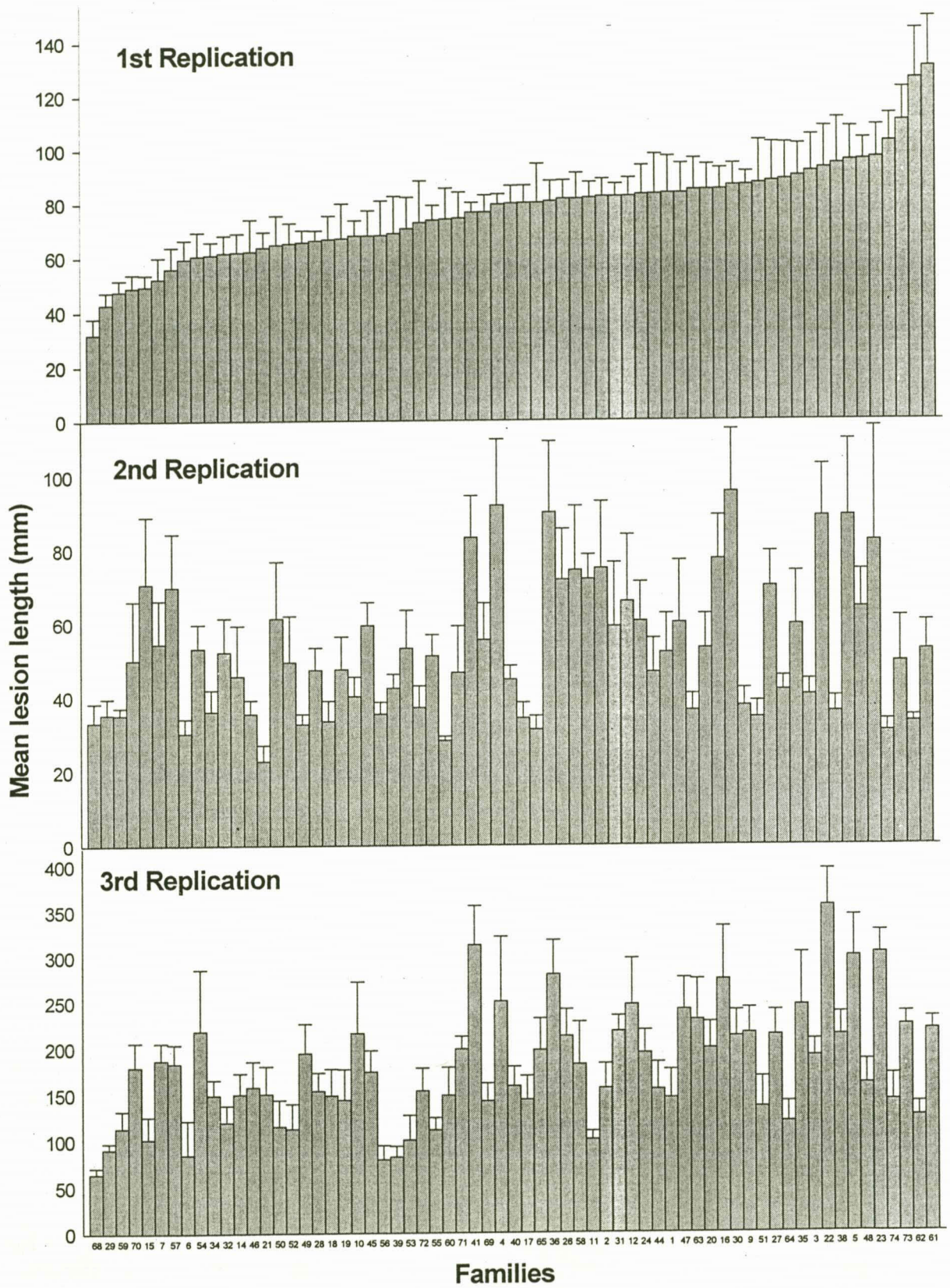


Figure 3: Three replicate trials of *Eucalyptus fraxinoides* families showing comparative lesion lengths following inoculation with *Phytophthora cinnamomi*. Bars represent the mean (\pm SE) lesion length (mm). Families are ranked from the least susceptible to the most susceptible family in the first replication only.





CHAPTER FOUR

PHYTOPHTHORA AND PYTHIUM SPP. ASSOCIATED WITH THE CITRUS ROOT ROT COMPLEX IN SOUTH AFRICA

Abstract

Root rot is one of the most common disease problems in all major citrus growing regions in South Africa. *Phytophthora nicotianae* and several *Pythium* spp. are commonly associated with this disease. Surveys were conducted in selected citrus nurseries and orchards in the Northern and Mpumalanga Provinces of South Africa, in order to assess the occurrence of *Phytophthora* and *Pythium* spp. associated with root rot. Diseased bark and root samples collected from these sites were plated directly on selective media. Soil samples collected from the rhizosphere of diseased trees were baited using citrus leaf pieces which were later plated on selective media. A total of 320 *Phytophthora* and *Pythium* isolates were recovered from diseased citrus trees. These included two *Phytophthora* and eight *Pythium* species. *Phytophthora nicotianae* and *Pythium irregulare* were the most frequently isolated species. Sixteen *P. citrophthora* isolates were successfully recovered, even though it had been assumed that this fungus was absent in the sampled region.

INTRODUCTION

Surveys of South African citrus nurseries (Wehner, Combrink & Kotzé, 1986) and orchards (Martin, 1960; Kotzé, 1984) indicate that citrus root rot is one of the major causes of tree decline. *Phytophthora* and *Pythium* spp. are commonly isolated from such declining citrus trees (Fatemi, 1972). *Phytophthora nicotianae* Breda de Haan, in particular, is one of the most common pathogens associated with citrus root rot in South Africa (Wehner *et al.*, 1986; Thompson, Phillips & Nel, 1995). Other *Phytophthora* spp. such as *P. citricola* Sawada (Von Malitz & Von Broemsen, 1985), and *P. cryptogea* Penthylbridge & Lafferty (Von Malitz & Von Broemsen, 1985; Thompson *et al.*, 1995) have been reported but are considered rare. *Phytophthora citrophthora* (Smith & Smith) Leonian, a common soil-borne pathogen found in most citrus growing regions in the world (Klotz, 1978; Timmer & Menge, 1988; Graham & Timmer, 1994), has not been recovered from South African nurseries (Wehner *et al.*, 1986) and orchards (Thompson *et al.*, 1995). Wager apparently isolated a *P. citrophthora* from a diseased grapefruit tree in 1942, but his description of the fungus is suggestive of *P. nicotianae* (Stamps *et al.*, 1990; Ho, Ann & Chang, 1995).

Although *Pythium* spp. are sometimes involved in damping-off of citrus seedlings in nurseries (DeWolfe, Calavan & Sufficool, 1954; Whiteside, 1988), they are often overlooked as serious pathogens of mature citrus trees. *Pythium* spp. have been reported as secondary root saprophytes on peach (*Prunus persica* (L.) Batsch) (Mircetich, 1971) or weak pathogens of citrus (Wager, 1942). A few *Pythium* spp. are known to play a protective role against *Phytophthora* spp. and other fungi (Deacon, 1976; Fang & Tsao, 1995). Their role in the development of citrus root rot has, however, not been clearly defined, although they have been shown to contribute to the decline of peach (Hine, 1961; Hendrix, Powell & Owens, 1966).

In South Africa, very little is known about the occurrence and pathogenicity of *Phytophthora* and *Pythium* spp. associated with diseased citrus trees. The aim of this study was, therefore, to isolate and identify *Pythium* and *Phytophthora* spp. associated with the citrus root rot complex in the Northern and Mpumalanga Provinces of South Africa.

MATERIALS AND METHODS

Sampling

Soil from the rhizosphere of diseased citrus trees was routinely collected from orchards and nurseries in the Northern and Mpumalanga Provinces of South Africa from October 1996 to February 1997. Identification of diseased trees was achieved by seeking irregular growth patterns of declining trees in orchards (Fig.1 A). The number of samples collected varied at each site depending on the number of infected trees. At sites where trees showed foot rot and gummosis symptoms (Fig.1 B), the bark was carefully removed (Fig.1 C), and soil with feeder roots was sampled. Rhizosphere soil was collected to a depth 3-5cm under the canopy (Tsao, 1983). Samples were stored in plastic bags, sealed and isolations were performed within 24 hours.

Direct plating on selective medium

In this study, methods recommended for collecting samples for disease analysis from citrus nurseries (Zitko, Timmer & Castle, 1987) and orchards (Timmer *et al.*, 1988) were used. Diseased bark samples were surface-sterilised using (70 %) ethanol. Diseased plant tissue was cut open and small pieces taken from the inner diseased tissue. These were then plated onto the selective media, PARP and PARPH (Tsao & Ocana, 1969). Diseased roots were surfaced disinfected by immersion into 70% ethanol for 60 seconds, then washed in 35% household bleach solution (sodium hypochlorite 3.5% m/v) for 5 min before being plated on selective isolation medium. Typical *Phytophthora* and *Pythium* hyphae emerged after 2-4 days incubation at room temperature in the dark. Small agar blocks with hyphae were removed and sub-cultured on corn meal agar (CMA) (17g/litre). Purification was achieved by placing an agar block with mycelium on Van Teigheim cells (Erwin & Ribeiro, 1996). Pure cultures were sub-cultured to potato-dextrose agar (PDA) plates and incubated at 20 °C. Small blocks of agar were removed from the edges of the colonies and transferred to screw-capped bottles containing 10 ml sterilised distilled water for storage (Boesewinkel, 1976; Ann & Ko, 1990).

Isolation of *Phytophthora* and *Pythium* spp. from the soil

Each soil sample of approximately 50 g was halved and placed in two 350 ml Styrofoam cups. The soil was saturated with distilled water and baited with citrus leaf pieces (Grimm & Alexander, 1973). The cups were incubated at room temperature for three days in the dark. Leaf pieces were collected and plated on selective media, PARP and PARPH (Tsao & Ocana, 1969) and incubated for three days in the dark. Small blocks of agar taken from the edge of growing colonies were aseptically transferred onto CMA and PDA for growth and identification. Plates were incubated at 20°C for two days for *Pythium* spp. and four days for *Phytophthora* spp., and colony characteristics were recorded to aid identification of isolates.

Morphology and Identification of species

Identification of isolates was achieved using taxonomic keys for *Phytophthora* (Stamps *et al.*, 1990; Ho *et al.*, 1995) and *Pythium* (Dick, 1990; Van der Plaats-Niterink, 1981). The identification was based on morphological and cultural characteristics of each *Phytophthora* and *Pythium* isolate collected.

Sporulation was induced by adding Petri's solution to Petri dishes containing a CMA block covered with mycelia (Ribeiro, 1978). Sexual and asexual structures which formed on the agar blocks were measured and means calculated for between 15-20 structures of each diagnostic character. The indices of oogonial structures (i.e. oospores, ooplasts) necessary for the oogonial criteria of identification were calculated using recommended formulae (Dick, 1990).

RESULTS

A total of 320 *Phytophthora* and *Pythium* isolates were recovered from soil and diseased plant material. One hundred and nineteen isolates were *Phytophthora* spp. and the remaining 201 *Pythium* spp. Two *Phytophthora* and eight *Pythium* species were recovered from plant material as well as from the rhizosphere soil. *Phytophthora nicotianae* and *Pythium irregulare* Buisman were the most frequently isolated species (Table 1). Measurement of oospores, oogonia, ooplasts and indices necessary for identification based on oogonial characteristics are listed in Table 2.

A total of 103 *P. nicotianae* and 16 *P. citrophthora* isolates were successfully recovered from rhizosphere soil and diseased plant material. *Phytophthora nicotianae* was isolated more frequently than *P. citrophthora* and the former species was also more prevalent in orchards than in nurseries. All *P. citrophthora* isolates were recovered from plant material and rhizosphere soil from orchards but the fungus was not recovered from nursery samples.

