Fungal diseases in *Eucalyptus* and *Acacia* nurseries in South Africa

Submitted by

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Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Magister Scientiae to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other University.

Lorenzo Lombard

February 2004

This thesis is dedicated to my parents, Lorenzo Z. C. Lombard and Lee E. Lombard

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Preface

The forestry nursery system in South Africa has undergone major changes in the past two decades, with the implementation of clonal forestry. Vegetative propagation of superior *Eucalyptus* hybrid clones have resulted in uniform, high – value plantations. However, losses to planting stock in nurseries can severely affect planting programmes. Most losses in South African forestry nurseries are caused by fungal diseases. *Acacia mearnsii* (black wattle) seedlings are severely affected by an unknown *Cylindrocladium* species in nurseries. Diseases of *Eucalyptus* cuttings and *A. mearnsii* seedlings are a serious threat in forestry in South Africa and require further study.

Chapter one of this dissertation presents a review on the pathogens affecting crops in hydroponics and possible implications of diseases in *Eucalyptus* hedge plants maintained in hydroponics. The aim of this review is to consider the prospect of *Eucalyptus* nursery pathogens becoming a limiting factor in hydroponic systems and it also treats the general topic of diseases in South African forestry nurseries. No information is available on pathogens related to *Eucalyptus* spp. in hydroponics. The possible pathogens, potential symptom expression and possible control measures are considered. This sets the stage for the rest of the dissertation and highlights the importance of nursery pathogens.

The experimental section of this dissertation focuses on nursery diseases of *Eucalyptus* and *Acacia mearnsii*. In chapter two, a survey of *Eucalyptus* hedge plants, maintained in an ebb and flow hydroponic system, was conducted to determine which pathogens are present. Fungal isolates obtained during the survey were characterized based on morphology and where appropriate, identifications were supported by DNA sequence data comparisons.

Chapter three of this dissertation considers the importance of pathogens in *Eucalyptus* cutting production. A survey was conducted to determine which pathogens influence productivity in cutting production nurseries in KwaZulu – Natal, South Africa. A dominant pathogen was identified based on morphological characteristics and DNA sequence data comparisons. A pathogenicity test was also conducted to determine the susceptibility of *Eucalyptus* hybrids to the pathogen.

In chapter four, the identity of an unknown *Cylindrocladium* sp. affecting *Acacia mearnsii* seedling production is identified. This fungus was characterized based on morphological characteristics and using DNA sequence data comparisons. The pathogenicity of this pathogen was also considered.

Chapter 1

An evaluation of the potential importance of pathogens to *Eucalyptus* plants propagated in hydroponics

Introduction

During the course of the past two decades, the South African forestry industry has made the major step of implementing clonal forestry (Van Wyk 1985, Denison & Quaile 1987). Thus, vegetatively propagated tropical and sub – tropical *Eucalyptus* spp. and their hybrids have been deployed widely in commercial plantations in South Africa (Denison & Kietzka 1993a,b). Clonal forestry allows for a small number of clones to be vegetatively propagated (White 1995) resulting in uniform plantations of selected, high value trees (Kulkarni & Lal 1995). The development of clonal hedge plants that are necessary for vegetative propagation is time consuming (Van Wyk 1985) and can take up to 18 months before they become productive (Wilson 1998, Aimers – Halliday *et al.* 1999). Mondi Forests in South Africa has thus embarked on the innovative step of speeding up clonal reproduction through the production of clonal – hedge plants using hydroponics.

Hydroponics represents the technology of growing plants in nutrient solution with or without the use of a substrate to provide mechanical support to the root system (Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Jensen 1997, Jensen 1999). In a liquid hydroponic system, no inert substrate is used, while in an aggregate hydroponic system inert substrates such as those composed of sand, gravel, peat, perlite, vermiculite and rock wool are utilized. Hydroponic systems are also further classified as being either open or closed systems. In closed systems, the nutrient solution is recovered, replenished and recycled following the direct delivery to the root systems. In open systems, the nutrient solution is not reused (Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Jensen 1997, Jensen 1999). Various types of hydroponic systems are commonly used in commercial nurseries. These include the nutrient film technique (NFT), deep flow technique, trough culture, ebb and flow, rock wool culture, sand culture and bag culture (Graves 1983, Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Jensen 1997, Paulitz & Belanger 2001).

Hydroponic crop production is, in combination with greenhouses, a high - technology and capital-intensive approach (Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Jensen 1997, Jensen 1999). It allows for high-density maximum crop yield, production of crops in areas where unsuitable soils exist, a lack of dependence on ambient temperature and seasons, more efficient use of water and fertilizers and minimal land use. Hydroponics systems lend themselves to mechanization, which also enhances production. The plants can be isolated from the soil, which is often associated with disease problems, salinity, poor structure and drainage (Stanghellini & Rasmussen 1994, Jensen 1999). Major disadvantages of hydroponics include the high costs of capital and energy inputs as well as requiring a high degree of competence in plant science and engineering skills. High operation costs of hydroponic systems have tended to limit this technology to high economic value crops (Stanghellini & Rasmussen 1994, Jensen 1999).

Hydroponics is currently employed worldwide to produce flower, foliage, bedding plants and high value vegetable crops (Jensen & Collins 1985, Stanghellini & Rasmussen 1994). In South Africa, *Eucalyptus* clonal-hedge plants are grown in an ebb and flow system for cutting production (Dr. B. Janse, personal communication). These systems are based on a "flood and drain" principle that allows for the rapid production of *Eucalyptus* clonal cuttings.

One of the problems that can be associated with hydroponics is that of root diseases and especially diseases that are suited to spread in liquid media. Given that hydroponics is new to forestry in South Africa, virtually nothing is known regarding the possible impact of diseases in this system.

Several reviews and surveys have been published on diseases of *Eucalyptus* spp. in nurseries (Sharma *et al.* 1984, Sharma *et al.* 1985, Crous *et al.* 1991, Sharma & Mohanan 1992, Viljoen *et al.* 1992, Brown & Ferrreira 2000). These have included diseases in both seedling and clonal cutting systems. To the best of our knowledge, there have, however, been no reviews of diseases in nurseries where *Eucalyptus* are grown in hydroponics. The aim of this review is not to repeat the contents of previous reviews on diseases in *Eucalyptus* nurseries, but rather to consider the prospect of *Eucalyptus* nursery pathogens becoming a limiting factor in plants grown in hydroponics. The possible pathogens and potential symptom expressions that might occur in and on the hydroponically grown plants will be discussed and possible control measures that might be applied considered.

Eucalyptus Nursery Diseases

The nursery system for a *Eucalyptus* planting programme is a key component of any plantation industry. The planting programme can be severely affected by the loss of planting stock or by the production of inferior plants (Brown & Ferreira 2000). Both abiotic and biotic agents can cause damage to planting stock, with conditions in nurseries being very conducive to disease (Viljoen *et al.* 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000).

Fungi are responsible for the majority of the diseases associated with *Eucalyptus* species in nurseries (Viljoen *et al.* 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000). These diseases include death of germinating seedlings, foliar, stem and root diseases (Alfenas *et al.* 1997, Brown & Ferreira 2000). The most important diseases include damping – off, root rot, seedling, shoot or web blight, leaf spots, mildew and rust (Peterson & Smith 1975, Sharma *et al.* 1984, Sharma *et al.* 1985, Sharma & Mohanan 1992, Viljoen *et al.* 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000).

Damping – off is a disease of young seedlings prior to lignification, and leads to the collapse of the seedling (Brown & Ferreira 2000). Infection takes place at the soil – air interface and will result in girdling diseases (Holliday 1990). Sharma *et al.* (1985) showed that a complex of pathogens could be responsible for the disease, which can occur prior to emergence (pre-emergence damping – off) or after seedlings have emerged (post-emergence damping – off). Damping – off of

Eucalyptus seedlings is caused by soilborne, seedborne or waterborne fungi belonging to genera *Botrytis* P. Micheli ex Pers., *Calonectria* de Not., *Cylindrocladium* Morgan, *Fusarium* Link, *Phytophthora* de Bary and *Rhizoctonia* DC. (Sharma *et al.* 1984, Sharma *et al.* 1985, Crous *et al.* 1991, Blum *et al.* 1992, Sharma & Mohanan 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000, Sanfuentes *et al.* 2002).

Root rot is the second most commonly found disease in forestry nurseries (Sharma *et al.* 1985, Sharma & Mohanan 1992, Alfenas *et al.* 1997). This disease leads to the slow wilting of seedlings and cuttings (Sharma *et al.* 1985, Sharma & Mohanan 1992). Root rot is characterized by a change of pigmentation in the leaves from green to light purple. Within a week of infection, the pigmentation change moves downwards and is followed by wilting and eventual death of plants. The roots of plants are completely damaged with the roots turning light or dark brown. The infection usually begins in the lateral roots and proceeds to the main root system. The pathogens can then move into the root collar region causing decay and death (Sharma *et al.* 1984, Sharma *et al.* 1985, Crous *et al.* 1991, Crous *et al.* 1993, Sharma & Mohanan 1992, Viljoen *et al.* 1992). Some genera of pathogens responsible for root rot of *Eucalyptus* in nurseries include *Rhizoctonia* (Sharma *et al.* 1985, Sharma & Mohanan 1992, Viljoen *et al.* 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000, Sanfuentes *et al.* 2002), *Phytophthora* spp., *Pythium* Nees spp. (Marks & Kassaby 1974, Von Broembsen 1984, Sharma & Mohanan 1992, Brown & Ferreira 2000) *Cylindrocladium* spp (Hodges & May 1972, Crous *et al.* 1991, Sharma &

Mohanan 1992, Crous *et al.* 1993, Alfenas *et al.* 1997, Brown & Ferreira 2000) and *Fusarium* spp. (Arya & Jain 1962, Sharma *et al.* 1985, Brown & Ferreira 2000).

Seedling and shoot blight are related diseases that share the same symptoms (Sharma *et al.* 1985, Crous *et al.* 1991). Seedling blight results from infection of either the stem of the seedlings or cuttings, or the leaves. Shoot blight is characterized by infection of the stem at soil level (Bolland *et al.* 1985, Sharma *et al.* 1985, Crous *et al.* 1991, Crous *et al.* 1993, Alfenas *et al.* 1997, Sanfuentes *et al.* 1999). Infection of the stem leads to the development of a grayish brown to dark brown lesion on the stem, which leads to desiccation of the seedlings or cuttings (Bolland *et al.* 1985, Sharma *et al.* 1985, Crous *et al.* 1991, Crous *et al.* 1993, Sanfuentes *et al.* 2002). Leaf infections usually begin at the tips of the leaves and extend down the length of the leaves towards the stems where lesions develop (Sharma *et al.* 1985, Sharma & Mohanan 1992). In conditions of high humidity, such as those encountered in greenhouses, these lesions can give rise to masses of spores and this increased inoculum significantly exacerbates disease conditions. Pathogens responsible for both seedling and shoot blight include *Rhizoctonia solani* Kuhn, *Botrytis cinerea* Pers., *Phytophthora* spp., *Cylindrocladium* spp. and *Cylindrocladiella* Boesew. spp. (Barnard 1984, Bolland *et al.* 1985, Sharma *et al.* 1985, Crous *et al.* 1991, Crous *et al.* 1993, Alfenas *et al.* 1997, Crous 2002, Sanfuentes *et al.* 2002).

Web blight is a common *Eucalyptus* seedling and cutting disease, occurring in conditions of over – crowding and high humidity (Sharma & Mohanan 1992, Alfenas *et al.* 1997). This disease is caused by *Rhizoctonia solani* and is characterized by mycelium emerging from the soil to grow epiphytically on the stems and leaves of plants. This mycelium gives rise to a cobweb – like profuse mycelial growth that entangles the infected seedlings or cuttings (Sharma *et al.* 1985, Sharma & Mohanan 1992, Alfenas *et al.* 1997, Sanfuentes *et al.* 1999, Sanfuentes *et al.* 2002).

Leaf spot diseases are commonly found on *Eucalyptus* seedlings and cuttings in nurseries. These leaf spots range in appearance from small, round water – soaked lesions, red to black in colour to larger irregular necrotic lesions. Several genera of pathogens are responsible for leaf spot and some of them are *Hainesia lythri* (Desm.) Hohn. (Palm 1991), *Mycosphaerella* Johanson spp., *Phaeophleospora* Rangel spp. (Crous *et al.* 1989a,b), *Cylindrocladium* spp. and *Cylindrolcadiella* spp. (Bolland *et al.* 1985, Sharma *et al.* 1985, Crous *et al.* 1991, Viljoen *et al.* 1992, Crous *et al.* 1993, Alfenas *et al.* 1997, Crous 2002).

Powdery mildew can cause significant nursery problems but rarely kills *Eucalyptus* seedlings and cuttings. Powdery mildews are responsible for symptoms that include leaf distortions, shoot discolouration and reduction in growth of nursery stock (Marks 1981, Marks *et al.* 1982, Brown & Ferreira 2000). Powdery mildews produce superficial mycelium on the surface of leaves and shoots and are regarded as obligate, biotrophic parasites on a wide host range

(Brown & Ferreira 2000). Infections are caused by air-dispersed conidia that germinate on dry surfaces in low humidity. The presence of free water inhibits germination (Sinclair *et al.* 1987). Seven species residing in the genera *Erysiphe* R. Hedw. Ex DC and *Sphaerotheca* Desv. cause powdery mildew of *Eucalyptus* (Brown & Ferreira 2000).

Eucalyptus rust, caused by *Puccinia psidii* Winter, is a potentially severe treat to *Eucalyptus* production worldwide (Alfenas *et al.* 1997, Coutinho *et al.* 1998, Alfenas *et al.* 1999). *Puccinia psidii* forms yellow uredinial pustules on juvenile *Eucalyptus* tissue that leads to necrosis and stunting of the infected tissues (Coutinho *et al.* 1998, Alfenas *et al.* 1999). Severe infections can occur under favourable conditions at mild temperatures and prolonged leaf wetness (Alfenas *et al.* 1999). Fortunately South Africa is still free of this pathogen but it has been reported from nurseries in South – and Central America and Asia (Coutinho *et al.* 1998).

Factors leading to disease development in hydroponics

The main driving force for the development of hydroponics in agriculture has been the avoidance of root diseases (Zinnen 1988, Stanghellini & Rasmussen 1994). Most common agricultural crop diseases are caused by soilborne pathogens (Stanghellini & Rasmussen 1994) infecting the roots and stems of these crops. Isolating the plants from these soilborne pathogens has become necessary for many intensively grown crops where enhanced productivity has been required (Jensen & Collins 1985).

Hydroponic cultivation has led to a decrease in the diversity of root-infecting microorganisms (Stanghellini & Rasmussen 1994, Zinnen 1988) but a few pathogens have become more prominent and devastating in hydroponic systems (Paulitz 1997). Stanghellini & Kronland (1986) found that *Pythium dissotocum* Drechs caused a yield loss of more than 50% in lettuce grown in hydroponics, while this pathogen is not a problem in soil grown lettuce. This indicates that disease losses of hydroponically grown crops can occasionally be greater than soil grown crops.

Several characteristics of hydroponics can increase the occurrence of disease (Paulitz & Berlanger 2001). Generally, clones of one genotype are grown in hydroponics, thereby reducing genetic diversity. Furthermore, these plants are planted at high densities and this can favour the movement of pathogens. A similar situation exists in conventional forestry practices where genotypic uniform hedges are cultivated in soil. However, soil provides biological "buffering", thereby limiting root-infecting pathogens by antagonism from other microorganisms and is subjected to nutrient competition and fungistatis (Paulitz 1997). In recirculating hydroponic systems, pathogens are not limited by biological "buffering" and can easily spread between plants. This is especially true for the zoosporic fungi that produce spores that are motile in liquid (Zinnen 1988, Stanghellini & Rasmussen 1994).

A small amount of inoculum in hydroponics systems can lead to infection and disease losses. The substrates used in hydroponics sometimes lack the microbial diversity and buffering capabilities of natural soil. Hydroponic systems also lack antagonists that can be important in reducing the impact of diseases in nurseries using soil or other forms of medium (Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Paulitz 1997, Alsanius *et al.* 2001). Without nutrient competition and fungistatis in the substrate, pathogens can rapidly become established and cause severe disease outbreaks.

The physical environment in hydroponic systems can be favourable for the growth of pathogens (Jensen & Collins 1985, Zinnen 1988, Stanghellini & Rasmussen 1994). To allow for optimal plant growth, the temperature and moisture regimes are strictly controlled and these regimes are similar to those optimal for infection by the pathogens. A study by Bates & Stanghellini (1984), for example, showed that specific pathogens become dominant in the nutrient solution at specific temperatures.

Diseases of crops grown in hydroponics

Reports of diseases of plants grown in soil culture far exceed those of plants grown in hydroponics (Paulitz 1997). Currently no information is readily available on diseases of *Eucalyptus* clonal hedge – plants grown in hydroponics. This is despite the fact that hydroponics have been widely used to propagate these plants in Brazil for more than a decade. In this section of the review, common pathogens causing diseases in hydroponically grown agricultural crops are considered in an attempt to illustrate their mechanism of spread and infection.

Zoosporic pathogens

Zinnen (1988) and Stanghellini & Rasmussen (1994) have reviewed many of the diseases occurring in agricultural hydroponic systems. They have shown that the most important pathogens of plants in hydroponics are zoosporic fungi, which is not surprising given the fact that these fungi spread by means of motile flagellate spores. These pathogens can cause root rot, seedling rot, stunting, yield loss and plant collapse. Reservoir and surface water, such as that from streams and rivers and inert substrates such as peat, provide a source of these pathogens in hydroponic systems. Some zoosporic fungi are also introduced by insects and these include *Pythium aphanidermatum* (Ebson) Fritzp. (Jenkins & Averre 1983, Bates & Stanghellini 1984, Stanghellini *et al.* 1988, Zinnen 1988, Moulin *et al.* 1994, Stanghellini & Rasmussen 1994, Stanghellini *et al.* 1996, Paulitz 1997, Wulff *et al.* 1998).

Most of the zoosporic pathogens reported in hydroponics are also well-recognized root pathogens of the same crops, grown in the field. There are more than 20 reported zoosporic pathogens in hydroponic systems (Zinnen 1988, Stanghellini & Rasmussen 1994). The most common species reside in the genera *Pythium*, *Phytophthora*, *Oplidium* Syd. and *Plasmopara* Schröt. (Zinnen 1988, Stanghellini & Rasmussen 1994).

The motile asexual zoospores encounter roots through chemotaxis; they attach, loose their flagella and encyst by forming a thick cell wall. A germ tube is produced from these encysted spores and this penetrates the roots. These events can take place within five minutes and depending on the fungus, the asexual life cycle can be completed within 12 hours. This can have serious consequences in a hydroponic system (Stanghellini & Rasmussen 1994, Paulitz 1997). Zoosporic pathogens rarely produce visible structures in the host tissue. The occurrence in plants is usually detected through the recognition of the macrosymptoms on the plants and is confirmed by laboratory analysis of the infected tissue (Bates & Stanghellini 1984, Zinnen 1988, O' Gara *et al.* 1997, Brown & Feirrera 2000).

Several *Pythium* and *Phytophthora* spp. are reported pathogens of *Eucalyptus* grown using traditional methods, e.g. soil culture. These include *P. aphanidermatum*, *P. debrayanum* R. Hesse, *P. intermedium* de Bary, *P. irregulare* Buisman, *P. myriotylum* Drechsler, *P. ultimum* Trow, *Ph. cinnamomii* Rands, *Ph. cryptogea* Pethybr. & Laff. and *Ph. nicotianae* de Haan (Marks & Kassaby 1974, Sharma *et al.* 1984, Von Broembsen 1984, Sharma *et al.* 1985, Sharma & Mohanan 1992, Belisario 1994, Brown & Ferreira 2000). All of these pathogens have also been reported from agricultural hydroponic crops (Jenkins & Averre 1983, Bates & Stanghellini 1984, Zinnen 1988, Stanghellini & Rasmussen 1994). Zoosporic pathogens usually attack only the young root tissue or undifferentiated root apices of *Eucalyptus* feeder roots. From these initial infections, the pathogens can spread to older roots and even the stems, resulting in root and stem lesions or cankers (Brown & Feirrera 2000). The primary symptoms are rot of the young and fine roots and subsequently the larger roots. The secondary symptoms result in decline of the plant that can lead to plant death. The lesions formed are usually dicoloured and water – soaked, although asymptomatic infections also occur. Infection by *Pythium* spp. generally does not proceed beyond this stage, as these fungi are usually restricted to non – lignified tissues in the young roots. *Phytophthora* spp. can produce secondary symptoms since they are not restricted to the non – lignified tissues (O' Gara *et al.* 1997, Brown & Feirrera 2000).

Non – zoosporic pathogens

Non – zoosporic fungi important in agricultural hydroponic systems reside in the genera *Fusarium*, *Colletotrichum* Corda and *Verticillium* Nees. These are well – known soilborne microorganisms that act mostly as wilt pathogens. They are introduced into hydroponic systems through air, for example *Fusarium oxysporum* f. sp. *radicis – lycopersici* Schlechtend.:Fr., and accidental introduction of soil into the nutrient solution (Paulitz 1997, Stanghellini & Rasmussen 1994, Zinnen 1988, Jenkins & Averre 1983, Mihuta – Grimm *et al.* 1990, Duffy & Defago 1999).

Amongst the non – zoosporic fungi, *Fusarium* species are the most common causal agents of disease hydroponics (Jenkins & Averre 1984, Zinnen 1988, Stanghellini & Rasmussen 1994). The diseases include wilting and root rot of specific hosts cultivated in a hydroponic system. The most predominant *Fusarium* species are *forma specialis* of *Fusarium oxysporum* Schlechtend. (Couteaudier & Alabouvette 1981, Jenkins & Averre 1984, Zinnen 1988, Stanghellini & Rasmussen 1994). *Fusarium oxysporum* f. sp. *radicis – lycopersici*, *F. o. lycopersici* Schlechtend.:Fr. and *F. o. cucumerinum* Schlechtend.:Fr. have been reported to cause wilting of several hydroponic crops that include tomatoes, cucumber and lettuce (Couteaudier & Alabouvette 1981, Jenkins & Averre 1984, Brammall & Lynch 1990).

Fusarium oxysporum f. sp. *eucalypti* Arya & G. L. Jain is the only form of *F. oxysporum* found to cause root rot and wilting of *Eucalyptus* seedlings in nurseries (Arya & Jain, 1962). This pathogen causes a brown discolouration of the vascular tissue of the stem and roots of the plants. This subsequently results in the roots becoming sticky and the tips of the plants start to droop. Leaves turn yellow and abscise and whole plants then begin to wilt (Arya & Jain 1962).

Colletotrichum coccodes Penz. has been found to cause significant losses of tomato seedlings because of root rot in hydroponics (Schneider *et al.* 1978, Daughtrey & Schippero 1980, Jenkins & Averre 1983). However, this pathogen has not yet been found on the roots of *Eucalyptus* seedlings or cuttings. The only pathogen in the same genus reported on *Eucalyptus* is *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Viljoen *et al.* 1992, Smith *et al.* 1998, Brown & Ferreira 2000). *Colletotrichum gloeosporioides* causes anthracnose of *Eucalyptus* seedlings and cuttings. The disease is characterized by discrete, round lesions surrounded by a red – purple border on *Eucalyptus* leaves. During wet weather, pink conidial masses exude from acervuli formed in the lesions (Viljoen *et al.* 1992).

Evans (1979) reported *Verticillium dahliae* Klebahn from the roots of wilted tomato plants grown using a nutrient – film technique. This pathogen has, however, not been reported on *Eucalyptus* plants. *Verticillium albo – astrum* Reinke & Berthold has been reported on *Eucalyptus* seedlings and is generally regarded as a seedborne pathogen that causes damping – off of seedlings (Harsh *et al.* 1992).

Disease control in hydroponics

Control of pathogens, once established in a hydroponic production system, is often difficult but can be successfully achieved. There are three primary categories of disease control available for use in hydroponics. They are biological methods, cultural and physical methods and chemical control.

Biological control

The most effective method of biological control is through use of resistant cultivars of plants. The choice of cultivar is dependant on the identification of the pathogens present (Stanghellini & Rasmussen 1994, Paulitz 1997, Paulitz & Bélanger 2001). Currently *Eucalyptus* breeding programmes select for resistance to plantation diseases but not for resistance to nursery pathogens (Denison & Kietzka 1993a). The use of disease free plants is the one alternative and in the case of hydroponics, they can be obtained through tissue culture (Mitha – Grimm *et al.* 1990). Even if the plant starts off disease free, it is impossible to maintain it under aseptic conditions, thus disease resistance is extremely important.

The use of antagonistic microorganisms is another approach to biological control. Unfortunately only one antagonist, *Streptomyces griseoviridis* (Mycostop, Kemira Biotech, Finland) has been registered for use against *Fusarium* spp. in commercial hydroponic systems. (Stanghellini & Rasmussen 1994). Investigations into antagonists that might be useful in hydroponics are, however, ongoing and are revealing positive results (Stanghellini & Rasmussen 1994, Berger *et al.* 1996, McCullagh *et al.* 1996, Paulitz 1997, Stanghellini & Miller 1997, Ongena *et al.* 1999, Paulitz & Bélanger 2001, Grosch *et al.* 2001).

The use of biocides has provided excellent control of root – infecting zoosporic pathogens in hydroponics. Several bacteria and fungi in the genera *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Rhodococcus*, *Acinetobacter*, *Corynebacterium*, *Candida* and *Torulopsis* produce these biocides. These microorganisms produce species-specific rhamnolipids responsible for the surfactant activity (Stanghellini & Miller 1997).

Cultural and physical control

Sanitation is the most important component of cultural control of pathogens. The removal of infected plants, sterilization of equipment and disinfection of recycled aggregate substrates is essential for a pathogen – free hydroponic system (Stanghellini & Rasmussen 1994). These principles do not only apply to hydroponics but should be part of management of any nursery.

There are numerous methods used for the elimination of pathogens from hydroponic nutrient solutions. These methods include filtration (van Os *et al.* 1999, Schwartzkopf *et al.* 1987, Runia 1995, Goldberg *et al.* 1992), ozonation (Vestergárd 1988, Vanachter *et al.* 1988, Runia 1995), ultraviolet irradiation (Zhang & Tu 2000, Schwartzkopf *et al.* 1987, Runia 1995, Acher *et al.* 1997, Sutton *et al.* 2000) and thermal inactivation (Runia & Amsing 2001, Schuerger & Mitchell 1992, Runia 1995).

Chemical control

The use of chemicals and particularly fungicides has typically been the preferred means of disease control in agriculture. This is because they can be used relatively easily and cheaply. Unfortunately no fungicides have yet been registered for use in hydroponics. The reason for this is that a lag time is required between application and harvest in order for fungicide residue to be at an acceptable level. This is impossible in hydroponics because harvesting of food crops is done daily. In forest crops such as *Eucalyptus*, a lag phase is not required since the products are not destined for human or animal consumption. However, the environmental impact of chemical application is a matter that is increasing in sensitivity.

Several investigators have found that the application of fungicides is very effective against some of the afore-mentioned pathogens. However, these chemicals could also have a negative effect on the crop grown in the hydroponic system. Price & Fox (1986) found that furalaxyl (Fongarid, Ciba – Geigy) inhibited the growth of *Phytophthora* and *Pythium* species in a NFT system. They also found a significant reduction in fruit development of cucumbers due to increased sodium levels but no disease development was noticed. The use of benomyl (Benlate, Du Pont) at very low concentrations (0.090 g a. i./ℓ) is very effective against *Fusarium* crown and root rot of tomatoes but can cause severe phytotoxic symptoms at higher concentrations (Mitha – Grimm *et al.* 1990).

Synthetic surfactants also occur in some fungicides, e. g. Manoxol, Triton X100, Sodium lauryl sulfate and Bavistin (Stanghellini & Miller 1997). Surfactants dissolve the unit membrane encasing zoospores and reduce their motility by disrupting the integrity and permeability of the plasma membrane (Stanghellini & Tomlinson 1987, Stanghellini & Rasmussen 1994, Stanghellini *et al.* 1996, Stanghellini & Miller 1997).

Addition of chitosan to nutrient solutions can increase the resistance of plants to infection by zoosporic pathogens. Chitosan (chitinase, chitosanase and β – 1,3 – glucanase) induce structural barriers in roots and stimulate the production of antifungal hydrolases in both roots and leaves. This chemical also causes wall loosening, vacuolation and protoplasm disintegration of zoosporic pathogens (Ghaouth *et al.* 1994).

Cherif & Belanger (1992) found that the addition of potassium silicate to nutrient solutions controls infections by zoosporic pathogens. This chemical also increases resistance in the plants by inducing structural barriers in the roots and also increases crop yields. However, Cherif & Belanger (1992) noted that the mechanism by which potassium silicate confers protection is still not fully understood.

Conclusions

Production using hydroponics is gradually increasing in both agriculture and horticulture. Hydroponics is an attractive yet over simplified technology, which is easier to promote than to sustain. This is indicated by the large number of failed applications of this technology (Jensen 1997). These failures can be attributed to several factors. One of these is disease caused by root infecting pathogens.

With the increase in demand for *Eucalyptus* products, forestry companies in South Africa must develop methods for the rapid production of plants. The new initiative of one of South Africa's major forestry companies to convert its

Eucalyptus production system to hydroponics is thus visionary. This system will clearly allow for the rapid production of large numbers of *Eucalyptus* cuttings in a small and environmentally controlled area.