Phytophthora nicotianae van Breda de Haan

Phytophthora nicotianae isolates formed tufted mycelial growth on PDA plates (Fig. 2 c). *P. nicotianae* had papillate and broadly ovoid zoosporangia (Fig. 4 a-d) with a length:breath ratio of between 1.2 and 1.4. Other characteristics observed under the light microscope were the presence of chlamydospores and hyphal swellings. *Phytophthora nicotianae* can be easily confused with other papillate and heterothallic species such as *P. citrophthora*. *Phytophthora citrophthora* differs

from *P. nicotianae* in its finely radiate cultural pattern (Fig. 2 a-b), its limoniform zoosporangia which are often irregular in shape and bifurcate (Fig. 5 a-b).

***Phytophthora citrophthora* (R.E Smith & E.H Smith) Leonian**

Phytophthora citrophthora isolates were recovered from two orchard sites in the Northern Province. The isolates were obtained only from diseased plant material. *Phytophthora citrophthora* isolates produced papillate zoosporangia with several distorted shapes. Hyphal swellings were absent and the sporangiophores were either sympodially or irregularly branched. One of the other distinguishing features of *P. citrophthora* is its longer, less conspicuous papillate zoosporangia with a shallow apical opening. The average length:breath ratio of various sporangial shapes ranged from between 1.6 and 1.7.

***Pythium* spp.**

Pythium isolates were divided into two groups based on whether they formed zoosporangia and oogonia (homothallic) or only zoosporangia and hyphal swellings (heterothallic). The first group was identified using the taxonomic key compiled by Dick, (1990) and based on oogonial criteria (Table 2). The second group of isolates was identified using the Van der Plaats-Niterink (1981) taxonomic key.

Pythium spp. were isolated more frequently than *Phytophthora* spp. and were recovered largely from diseased roots. A number of heterothallic isolates without oogonia could not be identified since their identification had to be verified using mating partners. These were grouped together in either the *Pythium* G-Group or *Pythium* F-Group complex (Van der Plaats-Niterink, 1981). The group G complex included isolates with globose and non-proliferating zoosporangia. In Group F all the isolates had filamentous hyphae that were not swollen. The cultural characteristics of some *Pythium* spp. on PDA are shown in Fig. 3.

***Pythium irregulare* Buisman.**

Pythium irregulare was the most commonly isolated species in orchards and nurseries throughout the survey. Colonies had a radiate and cotton-like aerial mycelium on PDA (Fig. 3b), and had marginally fluffy colonies on CMA. The hyphae ranged from 2-4 μm to 6 μm wide, often with spherical hyphal swellings. Several different zoosporangial shapes were observed, mainly spherical, pyriform and ellipsoid (Figs. 6 a-b). Oogonia were globose and intercalary; but sometimes terminal (Fig. 6 e). Isolates had smooth 'irregular' oogonial walls, often with blunt

projections of variable length (Fig. 6 h). Aplerotic oospores ranging from 18-38 μm (mean 28 μm) in diameter were observed.

Pythium aphanidermatum (Edson) Fitzp.

Cultures on CMA had a fluffy mycelium, and slightly submerged mycelia without a special pattern on PDA (Fig. 3g). The main hyphae were 6-10 μm wide. Zoosporangia formed complexes consisting of various inflated shapes. Oogonia were largely terminal, globose, with intercalary antheridia. Oogonial diameter ranged between 20-24 μm and had thin walled aplerotic oospores.

Pythium poroecandrum Drechsler

Cultures had no aerial mycelium on CMA and had only slightly submerged mycelia on PDA. The main hyphae were 4-8 μm in diameter. Zoosporangia were round, intercalary and had diameters of between 20-30 μm . Chains of round and intercalary oogonia were observed. *Pythium poroecandrum* can be easily confused with *P. irregulare*, but the former is distinguished by its larger oogonia which occur irregularly and lack projections.

Pythium rostratum Butler

Colonies on CMA had flat and coarsely radiate mycelium which had a distinctly chrysanthemum pattern on PDA (Fig. 9). *Pythium rostratum* grew slowly on both CMA and PDA. Main hyphae ranged between 4-8 μm in width. Non-proliferating and round zoosporangia with diameters between 20-28 μm were present. Smooth walled oogonia with diameters between 20-26 μm and aplerotic thick walled oospores.

Pythium ultimum Trow

Colonies on CMA were cotton to fluffy and on PDA had a radiate pattern with aerial mycelia (Fig. 3a). The hyphae ranged from 6 - 10 μm in width. Zoosporangia were not seen but globose and intercalary hyphal swellings were abundant. Smooth-walled oogonia were readily formed and were mostly globose and ranged from 20-24 μm in diameter. Oospores had characteristic thick walls, with mean diameters of 18 μm . *P. debaryanum* and *P. ultimum* were treated as conspecific.

Pythium vexans de Bary

Cultures on CMA were fairly fluffy and had no definite growth pattern on PDA (Fig. 6g). The main hyphae were between 2 - 5 μm in width. Both zoosporangia and oogonia were readily produced in culture. Proliferating zoosporangia were produced terminally and had round to pyriform shapes. Oogonia were produced on short lateral branches and were between 18 - 22 μm in diameter. Typical bell-shaped antherial cells were observed. Smooth walled, aplerotic oospores ranging from 16 - 18 μm in diameter were also observed.

DISCUSSION

Results obtained in this survey indicate that *Pythium* spp. occur in abundance in the rhizosphere of diseased citrus trees. *Pythium* spp. were also recovered more frequently than *Phytophthora* spp. from the rhizosphere soil. Despite the abundance of *Pythium* spp. in the rhizosphere of declining citrus trees, little attention has been paid to their role in citrus decline.

Phytophthora nicotianae was the most frequently isolated *Phytophthora* spp. and was recovered from both nurseries and orchards sampled. The isolates were recovered from the rhizosphere soil and infected rootlets. The dominance of *P. nicotianae* observed in the study has been reported previously in South Africa (Wehner *et al.*, 1986; Thompson *et al.*, 1995). *Phytophthora nicotianae* has also been reported to be a dominant species in citrus orchards in Florida (Zitko *et al.*, 1987; Timmer *et al.*, 1988) and Taiwan (Ann, 1984).

The successful recovery of *P. citrophthora* from orchards has great significance to the South African citrus industry since it has been presumed to be absent from these sites (Wehner *et al.*, 1986; Thompson *et al.*, 1995). *Phytophthora citrophthora* was last reported present from the Northern Province (Former Transvaal Region) in 1925 by Doidge. However, there have been unpublished reports on the occurrence of *P. citrophthora* in the Western Cape Province of South Africa (Le Roux, personal communication).

The low occurrence of *P. citrophthora* from the sampled regions indicates that it is rare and this may be attributed to the climate. The orchard and nurseries surveyed are situated in subtropical regions of South Africa. The climatic conditions in these areas are ideal for the growth of *P. nicotianae* since it is reported to withstand high temperatures (Stamps *et al.*, 1990). The adaptation of *P. nicotianae* to high temperatures could possibly explain its abundance in the sampled areas.

Results of this study indicate that several *Pythium* spp. occur in South African orchards and nurseries. *Pythium irregulare* is the most commonly isolated species from citrus orchards and nurseries. This observation confirms results obtained in previously conducted surveys (Wehner *et al.*, 1986; Thompson *et al.*, 1995). However, the role played by *P. irregulare* in the root rot of citrus remains unknown and deserves careful study.

Several *Pythium* spp. reported in this study have previously been found on host plants in South Africa (Darvas, Scott & Kotzé, 1978; Denman & Knox-Davis, 1992., Linde, Kemp & Wingfield, 1994; Botha & Coetzer 1996). Unfortunately, very little is known about the distribution, occurrence and pathogenicity of these *Pythium* spp. on citrus and this should also be considered in the future.

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Table 1. *Phytophthora* and *Pythium* spp. isolated from diseased citrus trees and rhizosphere soil

<i>Phytophthora/Pythium</i> sp.	No of isolates	Presence in Nurseries or Orchards
<i>Phytophthora nicotianae</i>	103	N, O
<i>Phytophthora citrophthora</i>	16	O
<i>Pythium irregulare</i>	108	N,O
<i>Pythium aphanidermatum</i>	9	O
<i>Pythium paroecandrum</i>	12	O
<i>Pythium vexans</i>	16	O
<i>Pythium rostratum</i>	18	N,O
<i>Pythium ultimum</i>	15	O
<i>Pythium</i> group F & G (non sporulating)	23	N,O

N = Nursery, O = orchard

Table 2. Morphological characteristics used to identify *Pythium* spp. associated with diseased citrus trees

Species	Oogonium Data (μm)		Oospore Data (μm)		Aplerotic Index (%)	Ooplast Data (μm)		Ooplast Index (%)	Modal position of oogonia and antheridia			
	Width	$\bar{x}(\pm \text{SE})$	Width	$\bar{x}(\pm \text{SE})$		Width	$\bar{x}(\pm \text{SE})$		PO	AN	AO	AP
<i>P. irregulare</i>	18-20	19 (± 0.32)	13-15	14 (± 0.20)	52	12-14	13 (± 0.92)	12	I+T	1	M	L
<i>P. aphanidermatum</i>	20-24	22 (± 0.15)	16-20	18	60	15-16	16 (± 0.54)	17	T	1	M	L
<i>P. paroecandrum</i>	20-26	23 (± 0.37)	18-24	21 (± 0.12)	56	14-18	16 (± 0.21)	20	I	1	M	L
<i>P. vexans</i>	18-22	20 (± 0.09)	16-20	18 (± 0.83)	56	14-15	15 (± 0.28)	28	T	1	M	BC
<i>P. rostratum</i>	18-24	21 (± 0.34)	14-15	15 (± 0.15)	65	12-14	13 (± 0.64)	16	I	1-2	CM	H
<i>P. ultimum</i>	20-23	22 (± 0.42)	17-19	18 (± 0.23)	54	15-16	15.5 (± 0.37)	24	T	1	CM	NA

a. All measurements are the ranges and means $\bar{x}(\pm \text{SE})$. for 15 measurements

b. Modal positions are those of oogonia and Antheridia, **PO**. Position of the oogonium (**I**. intercalary or **T**. terminal); **AN**. Number of antheridia per oogonium; **AO**. Antheridial branch of origin (**M**. monoclinal; **CM**. closely monoclinal); **AP**. Antheridial attachment (**BC**. broadly campanulate; **H**. Hypogynous origin; **L**. lateral ; **NA**. narrow apical).

Figure 1: Symptoms associated with citrus decline. **A.** Citrus tree with pale green leaves and stunted growth. **B.** Foot rot and gummosis symptoms on a citrus tree grafted on a susceptible rootstock. **C.** Exposed lesion after the bark was removed.

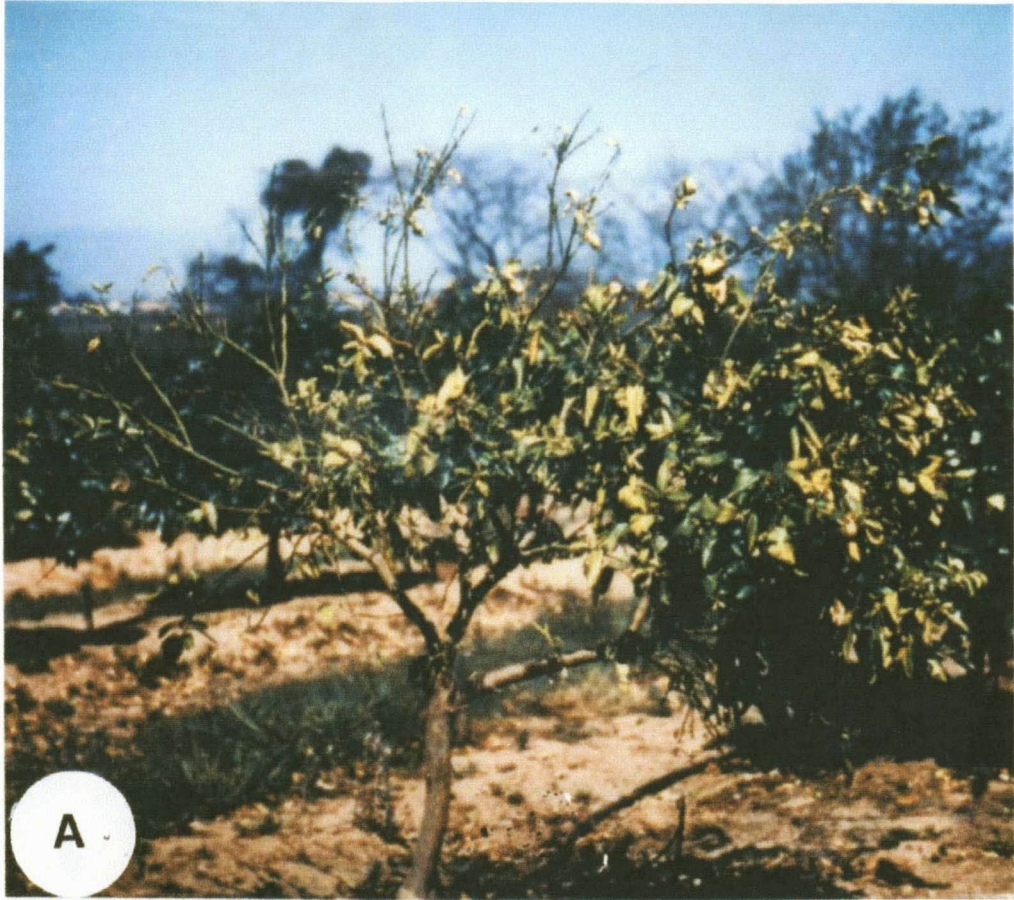


Figure 2: Colony morphology of *Phytophthora* spp. grown on Potato Dextrose Agar. **a.** and **b.** *Phytophthora citrophthora* isolates with a radiate pattern. **c.** *Phytophthora nicotianae* isolate with tufted mycelial growth.

Figure 3: Colony morphology of *Pythium* spp. grown on Potato Dextrose Agar. **a.** coarsely rosette; *P. ultimum*. **b** and **h.** Rosette; some members of *Pythium* F-Group. **c.** coarsely radiate; *P. rostratum*. **d.** Coarsely rosette, *P. paroecandrum*. **e.** Mixed rosette and chrysanthenum patterns. **f.** mixed rosette and radiate patterns; *P. irregulare*. **g.** No obvious pattern, e.g. *P. aphanidermatum* and *P. vexans*. **i.** Chrysanthenum pattern; some members of *Pythium* F-Group

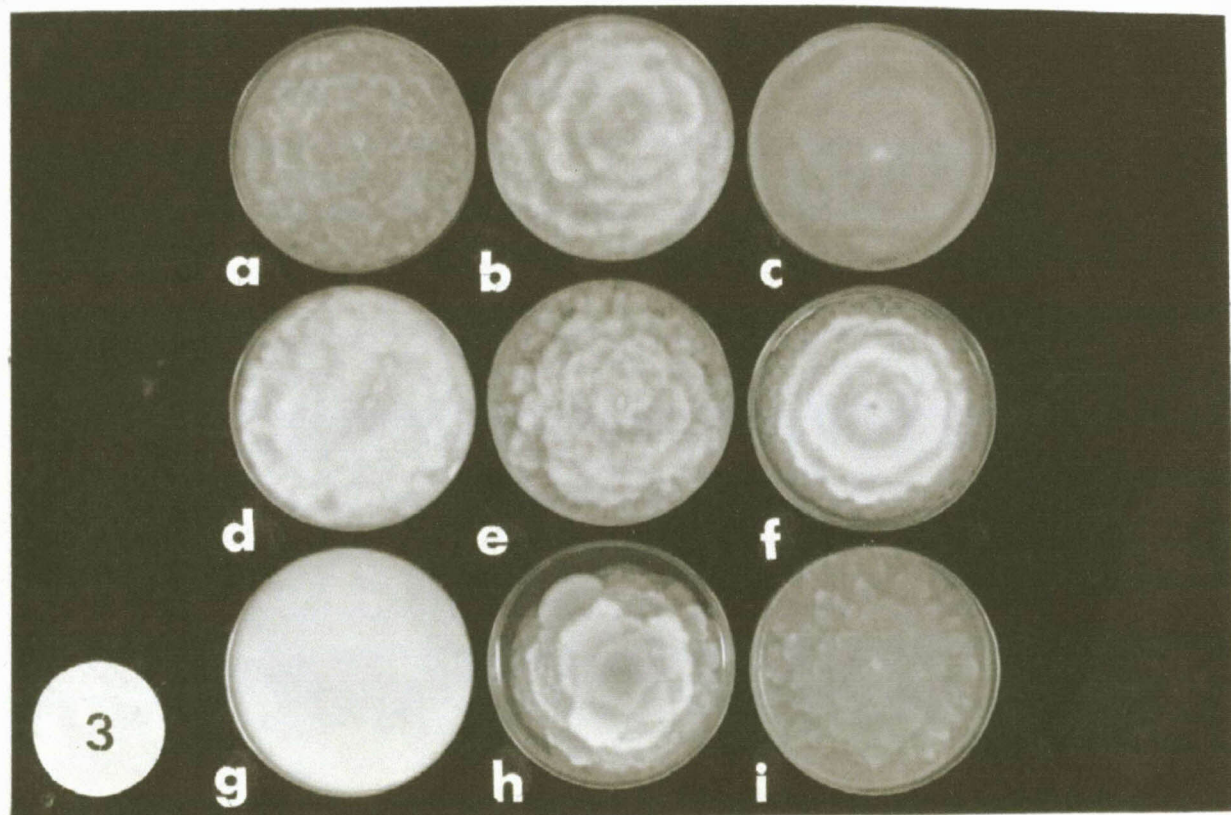
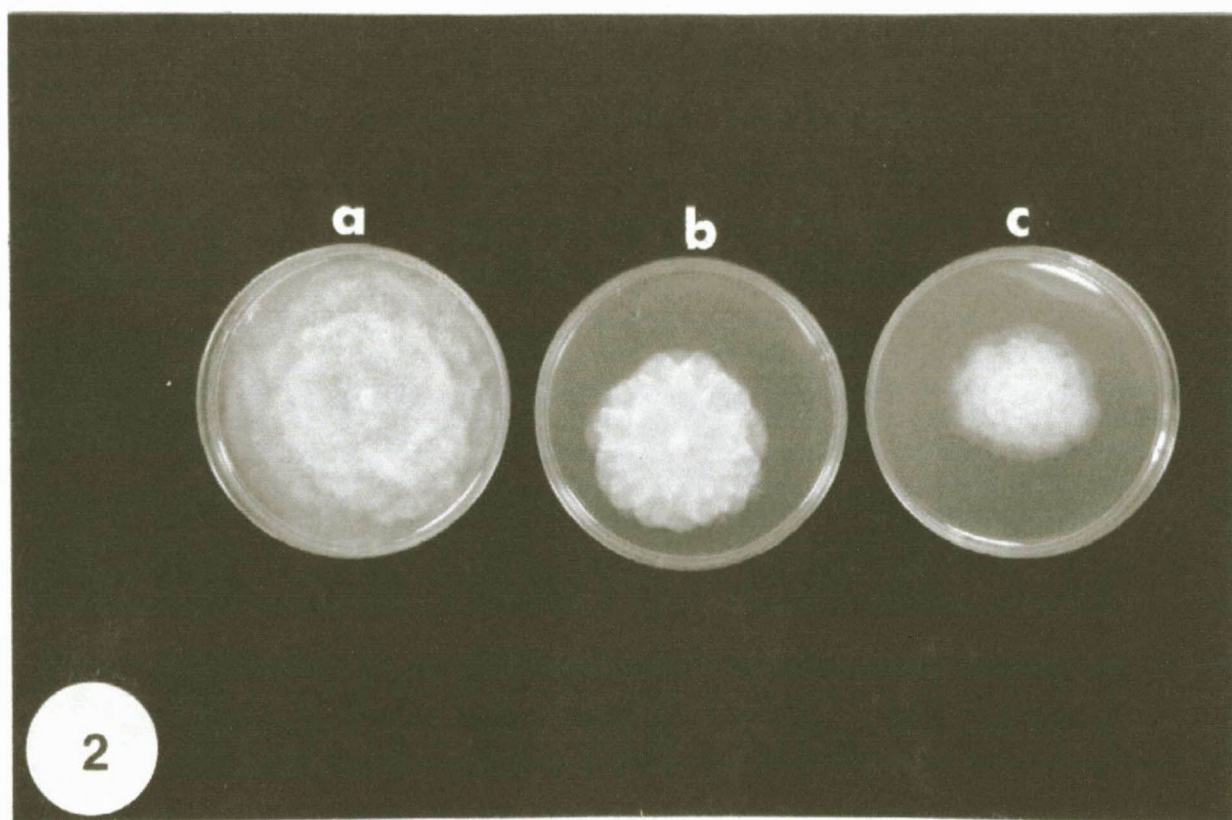


Figure 4: *Phytophthora nicotianae* sporangia. **a.** Sporangium, obpyriform and papillate in shape (Scale bar: 1 μm). **b.** Sporangia (Scale bar 2.5). **c.** Sporangia, obpyriform and spherical. **d.** Zoospore released in a sporangium (Scale bar: 2 μm)

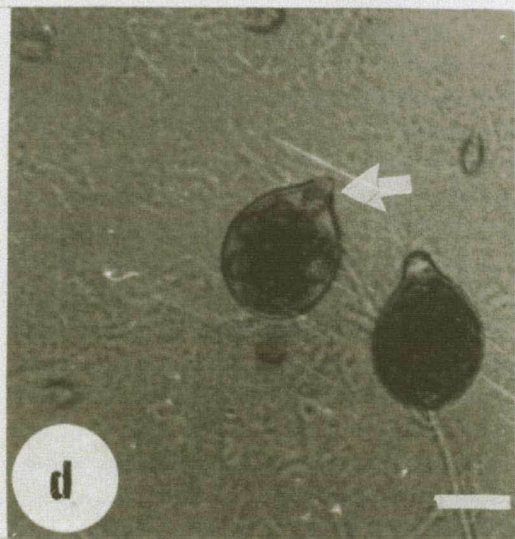
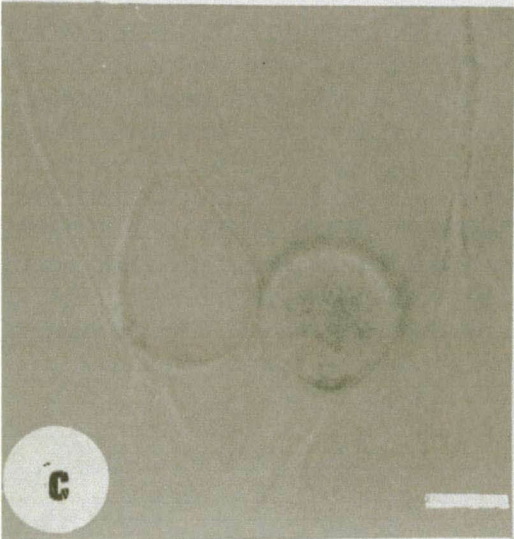
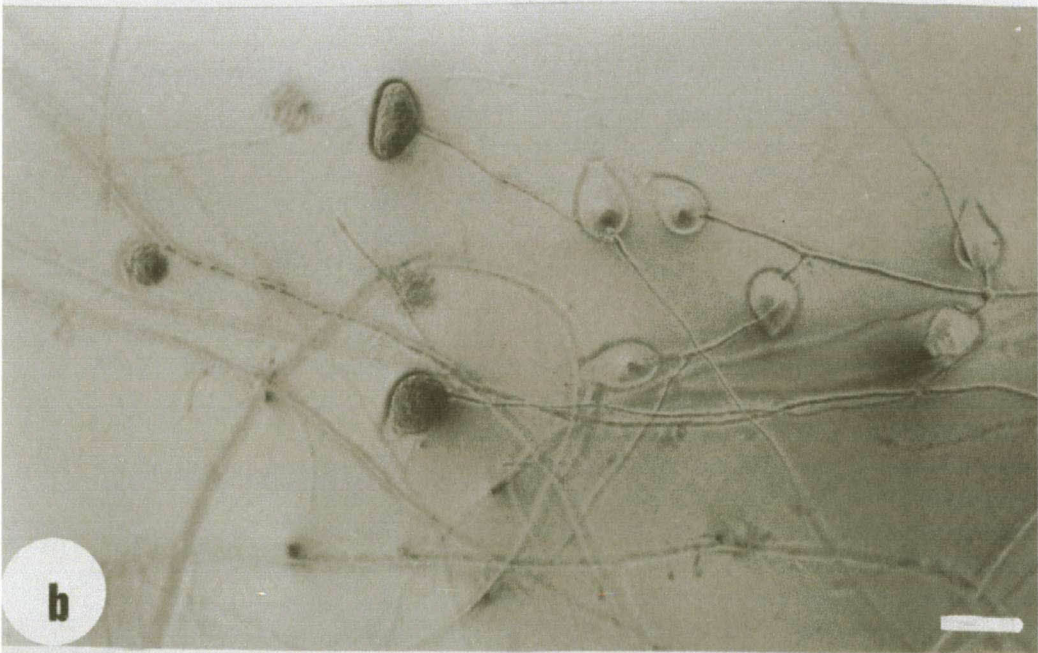