There have been no previous investigations regarding the role of diseases that might cause damage to *Eucalyptus* plants grown in hydroponics. Several surveys of *Eucalyptus* nursery diseases have been conducted in the past (Sharma *et al*. 1984, Sharma *et al*. 1985, Sharma & Mohanan 1992, Viljoen *et al*. 1992, Brown & Feirrera 2000). Using knowledge gained from these surveys as well as information from hydroponically grown agricultural crops, it should be possible to identify potential pathogens relatively easily. This will not be sufficient to ensure that pathogens do not become important, but it will provide a foundation for the research that will be needed to reduce the impact of diseases. What is now required is a thorough survey of diseases in the emerging hydroponics nurseries in South Africa and in addition, potential pathogens will need to be evaluated for their relative importance.

Literature cited

Acher, A. A., Heuer, B., Rubinskaya, E. & Fischer, E. (1997) Use of ultraviolet – disinfected nutrient solutions in greenhouses. *Journal of Horticultural Science* **72**: 117 – 123.

Aimers – Halliday, J., Dibley, M. J., Hong, S. O. & Menzies, M. I. (1999) Cutting propagation of hybrid *Eucalyptus grandis x E. nitens*. *New Zealand Journal of Forestry Science* **29**: 238 – 250.

Alfenas, A. C., Old, K. M. & do Valle, L. A. C. (1999) *Eucalyptus* rust (*Puccinia psidii*): A threat to *Eucalyptus* plantations and native Myrtaceae. *12th Bienal Conference Canberra 1999, Asia-Pacific Plant Pathology for the Millenium, pp. 321.*

Alfenas, A. C., Silveira, S. F. da & Stowasser, E. A. S. V. (1997) Current status and control strategies of diseases associated to clonal propagation of *Eucalyptus* in Brazil. *IUFRO Conference on Silviculture and Improvement of Eucalyptus, Salvador, Brazil, 24 – 29 August.*

Alsanius, B. W., Hultberg, M. & Khalil, S. (2001) Microorganisms – Tools for closed hydroponic systems? *Acta Horticultura* **548**: 207 – 214.

Arya, H. C. & Jain, G. L. (1962) *Fusarium* wilt of *Eucalytpus*. *Phytopathology* **52**: 638 – 642.

Barnard, E. L. (1984) Occurrence, impact, and fungicidal control of gridling stem cankers caused by *Cylindrocladium scoparium* on *Eucalyptus* seedlings in a South Florida nursery. *Plant Disease* **68**: 471 – 473.

Bates, M. L. & Stanghellini, M. E. (1984) Root rot of hydroponically grown spinach caused by *Pythium aphenidermatum* and *P. dissotocum*. *Plant Disease* **68**: 989 – 991.

Belisario, A. (1993) Identification of *Phytophthora nicotianae* on the aerial portions of eucalypt seedlings. *European Journal of Forest Pathology* **23**: 85 – 91.

Berger, F., Hong Li, White, D., Frazer, R. & Leifert, C. (1996) Effect of pathogen inoculum, antagonist density and plant species on biological control of *Phytophthora* and *Pythium* damping – off by *Bacillus subtilis* Cot1 in high – humidity fogging glasshouses. *Phytopathology* **86**: 428 – 433.

Blum, L. E. B., Dianese, J. C. & Costa, C. L. (1992) Comparative pathology of *Cylindrocladium clavatum* and *C. scoparium* on *Eucalyptus* spp. and screening on *Eucalyptus* provenances for resistance to *Cylindrocladium* damping – off. *Tropical Pest Management* **38**: 155 – 159.

Bolland, L. Teirney, J. W. & Teirney, B. J. (1985) Studies on leaf spot and shoot blight of *Eucalyptus* caused by *Cylindrocladium quinqueseptatum. European Journal of Forest Pathology* **15**: 385 – 397.

Brammall, R. & Lynch, K. (1990) Occurrence of *Fusarium* crown and root rot of tomato in New Brinswick, Canada. *Plant Disease* **74**: 1037.

Brown, B. N. & Ferreira, F. A. (2000) Disease during propagation of eucalypts. In *Diseases and pathogens of eucalypts*, Chapter 8: 119-151.(Ed. P. J. Keane, G. A. Kile, F. D. Podger, B. N. Brown). CSIRO Publishing, Collingwood, Australia.

Cherif, M. & Belanger, R. R. (1992) Use of potassium silicate amendments in recirculating nutrient solutions to suppress *Pythium ultimum* on long english cucumber. *Plant Disease* **76**: 1008 – 1011.

Couteaudier, Y. & Alabouvette, C. (1981) *Fusarium* wilt diseases in soilless cultures. *Acta Horticultura* **126**: 153 – 158.

Coutinho, T. A. Wingfield, M. J. Alfenas, A. C. & Crous P. W. (1998) *Eucalyptus* rust: A disease with the potential for serious international implications. *Plant Disease* **82**: 819 – 825.

Crous, P. W. (2002) Taxonomy and pathology of *Cylindrocladium* (*Calonectria*) and allied genera. APS Press, St. Paul, Minnesota, USA.

Crous, P. W., Knox – Davies, P. S. & Wingfield, M. J. (1989a) A summary of fungal leaf pathogens of *Eucalyptus* and the diseases they cause in South Africa. *South African Forestry Journal* **149**: 9 – 16.

Crous, P. W., Knox – Davies, P. S. & Wingfield, M. J. (1989b) A list of *Eucalyptus* leaf fungi and their potential importance to South African forestry. *South African Forestry Journal* **149**: 17 – 29.

Crous, P.W., Phillips, A. L. J. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Journal of Forestry* **157**: 69 – 85.

Crous, P.W., Phillips, A. L. J. & Wingfield, M. J. (1993) New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Plant Pathology* **42**: 302 – 305.

Daughtrey, M. L. & Shippero, P. A. (1980) Root death and associated problems. *Acta Horticultura* **98**: 283 – 291.

Denison, N. P. & Kietzka, J. E. (1993a) The use and importance of hybrid intensive forestry in South Africa. *South African Forestry Journal* **162**: 55 – 60.

Denison, N. P. & Kietzka, J. E. (1993b) The development and utilization of vegetative propagation in Mondi for commercial afforestation programmes. *South African Forestry Journal* **162**: 47 – 54.

Denison, N. P. & Quaile, D. R. (1987) The applied clonal eucalypt programme in Mondi Forests. *South African Forestry Journal* **142**: 60 – 67.

Duffy, B. K. & Defago, G. (1999) Macro – and microelement fertilizers influence the severity of *Fusarium* crown and root rot of tomato in a soilless production system. *HortScience*. **34**: 287 – 291.

Evans, S. G. (1979) Susceptibility of plants to fungal pathogens when grown by the nutrient – film technique. *Plant Pathology* **28**: 45 – 48.

Ghaouth, A. E., Arul, J., Grenier, J., Benhamou, N., Asselin, A. & Belanger, R. (1994) Effect of chitosan on cucumber plants: suppression of *Pythium aphanidermatum* and induction of defense reactions. *Phytopathology* **84**: 313 – 320.

Goldberg, N. P., Stanghellini, M. E. & Rasmussen, S. L. (1992) Filtration as a method for controlling *Pythium* root rot of hydroponically grown cucumbers. *Plant Disease* **76**: 777 – 779.

Graves, C. J. (1983) The nutrient film technique. *Horticultural Review* **5**: 1 – 44.

Grosch, R., Kofoet, A. & Junge, H. (2001) Biological control of root pathogens in soilless culture using bacteria. *Acta Horticultura* **548**: 393 – 400.

Harsh, N. S. K., Dadwal, V. S. & Jamaluddin. (1992) A new post – emergence damping – off disease of *Eucalyptus* seedlings. *Indian Forester* **118**: 279 – 283.

Hodges, C. S. & May, L. C. (1972) A root disease of pine, *Araucaria*, and *Eucalyptus* in Brazil caused by a new species of *Cylindrocladium*. *Phytopathology* **62**: 898 – 901.

Holliday, P. (1990) A dictionary of plant pathology. Cambridge University Press, Cambridge.

Jenkins, JR., S. F. & Averre, C. W. (1984) Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. *Plant Disease* **67**: 968 – 970.

Jensen, M. H. (1997) Hydroponics. *HortScience* **32**: 1018 – 1021.

Jensen, M. H. (1999) Hydroponics worldwide. *Acta Horticultura*. **481**: 719 – 729.

Jensen, M. H. & Collins, W. L. (1985) Hydroponic vegetable production. *Horticultural Review* **7**: 483 – 558.

Kulkarni, H. D. & Lal, P. (1995) Performance of *Eucalyptus* clones at ITC Bhadrachlm, India. CRC for Temperate Hardwood Forestry, IUFRO, Hobart.

Marks, G. C. (1981) Newly recorded diseases of Victorian forest trees. *Australasian Plant Pathology* **10**: 58 – 59.

Marks, G. C., Fuhrer, B. A. & Walters, N. E. M. (1982) Tree diseases in Victoria. Forests Commission Victoria, Handbook 1.

Marks, G. C. & Kassaby, F. Y. (1974) Pathogenicity of *Pythium* spp. and *Phytophthora drechsleri* to *Eucalyptus* spp. *Australian Journal of Botany* **22**: 661 – 668.

McCullagh, M., Utkhede, R., Menzies, J. G., Punja, Z. K. & Paulitz, T. C. (1996) Evaluation of plant growth – promoting rhizobacteria for biological control of *Pythium* root rot of cucumbers grown in rockwool and effects on yield. *European Journal of Plant Pathology* **102**: 747 – 755.

Mihta – Grimm, L., Erb, W. A. & Rowe, R. C. (1990) *Fusarium* crown and root rot of tomato in greenhouse rock wool systems: Source of inoculum and disease management with benomyl. *Plant Disease* **74**: 996 – 1002.

Moulin, F., Lemanceau, P. & Alabouvette, C. (1994) Pathogenicity of *Pythium* species on cucumber in peat – sand, rockwool and hydroponics. *European Journal of Plant Pathology* **100**: 3 – 17.

O' Gara, E., McComb, J. A., Colguhoun, I. J. & Hardy, G. E. St. J. (1997) The infection of non – wounded and wounded periderm tissue at the lower stem of *Eucalyptus marginata* by zoospores of *Phytophthora cinnamomi* from Australia, in a rehabilitated bauxite mine. *Australian Plant Pathology* **26**: 135 – 141.

Ongena, M., Daayf, F., Jacques, P., Thonart, P., Benhamou, N., Paulitz, T. C., Cornelis, P., Koedam, N. & Belanger, R. R. (1999) Protection of cucumber against *Pythium* root rot by fluorescent pseudomonads: predominant role of induced resistance over siderophores and antibiosis. *Plant Pathology* **48**: 66 – 76.

Palm, M. E. (1991) Taxonomy and morphology of the synanamorphs *Pilidium conavum* and *Hainesia lythri* (Coelomycetes). *Mycologia* **83**: 787 – 796.

Paulitz, T. C. (1997) Biological control of root pathogens in soilless and hydroponic systems. *HortScience* **32**: 193 – 196.

Paulitz, T. C. & Bélanger, R. R. (2001) Biological control in greenhouse systems. *Annual Review of Phytopathology* **39**: 103 – 133.

Peterson, G. W. & Smith, R. S. (1975) Forest nursery diseases in the United States. Forest Services, U. S. Department of Agriculture. Agriculture Handbook No. 470.

Price, T. V. & Fox, P. (1986) Studies on the behaviour of furalaxyl on pythiaceous fungi and cucumbers in recirculating hydroponic systems. *Australian Journal of Agricultural Research* **37**: 65 – 77.

Runia, W. T. (1995) A review of possibilities for disinfection of recirculation water from soilless cultures. *Acta Horticultura* **382**: 221 – 228.

Runia, W. T. & Amsing, J. J. (2001) Disinfection of recirculation water from closed cultivation systems by heat treatment. *Acta Horticultura* **548**: 215 – 222.

Sanfuentes, E. A. Alfenas, A. C., Maffia, L. A. & Penchel, R. (1999) Suppression of *Rhizoctonia* spp. in *Eucalyptus* clonal hedge soil. *12th Bienal Conference Canberra 1999, Asia-Pacific Plant Pathology for the Millenium, pp. 321.*

Sanfuentes, E. A. Alfenas, A. C., Maffia, L. A. & Silveira, S. F. (2002) Comparison of baits to quantify inoculum density of *Rhizoctonia* spp. in *Eucalyptus* clonal garden soils. *Australasian Plant Pathology* **31**: 177 – 183.

Schneider, R. W., Grogan, R. G. & Kimble, K. (1978) *Colletotrichum* root rot of greenhouse tomatoes in California. *Plant Disease Reporter* **62**: 969 – 971.

Schuerger, A. C. & Mitchell, D. J. (1992) Effects of temperature and hydrogen ion concentration on attachment of macroconidia of *Fusarium solani* f. sp. *phaseoli* to mung bean roots in hydroponic nutrient solution. *Phytopathology* **82**: 1311 – 1319.

Schwartzkopf, S. H., Dudzinski, D. & Minners, R. S. (1987) The effects of nutrient solution sterilization on the growth and yield of hydroponically grown lettuce. *HortScience* **22**: 873 – 874.

Sharma, J. K. & Mohanan, C. (1992) Effects of some nursery practices on incidence and severity of diseases, and growth of *Eucalyptus grandis* seedlings. *European Journal of Forest Pathology* **22**: 125-135.

Sharma, J. K. Mohanan, C. Florence, E. J. M. (1984) Nursery diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* **14**: 77-89.

Sharma, J. K. Mohanan, C. Florence, E. J. M. (1985) Disease survey in nurseries and plantations of forest tree species grown in Kerala. *Research report 36. (Kerala Forest Research Institute: Peechi, Kerala).*

Sinclair, W. A., Lyon, H. H. & Johnson, W. T. (1987) Diseases of trees and shrubs. *Comstock Publishing Associates, Cornell University Press: Ithaca.*

Smith, H., Wingfield, M. J. & Coutinho, T. A. (1998) *Eucalyptus* die – back in South Africa associated with *Colletotrichum gloeosporioides*. *South African Journal of Botany* **64**: 226 – 228.

Stanghellini, M. E., Kim, D. H., Rasmussen, S. L. & Rorabaugh, P. A. (1996) Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. *Plant Disease* **80**: 1113 – 1116.

Stanghellini, M. E. & Kronland, W. C. (1986) Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder rootlets by *Pythium dissotocum*. *Plant Disease* **70**: 1053 – 1056.

Stanghellini, M. E. & Miller, R. M. (1997) Biocides: their identity and potential efficacy in the biological control of zoosporic plant pathogens. *Plant Disease* **81**: 4 – 12.

Stanghellini, M. E. & Rasmussen, S. L. (1994) Hydroponics. A solution for zoosporic pathogens. *Plant Disease* **78**: 1129 – 1138.

Stanghellini, M. E. & Tomlinson, J. A. (1987) Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. *Phytopathology* **77**: 112 – 114.

Stanghellini, M. E., White, J. G., Tomlinson, J. A. & Clay, C. (1988) Root rot of hydroponically grown cucumbers caused by zoospore – producing isolates of *Pythium intermedium*. *Plant Disease* **72**: 358 – 358.

Sutton, J. C., Yu, H., Grodzinski, B. & Johnstone, M. (2000) Relationship of ultraviolet radiation dose and inactivation of pathogen propagules in water and hydroponic nutrient solutions. *Canadian Journal of Plant Pathology* **22**: 300 – 309.

Vanachter, A., Thys, L., Van Wambeke, E. & van Assche, C. (1988) Possible use of ozone for disinfestation of plant nutrient solutions. *Acta Horticultura* **221**: 295 – 302.

Van Os, E. A., Amsing, J. J., van Kuik, A. J. & Willers, H. (1999) Slow sand filtration: a potential method for the elimination of pathogens and nematodes in recirculating nutrient solutions from glasshouse – grown crops. *Acta Horticiltura* **481**: 519 – 526.

Van Wyk, G. (1985) Tree breeding in support of vegetative propagation of *Eucalyptus grandis* (Hill) Maiden. *South African Forestry Journal* **132**: 33 – 39.

Vestergárd, B. (1988) Sterilization of water and nutrient solutions. *Acta Horticultura* **221**: 303 – 313.

Viljoen, A., Wingfield, M. J. & Crous, P. W. (1992) Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: A review. *South African Forestry Journal* **161**: 45-51.

Von Broembsen, S. (1984) Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South - Western Cape Province. *Phytophylactica* **16**: 221 - 225.

White, T. L. (1995) Prototype breeding strategies for clonal forestry: A case study of *Eucalyptus grandis*. CRC for Temperate Hardwood Forestry, IUFRO, Hobart.

Wilson, P. J. (1998) Developing clones from *Eucalyptus globulus* and hybrid seedlings by stem cuttings propagation. *New Zealand Journal of Forestry Science* **28**: 293 – 303.

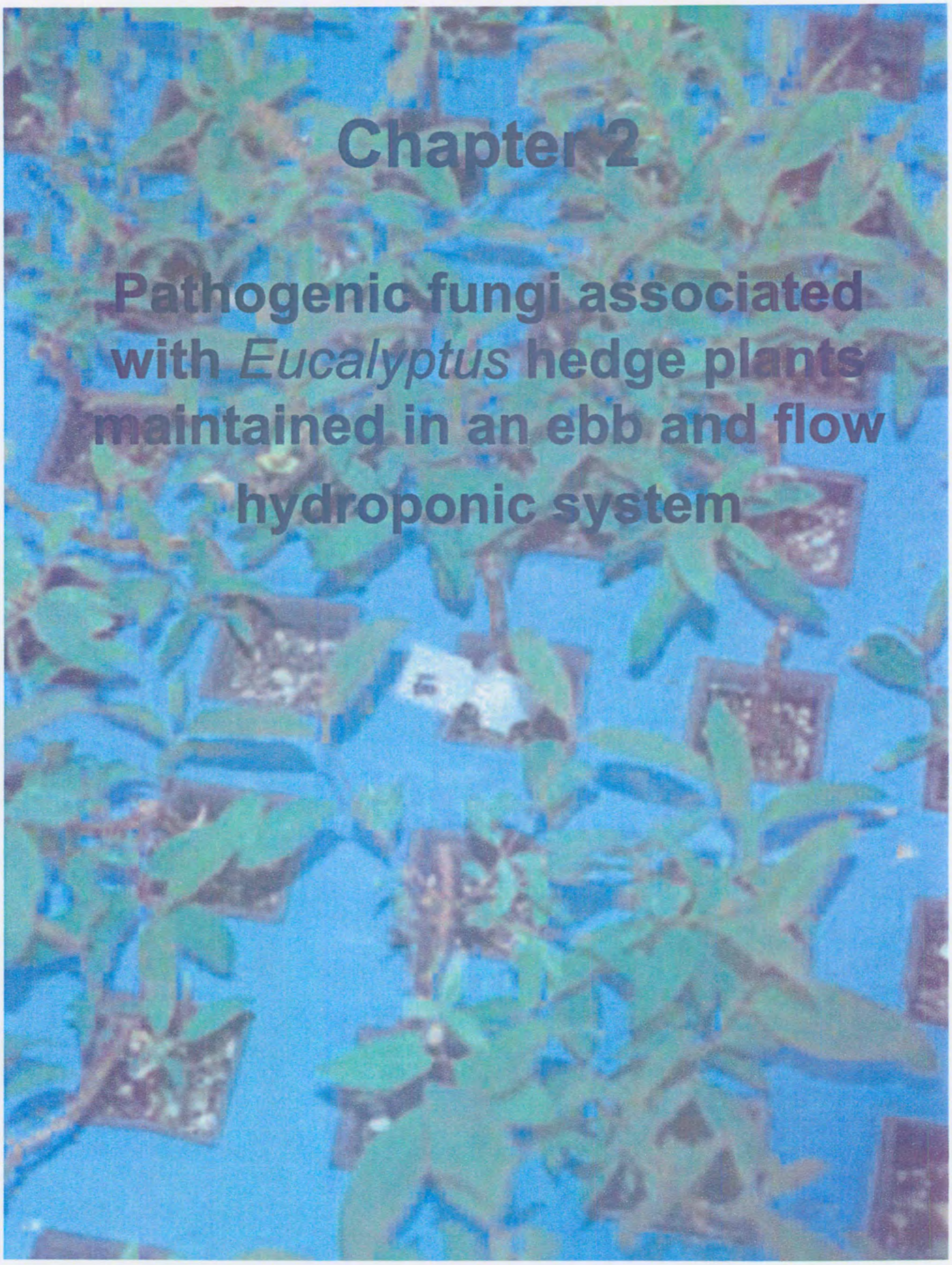
Wulff, E. G., Pham, A. T. H., Cherif, M., Rey, P., Tirilly, Y. & Hockenhull, J. (1998) Inoculation of cucumber roots with zoospores of mycoparasitic and plant pathogenic *Pythium* species: Differential zoospore accumulation, colonization ability and plant growth response. *European Journal of Plant Pathology* **104**: 69 – 76.

Zhang, W. & Tu, J. C. (2000) Effect of ultraviolet disinfection of hydroponic solutions on *Pythium* root rot and non – target bacteria. *European Journal of Plant Pathology* **106**: 415 – 421.

Zinnen, T. M. (1988) Assessment of plant disease in hydroponic culture. *Plant Disease* **72**: 96 – 99.

Chapter 2

Pathogenic fungi associated with *Eucalyptus* hedge plants maintained in an ebb and flow hydroponic system



Abstract

Vegetative propagation of *Eucalyptus* hybrid clones is a powerful tool contributing towards the establishment of uniform, high – value commercial plantations. However, the development and establishment of *Eucalyptus* hedge plants is a time consuming and space inefficient process. A new system to propagate and maintain these hedge plants in hydroponics has recently been developed for use in some South African forestry nurseries. No information is available on the presence of pathogens of *Eucalyptus* in hydroponics in this country. The aim of this study was, therefore, to determine which pathogens are responsible for the root rot symptoms observed on the hedge plants. A study was thus undertaken at a forestry nursery that maintains *Eucalyptus* hedge plants in an ebb and flow feeding system. Roots were randomly collected and direct isolations were done on standard and selective media. Nutrient solution was also collected, filtered and filters plated onto standard and selective media for the isolation of fungi. Several well – known fungal pathogens were collected that reside in the genera *Cylindrocladium*, *Fusarium*, *Pythium* and *Phytophthora*. All isolates were identified based on morphological characteristics and these identifications were confirmed using DNA sequence data comparisons.

Introduction

In the past two decades, the *Eucalyptus* nursery system in South Africa has undergone major changes. The most significant of these was the rapid development of macropropagation and micropropagation of *Eucalyptus* hybrid clones from stem cuttings and tissue culture (Denison & Kietzka 1993a). Macropropagation, commonly known as vegetative propagation, is a powerful tool to capture specific family and clonal gains, not available from conventional tree breeding strategies using seed orchards (Denison & Kietzka 1993a).

In South Africa, vegetatively propagated tropical, sub – tropical and cold tolerant *Eucalyptus* spp. and their hybrids have been deployed in commercial plantations (Denison & Kietzka 1993b). This has resulted in uniform plantations of selected, high value trees (Kulkarni & Lal 1995). The development and establishment of conventional clonal hedge plants is time consuming, taking up to 18 months before they become productive (Wilson 1998, Aimers – Halliday *et al.* 1999). Mondi Forests, South Africa, has thus introduced a system to maintain clonal hedge plants in hydroponics. This has significantly reduced the time required for establishment of plants as well as increasing productivity of cuttings (Dr. B. Janse, pers. comm.).

Hydroponics encompasses the technology of growing plants in nutrient solution with or without the aid of a root – supporting substrate (Stanghellini & Rasmussen 1994, Jensen 1999). Several types of hydroponic systems are available and used for a wide variety of plant products (Jensen & Collins 1985, Stanghellini & Rasmussen 1994, Jensen 1999). Currently in South African forestry, a recirculating liquid hydroponic system, known as an ebb and flow system, is used. This system is based on a "flood and drain" principle where the roots are submerged in nutrient solution for a few minutes then drained. This process is repeated approximately three to four times daily, depending on climatic conditions.

Although hydroponics has several advantages, one important disadvantage is that fungal pathogens capable of causing root diseases are easily spread in liquid medium. Several of these fungi are well – known plant pathogens while other fungi are only known to be pathogenic in a hydroponic system (Stanghellini & Rasmussen 1994, Paulitz 1997). Given that hydroponics is new to forestry in South Africa, little is known about the fungal pathogens present in this system. The aim of this study was, therefore, to determine which pathogens are present in newly established ebb and flow hydroponics systems used to grow *Eucalyptus* hedge plants for cutting production.

Materials and Methods

Ebb and flow system

The ebb and flow system used in this study is a liquid hydroponic system where the nutrient solution is re-circulated. The system consisted of four nutrient tanks (Batch 1 – 4; Fig. 1B) which each feed nutrient solution to eight shallow tanks. The shallow tanks have removable lids containing holes in which the hedge plants are suspended in a plug.

Nutrient solution is pumped into the shallow tanks until the plugs are halfway submerged in the nutrient solution. This level is maintained for three to four minutes and the nutrient solution is then drained. This process is repeated three to four times daily. The nutrient solution consists of tap water and commercially available hydroponics fertilizers and is replaced every 20 days.

Survey of Eucalyptus *hedge plants*

Roots of hybrid *E. grandis* x *urophylla* hedge plants, in the ebb and flow system, were collected from one forestry nursery in KwaZulu – Natal. A sample of 20 root plugs was randomly collected from each of the four batches of the hydroponics system. Healthy and diseased roots (Fig. 1E) were air – pruned from the plugs and placed separately in sterile plastic tubes and transferred to the laboratory for further study. The aerial parts of the hedge plants showed no obvious signs of disease. These collections were made on five different occasions at two – month intervals. Thus, 80 samples were collected on each of five occasions, resulting in a total sample of 400 roots. Statistical analysis (ANOVA) of the survey data as means for each pathogen in each batch was carried out using SAS analytical programmes (1990).

Roots were surface – sterilized for one minute in 1% sodium hypochlorite and rinsed twice in sterile water. Isolations were made randomly directly from both healthy and diseased roots onto 2% Malt Extract Agar (MEA; Biolab, Midrand, South Africa), *Fusarium* Selective Medium (FSM) (Nelson *et al.* 1983), Nystatin – Ampicillin – Rifampicin – Pentachloronitrobenzene Medium (NARP; 17g/l corn meal agar (Biolab, Midrand, South Africa) 100 000 units/ml, 0.1g/l ampicillin, 0.5g/l rifampicin, 0.1g/l, 0.1g/l pentachloronitrobenzene (Sigma, Steinheim, Germany) and NARP supplemented with Hymexazol (0.05g/l) (NARPH) as selective media for the isolation of *Fusarium* spp., *Pythium* spp. and *Phytophthora* spp. After isolation, the plates were incubated at 25°C in the dark for 3 – 5 days.

Nutrient solution was also collected from each batch of the hydroponics tanks, at regular intervals to determine the presence of pathogens. The nutrient solution (250ml) was filtered through four 0.45 μm cellulose – nitrate membrane filters (Whatman, Maidstone, England) for each batch. The filters were plated onto 2% MEA, FSM, NARP and NARPH and incubated at 25°C in the dark for 3 – 5 days.

The most commonly isolated fungi representing four different genera were selected for further study. Isolates were grouped based on their growth characteristics on the different media. Genera were then identified based solely on morphological characteristics. These cultures are maintained in the culture collection (CMW and FCC) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological characteristics of Cylindrocladium *isolates*

A large number of isolates resembling *Cylindrocladium* spp. were obtained from the roots. Twenty randomly selected single conidial isolates were transferred onto Carnation Leaf Agar (CLA) (Crous 2002) to induce production of both anamorph and teleomorph structures. The plates were incubated at 25°C under continuous near – ultraviolet light and examined after 14 days. Morphological characteristics, described by Crous (2002), were used to identify cultures. Macroconidiophores were mounted in lactophenol and twenty measurements of vesicles, stipes and conidia were made for each of the selected isolates, using a light microscope. Measurements for these structures are presented as (min-)(average – standard deviation) – (average + standard deviation)(-max).

Morphological characteristics of Fusarium *isolates*

A large number of isolates resembling *Fusarium* spp. were obtained from the roots. Single conidial isolates were transferred onto CLA, half strength Potato Dextrose Agar (PDA; Biolab, Midrand, South Africa) and Synthetic Low Nutrient Agar (SNA) (Nirenberg &O'Donell 1998). The plates were incubated at 25°C under continuous near – ultraviolet light and examined after 14 days. Mycelium from PDA plates and conidia and conidiophores produced on CLA plates were mounted in lactophenol and SNA plates were studied directly with a light microscope. Morphological characteristics described by Nelson *et al.* (1983) and Nirenberg &O'Donell (1998) were used to identify cultures.

Morphological characteristics of Pythium *and* Phytophthora *isolates*

Pythium and *Phytophthora* isolates were grown for five days on half strength PDA and Corn Meal Agar (CMA) at 25°C in the dark. Four 3 mm discs taken from the actively growing margins of the cultures, growing on PDA, were placed in 12 ml 20% clarified V8 broth and incubated at 25°C for three days with continuous light. Mycelial mats were washed twice with sterile Petri's salt solution (Ribeiro 1978) amended with 0.002g E.D.T.A. disodium salt (Roche, Indianapolis, USA) and 0.002g $MnSO_4$ (Sigma, Steinheim, Germany). Mycelial mats were then incubated in 12 ml Petri's salt solution at 25°C under continuous light. After 48h, the mycelial mats were harvested and examined using a light microscope for the presence of sporangia. Morphological characteristics described by Newhook *et al.* (1978) and Van der Plaats – Niterink (1981) were used to identify cultures.

DNA sequence comparisons

Seven *Cylindrocladium* isolates (Table 1) were randomly selected and utilized for the DNA sequence comparisons. Single conidial isolates were grown on 2% MEA plates from which mycelium was collected and freeze dried. The freeze – dried mycelium was ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted using the technique described by Möller *et al.* (1992).

Nine morphologically distinct *Fusarium* isolates (Table 1) morphologically distinct were randomly selected and utilized in the DNA sequence comparisons. The single conidial isolates were grown on half strength PDA plates from which mycelium was collected and freeze dried. The freeze – dried mycelium was ground to a fine powder in liquid nitrogen with a mortar and pestle. DNA was extracted using the technique described by Möller *et al.* (1992).

Three *Phytophthora* and ten *Pythium* isolates (Table 1) representing morphologically distinct groups were randomly selected and utilized in the DNA sequence comparisons. Cultures were grown in 20ml sterile 20% chlarified V8 broth for five days at 25°C. Mycelium was harvested and rinsed twice with sterile water before freeze drying. The freeze – dried mycelium was ground to a fine powder in liquid nitrogen with a mortar and pestle. DNA was extracted using the technique described by Möller *et al.* (1992).

A 507 base pairs (bp) fragment of the β - tubulin gene was amplified using primers T1 (O'Donell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995) for the *Cylindrocladium* isolates. For the *Fusarium* isolates a 280 bp fragment of the Elongation 1 - α gene (EF1 - α) was amplified using primers EFFF and EFFR (Dr. K. Jacobs, pers. comm.). For the *Phytophthora* and *Pythium* isolates a 877 bp. and 851 bp. fragment of the internal transcribed spacer regions were obtained, respectively using primers ITS4 (White *et al.* 1990) and ITS6 (Cooke & Duncan 1997). The PCR reaction of 25μl comprised of 2.5 units Taq enzyme (Roche Molecular Biochemicals, Almeda, California, USA), 10x buffer, 1mM $MgCl_2$ (as supplied by manufacturer), 0.25 mM deoxynucleoside triphosphate, 0.5 µm primers and approximately 30 ng of fungal DNA as target. The amplified fragments were purified using a High Pure PCR Product Purification Kit (Roche Molecular Biochemicals, Almeda, California, USA).

Each DNA strand of the PCR products was sequenced in both directions with the primers used for the PCR amplification. Sequence reactions were done using an ABI PRISM™3100 DNA Autosequencer (Applied BioSystems). Sequence data were processed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California, USA). The nucleotide sequences were aligned manually by inserting gaps where necessary and phylogenetic relationships were determined using PAUP version 4.0b10 (Swofford 2002). Gaps were treated as missing data and confidence intervals were determined using 1000 bootstrap replications.

Phylogenetic relationships and identities were established for the *Cylindrocladium* isolates by including 12 sequences of known *Cylindrocladium* spp. (Table 1) from GenBank, in the alignment. *Cylindrocladiella infestans* Boesew. (AF320190) was used as the out – group taxon in the analysis. The same was done for the *Fusarium* isolates by including nine sequences of known *Fusarium* spp. (Table 1) with *Botryosphaeria dothidea* Ces. & De Not (AY 236899) as the out – group taxon. To establish the phylogenetic relationships and identities of the *Phytophthora* isolates, five sequences of known *Phytophthora* spp. (Table 1) were obtained from GenBank and included in the alignment. *Pythium aphanidermatum* (Edson.) Fitzp. (AJ 233438) was used as out – group taxon in the analysis. For the *Pythium* isolates, eight sequences of known *Pythium* spp. (Table 1) were used from GenBank and included in the alignment. *Phytophthora cinnamomi* Rands (AF 266764) was used as out – group taxon in the analysis.

Results

Survey of Eucalyptus *hedge plants*

Several well – known *Eucalyptus* pathogens, represented by 717 fungal isolates, were collected from both healthy and diseased *Eucalyptus* hedge plant roots and nutrient solution at the forestry nursery surveyed (Table 2). *Cylindrocladium* isolates were the most commonly isolated pathogens (Fig. 1F) in the ebb and flow system and the next most common pathogens resided in the genus *Pythium* (Table 2). Several *Fusarium* isolates and *Phytophthora* isolates were also obtained in this study (Table 2). These possible pathogens were all isolated from diseased roots of the hedge plants and the nutrient solution. Significant differences existed ($P < 0.0001$) between the pathogens isolated in the survey (Table 3). No significant difference existed ($P > 0.6$) between the batches and pathogens isolated from each batch and indicate no changes ($P > 0.9$) over time (Table 3). However, the interactions between pathogens were significant (Table 3 - 4). Change in pathogen density over time and between batches was not significant ($P > 0.8$). Most of the fungi isolated in this study have been reported previously as pathogens of

Eucalyptus in South Africa, with exception of a few isolates in the genera *Fusarium* and *Pythium*.

Morphological characteristics of Cylindrocladium *isolates*

All *Cylindrocladium* isolates were morphologically similar. The stipes and extensions to the stipes (Fig. 2A) were septate, straight, hyaline, (79)104 – 149(200) µm in length and terminated in obpyriform to ellipsoidal vesicles, (11)16 – 23(31) x (2)5 – 7(9) µm (Fig.2 D & E). Each terminal branch of the conidiophores produced approximately 4 – 6 phialides (Fig. 2A). Phialides were doliiform to reniform, hyaline and aseptate. Conidia were cylindrical, straight, (37)45 – 57(65) x (3)4 – 6(7) µm, 1 – septate and held in parallel cylindrical clusters by colourless slime (Fig. 2B). These characteristics are typical of *C. pauciramosum* C. L. Schoch & Crous as described by Schoch *et al.* (1999).

Morphological characteristics of Fusarium *isolates*

Several *Fusarium* spp. were isolated from the *Eucalyptus* hedge plant roots (Table 2). Based on morphology, five *Fusarium* spp. were identified (Table 5, Fig. 3A - G) according to Nelson *et al.* (1983) and Nirenberg & O'Donnell (1998). They were *F. lateritium* Nees, *F. nygamai* Nirenberg & O'Donnell, *F. oxysporum* Schlect. Emend. Snyd. & Hans., *F. solani* (Mart.) Appel & Wollenw. Emend. Snyd. & Hans. and *F. verticillioides* Nirenberg & O'Donnell.

Morphological characteristics of Phytophthora *and* Pythium *isolates*

Two *Phytophthora* spp. were isolated from the hedge plant roots (Table 6) and identified following the suggestions of Newhook *et al.* (1978) (Fig. 4A – D). These were *P. cinnamomi*, characterized by large, non – papillate, ellipsoid sporangia (Fig. 4C), and *P. nicotianae* van Breda de Haas with papillate, obpyriform sporangia (Fig. 4A & B). Six *Pythium* spp. were isolated from the hedge plant roots (Table 6) and identified using the keys of Van der Plaats – Niterink (1981) (Fig.4E – I). These were *P. aphanidermatum*, *P. deliense* Meurs, *P. dissotocum* Drechsler, *P. intermedium* de Bary, *P. helicoides* Drechsler and *P. vexans* de Bary.

DNA sequence comparisons

***Cylindrocladium* spp.** A data set of 12 in-group taxa and one out-group taxon, *Cylindrocladiella infestans*, was analyzed for the *Cylindrocladium* isolates. The alignment of the β-tubulin gene fragments gave rise to a data set of 520 characters of which 372 were constant, 80 were parsimony – uninformative and 68 parsimony – informative (Appendix II). One parsimonious tree was generated and chosen for presentation (Fig.5). The tree had a consistency index (CI) = 0.852, retention index (RI) = 0.830, and rescaled consistency index (RC) = 0.707 while gaps were treated as missing data. The phylogenetic tree (Fig.5) clearly showed that all seven randomly selected *Cylindrocladium* isolates grouped in the clade representing *C. pauciramosum* (64% bootstrap support).

***Fusarium* spp.** A data set of nine in-group taxa and one out-group taxon, *Botryosphaeria dothidea*, was analyzed for the *Fusarium* isolates. The alignment of the EF1 – α gene fragments gave rise to a data set of 280 characters of which 82 were constant, 75 were parsimony – uninformative and 123 parsimony – informative (Appendix II). One tree from two most parsimonious trees was chosen for presentation (Fig.6). The trees had a CI = 0.867, RI = 0.926, and RC = 0.803 while gaps were treated as missing data. The phylogenetic tree (Fig.6) clearly showed that all nine selected *Fusarium* isolates grouped in clades representing the different *Fusarium* spp. identified (Table 5).

***Phytophthora* spp.** A data set of five in-group taxa and one out-group taxon, *Pythium aphanidermatum*, was analyzed for the *Phytophthora* isolates. The alignment of the ITS gene fragments gave rise to a data set of 814 characters of which 444 were constant, 235 were parsimony – uninformative and 135 parsimony – informative (Appendix II). One most parsimonious tree was generated and chosen for presentation (Fig.7). The trees had a CI = 0.885, RI = 0.754, and RC = 0.667 while gaps were treated as missing data. The phylogenetic tree (Fig.7) clearly showed that all three selected isolates grouped in the clades representing the different *Phytophthora* spp. identified (Table 6).

***Pythium* spp.** A data set of eight in-group taxa and one out-group taxon, *Phytophthora cinnamomi*, was analyzed for the *Pythium* isolates. The alignment of the ITS gene fragments gave rise to a data set of 851 characters of which 370 were constant, 106 were parsimony – uninformative and 375 parsimony – informative (Appendix II). One most parsimonious tree was generated and chosen for presentation (Fig.8). The tree had a CI = 0.748, RI = 0.819, and RC = 0.620 while gaps were treated as missing data. The phylogenetic tree (Fig.8) clearly showed that all ten selected *Pythium* isolates grouped in the clades representing the different but known *Pythium* spp. identified (Table 6).

Discussion

This study represents the first survey of pathogens to be conducted on *Eucalyptus* hedge plants maintained in a recirculating ebb and flow hydroponic system in South Africa. A relatively large number of potential pathogens or fungi previously shown to be pathogenic were isolated from the hedge plant roots. Some of these have not been reported previously on *Eucalyptus*. No disease symptoms were observed in the aerial parts of the plants but root rot was evident. The lack of symptoms could be attributed to the fact that the hedge plants were in a nutrient rich environment created by the hydroponic fertilizers that are used. Thus the plants were under less stress than those grown using conventional hedge bank methods and this could have resulted in lower levels of symptom development.

An interesting result of this study was finding the dominant presence of *Cylindrocladium pauciramosum* on *Eucalyptus* hedge plant roots. *Cylindrocladium pauciramosum* is an important *Eucalyptus* pathogen in forestry nurseries in South Africa (Crous 2002). It is a soilborne pathogen responsible for diseases such as root rot, stem cankers and leaf spots (Schoch *et al.* 1999, Crous 2002). This study showed that *C. pauciramosum* is present in a liquid hydroponic system and can spread to other plants through this solution. No stem cankers or leaf spots were observed on the hedge plants during the survey, even though they are commonly found elsewhere. However, *C. pauciramosum* was readily isolated from diseased roots. Other *Cylindrocladium* spp. might also become a limiting factor in hydroponic systems due to their large host range in the future (Crous 2002).

Fusarium spp. are the most common and destructive non – zoosporic pathogens in hydroponic systems (Stanghellini & Rasmussen 1994). *Fusarium oxysporum, F. solani* and *F verticilioides* (syn.= *F. moniliforme* J. Sheld.) are well – known pathogens of *Eucalyptus* seedlings responsible for damping – off, wilting and root rot (Sharma *et al.* 1985, Brown & Ferreira 2000). In this study, these *Fusarium* spp. were isolated together with *F. nygamai* and *F. lateritium,* neither of which have previously been reported as pathogens of *Eucalyptus*. Both *Fusarium* spp. are reported to occur on woody hosts (Nelson *et al.* 1983, Nirenberg & O'Donnell 1998).

Zoosporic fungi are known as the most important plant pathogens in hydroponics. This is because they are water – borne pathogens and they spread by means of motile flagellate spores (Zinnen 1988, Stanghellini & Rasmussen 1994). Both *Pythium* and *Phytophthora* spp. were isolated from the hedge plant roots during this study. *Phytophthora cinnamomi* and *P.*

nicotianae are both important pathogens of *Eucalyptus* causing root rot in nurseries and plantations (Sharma *et al.* 1985, Shearer & Smith 2000). All *Pythium* spp., with the exception of *P. dissotocum* and *P. helicoides*, are known pathogens of *Eucalyptus* seedlings responsible for damping – off and root rot (Sharma *et al.* 1985, Brown & Fereirra 2000, Shearer & Smith 2000). *Pythium helicoides* causes root diseases of ornamental plants (Van der Plaats – Niterink 1981) and *P. dissotocum* is responsible for stunting of lettuce in hydroponics (Stanghellini & Rasmussen 1994). Although pathogenicity tests have not been done at this stage, we expect that these fungi could contribute to reductions in tree health.

DNA sequence comparisons were used in this study to confirm the identity of the different species of pathogens associated with *Eucalytpus* hedge plants. This was necessary as species in these genera are morphologically similar and difficult to identify. The sequence data used in all cases confirmed identifications based on morphology when the β – tubulin gene was used for *Cylindrocladium* isolates (Schoch *et al.* 1999), EF1 – α for *Fusarium* isolates (Nirenberg & O'Donnell 1998) and ITS for *Pythium* and *Phytophthora* isolates (Wang & White 1997, Cooke *et al.* 2000).

Several well – known and possible new pathogens have been isolated from the *Eucalyptus* hedge plants in the ebb and flow hydroponics system considered in this study. Pathogenicity is suggested based on a knowledge of the fungi involved, but pathogenicity tests in an ebb and flow system need to be conducted in order to determine the impact of these pathogen on the hedge plants. Sound and cost effective management strategies can then be developed to reduce losses.

References

Aimers – Halliday, J., Dibley, M. J., Hong, S. O. & Menzies, M. I. (1999) Cutting propagation of hybrid *Eucalyptus grandis x E. nitens*. *New Zealand Journal of Forestry Science* **29**: 238 – 250.

Brown, B. N. & Ferreira, F. A. (2000) Disease during propagation of eucalypts. In *Diseases and pathogens of eucalypts*, Chapter 8: 119-151.(Ed. P. J. Keane, G. A. Kile, F. D. Podger, B. N. Brown). CSIRO Publishing, Collingwood, Australia.

Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. & Brasier, C. M. (2000) A molecular phylogeny of *Phytophthora* and related Oomycetes. *Fungal genetics and Biology* **30**: 17 – 32.

Cooke, D. E. L. & Duncan, J. M. (1997) Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. *Mycological Research* **101**: 667 – 677.

Crous, P. W. (2002) Taxonomy and pathology of *Cylindrocladium* (*Calonectria*) and allied genera. APS Press, St. Paul, Minnesota, USA.

Denison, N. P. & Kietzka, J. E. (1993a) The use and importance of hybrid intensitive forestry in South Africa. *South African Forestry Journal* **162**: 55 – 60.

Denison, N. P. & Kietzka, J. E. (1993b) The development and utilization of vegetative propagation in Mondi for commercial afforestation programmes. *South African Forestry Journal* **162**: 47 – 54.

Glass, N. L. & Donaldson, G. (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323 – 1330.

Jensen, M. H. (1999) Hydroponics worldwide. *Acta Horticultura* **481**: 719 – 729.

Jensen, M. H. & Collins, W. L. (1985) Hydroponic vegetable production. *Horticultural Review* **7**: 483 – 558.

Kulkarni, H. D. & Lal, P. (1995) Performance of *Eucalyptus* clones at ITC Bhadrachlm, India. CRC for Temperate Hardwood Forestry, IUFRO, Hobart.

Möller, E. M., Bahnweg, G., Sandermann, H. & Geiger, H. H. (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acid Research* **20**: 6115 – 6116.

Nelson, P. E., Tousson, T. A. & Marasas, W. F. O. (1983) *Fusarium* species: An illustrated manual for identification. The Pennsylvania State University Press, Pennsylvania, U. S. A.

Newhook, F. J., Waterhouse, G. M. & Stamps, D. J. (1978) Tabular key to the species of *Phytophthora* De Bary. *Mycological Papers* **43**. Commonwealth Mycological Institute, Kew, Surrey, England.

Nirenberg, H. I. & O' Donnell, K. (1998) New *Fusarium* species and combinations within the *Gibberalla fujikuroi* species complex. *Mycologia* **90**: 434 – 458.

O'Donnell, K. & Cigelnik, E. (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103 – 116.

Paulitz, T. C. (1997) Biological control of root pathogens in soilless and hydroponic systems. *HortScience* **32**: 193 – 196.

Ribeiro, O. K. (1978) A source book of the genus *Phytophthora*. Cramer, Vaduz, Liechtenstein. 417pp.

Stanghellini, M. E. & Rasmussen, S. L. (1994) Hydroponics. A solution for zoosporic pathogens. *Plant Disease* **78**: 1129 – 1138.

Schoch, C. L., Crous, P. W. Wingfield, B. D. & Wingfield, M. J. (1999) The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**: 286 – 298.

Sharma, J. K. Mohanan, C. Florence, E. J. M. (1985) Disease survey in nurseries and plantations of forest tree species grown in Kerala. *Research report 36. (Kerala Forest Research Institute: Peechi, Kerala).*

Shearer, B. L. & Smith, I. W. (2000) Diseases of eucalypts caused by soilborne species of *Phytophthora* and *Pythium*. In *Diseases and pathogens of eucalypts*, Chapter 11: 259-291.(Eds. P. J. Keane, G. A. Kile, F. D. Podger, B. N. Brown). CSIRO Publishing, Collingwood, Australia.

Swofford, D. L. (2002) PUAP*. Phylogenetic analysis using parsimony(* and other methods), 4.0b10. Computer programme. Sunderland, Massachusetts, U.S.A.: Sinauer Associates.

Van der Plaats – Niterink, A. J. (1981) Monograph of the genus *Pythium*. *Studies in Mycology* **21**. Centraal Bureau Voor Schimmelcultures, Baarn, Netherlands.

Wang, P. H. & White, J. G. (1997) Molecular characterization of *Pythium* species based on RFLP analysis of the internal transcribed spacer region of ribosomal DNA. *Physiological and Molecular Plant Pathology* **51**: 129 – 143.

White, T. J., Burns, T. L. S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols, A guide to Methods and Applications.* (Eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J.). Academic Press, San diego, U. S. A.

Wilson, P. J. (1998) Developing clones from *Eucalyptus globules* and hybrid seedlings by stem cuttings propagation. *New Zealand Journal of Forestry Science* **28**: 293 – 303.

Zinnen, T. M. (1988) Assessment of plant disease in hydroponic culture. *Plant Disease* **72**: 96 – 99.

Table 1. List of fungal isolates used in DNA sequence comparisons.

Species	Isolate number	GenBank Assencion number
Cylindrocladium candelabrum	STE-U 1677	AF210858
	STE-U 1674	AF210857
C. colhounii	STE-U 307	AF232855
	STE-U 705	AF232854
C. gracile	STE-U 623	AF333405
	IMI 167580	AF333404
C. insulare	STE-U 3211	AF44951
	STE-U 3219	AF44950
C. pauciramosum	**[a]CMW 12689**	
	[a]CMW 12697	
	[a]CMW 12702	
	[a]CMW 12707	
	[a]CMW 12709	
	[a]CMW 13039	
	STE-U 3207	AF449448
	CSL 2021133	AY162320
C. scoparium	STE-U 1722	AF210875
	STE-U 1720	AF210874
Fusarium avenaceum	MT – F 150	AY337423
F. equiseti	MT – F 151	AY337424
F. lateritium	MT – F140	AY337435
	[a]FCC 3117	
	[a]FCC 3119	
F. nygamai	FRC M 1374	AY337445
	[a]FCC 3113	
F. oxysporum	UG L6886#44	AY337428
	[a]FCC 3109	
	[a]FCC 3115	
F. proliferatum	MT – F14	AY337436
F. sambucinum	MT – F149	AY337422
F. solani	MT – F116	AY337438
	[a]FCC 3110	
	[a]FCC 3114	
F. verticillioides	FRC M 1325	AY337450
	[a]FCC 2957	
	[a]FCC 2964	
Phytophthora cinnamomi	UQ 881	AF266764
	CMW 13804	
P. cryptogea	IMI 045168	AF266796
P. infestans	IMI 66006	AF266779

Table 1. (Continued) List of fungal isolates used in DNA sequence comparisons.

Species	**Isolate number**	**GenBank Assencion number**
P. nicotianae	UQ 848	AF266776
	[a]**CMW 13800**	
	[a]**CMW 13816**	
P. palmivora	UQ 1294	AF266780
Pythium aphanidermatum	TOc 159	AJ233438
	[a]**CMW 13815**	
	[a]**CMW 13810**	
P. deliense	MAFF 305568	AJ233442
	[a]**CMW 13805**	
P. dissotocum	MAFF 305576	AJ233443
	[a]**CMW 13820**	
	[a]**CMW 13807**	
P. helicoides	RoPh3 C14	AB108061
	[a]**CMW 13808**	
	[a]**CMW 13817**	
P. intermedium	MAFF 305570	AJ233447
	[a]**CMW 13796**	
	[a]**CMW 13822**	
P. irregulare	MAFF 305572	AJ233448
P. ultimum	OF 231	AB108064
P. vexans	MAF 305905	AJ233462
	[a]**CMW 13819**	

[a] Isolates listed in bold were sequenced in this study. Other sequences originated from Genbank.

Table 2. Percentage of fungi isolated from *Eucalyptus* hedge plant roots and nutrient solutions from an ebb and flow hydroponic system.

Fungus	**[a]Roots**	**[b]Nutrient solution**
Cylindrocladium pauciramosum	17.2	12.1
Fusarium lateritium	1.5	0.3
F. nygamai	1.1	0.3
F. oxysporum	8.2	6.1
F. solani	5.2	4.0
F. verticillioides	4.0	2.8
Phytophthora cinnamomi	0.1	0
P. nicotianae	0.3	0
Pythium aphanidermatum	13.1	5.2
P. deliense	3.1	0.8
P. dissotocum	4.7	1.7
P. intermedium	1.8	1.3
P. helicoides	0.8	0.8
P. vexans	2.0	1.4
Total number of isolates		**717**

[a] Percentage isolated from the *Eucalyptus* hedge plant roots.

[b] Percentage isolated from the nutrient solution.

Table 3.: Combined ANOVA for interaction of the pathogens isolated from the *Eucalyptus* hedge plants and the interactions between the pathogens and the batches.

Source	**SS**	**d.f.**	**MS**	***F***	***P***
Pathogen	11.32	3	3.77	19.16	<0.0001
Batch	2.56	3	0.85	4.33	0.0183
Time	0.0019	4	1.37	0.01	0.995
Batch x Pathogen	1.28	9	0.14	0.72	0.682
Time x Batch x Pathogen	0.49	12	1.77	0.42	0.855

Table 4: The means of each pathogen in each batch to indicate differences between pathogens and batches.

Batch	Pathogen	Mean	Std. Dev.
1	*Cylindrocladium,*	2.60	0.36
	Fusarium	1.60	0.20
	Phytophthora	1.40	0.69
	Pythium	1.27	0.29
2	*Cylindrocladium,*	2.66	0.83
	Fusarium	2.07	0.13
	Phytophthora	1.89	0.44
	Pythium	0.92	0.80
3	*Cylindrocladium,*	2.69	0.22
	Fusarium	1.94	0.14
	Phytophthora	1.89	0.17
	Pythium	1.77	0.27
4	*Cylindrocladium,*	2.26	0.12
	Fusarium	1.25	0.64
	Phytophthora	1.36	0.59
	Pythium	0.90	0.82

Table 5. Morphological characteristics of *Fusarium* isolates obtained from the *Eucalyptus* hedge plants and nutrient solution.

***Fusarium* spp.**	**[a]Colony colour**	**Macroconidia**	**Microconidia**	**Conidiophores**	**Chlamydospores**
F. lateritium	Orange; tan	Abundant; 4 – 6 septate; cylindrical	Abundant in false heads; ellipsoidal	Branched and unbranched monophialides	Present; single or in chains
F. nygamai	White; light pink	Abundant; 4 – 6 septate	Abundant in false heads; oval to kidney shaped	Monophialides and polyphialides	Present; single
F. oxysporum	White/purple; purple	Abundant; 4 – 6 septate; sickle - shaped	Abundant in false heads; oval to kidney shaped	Branched and unbranched monophialides	Abundant; single and pairs
F. solani	White/blue; white	Abundant; 3 – 5 septate; cylindrical	Sparse; oval to kidney shaped	Branched and unbranched monophialides	Present; single
F. verticillioides	White/purple; purple	Absent	Abundant in false heads; oval to club - shaped	Branched and unbranched monophialides	Absent

[a]Colour of colony determined on half – strength PDA (Nelson *et al.* 1983). Indicated as aerial mycelium; below culture.

Table 6. Morphological characteristic of *Phytophthora* and *Pythium* isolates obtained from the *Eucalyptus* hedge plants and nutrient solution. ([a]Colony morphology described from CMA)

Species	[a]Colony morphology	Sporangia
Phytophthora cinnamomi	No aerial mycelium; rosette pattern; large hyphal swellings	Terminal; non – papillate; internally proliferating; ellipsoidal to ovoid
P. nicotianae	Scant aerial mycelium; hyphae uniform	Terminal and intercalary; papillate; obpyriform to obturbinate
Pythium aphanidermatum	Cottony aerial mycelium	Inflated; filamentous; complex of terminal swollen hyphae
P. deliense	Loose aerial mycelium	Terminal and intercalary; Inflated; filamentous
P. dissotocum	Submerged mycelium	Non – inflated; filamentous; dendroidly – shaped
P. intermedium	Submerged mycelium; terminal and intercalary hyphal swellings	None produced
P. helicoids	Cottony aerial mycelium	Terminal; subglobose to obovoid; papillate
P. vexans	Cottony aerial mycelium	Terminal and intercalary; ovoid; spherical; proliferating

Figure 1. The ebb and flow system used to maintain *Eucalyptus* hedge plants in a forestry nursery. A. The ebb and flow system. B. Nutrient solution tanks. C. Shallow tanks containing the hedge plants. D. Feeder roots of hedge plants extending from plugs in the shallow tank. E. Healthy and diseased roots extending from plug. F. Infected root tip with sporulating macroconidiophores of *Cylindrocladium pauciramosum*.

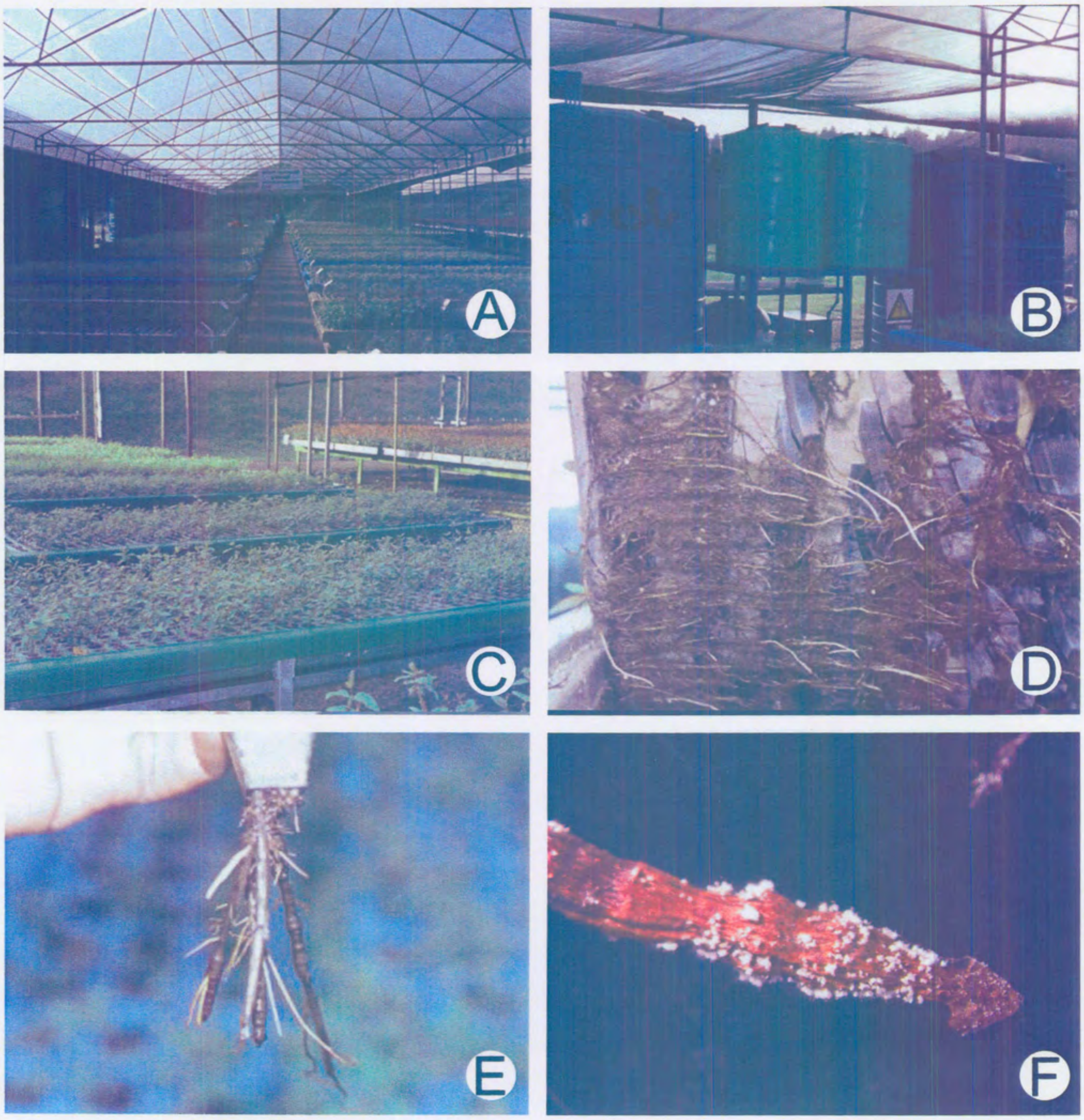

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Figure 2. Macroconidiophores, vesicles and conidia of *Cylindrocladium pauciramosum* isolated from *Eucalyptus* hedge plant roots. A. Macroconidiophores. B. Conidia. C. Chlamydospores forming a sclerotium. D & E. Vesicles (Scale bars = 10 μm).