Figure 5: *Phytophthora citrophthora* sporangia. a. Sporangium ovoid and papillate (Scale bar: 1 μm). b. Sporangium ellipsoidal and papillate (Scale bar: 2.5 μm). c. Sporangia with distorted shapes (Scale bar: 5 μm). d. Chlamydozoospores (Scale bar: 2.50 μm). e-f. Sporangium obpyriform, with elongated neck (Scale bar: 0.8 μm)

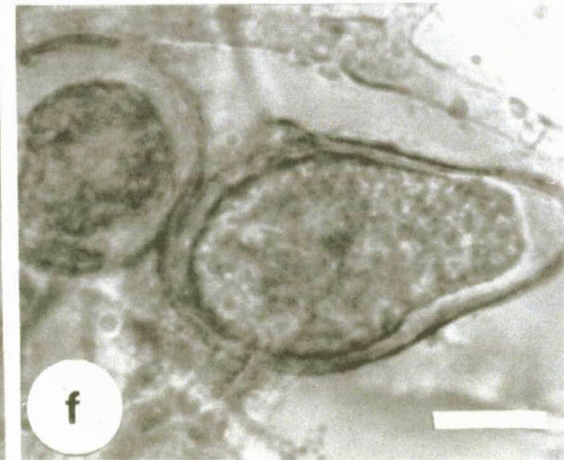
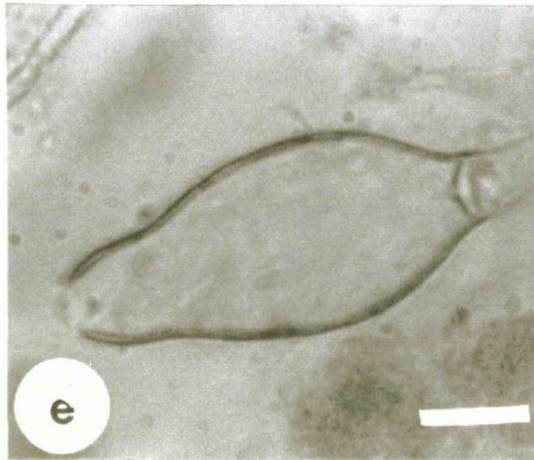
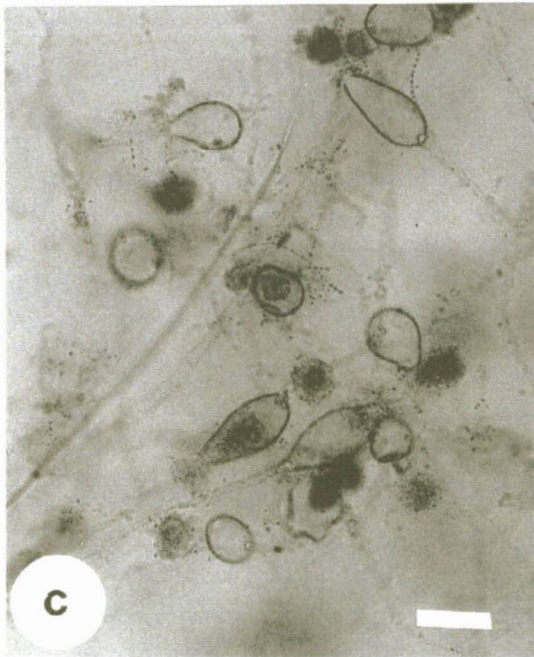
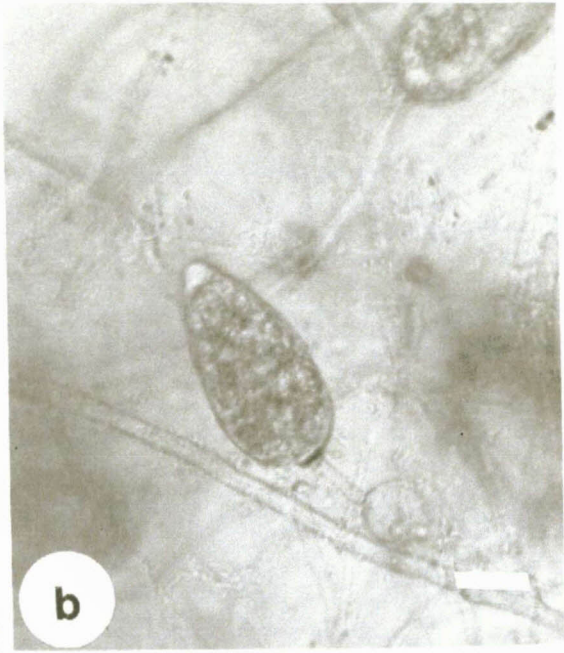
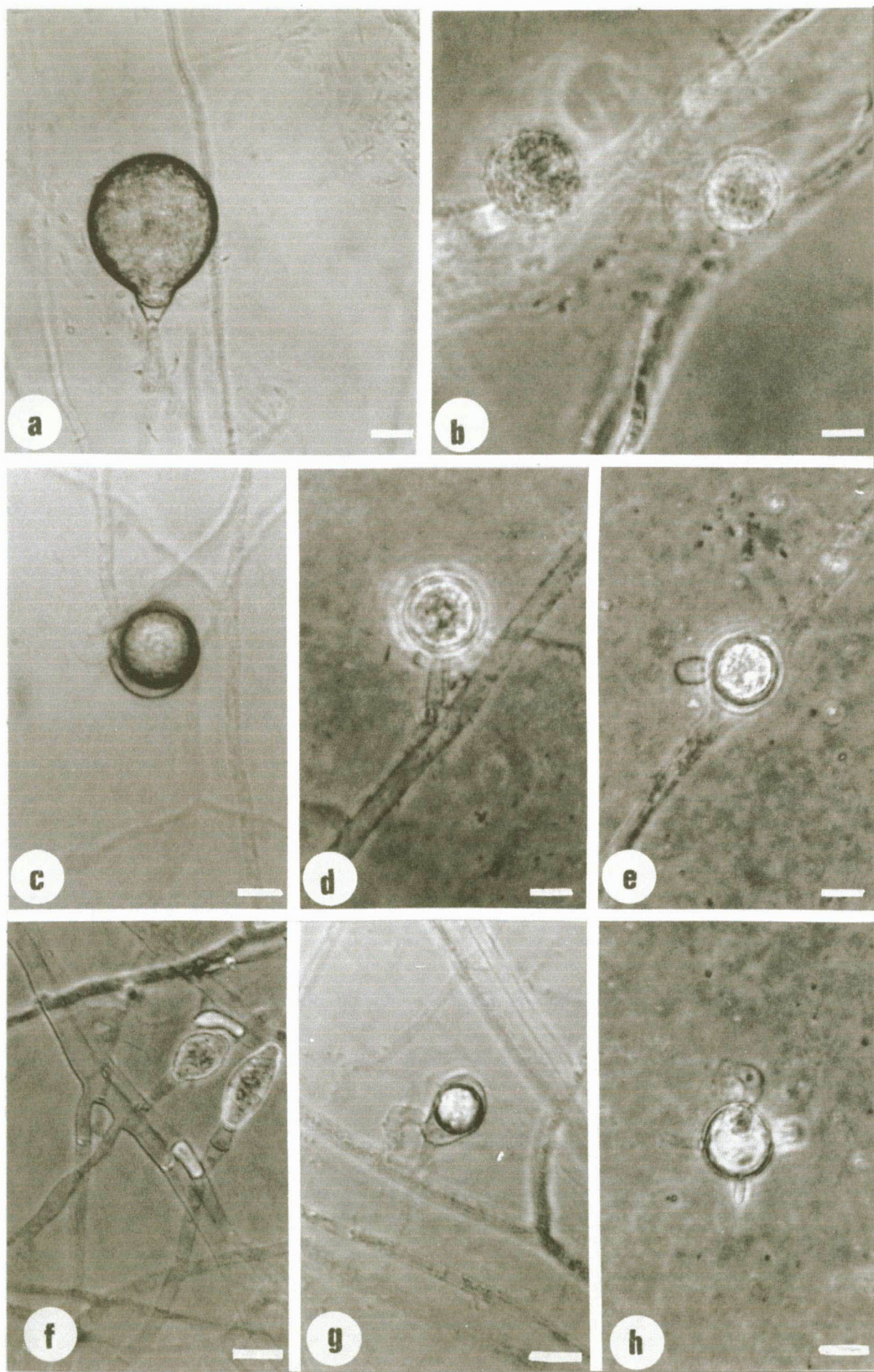


Figure 6: Some morphological characteristics of *Pythium* spp. associated with diseased citrus trees. **a.** Terminal globose zoosporangium with tapered base (Scale bar: 1.55 μm). **b.** Large spherical zoosporangia (thin-walled), intercalary and terminal, *P. irregulare*, **c.** Oogonium of *P. irregulare* with an irregular shape. **d.** Terminal oogonium, *P. poroecandrum*. **e.** An intercalary oogonium. **f.** Inflated hyphal swellings characteristic of *Pythium* Group F complex. **g.** Oogonium with an amphigynous antherium, distant from oogonial stalk, *P. irregulare*. **h.** Oogonium with finger-like projections and an subterminal amphigynous antherium, *P. irregulare*. Scale bar: 2.5 μm





CHAPTER FIVE

PATHOGENICITY OF *PHYTOPHTHORA* AND *PYTHIUM* SPP. ASSOCIATED WITH DISEASED CITRUS TREES IN THE MPUMALANGA AND NORTHERN PROVINCES OF SOUTH AFRICA

Abstract

Citrus root rot is one of the most serious problems facing the citrus industry in South Africa. Pythiaceous fungi, especially *Phytophthora* spp., are well known as causal agents of root diseases in citrus nurseries and orchards. However, little is known about the role played by *Pythium* spp. in the development of citrus root rot. Rapid screening techniques were used to determine the virulence of *Phytophthora* and *Pythium* spp. isolated from the rhizosphere of diseased citrus trees. A selected number of virulent *Phytophthora* and *Pythium* isolates were further inoculated into commercial citrus rootstocks, Rough Lemon and Troyer Citrange. Results obtained from the screening experiments were compared and analysed statistically. All *Phytophthora* isolates were found to be pathogenic on citrus fruit and rootstocks. Significant differences in pathogenicity amongst the different *Phytophthora* species and isolates were, however, found. When the same screening techniques were used for *Pythium* spp., these fungi were found to be either avirulent or only weakly pathogenic. Results obtained clearly indicate that *Pythium* spp. are not serious pathogens of citrus and probably do not play a significant role in the development of citrus root rot.