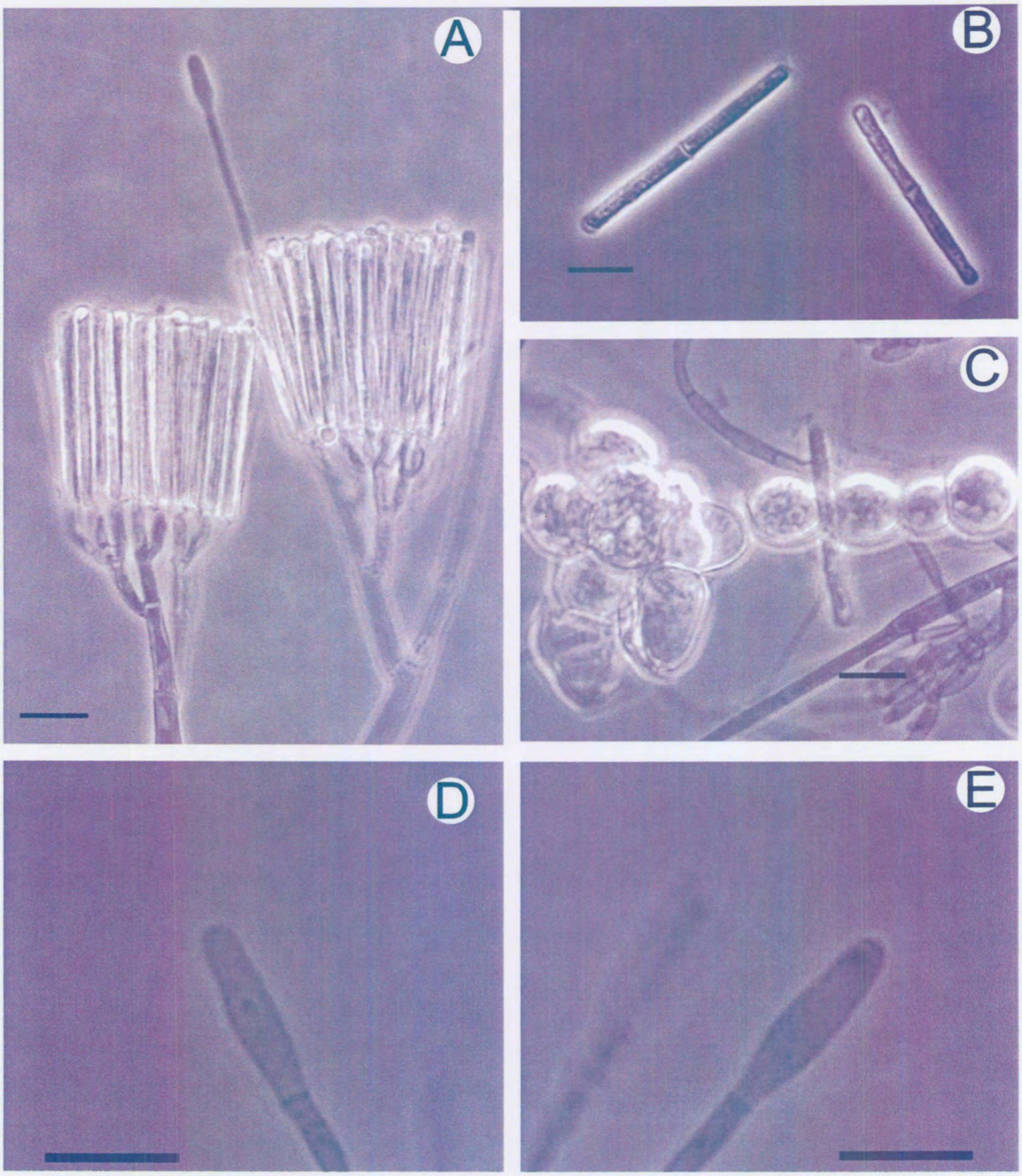

A
B
C
D
E

Figure 3. Some morphological structures of *Fusarium* spp. isolated from the roots of *Eucalyptus* hedge plants maintained in an ebb and flow hydroponic system. A. Microconidia of *F. lateritium* carried in false heads. B. Microconidia of *F. nygamai* carried in false heads. C. Monophialide of *F. solani*. D. Monophialide and polyphialide of *F. nygamai*. E. Intercalary chlamydospores of *F. oxysporum*. F. Sickle – shaped macroconidia of *F. oxysporum*. G. Microconidia of *F. verticillioides* (Scale bars = 10 µm).

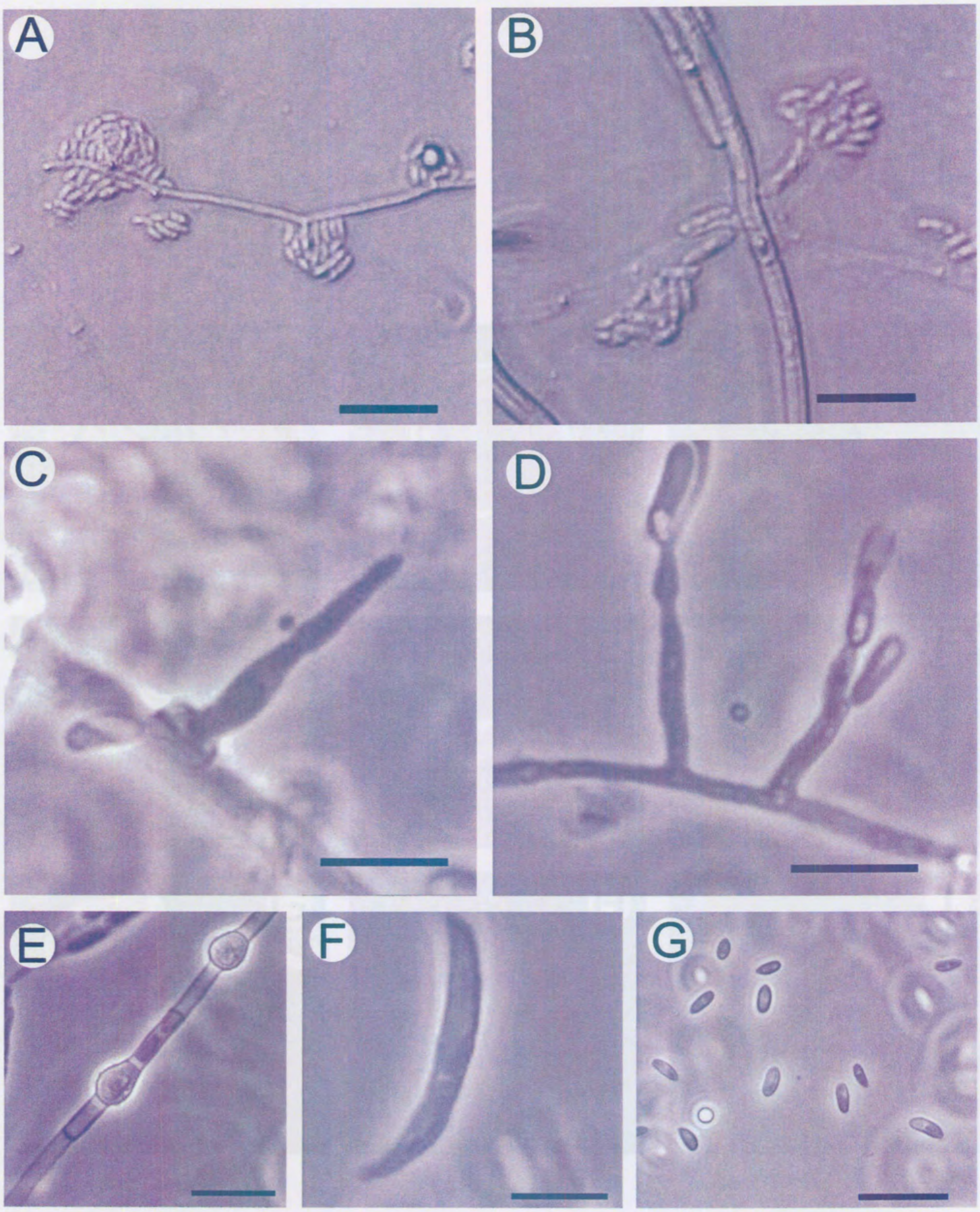

A
B
C
D
E
F
G

Figure 4. Sporangiophores of the Oomycetes isolated from the roots of Eucalyptus hedge plants maintained in an ebb and flow hydroponic system. A & B. Papillate sporangiophores of *Phytophthora nicotianae*. C. Sporangiophore of *P. cinnamomi*. D. Chlamydospores and oogonia of *P. cinnamomi*. E. Sporangiophore of *Pythium dissotocum*. F. Sporangiophore of *P. helicoids*. G. Sporangiophore of *P. aphanidermatum*. H. Sporangiophore of *P. deliense*. I. Sporangiophore of *P. vexans* (Scale bars = 10 µm).

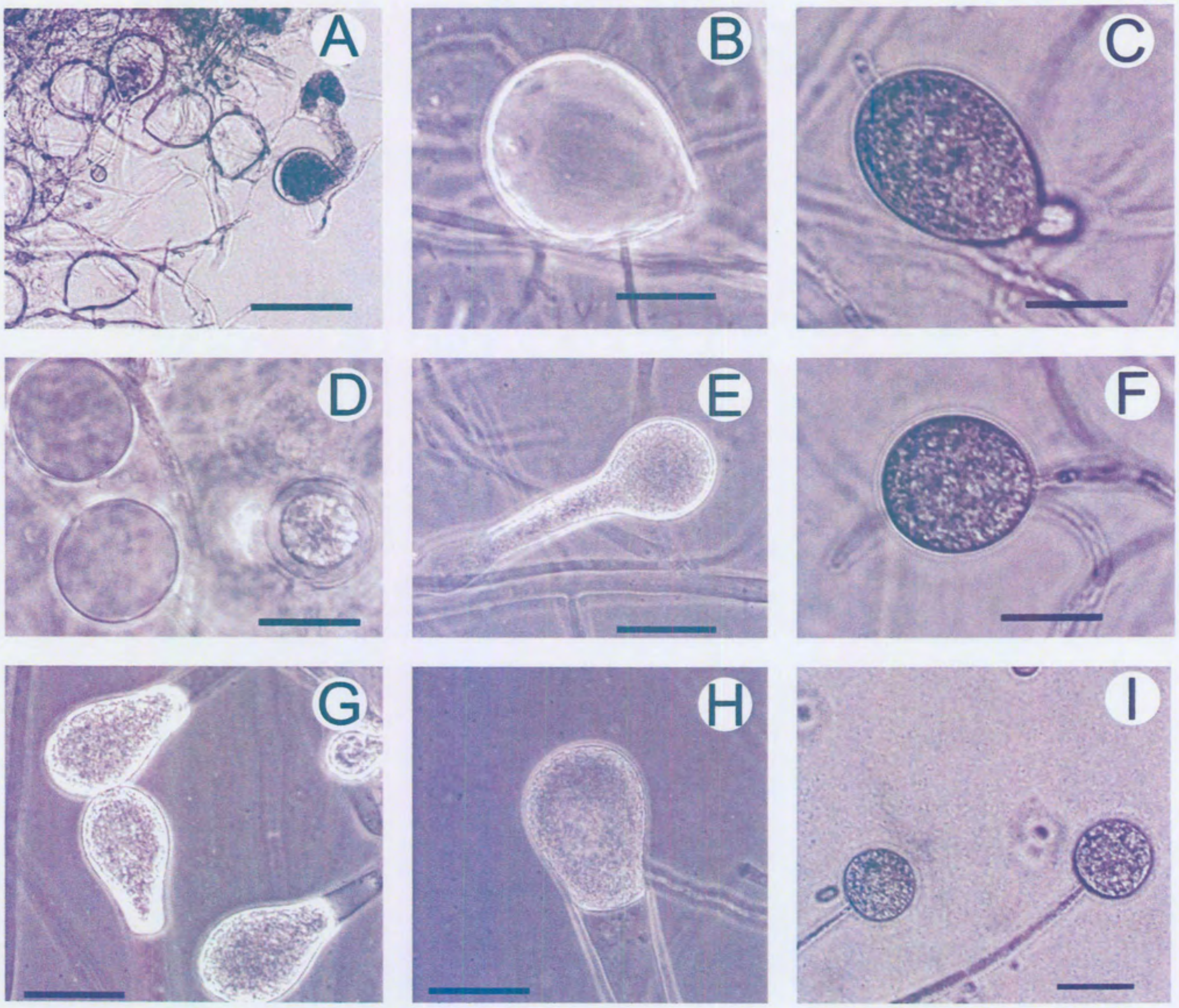

A
B
C
D
E
F
G
H
I

Figure 5. The most parsimonious tree obtained from a subset of *Cylindrocladium* isolates (520 steps, CI = 0.852, RC = 0.707, RI = 0.830) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of the 5' end of the β - tubulin gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown below branches and decay indices above. A *Cylindrocladiella infestans* (GenBank accession number AF320190) was used as out – group.

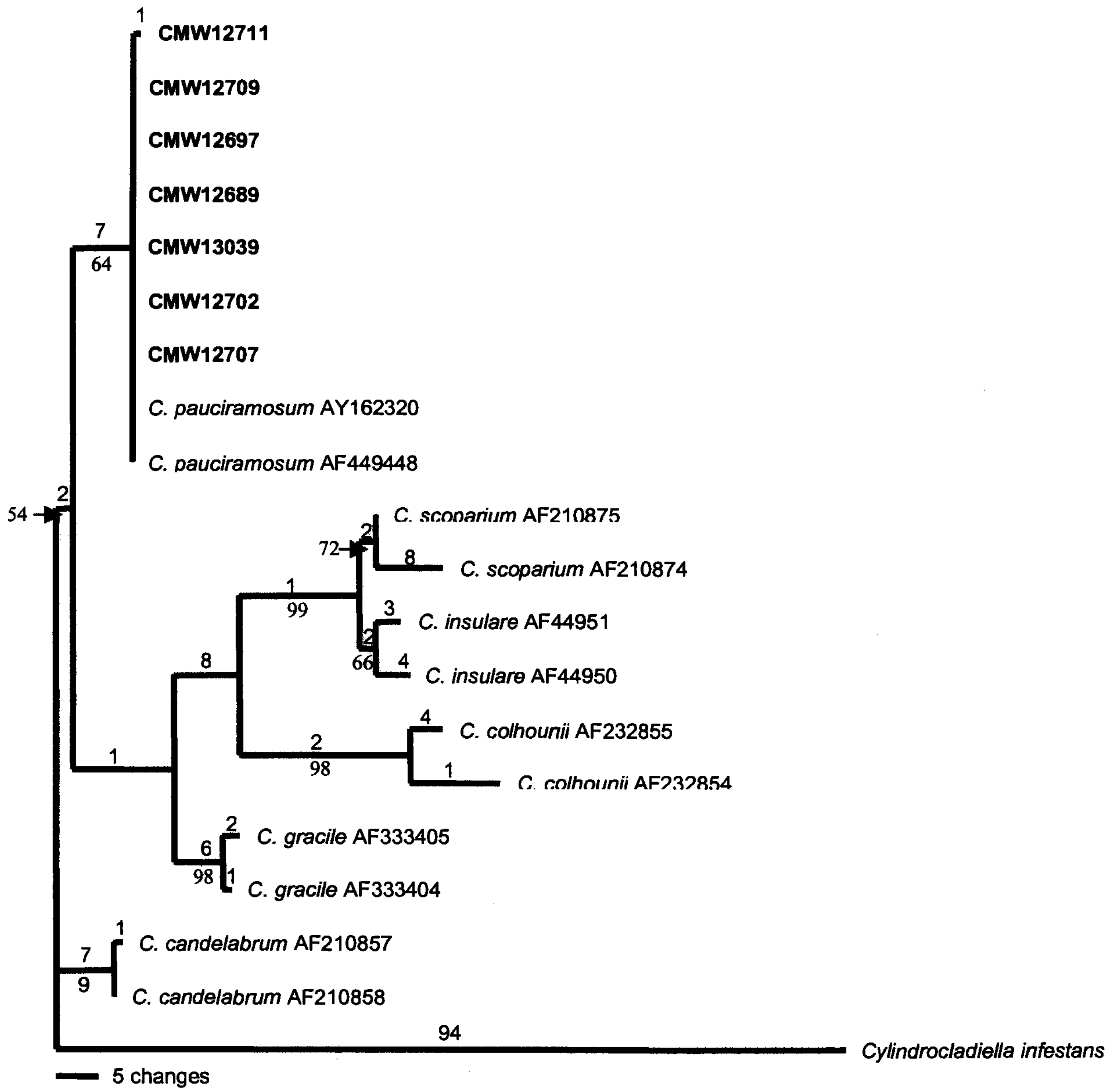

CMW12711
CMW12709
CMW12697
CMW12689
CMW13039
CMW12702
CMW12707
C. pauciramosum AY162320
C. pauciramosum AF449448
C. scoparium AF210875
C. scoparium AF210874
C. insulare AF44951
C. insulare AF44950
C. colhounii AF232855
C. colhounii AF232854
C. gracile AF333405
C. gracile AF333404
C. candelabrum AF210857
C. candelabrum AF210858
Cylindrocladiella infestans
5 changes

Figure 6. The most parsimonious tree obtained from a subset of *Fusarium* isolates (280 steps, CI = 0.867, RC = 0.803, RI = 0.926) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of the 5' end of the EF - 1α gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown below branches and decay indices above. A *Botryosphaeria dothidea* (GenBank accession number AY236899) was used as out – group.

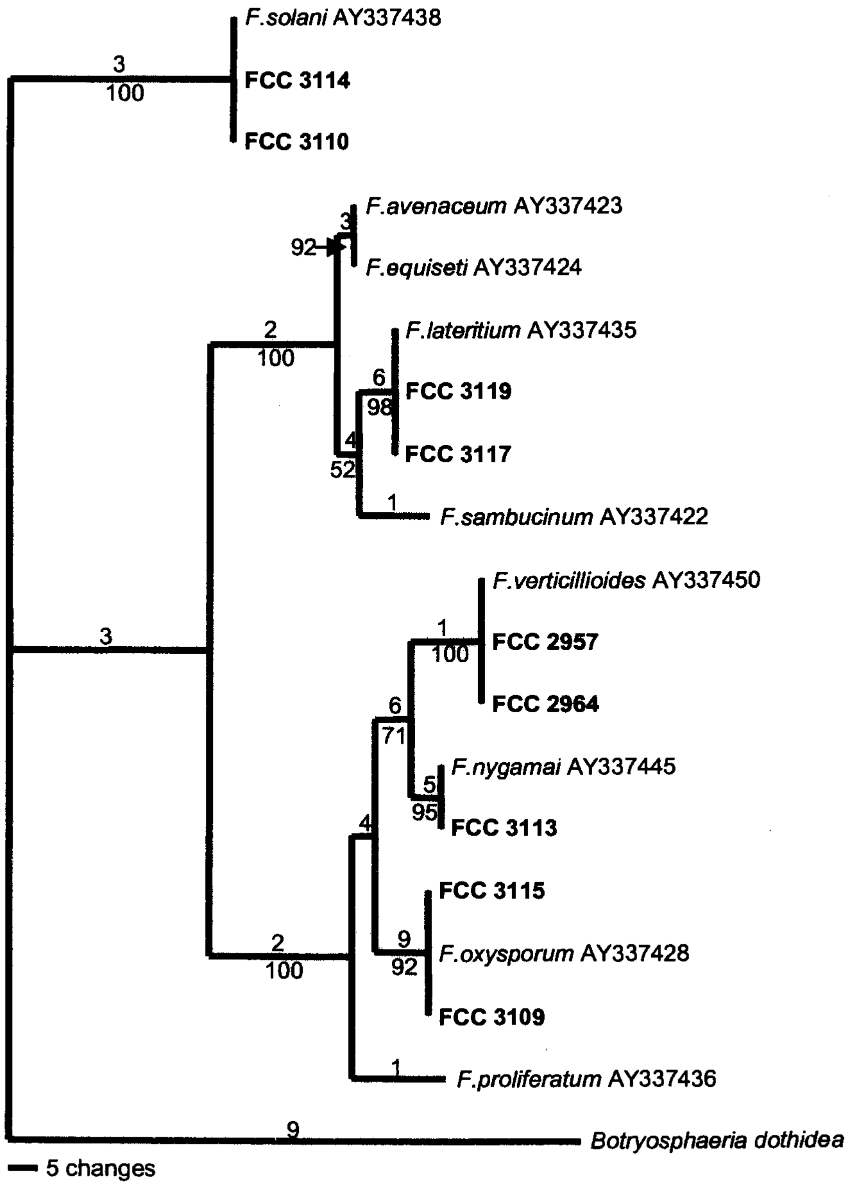

F.solani AY337438
3
100
FCC 3114
FCC 3110
F.avenaceum AY337423
3
92
F.equiseti AY337424
2
100
F.lateritium AY337435
6
FCC 3119
98
4
FCC 3117
52
1
F.sambucinum AY337422
F.verticillioides AY337450
1
FCC 2957
3
100
FCC 2964
6
71
F.nygamai AY337445
5
95
FCC 3113
4
FCC 3115
9
2
F.oxysporum AY337428
100
92
FCC 3109
1
F.proliferatum AY337436
9
Botryosphaeria dothidea
5 changes

Figure 7. The most parsimonious tree obtained from a subset of *Phytophthora* isolates (814 steps, CI = 0.885, RC = 0.667, RI = 0.754) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of combined ITS1, 5.8s subunit and ITS2 regions of the genomic RNA gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown below branches and decay indices above. A *Pythium aphanidermatum* (GenBank accession number AJ233438) was used as out – group.

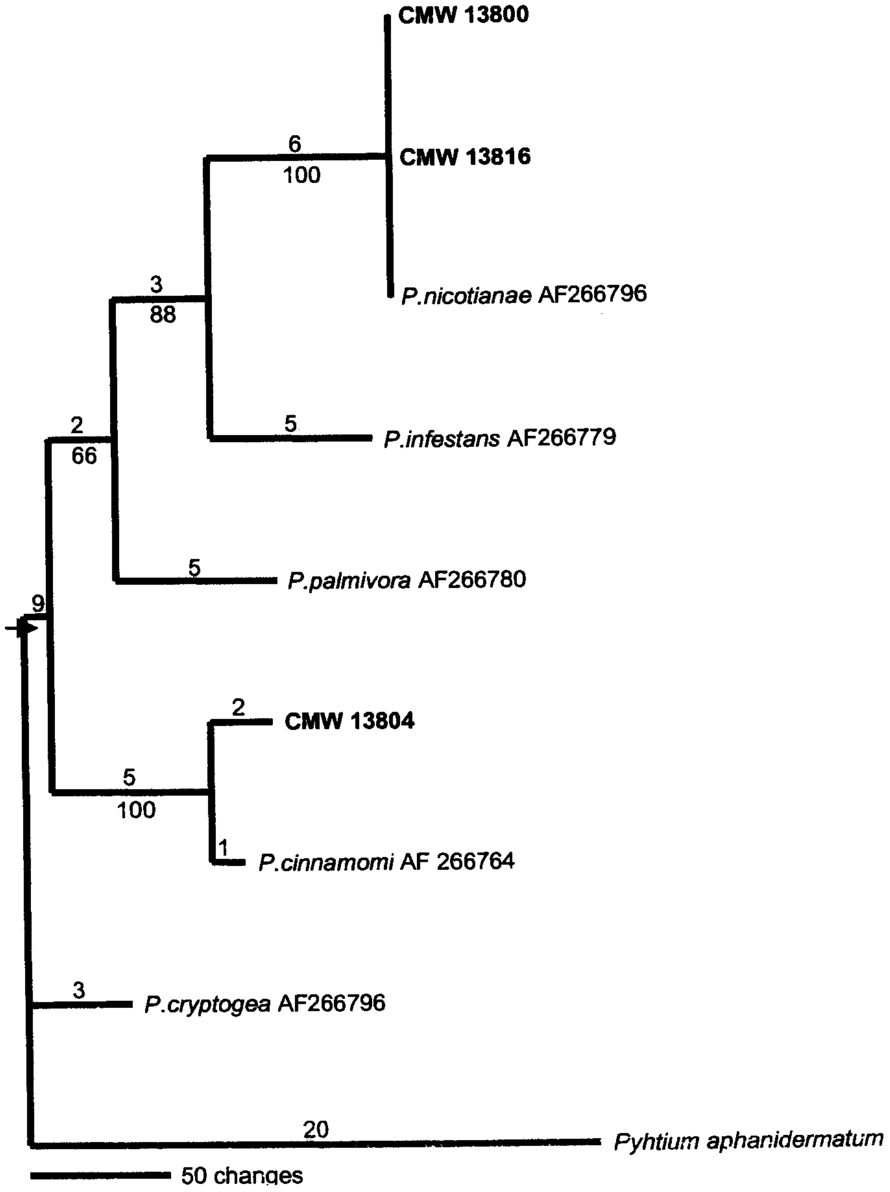
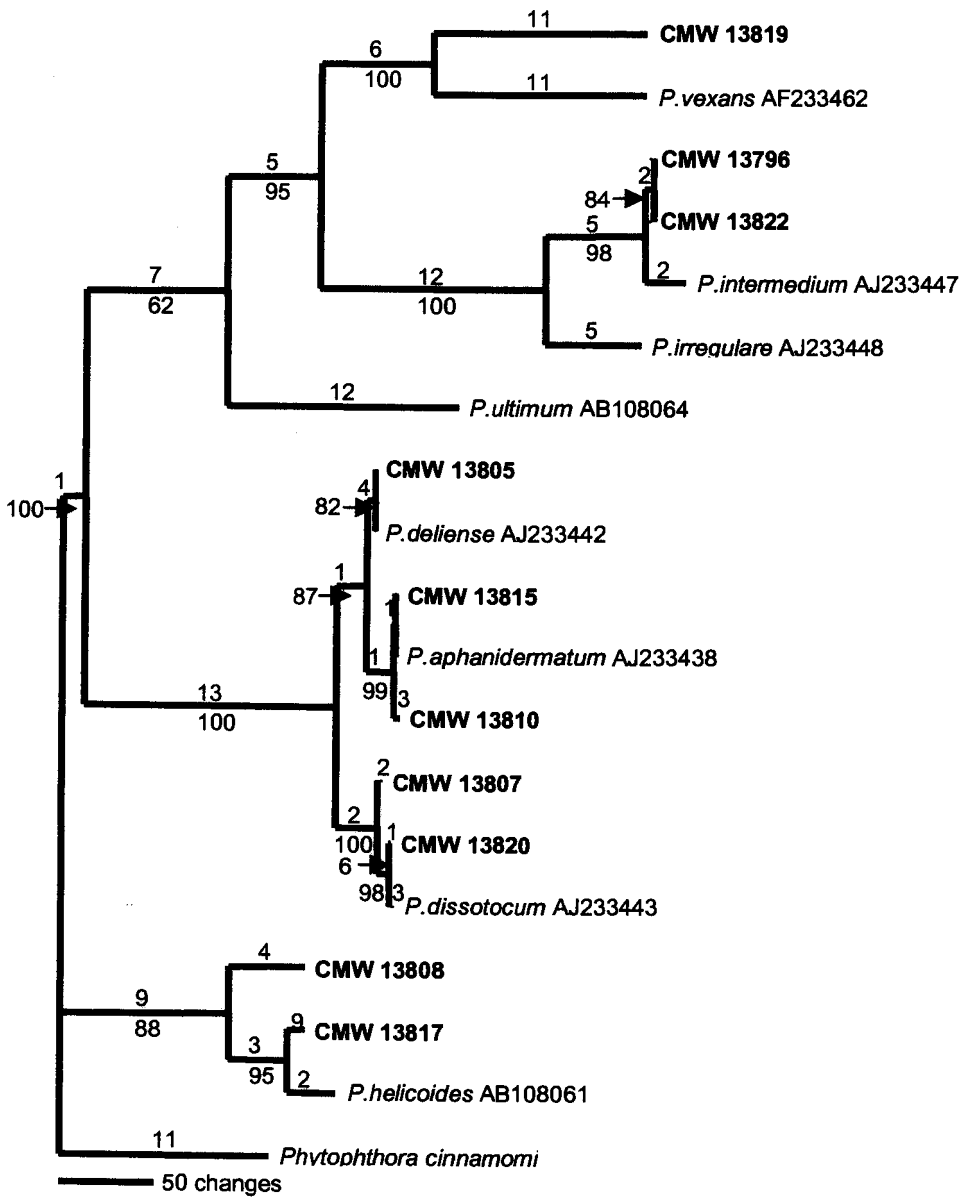
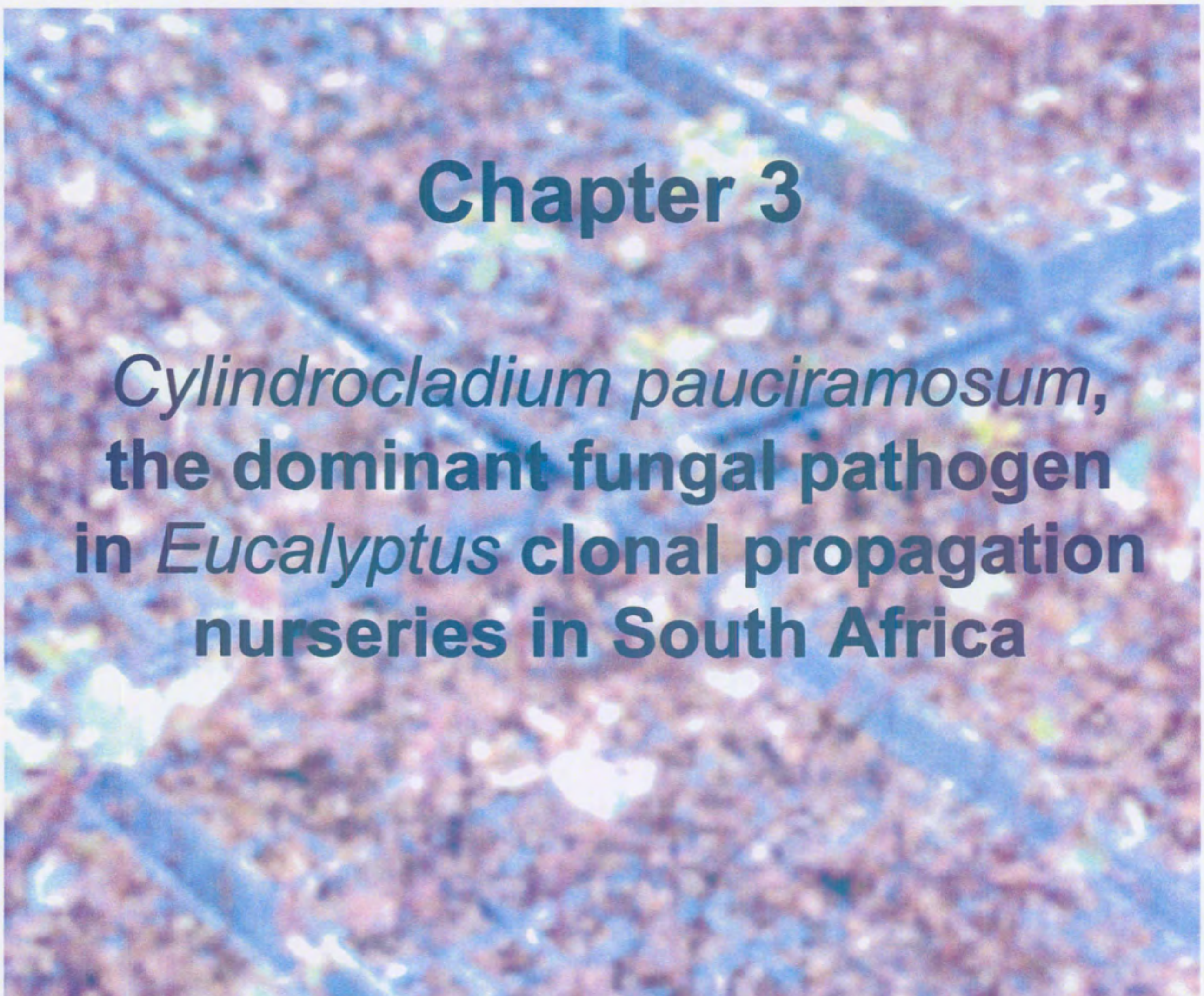

CMW 13800
6
100
CMW 13816
P.nicotianae AF266796
3
88
5
P.infestans AF266779
2
66
5
P.palmivora AF266780
9
2
CMW 13804
5
100
1
P.cinnamomi AF 266764
3
P.cryptogea AF266796
20
Pyhtium aphanidermatum
50 changes

Figure 8. The most parsimonious tree obtained from a subset of *Pythium* isolates (851 steps, CI = 0.748, RC = 0.620, RI = 0.819) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of combined ITS1, 5.8s subunit and ITS2 regions of the genomic RNA gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown below branches and decay indices above. A *Phytophthora cinnamomi* (GenBank accession number AF266764) was used as out – group.


CMW 13819
P.vexans AF233462
CMW 13796
CMW 13822
P.intermedium AJ233447
P.irregulare AJ233448
P.ultimum AB108064
CMW 13805
P.deliense AJ233442
CMW 13815
P.aphanidermatum AJ233438
CMW 13810
CMW 13807
CMW 13820
P.dissotocum AJ233443
CMW 13808
CMW 13817
P.helicoides AB108061
Phytophthora cinnamomi
50 changes



Chapter 3

Cylindrocladium pauciramosum, the dominant fungal pathogen in *Eucalyptus* clonal propagation nurseries in South Africa

Abstract

The implementation of clonal forestry in South Africa has resulted in substantial changes to the *Eucalyptus* nursery system. Thus, large clonal nurseries needed to be established. In these systems, fungal pathogens can be responsible for losses during the rooting of cuttings. The aim of this study was to identify the dominant fungal pathogens responsible for these losses in South African *Eucalyptus* clonal nurseries. A survey was conducted at four of the major nurseries that produce cuttings and which are located in KwaZulu Natal. At each nursery, five of the most commonly produced hybrid clones were selected for sampling. Cuttings were collected at each nursery and immediately examined for symptoms and then placed in moist chambers to enhance sporulation of fungi. Several well-known fungal pathogens were collected and amongst these, *Cylindrocladium* isolates were dominant. All *Cylindrocladium* isolates were identified as *C. pauciramosum* based on morphological characteristics and this was confirmed using DNA sequence data comparisons based on part of the β-tubulin gene. The pathogenicity of *C. pauciramosum* was tested on two – month – old plants representing four commercial hybrid clones. The pathogen gave rise to infection on all clones inoculated with little difference noted between these clones.

Introduction

During the course of the past two decades, the *Eucalyptus* nursery system in South Africa has undergone major changes to accommodate the implementation of clonal forestry. This forestry system involves the utilization of a limited number of selected clones and hybrids propagated by means of vegetative cuttings or tissue culture (Denison & Kietzka 1993b, White 1995). The result is large-scale uniform plantations of selected tropical and sub – tropical *Eucalyptus* clones and interspecific clonal hybrids (Kulkarni & Lal 1995, Wilson 1998). Generally, clones and hybrids are intensively selected for growth and form, disease resistance, wood properties and adaptation to environmental and climatic conditions (Denison & Kietzka 1993a, Aimers – Halliday *et al.* 1999).

Eucalyptus hybrid clones, along with *Pinus* spp., have emerged as amongst the most widely planted exotic forestry trees in South Africa (Denison & Kietzka 1993b). This is due to their rapid growth, adaptability and valuable end products. It is thus imperative to ensure an adequate supply of raw material, not influenced by factors such as diseases that could affect both quality and quantity of the end products. To support these clonal plantations, it is necessary to raise and maintain healthy *Eucalyptus* plants in nurseries.

Fungi cause most of the nursery diseases known on *Eucalyptus* species and include foliar, stem and root diseases (Viljoen *et al.* 1992, Brown & Ferreira

2000). Among the most important diseases are damping – off, root rot, shoot or web blight, stem cankers and leaf spots (Sharma *et al.* 1985, Viljoen *et al.* 1992, Brown & Ferreira 2000). Adequate control of these fungi relies on sound management strategies in nurseries that require knowledge of their development and spread.

Development of diseases in cuttings can be attributed to several factors during production. The optimal environmental requirements for rooting of *Eucalyptus* cuttings are similar to those required for fungal growth and infection (Alfenas *et al.* 1997). Close spacing of plants combined with regular watering and heavy use of fertilizers provides a microclimate between the plants that is ideal for the growth and infection of plants by pathogens. Wounding caused during cutting production or handling also facilitates infection.

Several reviews and surveys have been published on diseases of *Eucalyptus* spp. in nurseries (Sharma *et al.* 1985, Crous *et al.* 1991, Viljoen *et al.* 1992, Alfenas *et al.* 1997, Brown & Ferreira 2000). These have included diseases in both seedling and clonal cutting systems. There has, however, been no overall evaluation of diseases in clonal cutting nurseries in South Africa. The aim of this study was, therefore, to conduct a survey of *Eucalyptus* cutting nurseries and to determine which pathogens are most important in this environment.

Materials & Methods

Survey of Eucalyptus *hybrid clones*

Hybrid clonal *Eucalyptus* cuttings were collected from the four largest cutting nurseries in South Africa. All four forestry nurseries are located in KwaZulu Natal, South Africa. One nursery was situated in central KwaZulu Natal (Pietermaritzburg area) and the other three on the KwaZulu Natal north coast (Richards Bay area). At each nursery, five of the most commonly produced hybrid clones were selected for sampling. The hybrid clones included a pure *Eucalyptus grandis* Hill (TAG) clone, *E. grandis x camaldulensis* Dehnh. (GxC), *E. grandis x urophylla* Blake (GxU), *E. grandis x nitens* (Deane & Maiden) Maiden (GxN) and hybrid *E. nitens* x *grandis* (NxH). For each of the clones, 50 cuttings showing symptoms of disease were collected. Samples were placed in brown paper bags and transferred to the laboratory for further study. These collections were made on three different occasions at three-month intervals. Thus, 250 samples were collected from each nursery on each of three occasions, resulting in a total sample of 3000 cuttings.

Isolations were made directly from the cuttings on 2% Malt Extract Agar (MEA; Biolab, Midrand, South Africa) from half of the cuttings sampled. The remaining 25 cuttings were placed in moist chambers and incubated at room temperature for 48h in the dark to promote sporulation of fungi. Conidia and fungal structures produced on the cuttings were transferred to 2% MEA and incubated for seven days at 25ºC under continuous near – ultraviolet light. Preliminary identifications were made with light microscopy and the most commonly isolated fungal pathogen was selected for further study. The cultures derived from this study have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological characteristics

A large number of isolates resembling *Cylindrocladium* spp. were obtained from the cuttings and these were the most common probable pathogens isolated (Table 1). These *Cylindrocladium* isolates were grouped into five groups based on colony morphology and growth rate (results not shown). For each group, ten isolates were randomly selected for more comprehensive identification based on morphology.

Single conidial isolates were transferred onto Carnation Leaf Agar (CLA) plates (Crous 2002) to induce production of both anamorph and teleomorph structures. The plates were incubated at 25ºC under continuous near – ultraviolet light and examined after 14 days. Morphological characteristics described by Crous (2002) were used to identify cultures. Macroconidiophores were mounted in lactophenol and twenty measurements of vesicles, stipes and conidia were made for each of the selected isolates, using a light microscope. Measurements for these structures are presented as (min-)(average – standard deviation) – (average + standard deviation)(-max).

DNA sequence comparisons

One isolate (Table 2) was randomly selected from each of the five morphological groups of *Cylindrocladium* isolates for DNA sequence comparisons. The single conidial isolates were grown on 2% MEA plates from which mycelium was collected and freeze dried. The freeze – dried mycelium was ground to a fine powder in liquid nitrogen with a mortar and pestle. DNA was extracted using the technique described by Möller *et al.* (1992).

A 495 base pairs fragment of the β - tubulin gene was amplified using primers T1 (O'Donell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). The PCR reaction of 25µl comprised of 2.5 units Taq enzyme (Roche Molecular Biochemicals, Almeda, California, USA), 10x buffer, 1mM $MgCl_2$ (as supplied by manufacturer), 0.25 mM deoxynucleoside triphosphate (dNTPs), 0.5 µl primers and approximately 30 ng of fungal DNA as target. The amplified fragments were purified using a High Pure PCR Product Purification Kit (Roche Molecular Biochemicals, Almeda, California, USA).

Both DNA strands of the PCR products was sequenced using the primers used for the PCR amplification. Sequence reactions were done using an ABI

PRISM™3100 DNA Autosequencer (Applied BioSystems). Sequence data were processed using Sequence Navigator version 1.0.1 (Applied BioSystems). The nucleotide sequences were aligned manually by inserting gaps where necessary and phylogenetic relationships were determined using PAUP version 4.0b10 (Swofford 2002). Gaps were treated as missing data and confidence intervals were determined using 1000 bootstrap replications. To establish the phylogenetic relationships and identities of the *Cylindrocladium* isolates from the cuttings, 15 sequences of known *Cylindrocladium* species (Table 2) obtained by Schoch *et al.* (2001) and Crous (2002) were taken from GenBank and included in the alignment. *Cylindrocladiella infestans* Boesew. (AF320190) was used as the outgroup taxon in the analyses.

Evaluation of pathogenicity

Plants selected to assess pathogenicity of *Cylindrocladium* isolates represented four of the five hybrid clones included in the disease survey. These were thus TAG, GxC, GxU and GxN. The two-month-old plants were maintained in a greenhouse with a constant environment at 25°C prior to and during the trial. Thirty plants of each hybrid clone were used for the trial, which included ten controls for each clone.

One isolate of *C. pauciramosum* (CMW 9108), which was identified as the dominant potential pathogen from the surveys, was selected for inoculation of

Eucalyptus hybrid clones. This isolate was selected because of its rapid growth on MEA (results not shown) and its ability to produce abundant fruiting structures. The isolate was transferred to 100 2% MEA plates and incubated for seven days at 25°C under continuous near – ultraviolet light.

A spore suspension was prepared by adding 1 – 2 ml of sterile water to the plates and dislodging conidia with a sterile glass rod. The spore suspension was drained through a layer of cheesecloth and the concentration adjusted to 3.3 x 10^5 conidia/ml. Tween 80 (Merck – Schuchardt, Hohenburnn, Germany) was added to the suspension to ensure that conidia adhered to the plant tissue surface.

The spore suspension was sprayed onto the surface of the leaves to just before run – off. The plants were watered and covered with plastic bags for 48h to ensure sufficient humidity for infection. Controls were sprayed with sterile water and otherwise treated in the same manner as the treated plants. Lesions were scored after removing the plastic bags.

Scoring of symptoms was achieved using a scale of 0 – 4 with 0 representing 0% infection, 1 representing less than 25% infection, 2 representing 26 – 50% infection, 3 representing 51 – 75% infection and 4 representing above 76% infection. Statistical analyses of the infection data were carried out using SAS analytical programmes (1990). The analysis produced means for each scale with a 95% confidence limit for each mean using the General Linier Method. Re – isolations were made from the lesions to confirm the presence of the test fungus.

Results

Survey of Eucalyptus *hybrid clones*

Several well-known *Eucalyptus* nursery pathogens, represented by 2659 fungal isolates, were collected from the *Eucalyptus* cuttings at all four forestry nurseries surveyed (Table1). *Cylindrocladium* isolates were the most common potential pathogens at all four forestry nurseries and the next most common pathogen was *Botrytis cinerea* Pers. (Table 1). The pathogens were isolated from the roots, stems and leaves of the cuttings and all have been reported previously in South Africa.

Morphological characteristics of Cylindrocladium isolates

White macroconidiophores characteristic of those of *Cylindrocladium* spp. (Fig. 1A – B) were common on the surface of the *Eucalyptus* cuttings collected at the five forestry nurseries. Cultures on 2% MEA resulting from isolations from these structures differed in colony morphology. Thus, grouping according to colony morphology and growth rate was necessary.

All *Cylindrocladium* isolates were morphologically similar. The stipes and extensions to the stipes (Fig. 2 A – B) were septate, straight, hyaline, (85)103 – 130(147) µm in length and terminated in obpyriform to ellipsoidal vesicles, (11)14 – 19(23) x (4)5 – 7 µm (Fig. 2 A – D). Each terminal branch of the fertile branches produced approximately 4 – 6 phialides (Fig. 2B). Phialides were doliiform to reniform, hyaline and aseptate. Conidia were cylindrical, straight, (40)46 – 53(59) x 4 – 5(6) µm, 1 – septate and held in parallel cylindrical clusters by colourless slime (Fig. 2E). These characteristics are typical of *C. pauciramosum* C. L. Schoch & Crous as described by Schoch *et al.* 1999.

DNA sequence comparisons for Cylindrocladium isolates

A data set of 15 in-group taxa and one out-group taxon, *Cylindrocladiella infestans*, was analyzed. The alignment of the β-tubulin gene fragments gave rise to a data set of 495 characters of which 316 were constant, 62 were parsimony – uninformative and 17 parsimony – informative (Appendix II). One tree from two most parsimonious trees was chosen for presentation (Fig. 4). The trees had a consistency index (CI) = 0.817, retention index (RI) = 0.813, and rescaled consistency index (RC) = 0.678 while gaps were treated as missing data. The phylogenetic tree (Fig.4) clearly showed that all five randomly selected *Cylindrocladium* isolates grouped in the clade representing *C. pauciramosum* (74% bootstrap support).

Pathogenicity trial

First symptoms of infection were observed immediately after removal of the plastic bags at 48 hours (Fig. 3A – D). Initial symptoms on the leaves appeared as water – soaked lesions that caused a pink to purple discolouration of the leaf laminas. Lesions later became light brown and surrounded by a dark red to purple halo and varied in shape from round to irregular depending on the hybrid clone inoculated (Fig. 3A – D). Regression coefficients over the 22 days of evaluation were then determined for the mean of each scale (Table 3). All four clones were susceptible to *C. pauciramosum* infection (Fig. 3A – D) with the sub – tropical hybrid clone GxC (Fig. 3A) being the most susceptible. No symptoms were observed on the control plants.

Discussion

This study represents the first survey of pathogens in *Eucalyptus* clonal production nurseries in South Africa. A relatively large number of well – known pathogens were found and all have previously been recorded in the country (Lundquist & Baxter 1985, Crous *et al.* 1991, Viljoen *et al.* 1992). An interesting result was that a single species of *Cylindrocladium* was present and this was also the dominant pathogen in all nurseries surveyed.

Cylindrocladium spp. are well-known and important pathogens of *Eucalyptus* in nurseries around the world. In South Africa only *C. scoparium* Morgan, *C. candelabrum* Viégas, *C. clavatum* Hodges & May and *C. colhounii* Peerally have been reported from *Eucalyptus* spp. in forestry nurseries and plantations (Crous *et al.* 1991, Crous *et al.* 1993). This study, however, showed that only *C. pauciramosum* occurs in cutting production nurseries at the present time. It is possible that other *Cylindrocladium* spp. could be important on *Eucalyptus* cuttings in nurseries in the future, but the widespread occurrence of a single species suggests that *C. pauciramosum* is the dominant pathogen.

In this study, we used β – tubulin sequence data to confirm the identification of the *Cylindrocladium* isolates. Schoch *et al.* (1999) found that sequencing the Inter Transcribed spacer (ITS) including the 5.8S ribosomal DNA (rDNA) of *Cylindrocladium* spp. yielded very few variable characters in the region, to such an extent that the mating populations in the *C. candelabrum* species complex including *C. pauciramosum*, could not be distinguished from each other. DNA sequences obtained from part of the β – tubulin gene yielded more variable characters and distinguished between these fungi (Schoch *et al.* 2001). This was confirmed in the present study and supported our identifications made based on morphology.

Pathogenicity tests conducted in this study represent the first that have been done with *C. pauciramosum* on *Eucalyptus* hybrid plants. These tests showed that most of the commonly used hybrids in the forestry industry are susceptible to infection by this pathogen. The extent of leaf lesions varied between the hybrids as has been shown previously with other *Cylindrocladium* spp. (Brown & Ferreira 2000). This implies that it might be possible to select disease tolerant plants based on nursery screening, but many more clones have to be used in tests to verify this observation.

Several pathogens are responsible for diseases of *Eucalyptus* cuttings in forestry nurseries of South Africa. The most important pathogen of *Eucalyptus* cuttings in South Africa is clearly *C. pauciramosum*. In this study, *C. pauciramosum* was the only *Cylindrocladium* sp. present in the forestry nurseries where the surveys were conducted. This pathogen is capable of rapid infection and can thus cause severe epidemics in forestry nurseries, if it is not controlled. Future studies are thus planned to gain a greater understanding of the genetic structure of the pathogen in South Africa and to consider relative susceptibility of important planting stock.

References

Aimers – Halliday, J., Dibley, M. J., Hong, S. O. & Menzies, M. I. (1999) Cutting propagation of hybrid *Eucalyptus grandis x E. nitens*. *New Zealand Journal of Forestry Science* **29**: 238 – 250.

Alfenas, A. C., Silveira, S. F. da & Stowasser, E. A. S. V. (1997) Current status and control strategies of diseases associated to clonal propagation of *Eucalyptus* in Brazil. *IUFRO Conference on Silviculture and Improvement of Eucalyptus, Salvador, Brazil, 24 – 29 August.*

Brown, B. N. & Ferreira, F. A. (2000) Disease during propagation of eucalypts. In *Diseases and pathogens of eucalypts*, Chapter 8: 119-151.(Ed. P. J. Keane, G. A. Kile, F. D. Podger, B. N. Brown). CSIRO Publishing, Collingwood, Australia.

Crous, P. W. (2002) Taxonomy and pathology of *Cylindrocladium* (*Calonectria*) and allied genera. APS Press, St. Paul, Minnesota, USA.

Crous, P.W., Phillips, A. L. J. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Journal of Forestry* **157**: 69 – 85.

Crous, P.W., Phillips, A. L. J. & Wingfield, M. J. (1993) New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Plant Pathology* **42**: 302 – 305.

Denison, N. P. & Kietzka, J. E. (1993a) The use and importance of hybrid intensitive forestry in South Africa. *South African Forestry Journal* **162**: 55 – 60.

Denison, N. P. & Kietzka, J. E. (1993b) The development and utilization of vegetative propagation in Mondi for commercial afforestation programmes. *South African Forestry Journal* **162**: 47 – 54.

Glass, N. L. & Donaldson, G. (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323 – 1330.

Kulkarni, H. D. & Lal, P. (1995) Performance of *Eucalyptus* clones at ITC Bhadrachlm, India. CRC for Temperate Hardwood Forestry, IUFRO, Hobart.

Lundquist, J. E. & Baxter, A. P. (1985) Fungi associated with *Eucalyptus* in South Africa. *South African Forestry Journal* **138**: 9 – 19.

Möller, E. M., Bahnweg, G., Sandermann, H. & Geiger, H. H. (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acid Research* **20**: 6115 – 6116.

O'Donnell, K. & Cigelnik, E. (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics Evolution* **7**: 103 – 116.

Schoch, C. L., Crous, P. W. Wingfield, B. D. & Wingfield, M. J. (1999) The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**: 286 – 298.

Schoch, C. L., Crous, P. W. Wingfield, B. D. & Wingfield, M. J. (2001) Phylogeny of *Calonectria* based on comparisons of β - tubulin DNA sequences. *Mycological Research* **105**: 1045 – 1052.

Sharma, J. K. Mohanan, C. Florence, E. J. M. (1985) Disease survey in nurseries and plantations of forest tree species grown in Kerala. *Research report 36. (Kerala Forest Research Institute: Peechi, Kerala).*

Swofford, D. L. (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods), 4.0b10. Computer programme. Sunderland, Massachusetts, U.S.A.: Sinauer Associates.

Viljoen, A. Wingfield, M. J. & Crous, P. W. (1992) Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: A review. *South African Forestry Journal* **161**: 45-51.

White, T. L. (1995) Prototype breeding strategies for clonal forestry: A case study of *Eucalyptus grandis*. CRC for Temperate Hardwood Forestry, IUFRO, Hobart.

Wilson, P. J. (1998) Developing clones from *Eucalyptus globulus* and hybrid seedlings by stem cuttings propagation. *New Zealand Journal of Forestry Science* **28**: 293 – 303.

Table 1: List of 2659 fungal isolates obtained during the survey of *Eucalyptus* hybrid clone cuttings from all four forestry nurseries expressed as percentages.

FUNGUS	**Percentage of isolates**
Alternaria sp.	3
Botryosphaeria sp.	0.6
Botrytis cinerea Pers.	15
Cladosporium sp.	0.1
Colletotrichum gloeosporioides	8
Cylindrocladiella sp1.	2
Cylindrocladiella sp2.	1
Cylindrocladium sp.	21
Eppiccocum sp.	5
Fusarium sp1.	5
Fusarium sp2.	2
Fusarium sp3.	2
Fusarium sp4.	4
Gliocladium sp.	5
Hainesia lythri	4
Mycosphaerella spp.	a
Pestalatiopsis sp.	7
Phomopsis sp.	3
Pseudocercospera sp.	a
Rhizoctonia sp.	5
Trichoderma sp.	7
Verticillium sp.	0.3

(a) Observed but not isolated from *Eucalyptus* hybrid clone cuttings.

Table 2: List of *Cylindrocladium* spp. used in DNA sequence comparisons.

Species	**Isolate number**	**GenBank Assencion number**
Cylindrocladium candelabrum	STE-U 1677	AF210858
	STE-U 1674	AF210857
C. clavatum	ATCC 46300	AF232850
C. colhounii	STE-U 307	AF232855
	STE-U 705	AF232854
C. gracile	STE-U 623	AF333405
	IMI 167580	AF333404
C. ilicola	STE-U 723	AF333413
	CBS 190.50	AF333412
C. insulare	STE-U 3211	AF44951
	STE-U 3219	AF44950
C. pauciramosum	[a]**CMW 8741**	
	[a]**CMW 9108**	
	[a]**CMW 9109**	
	[a]**CMW 9189**	
	[a]**CMW 9190**	
	STE-U 3207	AF449448
	CSL 2021133	AY162320
C. scoparium	STE-U 1722	AF210875
	STE-U 1720	AF210874

[a] *Cylindrocladium* isolates from *Eucalyptus* cuttings that were sequenced.

Table 3: Regression coefficients for the means of each scale to indicate rate of infection (leaves/day) for each *Eucalyptus* hybrid clone used in the pathogenicity test with *C. pauciramosum*.

Hybrid clone	0*	1*	2*	3*	4*
G x C	1.90 [b]	-0.41 [a]	0.37 [b]	0.73 [a]	1.20 [a]
G x N	1.49 [a]	-0.69 [b]	-0.56 [a]	0.52 [b]	0.69 [a]
G x U	1.80 [a]	-0.15 [b]	-0.21 [b]	0.39 [b]	0.68 [b]
TAG	1.16 [a]	-0.43 [a]	0.68 [a]	0.72 [b]	-5.42 [b]

[a] Infection rate significant (P< 0.05).

[b] Infection rate not significant (P>0.05).

* Scales used to score disease symptoms.

Figure 1: Macroconidiophores of *Cylindrocladium pauciramosum*. A. Macroconidiophores on the stem of a *Eucalyptus* cutting collected during the survey. B. Macroconidiophores on a *Eucalyptus* leaf.

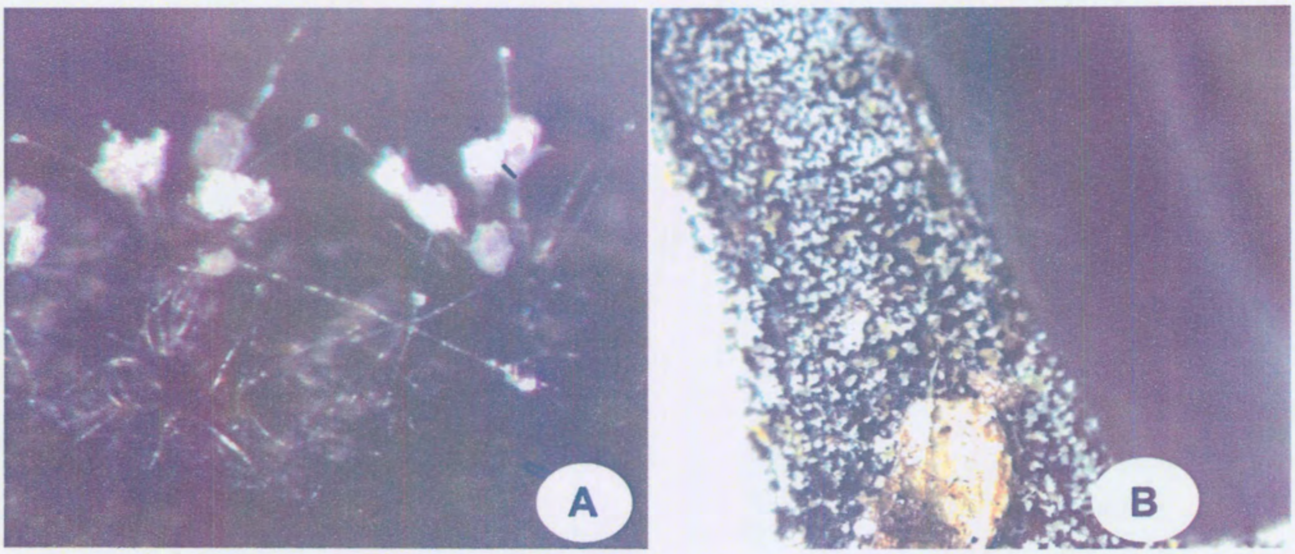

A
B

Figure 2: Macroconidiophores, vesicles and conidia of *Cylindrocladium pauciramosum*. A – B. Macroconidiophores. C – D. Vesicles. E. Conidia (Scale bars = 10 µm).