INTRODUCTION

The South African citrus industry is an important fruit industry, both locally and internationally (Mconie, 1991). South Africa produces and exports large quantities of citrus fruit to several countries (Cartwright, 1977). Root rot is one of the most serious diseases facing the citrus industry, causing substantial yield reduction (Kotzé, 1982) especially in old citrus orchards (Martin, 1960). According to Kotzé (1984), no area in South Africa is free of root rot. The disease symptoms are often complex since more than one soil-borne pathogen is reported to be involved (Kotzé, 1984)

Pythiaceous fungi, especially *Phytophthora* spp., are amongst the most notorious soil-borne pathogens associated with the citrus root rot complex throughout the world (Timmer & Menge, 1988). In nurseries, *Phytophthora* spp. are causal agents of damping-off, crown and root rot of citrus seedlings (Klotz, 1978; Graham & Timmer, 1994). In orchards, *Phytophthora* spp. are also responsible for brown rot of fruit (Feld, Menge & Pehrson, 1979; Brown & Eckert 1988), foot and fibrous root rot (Klotz, De Wolfe & Wong, 1958; Klotz, 1978; Timmer & Menge, 1988).

The role played by *Pythium* spp. in the citrus root rot complex is still not clearly defined. Despite reports of its occurrence as one of the most commonly isolated fungi from South African citrus orchards (Thompson, Phillips & Nel, 1995) and nurseries (Wehner, Combrink & Kotzé, 1986). *Pythium* spp. are seldom implicated as causal agents of damping-off in citrus nurseries (DeWolfe, Calavan & Sufficool, 1954; Whiteside, 1988). At certain poor orchard sites, root rot is often associated with a high population density of *Pythium* spp., and in the apparent absence of *Phytophthora* spp. and nematodes (Tsao, Martin & Davis, 1978; Thompson *et al.*, 1995). It is, therefore, apparent that *Pythium* spp. could play a role in the development of citrus root rot. In this study, an attempt was made to address the question of the role and significance of *Phytophthora* and *Pythium* spp. in causing root rot of citrus trees.

MATERIALS AND METHODS

Fruit Assay

Phytophthora and *Pythium* spp. isolated from the rhizosphere of diseased citrus trees in a previous study were screened for their relative pathogenicity (Table. 1). One *P. citrophthora* (Smith & Smith) Leonian isolate (MOC 370) from the culture collection maintained in the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, was also included. Pathogenicity tests were conducted on citrus fruit using the method of Wager (1942). Navel oranges were wounded using a 9mm-cork borer and artificially inoculated with a mycelium-covered agar plug. Wounds were sealed with parafilm and fruit incubated at 25° C for five days. Three fruit were inoculated with each isolate. Control inoculations were made with sterile agar. The extent of discolouration on the rind surface was used to evaluate the relative virulence of each isolate. A plastic grid, divided into blocks of 1-mm², was placed on the fruit surface. The lesions were measured by counting the number of blocks over the discoloured area. This method was used to determine the pathogenicity of each isolate.

To compare the relationship between the growth rate and pathogenicity of isolates relative to lesions produced on fruit, each isolate was plated on three Potato Dextrose Agar (PDA) plates. The plates were incubated at 25° C for five days and colony diameters were measured in two directions and means computed.

Lupin Assay

Since *Pythium* spp. are not known to infect fruit and proved either avirulent or weakly non pathogenic on citrus fruit, a more sensitive screening test using lupin (*Lupinus angustifolius* L.) seedlings was conducted. Lupin seeds were surface sterilised by immersion in 70% ethanol for 60 seconds, then 5 min in 35% household bleach solution (sodium hypochlorite 3.5% m/v) followed by overnight soaking with distilled water (Ribeiro, 1978). Seeds were planted in pre-moistened perlite, which had been autoclaved twice. When the seedlings were approximately 10 cm high, 20 seedlings were transplanted into plastic trays (200 x 150mm) containing pre-inoculated soil, mixed with perlite (1:3) ratio.

The inoculum for pathogenicity tests was prepared by growing each *Pythium* isolate on Corn Meal Agar (CMA). Two agar plugs (9mm in diameter) taken from the edge of a growing

colony, were aseptically transferred into 10 Erlenmeyer flasks (250-ml), each containing sterile 50 ml Potato-dextrose broth. The flasks were incubated at 20°C for 2 weeks until a dense mycelial mat was formed. The mycelial mat was washed three times with sterile distilled water. To induce sporulation, 50 ml of Petri solution (Ribeiro, 1978) was added to each flask. The inoculum was then thoroughly mixed with 5 kg steam sterilised perlite to give a final ratio of 1:8 (inoculum:perlite). This mixture was combined with steam pasteurised soil at 1:3 ratio (pre-inoculated perlite:soil) and used immediately. Control treatments were conducted using steam sterilised perlite without inoculum. Two isolates of each of the eight *Pythium* spp. isolated from citrus were tested for their ability to infect lupins. Two replicate trials for each experiment was conducted and the percentage mortality of the lupin seedlings was recorded.

Pathogenicity tests on citrus rootstocks

Seedlings of commercial rootstocks, Rough Lemon (*Citrus jambhiri* Lush.) and Troyer Citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osbeck), were grown for 12 months in a composted bark medium. Seedlings were kept in a shade house until they developed stems of between 5-7 mm in diameter. Seedlings were watered regularly and pruned to leave a single stem.

Four *Phytophthora* and sixteen *Pythium* isolates were inoculated on Rough Lemon and Troyer Citrange rootstocks. The most virulent and least virulent *Phytophthora* isolates, from the fruit assay results, were used for inoculation. In the case of *Pythium* spp., two pathogenic isolates from the lupin assay were selected. The inoculum was prepared by growing each isolate on PDA for five days at 20 °C. A 4 mm-cork borer was used to punch holes aseptically from the edge of the growing colony. Inoculations were conducted by removing the bark from the stems of seedlings using a sterilised 4 mm-cork borer. Mycelium-covered agar plugs (4 mm diameter) were attached to the wound and sealed with parafilm using a modification of the technique of Afek, Sztenberg & Solel (1990). Control trees were inoculated with sterile agar plugs. Trees were kept in a greenhouse and lesion lengths measured after three weeks. The day and night temperature ranged between 20 °C and 28 °C in the greenhouse.

Seedlings were kept in the greenhouse to acclimatise two weeks prior to the commencement of experiments. Ten seedlings of each citrus rootstock were inoculated with one of each selected fungal isolate. Rootstocks were arranged in a completely randomised block design.

The average lesion lengths of ten replicates from each experiment were analysed statistically and compared with those obtained from fruit inoculations. Pathogenicity of the isolates was evaluated by measuring and comparing the lesion lengths. Necrotic brown discolouration on the wounded stem tissue was used to evaluate the pathogenicity of the different isolates. Two replicate trials were conducted for each rootstock

RESULTS

Fruit Assay

Brown fruit rot symptoms developed within three days after inoculation and spread rapidly on the rind surface. The inoculated fruit initially showed a dull grey discolouration that gradually turned brown. White delicate mycelial strands later formed on the rind surface. *Phytophthora* isolates had varying levels of pathogenicity (Fig. 1A).

Phytophthora nicotianae van Breda de Haan isolates were associated with varying levels of pathogenicity. The mean diseased surface area resulting from inoculation with different *P. nicotianae* isolates ranged between 57 and 188 mm² (Fig. 2). The mean colony diameter ranged between 33 and 56 mm (Fig. 3). Significant differences in pathogenicity among the various *P. nicotianae* ($F = 7.18$ $P < 0.005$) was found. *Phytophthora citrophthora* isolates also had varying degrees of pathogenicity on citrus fruit. The mean diseased surface area on the fruit rind surface ranged between 102 and 361 mm² (Fig. 4) and mean colony diameter ranged between 52 and 77 mm on PDA (Fig. 5). Significant differences in growth diameter among the different *P. citrophthora* isolates was found ($F = 6.65$, $P = 0.001$). A positive correlation between the fruit assay and colony diameter of *Phytophthora* isolates was also evident (Fig. 6).

The majority of the *Pythium* spp. screened were either avirulent or only weakly pathogenic in this study (Table 1). A few *Pythium* isolates produced small brown necrotic lesions on the wounded rind surface. Lesions were slightly different from those of the *Phytophthora* spp. The lesions did not spread rapidly and were restricted to the wounded rind surface. In most fruit, the mycelial growth was also completely restricted to the rind surface.

Lupin Assay

Pythium isolates screened were found to be pathogenic on the lupin seedlings. The variation in pathogenicity and the extent of damping-off caused by *Pythium* isolates screened was evident after one week (Table 2). The percent mortality ranging between 40 to 80 % was recorded. Severe damping-off of the remaining lupin seedlings was observed after two weeks. *Pythium irregulare* Buisman. and *P. ultimum* Trow in particular, were found to cause severe damping-off of the lupin seedlings.

Pathogenicity of Citrus rootstocks

Phytophthora nicotianae and *P. citrophthora* isolates were found to be pathogenic on Rough lemon and Troyer Citrange rootstocks. *Phytophthora citrophthora*, in particular, was found to be more pathogenic than *P. nicotianae*. The inoculated rootstocks showed typical foot rot symptoms. Gum exudation was noticed within four days after inoculation, especially on seedlings inoculated with *P. citrophthora* (Fig. 7A).

No gum exudation was observed in the seedlings inoculated with *Pythium* spp. Lesion were largely superficial and did not grow readily such as those found with *Phytophthora* inoculations (Fig. 7B). In most seedlings, lesion development was totally overcome by callus formation around the wounded stem tissue. Only three of sixteen *Pythium* spp. isolates screened were weakly pathogenic on the citrus rootstocks. *Pythium irregulare*, *P. ultimum* and *P. paroecandrum* Drechsler isolates produced superficial lesions on the wounded stems of citrus rootstocks. No significant differences in pathogenicity were found between these *Pythium* isolates on either rootstocks (Fig. 8). However, there was variation in virulence among the *Phytophthora* isolates screened on both rootstock. No significant differences in susceptibility were found between the Rough Lemon and Troyer Citrange rootstocks. Results obtained from the first trial correlated well with the second trial for both rootstocks (Fig. 8).

DISCUSSION

The fruit inoculation technique used in this study was found to provide a rapid method for screening *Phytophthora* and *Pythium* isolates for pathogenicity. The high moisture content in fruit plus the incubation temperature were ideal for growth of *Phytophthora* and *Pythium* spp. This technique works particularly well for *Phytophthora* spp., since brown rot symptoms are readily induced through artificial inoculation. It may be possible to measure and determine various levels of pathogenicity amongst different isolates using this technique. The technique

is inexpensive, reliable and makes it possible for one to screen a large number of isolates with accuracy under laboratory conditions.

The results obtained from the fruit assay correlated well with growth studies conducted using *Phytophthora* isolates. Thus the pathogenic isolates also grew most rapidly on PDA. However, there was one exception, for example, MOC 370 which grew faster on PDA but was the least virulent of all *Phytophthora* isolates tested. The loss of pathogenicity of this isolate could be attributed to long storage. *Phytophthora* spp. are known to lose their pathogenicity after prolonged storage (Apple, 1957; Goth, 1981).

Results obtained from the lupin assay were contrary to those obtained from the fruit assay, since all *Pythium* isolates were found to be pathogenic. The lupin assay was thus found to be less accurate in determining the virulence of *Pythium* isolates. This was because the scale used for ranking seedling mortality was largely based on visual observations. This made it difficult to quantify the pathogenicity of each *Pythium* isolate.

A number of citrus rootstock-screening methods have been reported. These methods include immersing intact roots system in a concentrated zoospore suspension (Tsao & Garber, 1960; Carpenter & Furr, 1962; Cameron *et al.*, 1972) or artificially inserting the inoculum (hyphae, sporangia, and zoospores) on wounded stem tissue (Grimm & Hutchison, 1973; Smith, Hutchison & Henderson 1987; Afek *et al.*, 1990). In this study, rootstocks were used to determine the pathogenicity of *Phytophthora* and *Pythium* spp., and to compare the response of these rootstocks to artificial inoculation with the fungi. Methods with similar objectives have been reported for apples (Borecki & Millikan, 1969; Jeffers *et al.*, 1981) and avocado (Dolan & Coffey, 1985).