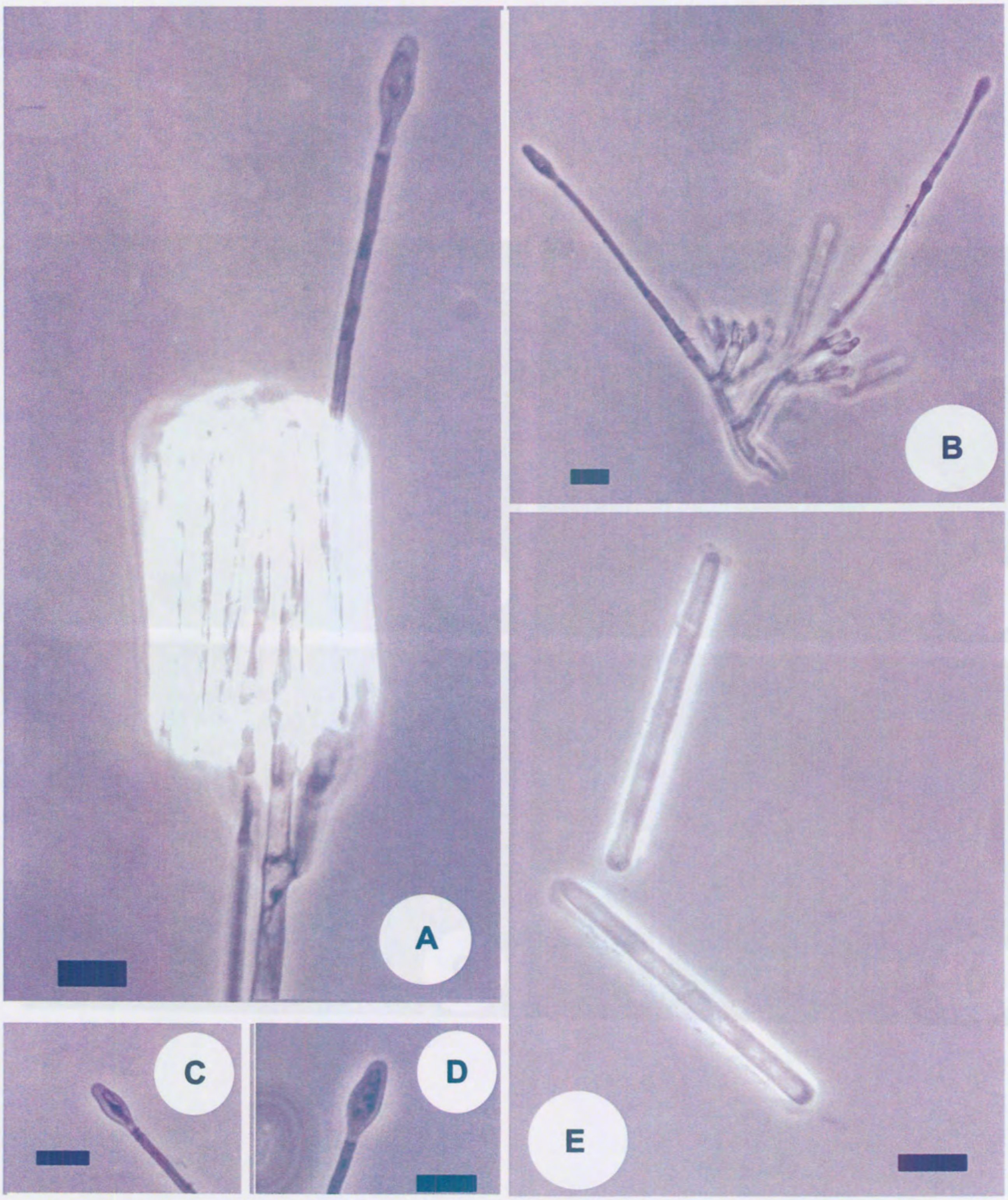

A
B
C
D
E

Figure 3: Leaf lesions on *Eucalyptus* hybrid clones used in pathogenicity trial.

A. *Eucalyptus grandis* x *camaldulensis;* B. *E. grandis* x *nitens;* C. *E. grandis* x *urophylla;* D. *E.grandis*

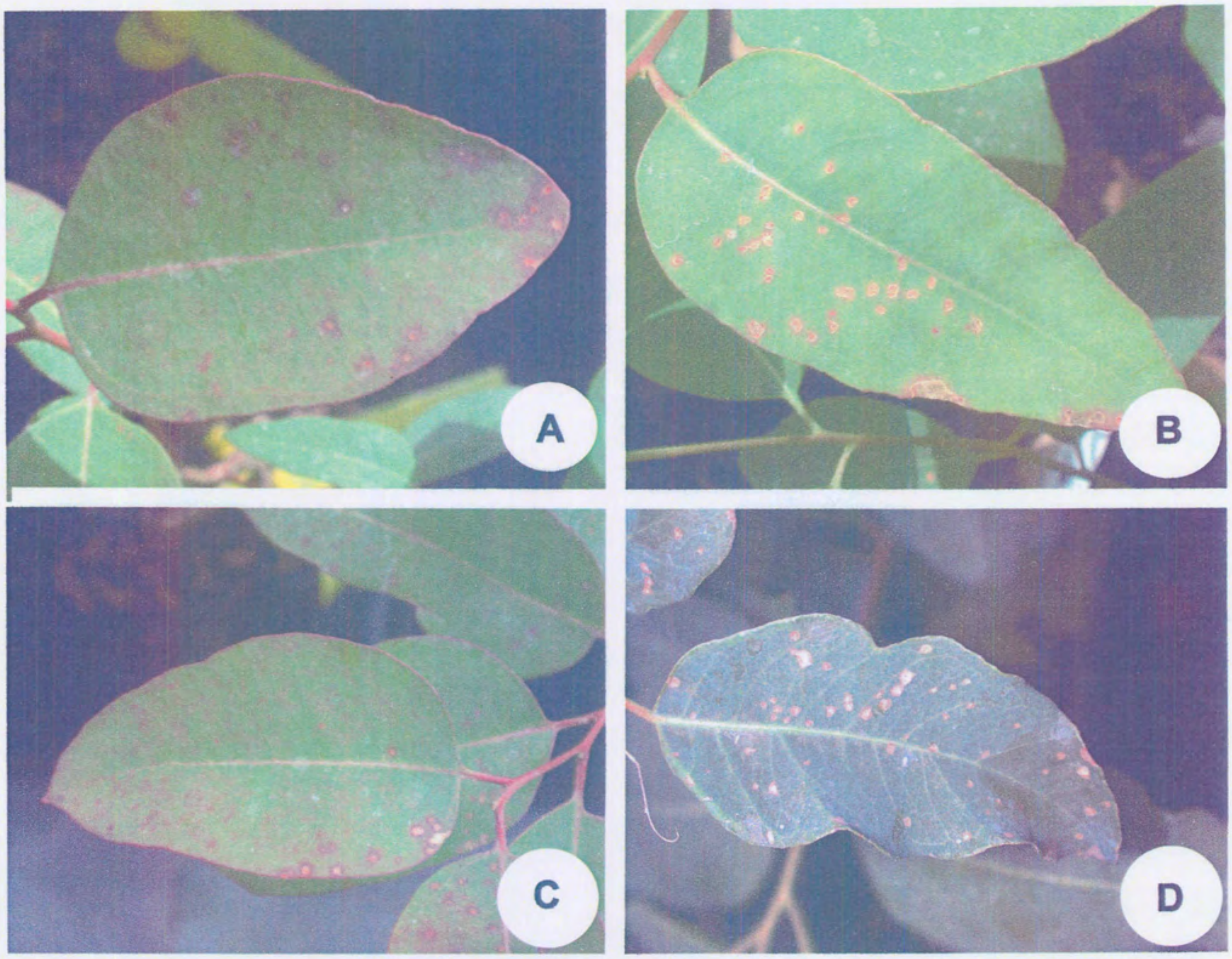
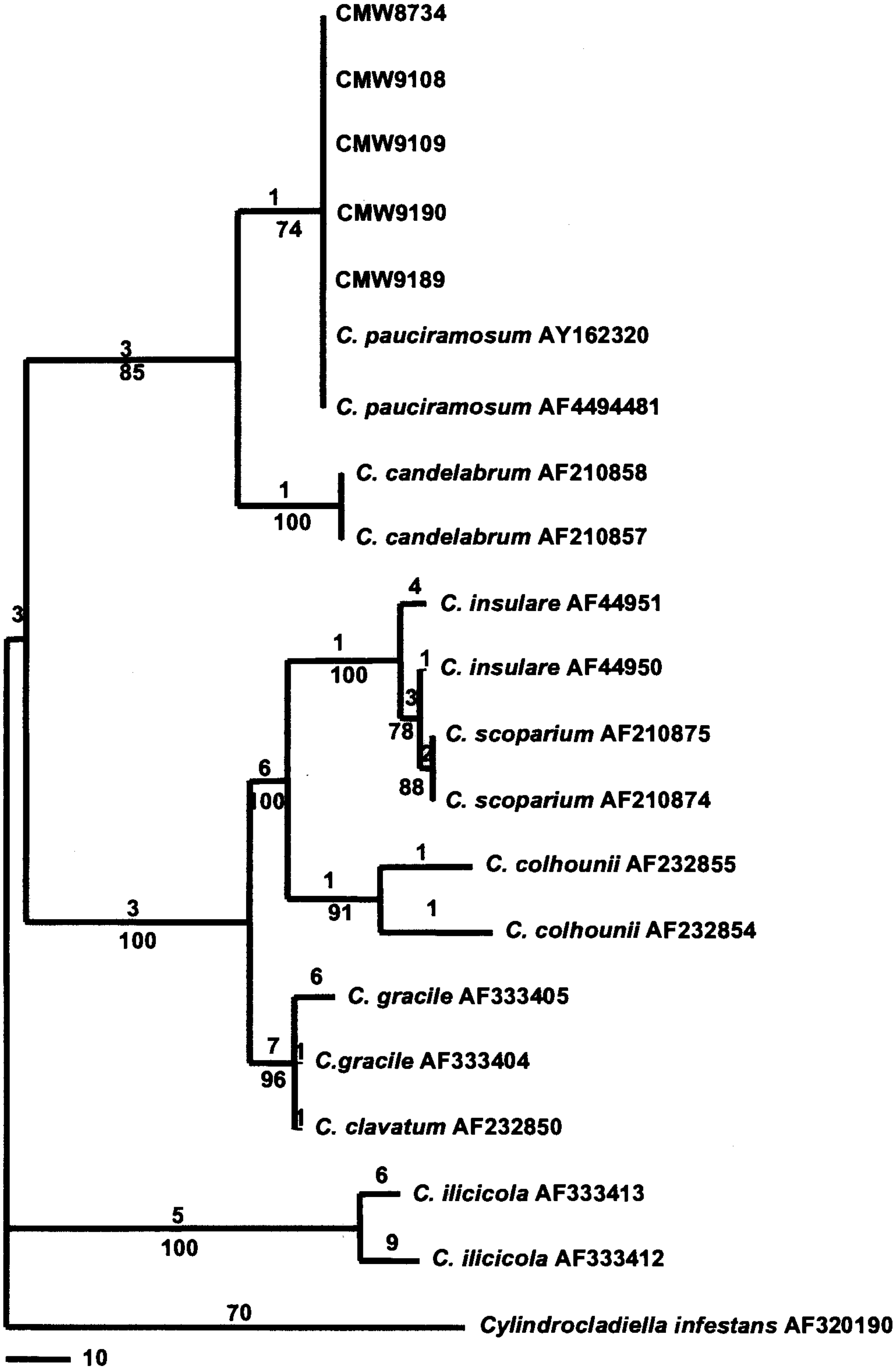

A
B
C
D

Figure 4: The most parsimonious tree obtained from a subset of *Cylindrocladium* isolates (495 steps, CI = 0.817, RC = 0.678, RI = 0.813) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of the 5' end of the β - tubulin gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown above branches and decay indices below. A *Cylindrocladiella infestans* (GenBank accession number AF320190) was used as outgroup.


CMW8734
CMW9108
CMW9109
CMW9190
CMW9189
C. pauciramosum AY162320
C. pauciramosum AF4494481
C. candelabrum AF210858
C. candelabrum AF210857
C. insulare AF44951
C. insulare AF44950
C. scoparium AF210875
C. scoparium AF210874
C. colhounii AF232855
C. colhounii AF232854
C. gracile AF333405
C.gracile AF333404
C. clavatum AF232850
C. ilicicola AF333413
C. ilicicola AF333412
Cylindrocladiella infestans AF320190
10

Chapter 4

Cylindrocladium pauciramosum infecting *Acacia mearnsii* seedlings in South Africa

Abstract

The versatility of *Acacia mearnsii* makes this a popular tree to cultivate in commercial plantations in South Africa. The timber has a wide variety of uses and the bark contains vegetable tannins that are important in the leather and adhesives industry. The tree is native to Australia and in South Africa has been affected by a number of serious diseases in plantations and nurseries. This study arose from an outbreak of *Cylindrocladium* blight in an *A. mearnsii* nursery. The aim was thus to identify the causal agent of the disease. *Cylindrocladium* isolates were collected from diseased seedlings and identified as *C. pauciramosum*, based on morphological characteristics as well as DNA sequence comparisons. A pathogenicity test was conducted, where *A. mearnsii* seedlings were inoculated using three different isolates. Comparison of lesion lengths and re-isolation of the inoculated fungus from lesions showed that the fungus was pathogenic and there was little difference between isolates.

Introduction

Acacia mearnsii de Wild (black wattle) was first introduced into South Africa from Australia in 1864 (Sherry 1971) to provide firewood, shelterbelts and shade (Dunlop 2002). The first plantations of *A. mearnsii* were established in South Africa in 1888 after it was discovered that the bark was rich in vegetable tannins that could be used in the leather industry (Dunlop 2002). The demand for leather products during World War II led to increased wattle plantings in the mid 1900's and these now make up about 7% of the South African commercial forestry areas (Dunlop 2002, Anonymous 2003).

In South Africa, *A. mearnsii* has been damaged by several pathogens and pests (Wingfield & Roux 2000). Several reviews on this topic have been provided (Roux *et al.* 1995, Old *et al.* 2003, Roux 2002). There is, however, a general lack of knowledge regarding disease problems associated with *A. mearnsii* in forestry nurseries (Roux 2002).

Roux (2002) reported that the most common disease problems of *A. mearnsii* in nurseries are caused by a *Cylindrocladium* sp. Symptoms included root rot (Crous *et al.* 1991), stem cankers and shoot blight (Roux 2002). These symptoms were initially believed to be caused by *C. scoparium* Morgan (Crous *et al.* 1991, Roux & Wingfield 1997) but recent studies indicate that this pathogen is restricted to the Northern hemisphere (Overmeyer *et al.* 1996, Crous 2002). Other *Cylindrocladium* spp. that have been reported on *A. mearnsii* and other *Acacia* spp. elsewhere in the world include *C. crotolariae* (Loos) Bell & Sobers, *C. candelabrum* Viegas, *C. ilicicola* (Hawley) Boedjin &

Reitsma, *C. quinqueseptatum* Boedjin & Reitsma, *C. reteaudii* (Bugn.) Boesew. and *C. theae* (Petch) Subramanian (Old *et al.* 2003).

In South Africa, it has been suggested that the *Cylindrocladium* sp. responsible for diseases of *A. mearnsii* in nurseries resides in the *C. candulabrum* species complex (Schoch *et al.* 1999) and could be *C. pauciramosum* Schoch & Crous (Roux 2002). The aim of this study was to identify the *Cylindrocladium* sp. responsible for losses of *A. mearnsii* in nurseries in South Africa.

Material & Methods

Plant material and isolations

Acacia mearnsii seedlings were collected at a forestry nursery in KwaZulu Natal, South Africa. The 25 plants selected for study were girdled and had stem cankers. Isolations were made from symptomatic tissue directly on 2% Malt Extract Agar (MEA; Biolab, Midrand, South Africa) and incubated for seven days at 25ºC under continuous near – ultraviolet light. All *Cylindrocladium* isolates obtained are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological characteristics

Single conidial isolates were transferred onto Carnation Leaf Agar (CLA) plates (Crous 2002) to induce production of both anamorph and teleomorph structures. The plates were incubated at 25°C under continuous near – ultraviolet light and examined after 14 days. Morphological characteristics described by Crous (2002) were used to identify cultures. Macroconidiophores were mounted in lactophenol and 20 measurements of vesicles, stipes and conidia were made for each isolate using a light microscope. Measurements are presented as (min-)(average – standard deviation) – (average + standard deviation)(-max).

DNA sequence comparisons

Five isolates (Table 1) were randomly selected and utilized in the DNA sequence comparisons. Single conidial isolates were grown on 2% MEA plates from which mycelium was collected and freeze dried. The freeze – dried mycelium was ground to a fine powder in liquid nitrogen using a mortar and pestle with a mortar. DNA was extracted using the technique described by Möller *et al.* (1992).

A 507 bp. fragment of the β - tubulin gene was amplified using primers T1 (O'Donell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). The PCR reaction of 25μl comprised of 2.5 units Taq enzyme (Roche Molecular Biochemicals, Almeda, California, USA), 10x buffer, 1mM $MgCl_2$ (as supplied by manufacturer), 0.25 mM deoxynucleoside triphosphate, 0.5 μm primers and approximately 30 ng of fungal DNA as target. The amplified fragments were purified using a High Pure PCR Product Purification Kit (Taq (Roche Molecular Biochemicals, Almeda, California, USA).

The PCR product was sequenced in both directions using the same primers used for the PCR amplification. Sequence reactions were done using the ABI PRISM™3100 DNA Autosequencer (Applied BioSystems). Sequence data were processed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California, USA). The nucleotide sequences were aligned manually by inserting gaps where necessary and phylogenetic relationships were determined using PAUP version 4.0b10 (Swofford 2002). Gaps were treated as missing data and confidence intervals were determined using 1000 bootstrap replications. To establish the phylogenetic relationships and identities of the *Cylindrocladium* isolates from the *A. mearnsii* seedlings, 12 sequences of known *Cylindrocladium* spp. (Table 1) were obtained from GenBank and included in the alignment. *Cylindrocladiella infestans* Boesew. (AF320190) was used as the out – group taxon in the analysis.

Evaluation of Pathogenicity

Cylindrocladium isolates CMW 9156, CMW 9164 and CMW 9171 were randomly selected for use in glasshouse inoculations on *A. mearnsii*. Isolates were grown on 2% MEA for seven days prior to inoculation. Glasshouse conditions were set with day/night lighting and with an average temperature of approximately 25°C.

Twenty *A. mearnsii* seedlings were inoculated for each isolate and an equal number were used for controls. A 5 mm diameter wound was made on the stem of each seedling by removing the bark with a cork borer and exposing the cambium. Plugs (5mm diam) were cut from the surface of Petri dishes, overgrown with the test isolates and placed in the wounds with the mycelial surface facing the cambium. All inoculations were covered with parafilm to prevent desiccation of the inoculum and wounds. For control inoculations, sterile 2% MEA plugs were used. Lesion lengths were assessed after six weeks and statistical differences in lesion length for each isolate were determined using SAS analytical programmes (2002). Re – isolations were made from the lesions to confirm the presence of the test fungus.

Results

Morphological characteristics

All *Cylindrocladium* isolates were morphologically similar. The stipes and extensions to the stipes (Fig. 1A) were septate, straight, hyaline, (84)102 – 140(179) μm in length and terminated in obpyriform to ellipsoidal vesicles, (10)14 – 22(32) x (3)5 – 7 μm (Fig. 2D - F). Each terminal branch of the conidiophores produced approximately 4 – 6 phialides (Fig. 1A). Phialides were doliiform to reniform, hyaline and aseptate. Conidia were cylindrical, straight, (37)46 – 55(64) x (3)4 – 5(7) μm, 1 – septate and held in parallel cylindrical clusters by colourless slime (Fig. 1B). These characteristics are typical of *C. pauciramosum* C. L. Schoch & Crous as described by Schoch *et al.* (1999).

DNA sequence comparisons

A data set of 17 in-group taxa and one out-group taxon, *Cylindrocladiella infestans*, was analyzed. The alignment of the β-tubulin gene fragments (Fig. 4) gave rise to a data set of 507 characters of which 344 were constant, 70 were parsimony – uninformative and 93 parsimony – informative (Fig.2). One tree from 4 most parsimonious trees was chosen for presentation (Fig.2). The trees had a consistency index (CI) = 0.917, retention index (RI) = 0.929, and rescaled consistency index (RC) = 0.852 while gaps were treated as missing data. The phylogenetic tree (Fig.2) clearly showed that all five randomly selected *Cylindrocladium* isolates from *A. mearnsii* grouped in the clade representing *C. pauciramosum* (94% bootstrap support).

Pathogenicity tests

After six weeks, inoculated seedlings displayed symptoms of die-back and wilting. Exposure of the cambium showed that the vascular tissue had a streaked appearance (Fig. 3). Isolations from the lesions on the inoculated plants consistently yielded isolates of *Cylindrocladium* and no pathogens were re-isolated from the control plants.

Cylindrocladium isolate CMW 9164 produced the longest lesions on *A. mearnsii* seedlings (X = 105.7 mm). Isolates CMW 9156 and CMW.9171 produced lesions with mean lengths of 80.4 mm and 102.7mm respectively. (Fig.3). However, there was no significant difference between the three isolates used in the trial (Table 2). All isolates produced lesions significantly (P=0.0001) larger than those of the controls that had an average lesion length of 25.5 mm (Fig. 3).

Discussion

In this study, we have shown that *Cylindrocladium pauciramosum* is the species responsible for disease of *A. mearnsii* in South African nurseries. Identification of the fungus was based on morphological characteristics and this was further confirmed using DNA sequence comparisons. Although *Cylindrocladium* has previously been recognized as a pathogen *of A. meamsii* seedlings in nurseries, this is the first robust identification of the pathogen. This study also provides the first pathogenicity tests using *C. pauciramosum* on *A. mearnsii*.

DNA sequence comparisons were used in this study to confirm the identity of the *Cylindrocladium* sp. associated with *A. mearnsii* infections. This was necessary because species in the *Cylindrocladium candelabrum* complex including *C. pauciramosum* are morphologically similar and difficult to identify (Crous 2002). It is possible that other species in this group might appear on *A. mearnsii* in the future. However, recent surveys in *Eucalyptus* nurseries have shown that *C. pauciramosum* is the dominant fungus in those situations (Chapter 3, this thesis).

To the best of our knowledge, pathogenicity tests have not previously been conducted on *A. mearnsii,* using *C. pauciramosum*. Our tests clearly showed that this fungus is pathogenic to this tree species and that the three isolates chosen for the test have similar levels of pathogenicity. *Cylindrocladium pauciramosum* appears to be one of the most common species of *Cylindrocladium* causing plant diseases in South Africa (Crous 2002) and it is particularly important in forest nurseries.

Cylindrocladium spp. are soil-borne and produce microsclerotia that enable them to survive in soil and potting medium for long periods of time. To reduce the impact of *C. pauciramosum* in *A. mearnsii* nurseries, it will be necessary to eliminate sources of inoculum. In this regard, it will be especially important to ensure that planting substrate is free of the pathogen and that inoculum does not build up in nurseries.

References

Anonymous. (2003) Abstract of South African Forestry Facts for the year 2001/2002. Forestry South Africa, Department Water Affairs & Forestry, South Africa.

Crous, P. W. (2002) Taxonomy and pathology of *Cylindrocladium* (*Calonectria*) and allied genera. APS Press, St. Paul, Minnesota, USA.

Crous, P.W., Phillips, A. L. J. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Journal of Forestry* **157**: 69 – 85.

Dunlop, R. W. (2002) Introduction. In *Black Wattle: The South African research experience*, Chapter 1: 1 – 2 (Ed. R. W. Dunlop & L. A. Maclennan) Institute for Commercial Forestry Research, Pietermaritzburg, South Africa.

Glass, N. L. & Donaldson, G. (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323 – 1330.

Möller, E. M., Bahnweg, G., Sandermann, H. & Geiger, H. H. (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acid Research* **20**: 6115 – 6116.

O'Donnell, K. & Cigelnik, E. (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics Evolution* **7**: 103 – 116.

Old, K. M., Vercoe, T. K., Floyd, R. B., Wingfield, M. J., Roux, J. & Neser, S. (2003) *Acacia* spp. *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm* No. **20**.

Overmeyer, C. Lünnemann, S., Von Wallbrünn, C. & Meinhardt F. (1996) Genetic variability among isolates and sexual offspring of the plant pathogenic fungus *Calonectria morganii* on the basis of random amplification of polymorphic DNA (RAPD) and restriction fragment length polymorphisms (RFLP). *Current Microbiology* **33**: 249 – 255.

Roux, J. (2002) Threats to wattle plantations III: Diseases. In *Black Wattle: The South African research experience*, Chapter 12: 121 – 126. (Ed. R. W. Dunlop & L. A. Maclennan) Institute for Commercial Forestry Research, Pietermaritzburg, South Africa.

Roux, J., Kemp, G. H. J. & Wingfield, M. J. (1995) Diseases of black wattle in South Africa – A review. *South African Journal of Forestry* **174**: 35 – 40.

Roux, J. & Wingfield, M. J. (1997) Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* **99**: 327 – 336.

Sherry, S. P. (1971) *The black wattle* (Acacia mearnsii *de Wild*). University of Natal Press, Pietermaritzburg, South Africa. 402p.

Schoch, C. L., Crous, P. W. Wingfield, B. D. & Wingfield, M. J. (1999) The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**: 286 – 298.

Swofford, D. L. (2002) PAUP*. Phylogenetic analysis using parsimony(* and other methods), 4.0b10. Computer programme. Sunderland, Massachusetts, U.S.A.: Sinauer Associates.

Wingfield, M. J. & Roux, J. (2000) Diseases of Forest Plantations. In *South African Forestry Handbook 2000*. South African Institute of Forestry, Pretoria, South Africa, pp. 241 – 252.

Table 1. List of *Cylindrocladium* isolates used in DNA sequence comparisons

Species	Isolation number	GenBank Assencion number
Cylindrocladium candelabrum	STE-U 1677	AF210858
	STE-U 1674	AF210857
C. pauciramosum	STE-U 3207	AF449448
	CSL 2021133	AY162320
	[a,b]CMW9156	
	[a]CMW9159	
	[a,b]CMW9164	
	[a]CMW9169	
	[a,b]CMW9171	
C. quinqueseptatum	STE-U 759	AF232869
	STE-U 516	AF232870
C. reteaudii	STE-U 758	AF389847
	STE-U 602	AF389846
C. scoparium	STE-U 1722	AF210875
	STE-U 1720	AF210874
C. theae	UFV 16	AF232862
	ATCC 48895	AF232861

[a] Isolates sequenced in this study. All other sequences are those from GenBank.

[b] Isolates used in the pathogenicity test.

Table 2. Mean lesion lengths on *A. mearnsii* seedlings 6 weeks after inoculation with three isolates of *C. pauciramosum* and a control in the greenhouse.

Isolates	**[a]Mean lesion length**
CMW 9156	80.4
CMW 9164	105.7
CMW 9171	102.7
Control	25.5

[a]Each value is the average of 20 measurements for each isolate.

$P < 0.0001$

CV = 35.94

R – square = 0.517

F = 22.48

Figure 1. Macroconidiophores, vesicles and conidia of *Cylindrocladium pauciramosum* isolated from *Acacia mearnsii* seedlings. A. Macroconidiophore with fertile branches (phialides); B. Conidia; C. Chlamydospores; D – F. Vesicles (Scale bars = 10 μm).

p.51

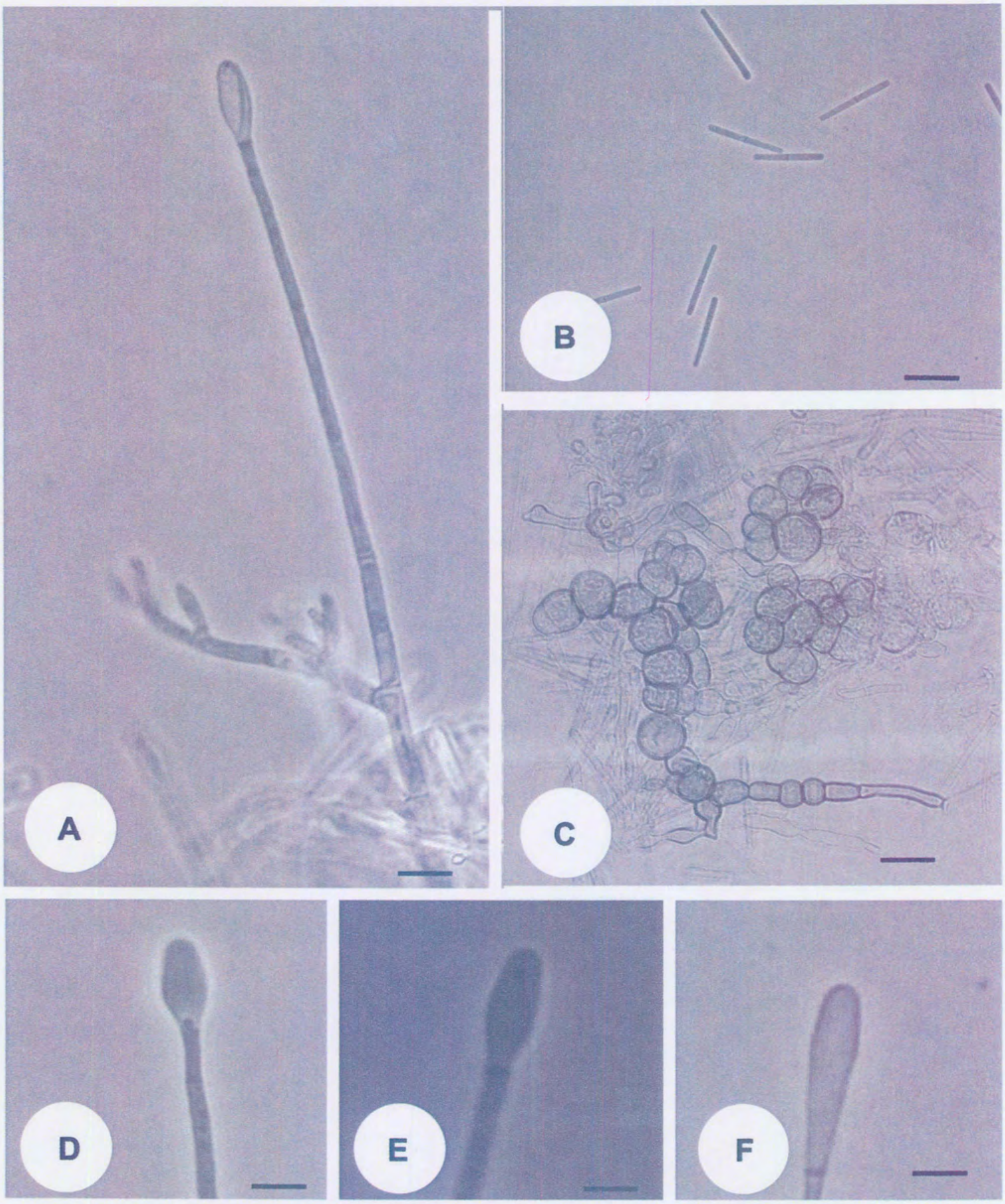


Figure 2. Most parsimonious tree obtained from a subset of *Cylindrocladium* isolates. The most parsimonious tree (507 steps, CI = 0.917, RC = 0.852, RI = 0.929) generated with a heuristic search in PAUP* version 4.0b1 from aligned sequences of the 5' end of the β - tubulin gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown above branches and decay indices below. A *Cylindrocladiella infestans* (GenBank accession number AF320190) was used as outgroup.

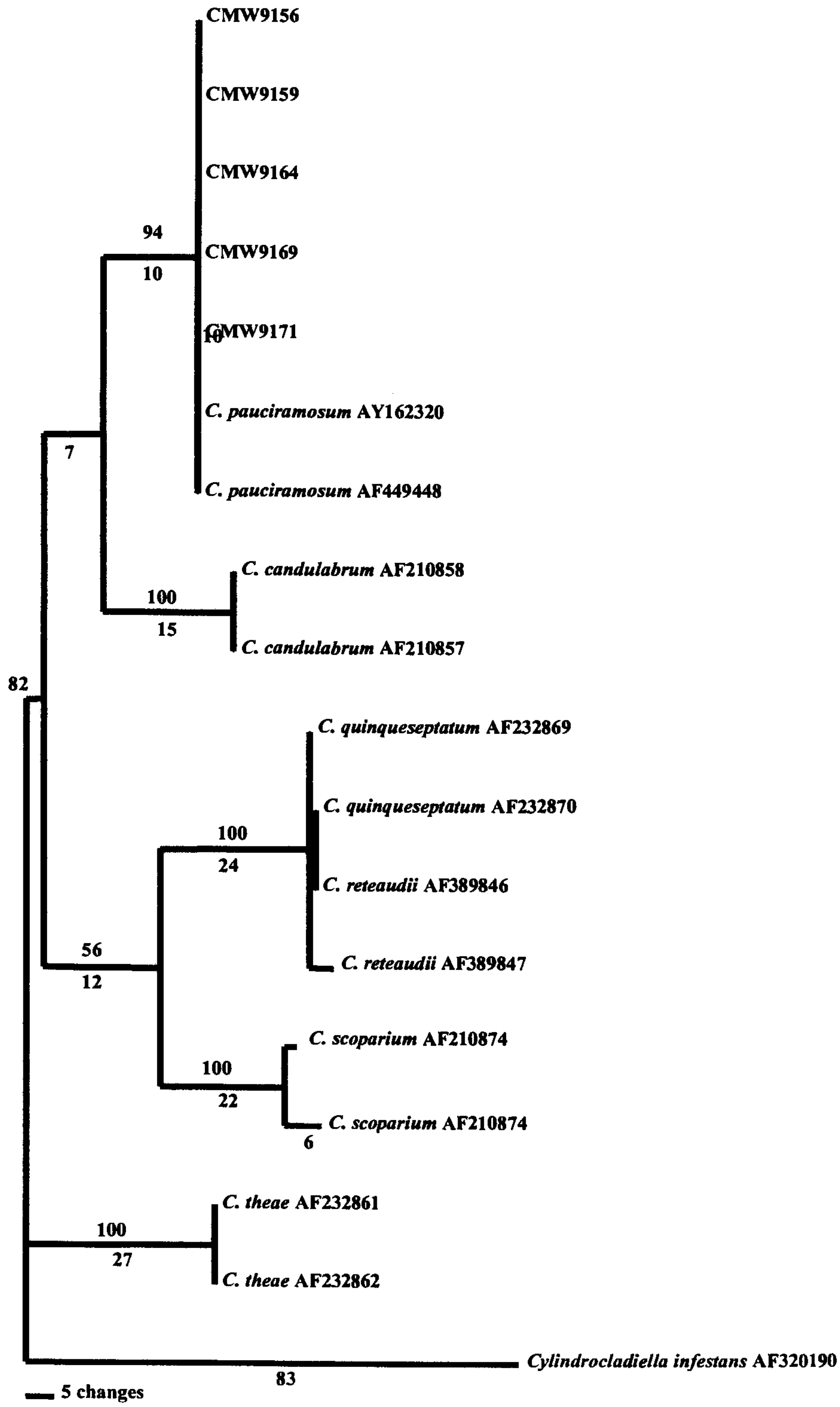

CMW9156
CMW9159
CMW9164
94
CMW9169
10
CMW9171
10
C. pauciramosum AY162320
7
C. pauciramosum AF449448
C. candulabrum AF210858
100
15
C. candulabrum AF210857
82
C. quinqueseptatum AF232869
C. quinqueseptatum AF232870
100
24
C. reteaudii AF389846
56
12
C. reteaudii AF389847
C. scoparium AF210874
100
22
C. scoparium AF210874
6
C. theae AF232861
100
27
C. theae AF232862
Cylindrocladiella infestans AF320190
83
5 changes

Figure 3. Streaked appearance of the vascular tissue of *A. mearnsii* stems after inoculation with *C. pauciramosum* including control. A. Control; B. CMW 9164; C. CMW 9171; D. CMW 9156.


A
B
C
D

Summary

Studies presented in this dissertation highlight the importance of fungal pathogens in forestry nurseries in South Africa. Both *Acacia mearnsii* seedlings and *Eucalyptus* hybrid cuttings are shown to be affected by important nursery pathogens.

Chapter one presents an evaluation of the potential importance of pathogens to *Eucalyptus* hedge plants maintained in hydroponics. Hydroponics is a new technology being used in South African forest nurseries, which allows for the rapid establishment of *Eucalyptus* hedge plants. However, no information is available on pathogens affecting *Eucalyptus* in hydroponics. By applying information on pathogens of other hydroponic crops, several potentially important pathogens were identified and these reside in the genera *Phytophthora*, *Pythium* and *Fusarium*. Possible disease symptoms in *Eucalyptus* caused by these pathogens include wilting, stem cankers and root rots. Implementation of appropriate control measures that include cultural, biological and chemical practices could prevent and/or reduce disease impact in hydroponics.

Chapter two presents the results of a survey of the roots of *Eucalyptus* hedge plants grown in an ebb and flow hydroponic system. An interesting result of the survey was the discovery of *Cylindrocladium pauciramosum* in the hydroponic system. This is the first report of the pathogen in a hydroponic system. Other important pathogens in the genera *Phytophthora* and *Pythium*

were also isolated. Two *Pythium* species, namely *P. dissotocum* and *P. helicoids*, found in the roots and nutrient solution are new to *Eucalyptus*. Several *Fusarium* species were also isolated of which two, namely *F. nygamai* and *F. lateritium,* are also new to *Eucalyptus*.

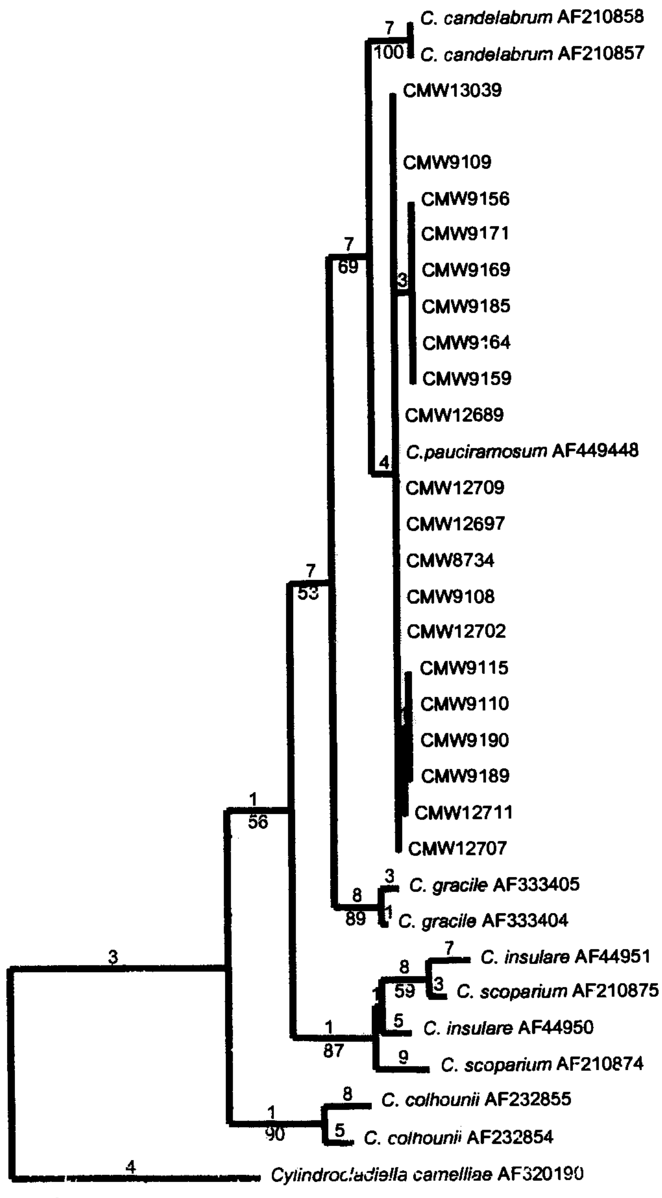
Chapter three of this dissertation presents the results of a survey of *Eucalyptus* cuttings conducted at four forestry nurseries in KwaZulu – Natal, South Africa. Several well – known *Eucalyptus* nursery pathogens were isolated. *Cylindrocladium pauciramosum* was identified as the dominant pathogen on *Eucalyptus* cuttings. This was confirmed based on morphological characteristics and DNA sequence comparisons. Pathogenicity tests conducted using a spore suspension of *C. pauciramosum* indicated that this pathogen is capable of infecting most commercial *Eucalyptus* clones used in South Africa.

Chapter four considers a serious disease of *Acacia mearnsii* seedlings caused by an unidentified species of *Cylindrocladium. Cylindrocladium pauciramosum* was isolated from *A. mearnsii* seedlings showing girdling and stem canker symptoms. The pathogen was identified based on morphological characteristics and DNA sequence comparisons. Pathogenicity tests with *Acacia* seedlings confirmed the susceptibility of this tree to *C. pauciramosum* infection.

This dissertation clearly indicates that *Cylindrocladium pauciramosum* is an important nursery pathogen in South African forestry nurseries. This pathogen has already been shown to be limiting during production of planting stock. I hope to have highlighted the importance of *C. pauciramosum* and other nursery pathogens in forestry nurseries in South Africa. This study will also hopefully provide information to forestry nursery managers and help them improve production.

Appendix I – A combined phylogenetic tree of *Cylindocladium pauciramosum* isolates used.

The most parsimonious tree obtained from a subset of *Cylindrocladium* isolates (560 steps, CI = 0.774, RC = 0.602, RI = 0.777) generated with a heuristic search in PAUP version 4.0b1 from aligned sequences of the 5' end of the β - tubulin gene. Gaps were treated as missing. Clade stability was assessed with 1000 bootstrap replications and values above 50% are shown below branches and decay indices above. A *Cylindrocladiella infestans* (GenBank accession number AF320190) was used as out – group.


C. candelabrum AF210858
C. candelabrum AF210857
CMW13039
CMW9109
CMW9156
CMW9171
CMW9169
CMW9185
CMW9164
CMW9159
CMW12689
C.pauciramosum AF449448
CMW12709
CMW12697
CMW8734
CMW9108
CMW12702
CMW9115
CMW9110
CMW9190
CMW9189
CMW12711
CMW12707
C. gracile AF333405
C. gracile AF333404
C. insulare AF44951
C. scoparium AF210875
C. insulare AF44950
C. scoparium AF210874
C. colhounii AF232855
C. colhounii AF232854
Cylindrocladiella camelliae AF320190
5

The most parsimonious tree obtained from the 5' end of the β – tubulin gene DNA sequence of the *C. pauciramosum* isolates used in this dissertation indicates that isolates are similar irrespective of host or environment. A slight difference is evident between the *Eucalyptus* and the *Acacia mearnsii* isolates but this cannot be supported by these DNA sequence comparisons (bootstrap < 50%). The application of further molecular population studies and multigene phylogeny could support and differentiate between *C. pauciramosum* strains.

Appendix II – DNA alignments used.

Chapter 2

Alignment 1. 5' end of the β - tubulin gene of the *Cylindrocladium pauciramosum* isolates obtained from the *Eucalyptus* hedge plants.

30

CMW 12711		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 12709		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 12697		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 12689		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 13039		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 12702		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 12707		GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. pauciramosum	AY162320	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. pauciramosum	AF449448	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. candelabrum	AF210857	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. candelabrum	AF210858	GCTTGCCCCT	GATTCTACCC	CGCCGCCCCG
C. scoparium	AF210875	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. scoparium	AF210874	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. colhounii	AF232855	GCT-GCCCCT	GATTCTACCC	CGCCGACCCG
C. colhounii	AF232854	GCT-GCCCCT	GATTCTACCC	CGCCGACCCG
C. insulare	AF44951	GCTTGCCCCT	GATTCTACCC	CGCCGCCCCG
C. insulare	AF44950	GCTTGCCCCT	GATTCTACCC	CGCCGCCCCG
C. gracile	AF333405	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
C. gracile	AF333404	GCT-GCCCCT	GATTCTACCC	CGCCGCCCCG
Cylindrocladiella infestans	AF320190	GCT-GCCCCT	- ATTCTATCC	CGCCGCCCCG

60

CMW 12711		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 12709		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 12697		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 12689		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 13039		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 12702		GTTTCCACC-	---GCTTCGA	CGACAACAAA
CMW 12707		GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. pauciramosum	AY162320	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. pauciramosum	AF449448	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. candelabrum	AF210857	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. candelabrum	AF210858	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. scoparium	AF210875	GTTTCCACC-	---ACATCGA	CGAAAACAAA
C. scoparium	AF210874	GTTTCCACC-	---ACATCGA	CGAAAACAAA
C. colhounii	AF232855	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. colhounii	AF232854	GTTTCCACC-	---GCTTCGA	CGACAACAAA
C. insulare	AF44951	GTTTCCACC-	---ACCTCGA	CGACAACAAA
C. insulare	AF44950	GTTTCCACC-	---ACCTCGA	CGACAACAAA
C. gracile	AF333405	GTTTCCACC-	---GCTCCGA	CGACAACAAA
C. gracile	AF333404	GTTTCCACC-	---GCTCCGA	CGACAACAAA
Cylindrocladiella infestans	AF320190	TTTCCCACCC	ACCGCCTCGA	CGACAACAAA

90

CMW 12711		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 12709		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 12697		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 12689		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 13039		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 12702		GCCGCAGCCT	CACGATCATA	ACGAGATATC
CMW 12707		GCCGCAGCCT	CACGATCATA	ACGAGATATC
C. pauciramosum	AY162320	GCCGCAGCCT	CACGATCATA	ACGAGATATC
C. pauciramosum	AF449448	GCCGCAGCCT	CACGATCATA	ACGAGATATC
C. candelabrum	AF210857	GCCGCAGCCT	CACGATCATG	ACGAGATATC
C. candelabrum	AF210858	GCCGCAGCCT	CACGATCATG	ACGAGATATC
C. scoparium	AF210875	GCCGCAGCCT	CACGAACATG	ATGTGATATC
C. scoparium	AF210874	GCCGCAGCCT	CACGAACATG	ATGTGATATC
C. colhounii	AF232855	GCCGCAGCCT	CACGAGCATG	ACGAGATATC
C. colhounii	AF232854	GCCGCAGCCT	CACGAGCATG	ACGAGATATC
C. insulare	AF44951	GCCGCAGCCT	CACGAACATG	ATGTGATATC
C. insulare	AF44950	GCCGCAGCCT	CAACAACAAA	ATGTGATATC
C. gracile	AF333405	GCCGCAGCCT	CACGAGCATG	GCGAGATATC
C. gracile	AF333404	GCCGCAGCCT	CACGAGCATG	GCGAGATATC
Cylindrocladiella infestans	AF320190	GCTCGCGATG	CCCACCCACA	TCGTGATATC

				120
CMW 12711		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 12709		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 12697		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 12689		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 13039		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 12702		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
CMW 12707		AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. pauciramosum	AY162320	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. pauciramosum	AF449448	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. candelabrum	AF210857	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. candelabrum	AF210858	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. scoparium	AF210875	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. scoparium	AF210874	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. colhounii	AF232855	GAAACAAGAT	TTGCTGACCA	TGTGCTTCTT
C. colhounii	AF232854	GAAACAAGAT	TTGCTGACCA	TGTGCTTCTT
C. insulare	AF44951	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. insulare	AF44950	AGAACAAGAT	T– GCTAACCG	TGTGCTTCTT
C. gracile	AF333405	AGAACAAGAT	T– GCTAACCG	TGTCCTTCTT
C. gracile	AF333404	AGAACAAGAT	T– GCTAACCG	TGTCCTTCTT
Cylindrocladiella infestans	AF320190	TGAAGACAAT	G– GCTAATTT	TGTG–TGTTT

				150
CMW 12711		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 12709		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 12697		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 12689		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 13039		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 12702		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
CMW 12707		TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. pauciramosum	AY162320	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. pauciramosum	AF449448	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. candelabrum	AF210857	TTTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. candelabrum	AF210858	TTTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. scoparium	AF210875	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. scoparium	AF210874	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. colhounii	AF232855	TTTCAATTAT	AGGTCCACCT	CCAGACCGGT
C. colhounii	AF232854	TTTCAATTAT	AGGTCCACCT	CCAGACCGGC
C. insulare	AF44951	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. insulare	AF44950	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. gracile	AF333405	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
C. gracile	AF333404	TCTCGATTAT	AGGTCCACCT	CCAGACCGGT
Cylindrocladiella infestans	AF320190	CTGCGAATAT	AGGTCCACCT	CCAGACCGGT

				180
CMW 12711		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 12709		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 12697		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 12689		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 13039		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 12702		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
CMW 12707		AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. pauciramosum	AY162320	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. pauciramosum	AF449448	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. candelabrum	AF210857	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. candelabrum	AF210858	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. scoparium	AF210875	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. scoparium	AF210874	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. colhounii	AF232855	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. colhounii	AF232854	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. insulare	AF44951	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. insulare	AF44950	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. gracile	AF333405	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
C. gracile	AF333404	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA
Cylindrocladiella infestans	AF320190	AGGTCCACCT	CCAGACCGGT	CAGTGCGTAA

					210
CMW 12711		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 12709		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 12697		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 12689		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 13039		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 12702		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
CMW 12707		GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
C. pauciramosum	AY162320	GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
C. pauciramosum	AF449448	GTACTCTTCT	CAACTCCAAC	AAAATTCTCA	
C. candelabrum	AF210857	GTACTCTTCT	CAACTCCGAC	CAAATTCTCA	
C. candelabrum	AF210858	GTACTCTTCT	CAACTCCGAC	CAAATTCTCA	
C. scoparium	AF210875	GTGCTCTTC–	– AACTCCAAC	AAAATTCTCA	
C. scoparium	AF210874	GTGCTCTTC–	– AACTCCAAC	AAAATTCTCA	
C. colhounii	AF232855	GTGCTCTTGT	CAACTCCAAC	AATATTATCA	
C. colhounii	AF232854	GTGCTCTTGT	CAACTCCAAC	AATATTATCA	
C. insulare	AF44951	GTGCTCTTC–	– AACTCCAAC	AAAATTCTCA	
C. insulare	AF44950	GTGCTCTTC–	– AACTCCAAC	AAAATTCTCA	
C. gracile	AF333405	GTGATATTCT	CAACTCCAAC	AAAATTCTCA	
C. gracile	AF333404	GTGATATTCT	CAACTCCAAC	AAAATTCTCA	
Cylindrocladiella infestans	AF320190	GTACATTCCT	CACCTC–GAC	AAGCTT– – – –	

					240
CMW 12711		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 12709		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 12697		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 12689		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 13039		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 12702		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
CMW 12707		CGATGAGATT	CACTGACAGT	–TGTCGATAG	
C. pauciramosum	AY162320	CGATGAGATT	CACTGACAGT	–TGTCGATAG	
C. pauciramosum	AF449448	CGATGAGATT	CACTGACAGT	–TGTCGATAG	
C. candelabrum	AF210857	CGATGAGATT	CACTGACAGT	–TGTCGATAG	
C. candelabrum	AF210858	CGATGAGATT	CACTGACAGT	–TGTCGATAG	
C. scoparium	AF210875	CGACCGGATT	CACTGACAGT	–TATCGACAG	
C. scoparium	AF210874	CGACCGGATT	CACTGACAGT	–TATCGACAG	
C. colhounii	AF232855	CGA– – –GATT	TACTGACACA	–TGTCGATAG	
C. colhounii	AF232854	CGA– – –GATT	TACTGACACA	–TGTCGATAG	
C. insulare	AF44951	CGACCAGATT	CACTGACAGT	–TATCGACAG	
C. insulare	AF44950	CGACCAGATT	CACTGACAGT	–TATCGACAG	
C. gracile	AF333405	CGACCAGATT	CACTGACATT	–TGTCGATAG	
C. gracile	AF333404	CGACCAGATT	CACTGACATT	–TGTCGATAG	
Cylindrocladiella infestans	AF320190	CGTCAACGGC	TGCTAACGGT	GTCTCGATAG	

					270
CMW 12711		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 12709		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 12697		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 12689		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 13039		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 12702		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
CMW 12707		GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. pauciramosum	AY162320	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. pauciramosum	AF449448	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. candelabrum	AF210857	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. candelabrum	AF210858	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. scoparium	AF210875	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. scoparium	AF210874	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. colhounii	AF232855	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. colhounii	AF232854	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. insulare	AF44951	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. insulare	AF44950	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. gracile	AF333405	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
C. gracile	AF333404	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	
Cylindrocladiella infestans	AF320190	GGTAACCAAA	TTGGTGCTGC	TTTCTGGCAG	

				300	
CMW 12711		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 12709		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 12697		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 12689		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 13039		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 12702		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
CMW 12707		ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. pauciramosum	AY162320	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. pauciramosum	AF449448	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. candelabrum	AF210857	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
C. candelabrum	AF210858	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
C. scoparium	AF210875	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. scoparium	AF210874	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. colhounii	AF232855	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
C. colhounii	AF232854	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
C. insulare	AF44951	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. insulare	AF44950	ACCATTTCTG	GCGAGCACGG	TCTCGACAGC	
C. gracile	AF333405	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
C. gracile	AF333404	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	
Cylindrocladiella infestans	AF320190	ACCATCTCTG	GCGAGCACGG	TCTCGACAGC	

				330	
CMW 12711		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 12709		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 12697		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 12689		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 13039		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 12702		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
CMW 12707		AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. pauciramosum	AY162320	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. pauciramosum	AF449448	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. candelabrum	AF210857	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. candelabrum	AF210858	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. scoparium	AF210875	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. scoparium	AF210874	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. colhounii	AF232855	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. colhounii	AF232854	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. insulare	AF44951	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. insulare	AF44950	AATGGTGTCT	ACGCTGGTAC	CTCCGAGCTC	
C. gracile	AF333405	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
C. gracile	AF333404	AATGGTGTCT	ACGCCGGTAC	CTCCGAGCTC	
Cylindrocladiella infestans	AF320190	AATGGTGTCT	ACAACGGCAG	CTCCGAGCTC	

				360	
CMW 12711		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 12709		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 12697		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 12689		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 13039		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 12702		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
CMW 12707		CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. pauciramosum	AY162320	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. pauciramosum	AF449448	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. candelabrum	AF210857	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. candelabrum	AF210858	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. scoparium	AF210875	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. scoparium	AF210874	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. colhounii	AF232855	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. colhounii	AF232854	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. insulare	AF44951	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. insulare	AF44950	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. gracile	AF333405	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
C. gracile	AF333404	CAGCTCGAGC	GTATGAACGT	CTACTTCAAC	
Cylindrocladiella infestans	AF320190	CAGCTCGAGC	GCATGAGCGT	CTACTTCAAC	

390

CMW 12711		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 12709		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 12697		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 12689		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 13039		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 12702		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
CMW 12707		GAGGTATGTG	AAAACCACTC	GAAGCACTCC
C. pauciramosum	AY162320	GAGGTATGTG	AAAACCACTC	GAAGCACTCC
C. pauciramosum	AF449448	GAGGTATGTG	AAAACCACTC	GAAGCACTCC
C. candelabrum	AF210857	GAGGTATGTG	AAAACCACTC	GAAGCACTCC
C. candelabrum	AF210858	GAGGTATGTG	AAAACCACTC	GAAGCACTCC
C. scoparium	AF210875	GAGGTATGTG	AAAACCACGC	GGTGTACTCA
C. scoparium	AF210874	GAGGTATGTG	AAAACCACGC	GGTGTTCTCA
C. colhounii	AF232855	GAGGTATGTG	AAAGCTGCTT	GGTGTACCCC
C. colhounii	AF232854	GAGGTATGTG	AAAGCTGCTT	G– –GTGTCCC
C. insulare	AF44951	GAGGTATGTG	AAAACCGCGC	GGTGTACCCA
C. insulare	AF44950	GAGGTATGTG	AAAATCACGC	GGTGTACCCA
C. gracile	AF333405	GAGGTATGTG	AAAACCACGC	GGAGCACTCC
C. gracile	AF333404	GAGGTATGTG	AAAACCACGC	GGAGCACTCC
Cylindrocladiella infestans	AF320190	GAGGTATGTG	ACTATGGCAC	TCA – CATTTG

420

CMW 12711		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 12709		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 12697		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 12689		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 13039		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 12702		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
CMW 12707		CTTGACCGAG	AAGCACAAGC	CAACTCACAC
C. pauciramosum	AY162320	CTTGACCGAG	AAGCACAAGC	CAACTCACAC
C. pauciramosum	AF449448	CTTGACCGAG	AAGCACAAGC	CAACTCACAC
C. candelabrum	AF210857	CTTGACCGAG	AAGCACAATC	CGACTCACAC
C. candelabrum	AF210858	CTTGACCGAG	AAGCACAATC	CGACTCACAC
C. scoparium	AF210875	CACG–CCGAG	AGGCACAAGC	AAACTGACAC
C. scoparium	AF210874	CACG–CCGAG	AGGCACAAGC	AAACTGACAC
C. colhounii	AF232855	– TATGTCGAG	AGACGCAAGC	AAACTGACAC
C. colhounii	AF232854	CTATGTCGAG	AGACGCAACT	AAACTGACAC
C. insulare	AF44951	CACG–CCGAG	AGGCACAAGC	AAACTGACAC
C. insulare	AF44950	CACG–CCGAG	AGGCACAAGC	AAACTGACAC
C. gracile	AF333405	CTTGATCGAG	AGGCACAAGC	AAACTGACAC
C. gracile	AF333404	CTTGATCGAG	AGGCACAAGC	AAACTGACAC
Cylindrocladiella infestans	AF320190	CTACACTGTG	AAATCAGAAT	GTACTCACGC

450

CMW 12711		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 12709		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 12697		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 12689		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 13039		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 12702		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
CMW 12707		CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
C. pauciramosum	AY162320	CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
C. pauciramosum	AF449448	CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
C. candelabrum	AF210857	CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
C. candelabrum	AF210858	CATCATGTAG	GCTTCCGGCA	ACAAGTTCGT
C. scoparium	AF210875	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
C. scoparium	AF210874	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
C. colhounii	AF232855	CAT– – –GTAG	GCTTCCGGCA	ACAAGTTCGT
C. colhounii	AF232854	CAT– – –GTAG	GCCTTCGGCA	ACAAGTTCGT
C. insulare	AF44951	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
C. insulare	AF44950	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
C. gracile	AF333405	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
C. gracile	AF333404	CAT– – –GTAG	GCTTCTGGCA	ACAAGTTCGT
Cylindrocladiella infestans	AF320190	– – –TCCGTAG	GCTTCTGGCA	ACAAGTATGT

480

CMW 12711		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 12709		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 12697		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 12689		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 13039		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 12702		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
CMW 12707		TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. pauciramosum	AY162320	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. pauciramosum	AF449448	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. candelabrum	AF210857	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. candelabrum	AF210858	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. scoparium	AF210875	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. scoparium	AF210874	TCCTTGTGCT	GTCCTCGTCG	ATCTTGAGCC
C. colhounii	AF232855	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. colhounii	AF232854	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. insulare	AF44951	TCCTCGCGCT	GTCCTCGTCG	ATCTCGAGCC
C. insulare	AF44950	TCCTCGCGCT	GTCCTCGTCG	ATCTCGAGCC
C. gracile	AF333405	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
C. gracile	AF333404	TCCTCGCGCT	GTCCTCGTCG	ATCTTGAGCC
Cylindrocladiella infestans	AF320190	CCCTCGCGCC	GTCCTCGTCG	ATCTTGAGCC

510

CMW 12711		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 12709		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 12697		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 12689		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 13039		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 12702		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
CMW 12707		CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. pauciramosum	AY162320	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. pauciramosum	AF449448	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. candelabrum	AF210857	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. candelabrum	AF210858	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. scoparium	AF210875	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. scoparium	AF210874	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. colhounii	AF232855	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. colhounii	AF232854	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. insulare	AF44951	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. insulare	AF44950	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. gracile	AF333405	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
C. gracile	AF333404	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC
Cylindrocladiella infestans	AF320190	CGGTACCATG	GACGCCGTCC	GTGCCGGTCC

520

CMW 12711		TTTCGGTCAG	CTCTTCCGCC
CMW 12709		TTTCGGTCAG	CTCTTCCGCC
CMW 12697		TTTCGGTCAG	CTCTTCCGCC
CMW 12689		TTTCGGTCAG	CTCTTCCGCC
CMW 13039		TTTCGGTCAG	CTCTTCCGCC
CMW 12702		TTTCGGTCAG	CTCTTCCGCC
CMW 12707		TTTCGGTCAG	CTCTTCCGCC
C. pauciramosum	AY162320	TTTCGGTCAG	CTCTTCCGCC
C. pauciramosum	AF449448	TTTCGGTCAG	CTCTTCCGCC
C. candelabrum	AF210857	TTTCGGTCAG	CTCTTCCGCC
C. candelabrum	AF210858	TTTCGGTCAG	CTCTTCCGCC
C. scoparium	AF210875	TTTCGGTCAG	CTCTTCCGCC
C. scoparium	AF210874	TTTCGGTCAG	CTCTTCCGCC
C. colhounii	AF232855	TTTCGGTCAG	CTCTTCCGCC
C. colhounii	AF232854	TTTCTGTCAG	CTCTTCCGCC
C. insulare	AF44951	TTTCGGTCAG	CTCTTCCGCC
C. insulare	AF44950	TTTCGGTCAG	CTCTTCCGCC
C. gracile	AF333405	TTTCGGTCAG	CTCTTCCGTC
C. gracile	AF333404	TTTCGGTCAG	CTCTTCCGCC
Cylindrocladiella infestans	AF320190	TTTCGGTCAG	CTCTTCCGTC

Alignment 2. The EF1 – α gene DNA sequence alignment of the *Fusarium* isolates obtained from the *Eucalyptus* hedge plants.