Phytophthora nicotianae and *P. citrophthora* isolates were pathogenic on Rough Lemon and Troyer Citrange rootstocks. Variation in pathogenicity among the different *Phytophthora* isolates was observed. *Phytophthora citrophthora* isolates were more pathogenic than *P. nicotianae* isolates. This observation may imply that foot rot symptoms could be more severe if caused by *P. citrophthora*. No significant differences in susceptibility were found between the two rootstocks. These results suggest that this technique is more suitable in determining the virulence of different *Phytophthora* isolates rather than as a rootstock screening technique,

since it is known that Rough Lemon is more susceptible than Troyer Citrange to *P. cinnamomi* (Rabe & Von Broembsen, 1991).

Pythium isolates were either avirulent or only weakly pathogenic. Lesions produced by some *Pythium* isolates were largely superficial and did not produce foot rot symptoms. Results obtained from this study indicate that although *Pythium* spp. occur in abundance in the rhizosphere of diseased citrus trees, they may not play a significant role in the development of the citrus root rot. Thus the relationship between the high *Pythium* population in the rhizosphere of diseased citrus trees and decline is still unclear.

Screening for virulent *Phytophthora* isolates must be a prerequisite for any project aimed at developing resistant citrus rootstocks. The fruit assay and rootstock screening technique used in this study proved to be rapid and reliable in determining the virulence and non-virulence of different *Phytophthora* and *Pythium* isolates. The use of such rapid techniques is thus recommended for determining the virulence of *Phytophthora* isolates in the future.

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Table 1. Pathogenicity of *Phytophthora* and *Pythium* isolates following inoculation into oranges.

Fungus		Source	No. of isolates screened	Mean diseased surface area (mm ²)
<i>Phytophthora</i>	<i>nicotianae</i>	r, rz	103	123
<i>P.</i>	<i>citrophthora</i>	r, rz, #	17	231
<i>Pythium</i>	<i>irregulare</i>	r, rz	108	13
<i>P.</i>	<i>aphanidermatum</i>	r, rz	9	10
<i>P.</i>	<i>paroecandrum</i>	rz	12	12
<i>P.</i>	<i>vexans</i>	rz	16	0
<i>P.</i>	<i>rostratum</i>	rz	18	0
<i>P.</i>	<i>ultimum</i>	r, rz	15	11
<i>Pythium</i>	group G & F	rz	23	0

p = pathogenic, wp = weakly pathogenic, np = non-pathogenic, c = culture collection, r = directly isolated from diseased roots, rz = rhizosphere, # One isolate from the FABI culture collection.

Table 2. Pathogenicity of *Pythium* spp. on lupin seedlings.

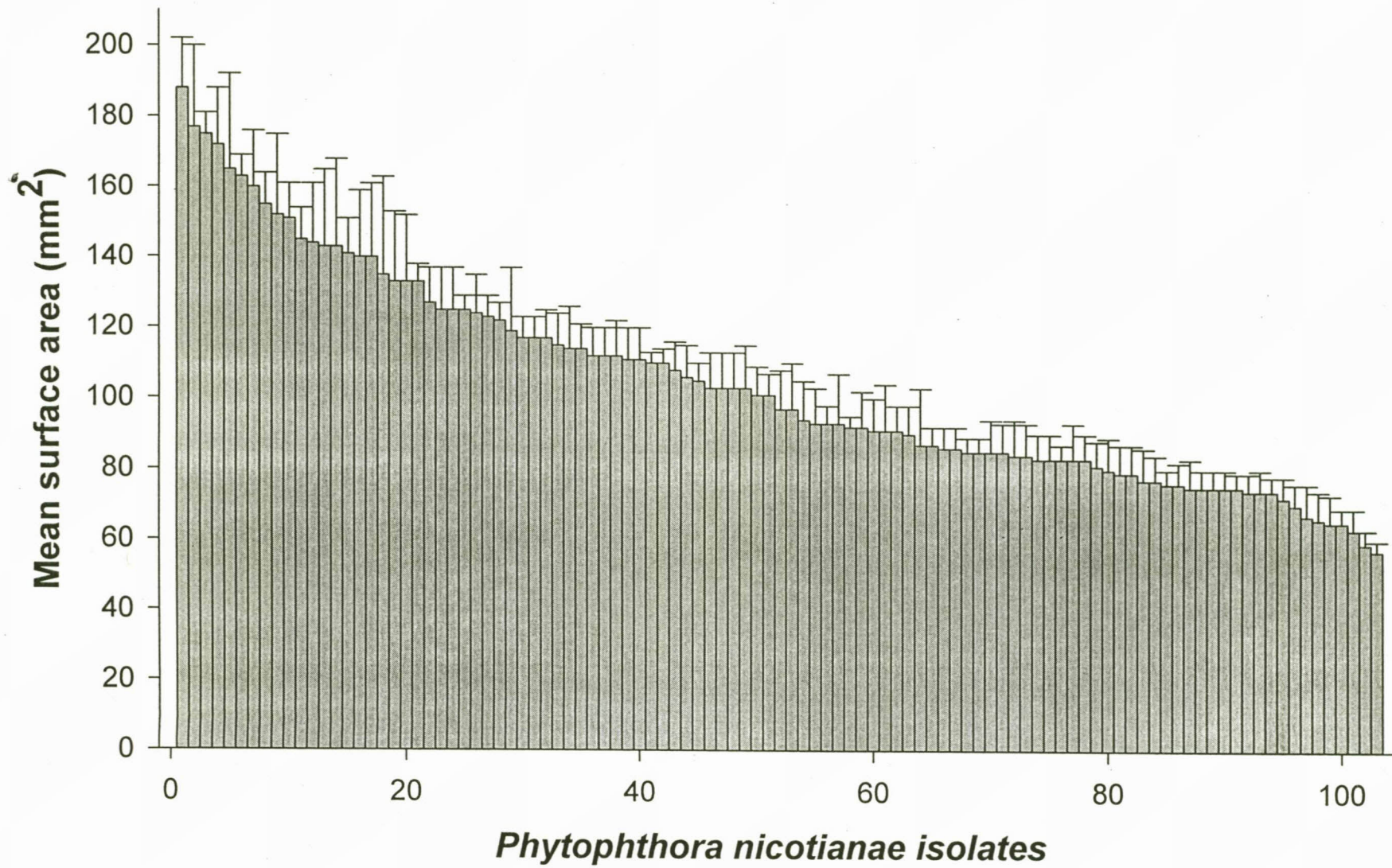
<i>Pythium</i> species	Source of the 2 isolates used	% Mortality after 1 week	%Mortality after 2 weeks
# <i>P. acanthicum</i> Drechsler	Soil ^b	45	80
* <i>P. debaryanum</i> Hesse	Soil ^a , Soil ^b	55	90
* <i>P. irregulare</i> Buisman	Roots ^a , Soil ^b	80	100
* <i>P. paroecandrum</i> Hesse	Soil ^b , Soil ^a	50	100
# <i>P. rostratum</i> Butler	Soil ^b	40	75
* <i>P. ultimum</i> Trow	Roots ^a , Soil ^b	70	100
# <i>P. vexans</i> de bary	Soil ^a	65	95
<i>P.</i> group G	Soil ^b	40	80
Control	-	0	0

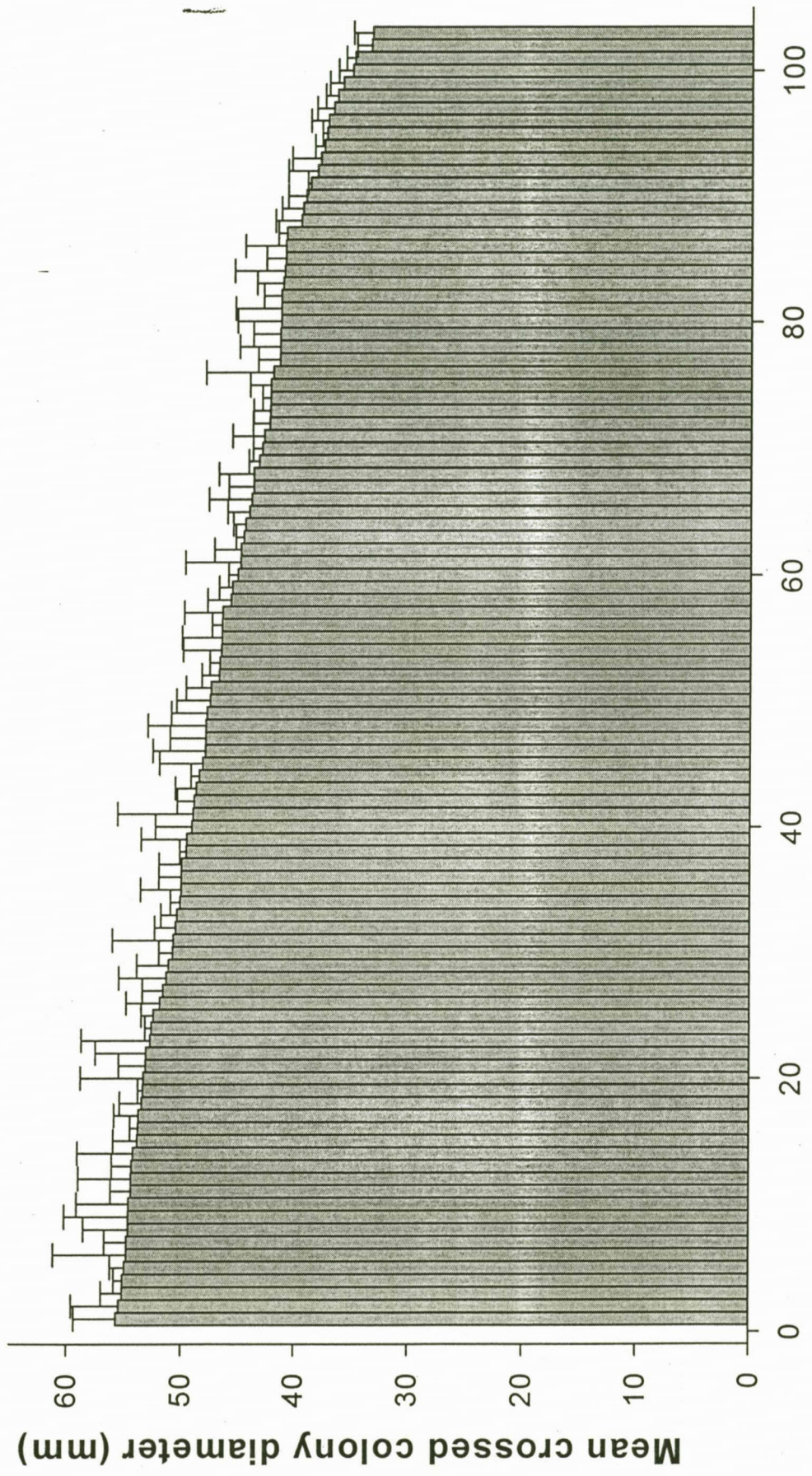
^a Isolates obtained from the nursery, ^b Isolates obtained from the orchards, *Weakly pathogenic species based on the fruit assay. #Non pathogenic species based on the fruit assay

Figure 1: Results obtained following artificial inoculation of citrus fruit with different *Phytophthora* and *Pythium* isolates. **A1.** Control. **A2** and **5** *Phytophthora citrophthora* and *P. nicotianae*, respectively. **A3** and **4** *Pythium irregulare* and *P. rostratum*, respectively



Figure 2: Results of the citrus fruit inoculation with 103 *Phytophthora nicotianae* isolates. Bars with standard errors represent means of lesions produced on the rind surface, isolates were ranked from the most to least pathogenic.





Phytophthora nicotianae isolates

Figure 4: Results of the citrus fruit inoculation with 17 *Phytophthora citrophthora* isolates. Bars with standard errors represent means of lesions produced on the rind surface. Isolates were ranked from least pathogenic to most pathogenic isolate.

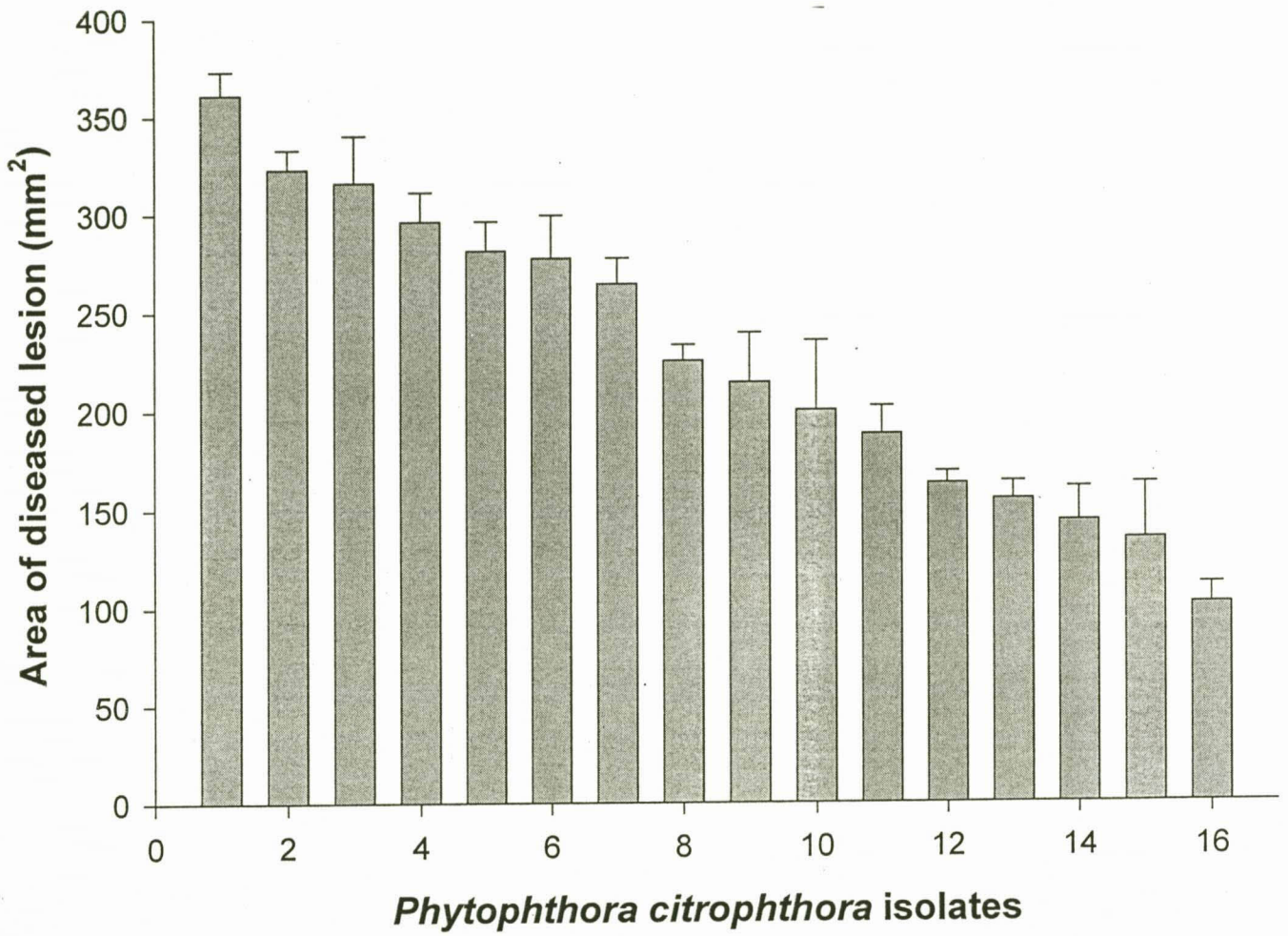


Figure 5: The results of a growth study conducted on 17 *Phytophthora citrophthora* isolates associated with declining citrus trees. Bars with standard errors represent means of the colony diameters (mm) on PDA plates incubated at 25 ° C. Isolates were ranked from the fastest to slowest growing isolates.

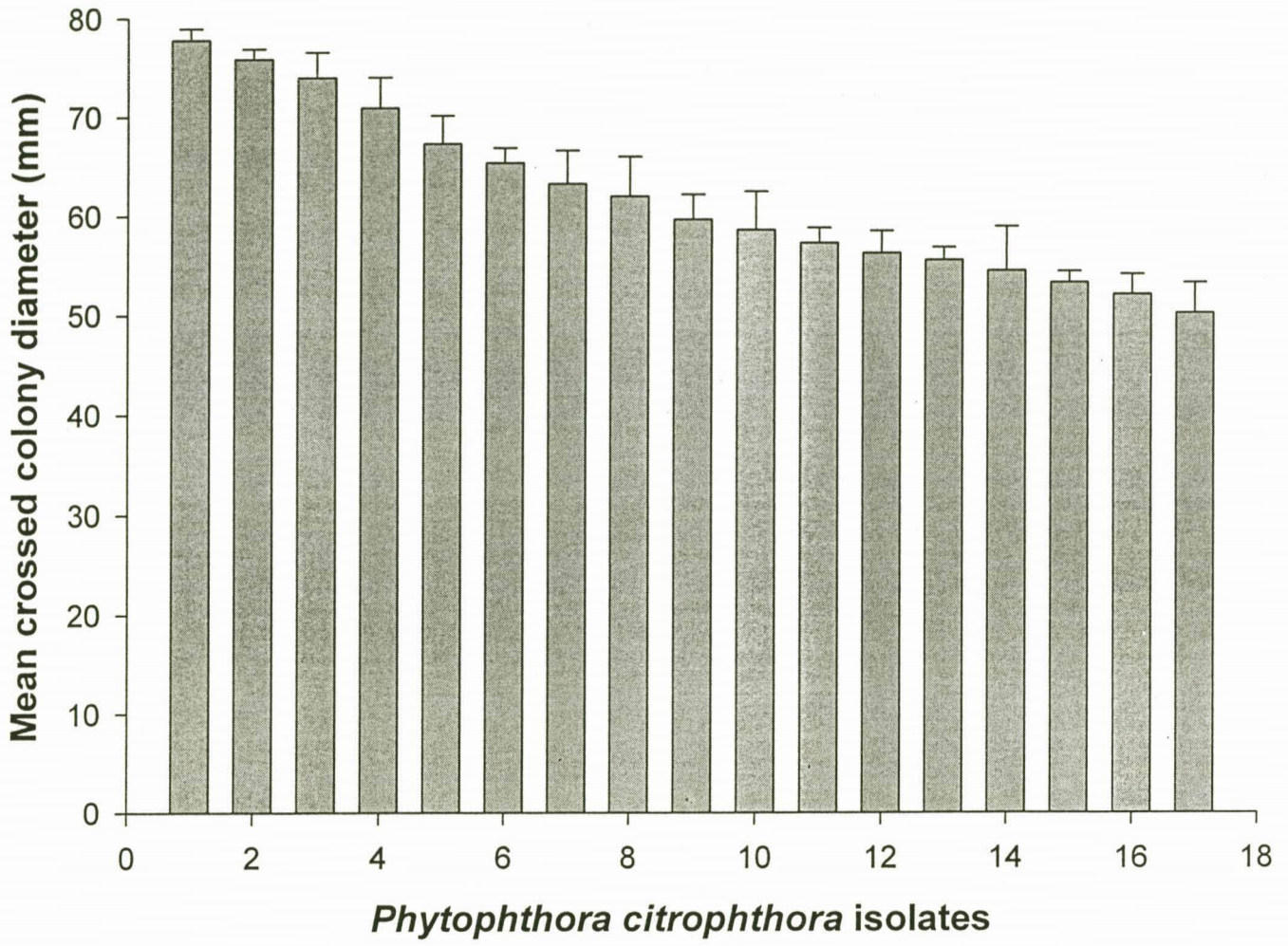


Figure 6: Correlation between *Phytophthora* lesions on fruit surface versus colony diameter on PDA

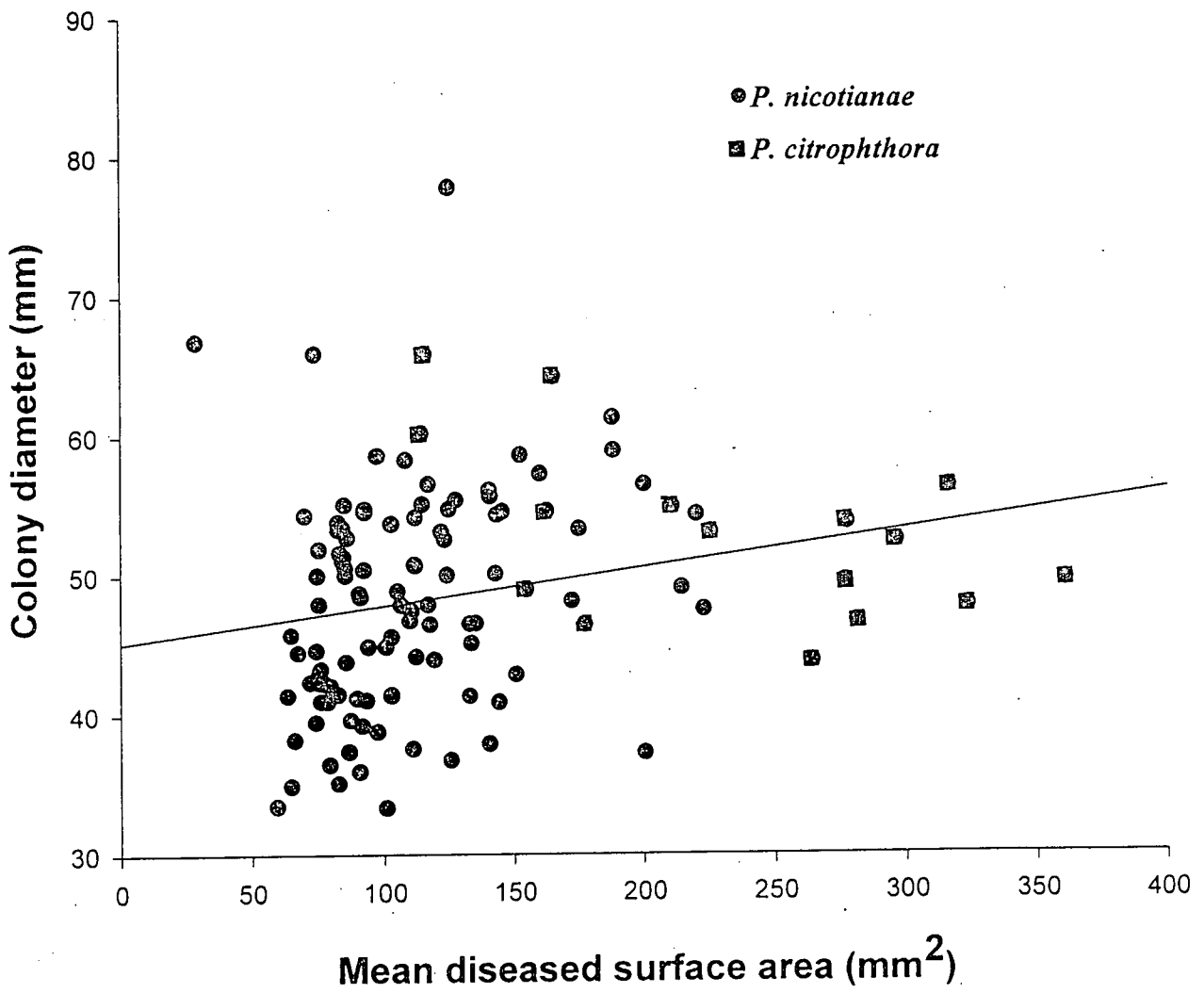
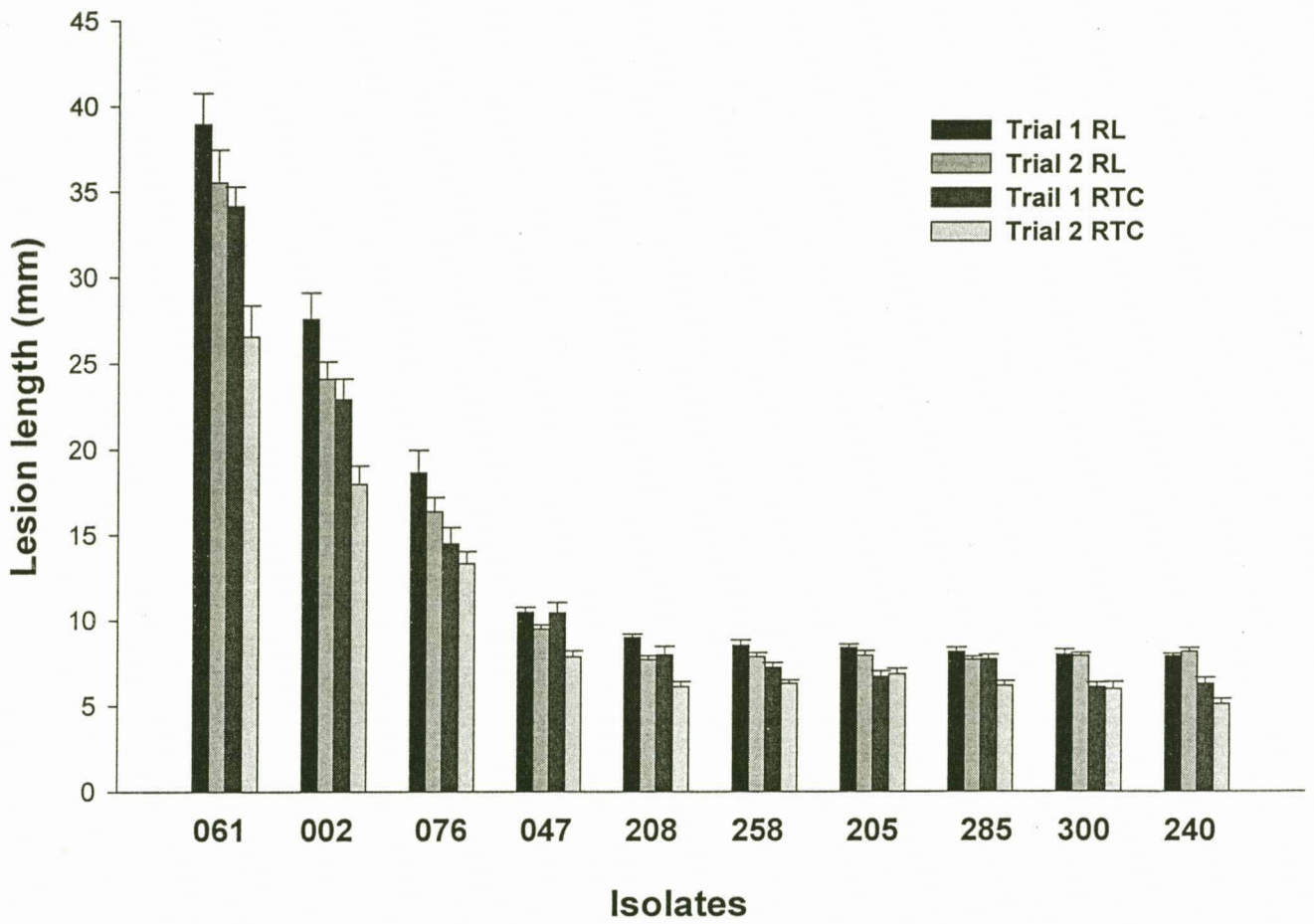


Figure 7: Results obtained following artificial inoculation of Rough Lemon rootstock with different *Phytophthora* and *Pythium* isolates after three weeks. **A.** Stems of Rough Lemon with and without the bark removed. The arrow shows brown discoloured lesion. **B.** Varying lesions produced by *Phytophthora* and *Pythium* isolates. **1.** *Pythium irregulare* lesion. **2** and **3** *Phytophthora citrophthora* lesions. **4.** *Phytophthora nicotianae* lesion. Scale bar: 10mm.



Figure 8: Comparison between Troyer Citrange (RTC) and Rough Lemon(RL) rootstocks after inoculation with isolates of *Phytophthora* and *Pythium* spp.



Summary

Research conducted in this thesis explores the role that *Phytophthora* and *Pythium* species play in citrus and eucalypt root rot in South Africa. The first chapter presents an extensive literature review with special emphasis on the importance of *Phytophthora* and *Pythium* spp. to the South African Citrus and Forestry Industries. This chapter is divided into two parts. Part one deals with biotic and abiotic factors that contribute to citrus decline. Part two focuses on recently published work on root diseases of exotic forest trees species planted commercially in South Africa. Special emphasis was placed on *P. cinnamomi* since it is thought to be the most important pathogen in the forestry industry.

Results obtained in Chapter Two indicate that a wide variation in tolerance and susceptibility to *P. cinnamomi* exists amongst half-sib families of *E. fraxinoides*. Field mortality of 52 % and 30% was recorded at two trial sites following natural infection. Seven disease tolerant families were identified with the potential to be used for commercial propagation. *Eucalyptus fraxinoides* families tolerant to *P. cinnamomi* were more reliably identified using stem inoculation of young trees relative to selecting families that survived the disease after planting in the field.

In Chapter Three half-sib families of *E. fraxinoides* and *E. smithii* were evaluated for tolerance to *P. cinnamomi* using stem inoculations in the greenhouse. Three replicate trials for each species were conducted at different times of the year. Disease tolerance and susceptibility varied between and among the different *E. fraxinoides* and *E. smithii* families. No correlation was found between the results obtained from the replicate trials of *E. fraxinoides* and *E. smithii*. The technique used was found to be unreliable for screening *E. fraxinoides* and *E. smithii* families for tolerance to *P. cinnamomi*.

Results of a preliminary survey in selected citrus nurseries and orchards in the Northern and Mpumalanga provinces of South Africa are presented in Chapter Five. A total of 320 *Phytophthora* and *Pythium* isolates were recovered from the rhizosphere of diseased citrus trees and diseased plant material. *Phytophthora nicotianae* and *Pythium irregulare* were the most frequently isolated species. Sixteen isolates of *P. citrophthora* were successfully recovered. This species has previously been presumed to be absent from the sampled regions.

In the last Chapter of this thesis, pathogenicity tests were conducted on *Phytophthora* and *Pythium* spp. associated with citrus root rot. *Phytophthora* isolates proved to be pathogenic when inoculated into citrus fruit while *Pythium* spp. were either weakly or non pathogenic. Contrary results were

found when the lupin assay was conducted since all *Pythium* spp. proved pathogenic. *Phytophthora* isolates were also found to be pathogenic on Rough Lemon (RL) and Troyer Citrange (RTC) rootstocks whereas *Pythium* spp. were either weakly pathogenic or avirulent. No difference in susceptibility was observed between RL and RTC. The fruit assay and stem inoculation techniques were found to be reliable, inexpensive and rapid results were obtained.

Results obtained in this study indicated that *Phytophthora* spp. play an important role in citrus and eucalypt root rot. Artificial stem inoculation using a single virulent isolate of *P. cinnamomi* proved to be a reliable technique for screening young eucalyptus trees in the field. However, contrary results were obtained when this technique was used to screen half-sib seedlings of *E. fraxinoides* and *E. smithii* in the greenhouse.

Eight *Pythium* and two *Phytophthora* spp. were isolated from the rhizosphere soil and declining citrus trees. *Phytophthora* species proved to be pathogenic when inoculated into citrus fruit and on Rough Lemon and Troyer Citrange rootstocks. On the other hand *Pythium* species screened proved to be nonpathogenic or only weakly pathogenic and thus probably play an insignificant role in citrus decline.

OPSOMMING

Die navorsing in hierdie tesis handel oor die rol wat *Phytophthora* en *Pythium* spesies in sitrus en *Eucalyptus* wortelvrot speel. Die eerste hoofstuk behels 'n uitgebreide literatuur-oorsig met spesifieke verwysing na die belangrikheid van *Phytophthora* en *Pythium* spesies in die Suid-Afrikaanse sitrus- en bosbouindustrie. Hierdie hoofstuk bestaan uit twee dele. Die eerste deel fokus op die biotiese en abiotiese faktore wat aanleiding gee tot die agteruitgang van sitrusbome. Die tweede deel fokus op onlangse publikasies oor wortelsiektes van eksotiese bome wat kommersieel in Suid-Afrika geplant word. Spesiale aandag is aan *P. cinnamomi* gegee, aangesien dit as die belangrikste wortelpatogeen in die bosbou-industrie gesien word.

Resultate in hoofstuk twee bewys dat daar aansienlike variasie in toleransie en vatbaarheid is in half-sib families van *Eucalyptus fraxinoides* teenoor *P. cinnamomi*. In twee *E. fraxinoides* proewe is onderskeidelik 52% en 30 % mortaliteit aangeteken na natuurlike infeksie deur *P. cinnamomi*. Sewe weerstandbiedende families is vir potensiële gebruik in kommersiële propagering geïdentifiseer. Weerstandbiedendheid van *E. fraxinoides* families teenoor *P. cinnamomi* is beter geïdentifiseer met stam-inokulasies van jong bome, as met oorlewing na natuurlike infeksie in die veld.

In hoofstuk drie word half-sib families van *E. fraxinoides* en *E. smithii* geëvalueer vir hul toleransie vir *P. cinnamomi* met behulp van stam-inokulasies. Drie herhalings van die proewe is gedoen vir elke spesie op verskillende tye van die jaar. Siekte toleransie en vatbaarheid het gevarieer tussen en binne *E. fraxinoides* en *E. smithii* families. Geen korrelasie kon egter gevind word tussen die resultate van die herhalings van die proewe met *E. fraxinoides* en *E. smithii*. Inokulasies in glashuise was dus nie betroubaar vir selektering van *E. fraxinoides* en *E. smithii* families vir toleransie vir *P. cinnamomi* nie.

Die resultate van 'n voorlopige ondersoek na die invloed van wortelsiektes in geselekteerde sitruskwekerie en -boorde in die Noordelike Provinsie en Mpumalanga in Suid Afrika word in hoofstuk vyf gegee. 'n Totaal van 320 *Phytophthora* en *Pythium* isolate is geïsoleer vanuit die risosfeer van siek sitrusbome en plant-materiaal. *Phytophthora nicotianae* en *Pythium irregulare* was die mees algemeen geïsoleerde spesies. Sestien isolate van *P. citrophthora* is ook gevind. Hierdie spesie is vroeër as afwesig in die gemelde gebiede aangeteken.

In die laaste hoofstuk van die tesis is patogenisiteits toetse gedoen met *Phytophthora* en *Pythium* spesies wat geassosieer word met sitrus wortelvrot. *Phytophthora* isolate was patogenies wanneer

hulle op sitrusvrugte geïnkuleer is, terwyl *Pythium* spesies swak of glad nie patogenies was nie. Teenstrydig met lg. resultate het die lupin analiese egter gewys dat alle *Pythium* spesies patogenies is. *Phytophthora* isolate was patogenies op Rough Lemon (RL) en Troyer Citrange (RTC) wortelstok variëteite, terwyl *Pythium* spesies swak patogenies of avirulent was op hierdie variëteite. Daar was geen verskil in vatbaarheid tussen RL en RTC nie. Die vrugte- en stam-inokulasie tegnieke het betroubare, ekonomiese en vinnige resultate gelewer.

Die resultate van hierdie studie het gewys dat *Phytophthora* spesies 'n belangrike rol speel in sitrus en *Eucalyptus* wortelvrot. Stam-inokulasies met 'n enkele virulente *P. cinnamomi* isolaat kon suksesvol gebruik word om te selekteer vir toleransie in jong bome in die veld. Die tegniek het egter onbetroubare resultate gelewer tydens die ondersoek na toleransie in *E. fraxinoides* en *E. smithii* in glashuise.

Agt *Pythium* en *Phytophthora* spesies is geïsoleer van die risosfeer van siek sitrus-plante en die plantmateriaal self. Stam-inokulasies het gewys dat *Phytophthora* spesies patogenies is op Rough Lemon en Troyer Citrange wortelstok variëteite. *Pythium* spesies aan die ander kant was nie patogenies nie of swak patogenies op hierdie wortelstok variëteite en speel waarskynlik 'n minder belangrike rol in sitrusboom agteruitgang.