				30
FCC 3110		GAAGTTCGAG	AAGGTTGGT-	CACATCTCCC
FCC 3114		GAAGTTCGAG	AAGGTTGGT-	CACATCTCCC
FCC 3119		GAAGTTCGAG	AAGGTTAGT-	CAATATCCCT
FCC 3109		GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
FCC 3117		GAAGTTCGAG	AAGGTTAGT-	CAATATCCCT
FCC 2957		GAAGTTCGAG	AAGGTTAGT-	CACTTTTCCT
FCC 3115		GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
FCC 2964		GAAGTTCGAG	AAGGTTAGT-	CACTTTTCCT
FCC 3113		GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
F. avenaceum	AY337423	GAAGTTCGAG	AAGGTTAGT-	CAATATCCCT
F .equiseti	AY337424	GAAGTTCGAG	AAGGTTAGT-	CAATATCCCT
F .lateritium	AY337435	GAAGTTCGAG	AAGGTTAGT-	CAATATCCCT
F. nygamai	AY337445	GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
F. oxysporum	AY337428	GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
F. proliferatum	AY337436	GAAGTTCGAG	AAGGTTAGT-	CACTTTCCCT
F. sambucinum	AY337422	GAAGTTCGAG	AAGGTTAGT-	CAAAATCCCT
F. solani	AY337438	GAAGTTCGAG	AAGGTTGGT-	CACATCTCCC
F. verticillioides	AY337450	GAAGTTCGAG	AAGGTTAGT-	CACTTTTCCT
Botryosphaeria_dothidea	AY236899	GAAGTTCGAG	AAGGTAAGCA	CACATTTTCT

				60
FCC 3110		CCGATCGCGC	CT----TGCT	ATCCCACATC
FCC 3114		CCGATCGCGC	CT----TGCT	ATCCCACATC
FCC 3119		TCGATTACGC	-------GCG	CTCCCA--TC
FCC 3109		TCAATCGCGC	GT----CCTT	TGCCCA--TC
FCC 3117		TCGATTACGC	-------GCG	CTCCCA--TC
FCC 2957		TCTATCGCGC	GT----TCTT	TGCCCA--TC
FCC 3115		TCAATCGCGC	GT----CCTT	TGCCCA--TC
FCC 2964		TCTATCGCGC	GT----TCTT	TGCCCA--TC
FCC 3113		TCGATCGCGC	GT----CCTT	TGTCCA--TC
F. avenaceum	AY337423	TCGATTACGC	-------GCG	CTCCCA--TC
F .equiseti	AY337424	TCGATTACGC	-------GCG	CTCCCA--TC
F .lateritium	AY337435	TCGATTACGC	-------GCG	CTCCCA--TC
F. nygamai	AY337445	TCGATCGCGC	GT----CCTT	TGTCCA--TC
F. oxysporum	AY337428	TCAATCGCGC	GT----CCTT	TGCCCA--TC
F. proliferatum	AY337436	TCGATCGCGC	GT----CCTC	TGCCCA--CC
F. sambucinum	AY337422	TCGATTGCGC	-------GCG	CTCCCA--TC
F. solani	AY337438	CCGATCGCGC	CT----TGCT	ATCCCACATC
F. verticillioides	AY337450	TCTATCGCGC	GT----TCTT	TGCCCA--TC
Botryosphaeria_dothidea	AY236899	GTGCCTGCAC	GTGTGCTGGG	TTCCTGCGCC

				90
FCC 3110		GAATTCCCCG	TCGAATTCCC	TCCTCCGCGA
FCC 3114		GAATTCCCCG	TCGAATTCCC	TCCTCCGCGA
FCC 3119		GATTCCCACG	ATTCGCTCCC	TCACTCGAAA
FCC 3109		GATTTCCCC-	--------TA	CGACTCGAAA
FCC 3117		GATTCCCACG	ATTCGCTCCC	TCACTCGAAA
FCC 2957		GATTCCCCCC	--------TA	CGACTCGAAA
FCC 3115		GATTTCCCC-	--------TA	CGACTCGAAA
FCC 2964		GATTCCCCCC	--------TA	CGACTCGAAA
FCC 3113		GATTTCCCCC	--------TC	CGACTCGAAA
F. avenaceum	AY337423	GATTCCCACG	ACTCGCTCCC	TCATTCGAAA
F .equiseti	AY337424	GATTCCCACG	ACTCGCTCCC	TCATTCGAAA
F .lateritium	AY337435	GATTCCCACG	ATTCGCTCCC	TCACTCGAAA
F. nygamai	AY337445	GATTTCCCCC	--------TC	CGACTCGAAA
F. oxysporum	AY337428	GATTTCCCC-	--------TA	CGACTCGAAA
F. proliferatum	AY337436	GATTTCACT-	--------TG	CGATTTGAAA
F. sambucinum	AY337422	GATTCATACG	ACTCGCTCCC	TCACTCGAAA
F. solani	AY337438	GAATTCCCCG	TCGAATTCCC	TCCTCCGCGA
F. verticillioides	AY337450	GATTCCCCCC	--------TA	CGACTCGAAA
Botryosphaeria_dothidea	AY236899	GAATTTGCCT	TATCA--CTC	TGGTGAGGGG

				120
FCC 3110		CACGCTCTGC	GCCCGCTTCT	CCCGAGTCCC
FCC 3114		CACGCTCTGC	GCCCGCTTCT	CCCGAGTCCC
FCC 3119		CACATCCATT	ACCC— —CG	CTCGAGTCC–
FCC 3109		CGTGCCCGCT	ACCC— —CG	CTCGAGACC–
FCC 3117		CACATCCATT	ACCC— —CG	CTCGAGTCC–
FCC 2957		CGTACCCGCT	ACCC— —CG	CTCGAGCCC–
FCC 3115		CGTGCCCGCT	ACCC— —CG	CTCGAGACC–
FCC 2964		CGTACCCGCT	ACCC— —CG	CTCGAGCCC–
FCC 3113		CGTGCCCGCT	ACCC— —CG	CTCGAATTC–
F. avenaceum	AY337423	CGCATTCATT	ACCC— —CG	CTCAAGTCC–
F .equiseti	AY337424	CGCATTCATT	ACCC— —CG	CTCAAGTCC–
F .lateritium	AY337435	CACATCCATT	ACCC— —CG	CTCGAGTCC–
F. nygamai	AY337445	CGTGCCCGCT	ACCC— —CG	CTCGAATTC–
F. oxysporum	AY337428	CGTGCCCGCT	ACCC— —CG	CTCGAGACC–
F. proliferatum	AY337436	CGTGCCTGCT	ACCC— —CG	CTCGAGACC–
F. sambucinum	AY337422	CGCATCCATT	ACCC— —CG	CTCGAGCCC–
F. solani	AY337438	CACGCTCTGC	GCCCGCTTCT	CCCGAGTCCC
F. verticillioides	AY337450	CGTACCCGCT	ACCC— —CG	CTCGAGCCC–
Botryosphaeria_dothidea	AY236899	CAATTTCTTG	GTGGGGCTGG	CCCGCGCTAA

				150
FCC 3110		AAAAATTTTG	CGGTTCGACC	GTAATTTTTT
FCC 3114		AAAAATTTTG	CGGTTCGACC	GTAATTTTTT
FCC 3119		GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
FCC 3109		AAAAATTTTG	CAATATGACC	GTAATTTTTT
FCC 3117		GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
FCC 2957		AAAAATTTTG	CGATACGACC	GTAATTTTTC
FCC 3115		AAAAATTTTG	CAATATGACC	GTAATTTTTT
FCC 2964		AAAAATTTTG	CGATACGACC	GTAATTTTTC
FCC 3113		AAAAATTTTG	CGATATGACC	GTAATTTTTT
F. avenaceum	AY337423	GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
F .equiseti	AY337424	GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
F .lateritium	AY337435	GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
F. nygamai	AY337445	AAAAATTTTG	CGATATGACC	GTAATTTTTT
F. oxysporum	AY337428	AAAAATTTTG	CAATATGACC	GTAATTTTTT
F. proliferatum	AY337436	AAAAATTTTG	CGATATGACC	GTAATTTTTT
F. sambucinum	AY337422	GAAAATTTTG	CGGTGCGACC	GTGATTTTTT
F. solani	AY337438	AAAAATTTTG	CGGTTCGACC	GTAATTTTTT
F. verticillioides	AY337450	AAAAATTTTG	CGATACGACC	GTAATTTTTC
Botryosphaeria_dothidea	AY236899	GCCTCGTTTG	GTCTTCGGCA	A–AATCTCCG

				180
FCC 3110		TTGGTGGGGC	AT–TTACCCC	GCCACTCGGG
FCC 3114		TTGGTGGGGC	AT–TTACCCC	GCCACTCGGG
FCC 3119		CTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
FCC 3109		–TGGTGGGGC	AC–TTACCCC	GCCACTTGAG
FCC 3117		CTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
FCC 2957		–TGGTGGGGC	AT–TTATCCC	GCCACTCGAG
FCC 3115		–TGGTGGGGC	AC–TTACCCC	GCCACTTGAG
FCC 2964		–TGGTGGGGC	AT–TTATCCC	GCCACTCGAG
FCC 3113		–TGGTGGGGC	AT–TTACCCC	GCCACTCGAG
F. avenaceum	AY337423	TTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
F .equiseti	AY337424	TTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
F .lateritium	AY337435	CTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
F. nygamai	AY337445	–TGGTGGGGC	AT–TTACCCC	GCCACTCGAG
F. oxysporum	AY337428	–TGGTGGGGC	AC–TTACCCC	GCCACTTGAG
F. proliferatum	AY337436	–TGGTGGGGC	AT–TTACCCC	GCCACTCGAG
F. sambucinum	AY337422	TTGGTGGGGT	ATCTTACCCC	GCCACTCGAG
F. solani	AY337438	TTGGTGGGGC	AT–TTACCCC	GCCACTCGGG
F. verticillioides	AY337450	–TGGTGGGGC	AT–TTATCCC	GCCACTCGAG
Botryosphaeria_dothidea	AY236899	CATCTGGATT	TTTTGTGACC	GGCGTGCGAC

210

FCC 3110		CGACGTTGGA	CAAAGCCCTG	ATCCCTGCAC
FCC 3114		CGACGTTGGA	CAAAGCCCTG	ATCCCTGCAC
FCC 3119		TCACGGATGC	GCTTGCCCTG	TTCCC-----
FCC 3109		CGACGGGAGC	GTTTGCCCTC	TTACC-ATTC
FCC 3117		TCACGGATGC	GCTTGCCCTG	TTCCC-----
FCC 2957		CGGCGC--GT	TTCTGCCCTC	TC-CC-ATTC
FCC 3115		CGACGGGAGC	GTTTGCCCTC	TTACC-ATTC
FCC 2964		CGGCGC--GT	TTCTGCCCTC	TC-CC-ATTC
FCC 3113		CGGCGC--GT	TTTTGCCCTC	TTCCC-ATTC
F. avenaceum	AY337423	TGACGGATGC	GCTTGCCCTG	TTCCC-----
F .equiseti	AY337424	TGACGGATGC	GCTTGCCCTG	TTCCC-----
F .lateritium	AY337435	TCACGGATGC	GCTTGCCCTG	TTCCC-----
F. nygamai	AY337445	CGGCGCG--T	TTTTGCCCTC	TTCCC-ATTC
F. oxysporum	AY337428	CGACGGGAGC	GTTTGCCCTC	TTACC-ATTC
F. proliferatum	AY337436	CGATGGGCGC	GTTTTTGCCC	TTTCC-TGTC
F. sambucinum	AY337422	TGACGGATGC	GCTTGCCCTG	TTCCC-----
F. solani	AY337438	CGACGTTGGA	CAAAGCCCTG	ATCCCTGCAC
F. verticillioides	AY337450	CGGCGCG--T	TTCTGCCCTC	TC-CC-ATTC
Botryosphaeria_dothidea	AY236899	CGACGCGAAC	A-----CCCC	TCACC-----

240

FCC 3110		ACAAAAACAC	CA-AACCCTC	TTGGCGCGCA
FCC 3114		ACAAAAACAC	CA-AACCCTC	TTGGCGCGCA
FCC 3119		ACAAAACCTT	----ACCACC	CTGTCGCGCA
FCC 3109		TCAGAACCTC	AATGAGTGCG	TCGTCACGTG
FCC 3117		ACAAAACCTT	----ACCACC	CTGTCGCGCA
FCC 2957		-CACAACCTC	ACTGAGCTCA	TCGTCACGTG
FCC 3115		TCAGAACCTC	AATGAGTGCG	TCGTCACGTG
FCC 2964		-CACAACCTC	ACTGAGCTCA	TCGTCACGTG
FCC 3113		-CACAACCTC	ACTGAGCGCA	TCGTCACGTG
F. avenaceum	AY337423	ACAAAACCTC	----ACCACA	CTGTCGCGCA
F .equiseti	AY337424	ACAAAACCTC	----ACCACA	CTGTCGCGCA
F .lateritium	AY337435	ACAAAACCTT	----ACCACC	CTGTCGCGCA
F. nygamai	AY337445	-CACAACCTC	ACTGAGCGCA	TCGTCACGTG
F. oxysporum	AY337428	TCAGAACCTC	AATGAGTGCG	TCGTCACGTG
F. proliferatum	AY337436	-CACAACCTC	AATGAGCGCA	TTGTCACGTG
F. sambucinum	AY337422	ACAAAACCTT	----ACTACC	CTGTCGCGCA
F. solani	AY337438	ACAAAAACAC	CA-AACCCTC	TTGGCGCGCA
F. verticillioides	AY337450	-CACAACCTC	ACTGAGCTCA	TCGTCACGTG
Botryosphaeria_dothidea	AY236899	--AACGCTTC	CAGCCACTCA	CGTTCGTCTA

270

FCC 3110		TCACGTGGTT	CACAACAGAC	ACTGACTGGT
FCC 3114		TCACGTGGTT	CACAACAGAC	ACTGACTGGT
FCC 3119		CTACATGTCT	T---GCAGTC	ACTAACCA-C
FCC 3109		TCAAGC----	------AGTC	ACTAACCA-T
FCC 3117		CTACATGTCT	T---GCAGTC	ACTAACCA-C
FCC 2957		TCAAGC----	------AGTC	ACTAACCA-T
FCC 3115		TCAAGC----	------AGTC	ACTAACCA-T
FCC 2964		TCAAGC----	------AGTC	ACTAACCA-T
FCC 3113		TCAAGC----	------AGTC	ACTAACCA-T
F. avenaceum	AY337423	CTATGTCTT-	----GCAGTC	ACTAACCA-C
F .equiseti	AY337424	CTATGTCTT-	----GCAGTC	ACTAACCA-C
F .lateritium	AY337435	CTACATGTCT	T---GCAGTC	ACTAACCA-C
F. nygamai	AY337445	TCAAGC----	------AGTC	ACTAACCA-T
F. oxysporum	AY337428	TCAAGC----	------AGTC	ACTAACCA-T
F. proliferatum	AY337436	TCAAGC----	------AGCG	ACTAACCA-T
F. sambucinum	AY337422	CTATCATATG	TCTTCCAGTC	ACTAACCA-C
F. solani	AY337438	TCACGTGGTT	CACAACAGAC	ACTGACTGGT
F. verticillioides	AY337450	TCAAGC----	------AGTC	ACTAACCA-T
Botryosphaeria_dothidea	AY236899	TGCGAC----	-----CATAT	GCTAACCACC

		280
FCC 3110		TCAACAATAG
FCC 3114		TCAACAATAG
FCC 3119		TGGACAATAG
FCC 3109		TCAACAATAG
FCC 3117		TGGACAATAG
FCC 2957		CCGACAATAG
FCC 3115		TCAACAATAG
FCC 2964		CCGACAATAG
FCC 3113		TCGACAATAG
F. avenaceum	AY337423	TGGACAATAG
F .equiseti	AY337424	TGGACAATAG
F .lateritium	AY337435	TGGACAATAG
F. nygamai	AY337445	TCGACAATAG
F. oxysporum	AY337428	TCAACAATAG
F. proliferatum	AY337436	TCGACAATAG
F. sambucinum	AY337422	TGGACAATAG
F. solani	AY337438	TCAACAATAG
F. verticillioides	AY337450	CCGACAATAG
Botryosphaeria_dothidea	AY236899	GCCACAACAG

Alignment 3. The combined ITS1, 5.8s subunit and ITS2 regions of the genomic RNA gene DNA sequence of the *Phytophthora* isolates obtained from the *Eucalyptus* hedge plants .

30

CMW 13800		GTTGGGGGTC	TTATTT–GGC	GGCGGCTGCT
CMW 13816		GTTGGGGGTC	TTATTT–GGC	GGCGGCTGCT
CMW 13804		GTTGGGGGCC	TGCTCTGGGC	GGCGGTTGTC
P. cinnamomi	AF266764	GTTGGGGGCC	TGCTCTGGGC	GGCGGTTGTC
P. cryptogea	AF266796	ATTTGGGGGC	TTCCGTCTG–	GCCGGCCG—
P. infestans	AF266779	GTTGGGGGTC	T–TACTTGGC	GGCGGCTGCT
P. nicotianae	AF266776	GTTGGGGGTC	TTATTT–GGC	GGCGGCTGCT
P. palmivora	AF266780	TTTGGGGGTC	T–CTTTCGGC	GGCGGCTGCT
Pyhtium aphanidermatum	AJ233438	GTTCTGTGCT	CTCTTTCGG–	GAGGGCTG—

60

CMW 13800		GGCTTAATTG	TTGGCGGCTG	CTGCTGAGTG
CMW 13816		GGCTTAATTG	TTGGCGGCTG	CTGCTGAGTG
CMW 13804		GATGTCAAAG	TCGACGGTTG	CTGTTGCGTG
P. cinnamomi	AF266764	GATGTCAAAG	TCGACGGTTG	CTGTTGCGTG
P. cryptogea	AF266796	— — — — — –G	TTTTCGGCTG	—GCTGGGTG
P. infestans	AF266779	GGCTTTATTG	CTGGCGGCTA	CTGCTG— G
P. nicotianae	AF266776	GGCTTAATTG	TTGGCGGCTG	CTGCTGAGTG
P. palmivora	AF266780	GGCTTCATTG	CTGGCGGCTG	CTGTTG — G
Pyhtium aphanidermatum	AJ233438	— — — — — –A	ACGAAGGTGG	— GCTG —

90

CMW 13800		AG — – CCCTA	TCAAAAAAAA	GGCGAACGTT
CMW 13816		AG — – CCCTA	TCAAAAAAAA	GGCGAACGTT
CMW 13804		GGGGGCCCTA	TCAC —— T	GGCGAGCGTT
P. cinnamomi	AF266764	GGGGGCCCTA	TCAC —— T	GGCGAGCGTT
P. cryptogea	AF266796	GCGG– CTCTA	TCA —— T	GGCGACCGCT
P. infestans	AF266779	GCGAGCCCTA	TCAAA ——A	GGCGAGCGTT
P. nicotianae	AF266776	AG — CCCTA	TCAAAAAAAA	GGCGAACGTT
P. palmivora	AF266780	GAGAGCTCTA	TCA —— T	GGCGAGCGTT
Pyhtium aphanidermatum	AJ233438	– – – – CTTAA	TTG —— T	AGT – — — —

120

CMW 13800		TGGGCTTC —	—— GGCC	TGATTTAGTA
CMW 13816		TGGGCTTC —	—— GGCC	TGATTTAGTA
CMW 13804		TGGGTCCCCC	TCGGGGGAAC	TGAGCTAGTA
P. cinnamomi	AF266764	TGGGTCCCCC	TCGGGGGAAC	TGAGCTAGTA
P. cryptogea	AF266796	TGGGCCTC —	—— GGCC	TGGGCTAGTA
P. infestans	AF266779	TGGACTTC —	—— GGTC	TGAGCTAGTA
P. nicotianae	AF266776	TGGGCTTC —	—— GGCC	TGATTTAGTA
P. palmivora	AF266780	TGGGCTTC —	—— GGTC	TGAACTAGTA
Pyhtium aphanidermatum	AJ233438	– – – – – – —	— — —	— CTGCCG

150

CMW 13800		GTCTTTTTTT	CTTTTAAACC	CATTC–CTTA
CMW 13816		GTCTTTTTTT	CTTTTAAACC	CATTC–CTTA
CMW 13804		GCCTCTCTTT	T—— AAACC	CATCC–TGTA
P. cinnamomi	AF266764	GCCTCTCT—	— TTTAAACC	CATCC–TGTA
P. cryptogea	AF266796	GCGTATTTTT	—— AAACC	CATTC–CTAA
P. infestans	AF266779	GCTTTTTTAT	— TTTAAACC	CTTTA–CTTA
P. nicotianae	AF266776	GTCTTTTTTT	CTTTTAAACC	CATTC–CTTA
P. palmivora	AF266780	GC—TTT—	— TTTAAACC	CATTC–TTTA
Pyhtium aphanidermatum	AJ233438	ATGTATTTTT	C ——AAACC	CATTTACCTA

180

CMW 13800		ATACTGAA–T	ATACTGTGGG	GACGAAAGTC
CMW 13816		ATACTGAA–T	ATACTGTGGG	GACGAAAGTC
CMW 13804		ATACTGAA–C	ATACTGTGGG	GACGAAAGTC
P. cinnamomi	AF266764	ATACTGAA–C	ATACTGTGGG	GACGAAAGTC
P. cryptogea	AF266796	TTACTGAA–T	ATACTGTGGG	GACGAAAGTC
P. infestans	AF266779	ATACTGAT–T	ATACTGTGGG	GACGAAAGTC
P. nicotianae	AF266776	ATACTGAA–T	ATACTGTGGG	GACGAAAGTC
P. palmivora	AF266780	TAACTGAT–T	ATACTGTAGG	GACGAAAGTC
Pyhtium aphanidermatum	AJ233438	ATACTGATCT	ATACTCCAAA	AACGAAAGTT

210

CMW 13800		TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
CMW 13816		TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
CMW 13804		TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
P. cinnamomi	AF266764	TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
P. cryptogea	AF266796	TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
P. infestans	AF266779	TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
P. nicotianae	AF266776	TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
P. palmivora	AF266780	TCTGCTTTTA	ACTAGATAGC	AACTTTCAGC
Pyhtium aphanidermatum	AJ233438	TATGGTTTTA	ATCT – ATAAC	AACTTTCAGC

240

CMW 13800		AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
CMW 13816		AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
CMW 13804		AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
P. cinnamomi	AF266764	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
P. cryptogea	AF266796	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
P. infestans	AF266779	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
P. nicotianae	AF266776	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
P. palmivora	AF266780	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA
Pyhtium aphanidermatum	AJ233438	AGTGGATGTC	TAGGCTCGCA	CATCGATGAA

270

CMW 13800		GAACGCTGCG	AACTGCGATA	CGTAATGCGA
CMW 13816		GAACGCTGCG	AACTGCGATA	CGTAATGCGA
CMW 13804		GAACGCTGCG	AACTGCGATA	CGTAATGCGA
P. cinnamomi	AF266764	GAACGCTGCG	AACTGCGATA	CGTAATGCGA
P. cryptogea	AF266796	GAACGCTGCG	AACTGCGATA	CGTAATGCGA
P. infestans	AF266779	GAACGCTGCG	AACTGCGATA	CGTAATGCGA
P. nicotianae	AF266776	GAACGCTGCG	AACTGCGATA	CGTAATGCGA
P. palmivora	AF266780	GAACGCTGCG	AACTGCGATA	CGTAATGCGA
Pyhtium aphanidermatum	AJ233438	GAACGCTGCG	AACTGCGATA	CGTAATGCGA

300

CMW 13800		ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
CMW 13816		ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
CMW 13804		ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
P. cinnamomi	AF266764	ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
P. cryptogea	AF266796	ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
P. infestans	AF266779	ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
P. nicotianae	AF266776	ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
P. palmivora	AF266780	ATTGCAGGAT	TCAGTGAGTC	ATCGAAATTT
Pyhtium aphanidermatum	AJ233438	ATTGCAGAAT	TCAGTGAGTC	ATCGAAATTT

330

CMW 13800		TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
CMW 13816		TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
CMW 13804		GGAACGCATA	TTGCACTTCC	GGGTTAGTCC
P. cinnamomi	AF266764	GGAACGCATA	TTGCACTTCC	GGGTTAGTCC
P. cryptogea	AF266796	TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
P. infestans	AF266779	TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
P. nicotianae	AF266776	TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
P. palmivora	AF266780	TGAACGCATA	TTGCACTTCC	GGGTTAGTCC
Pyhtium aphanidermatum	AJ233438	TGAACGCACA	TTGCACTTTC	GGGTTATGCC

360

CMW 13800		TGGAAGTATG	CCTGTATCAG	TGTCCGTACA
CMW 13816		TGGAAGTATG	CCTGTATCAG	TGTCCGTACA
CMW 13804		TGGGAGTATG	CCTGTATCAG	TGTCCGTACA
P. cinnamomi	AF266764	TGGGAGTATG	CCTGTATCAG	TGTCCGTACA
P. cryptogea	AF266796	TGGGAGTATG	CCTGTATCAG	TGTCCGTACA
P. infestans	AF266779	TGGAAGTATG	CCTGTATCAG	TGTCCGTACA
P. nicotianae	AF266776	TGGAAGTATG	CCTGTATCAG	TGTCCGTACA
P. palmivora	AF266780	TGGGAGTATG	CCTGTATCAG	TGTCCGTACA
Pyhtium aphanidermatum	AJ233438	TGGAAGTATG	CCTGTATCAG	TGTCCGTACA

					390
CMW 13800		TTAAACTTGA	CTTTCTTCCT	TCCGTGTAGT	
CMW 13816		TTAAACTTGA	CTTTCTTCCT	TCCGTGTAGT	
CMW 13804		TCAAACTTGG	CTCTCTTCCT	TCCGTGTAGT	
P. cinnamomi	AF266764	TCAAACTTGG	CTCTCTTCCT	TCCGTGTAGT	
P. cryptogea	AF266796	CTAAACTTGG	CTCCCTTCCT	TCCGTGTAGT	
P. infestans	AF266779	ACAAACTTGG	CTTTCTCCCT	TCCGTGTAGT	
P. nicotianae	AF266776	TTAAACTTGA	CTTTCTTCCT	TCCGTGTAGT	
P. palmivora	AF266780	TCAAACTTGG	TTTTCTTCCT	TCCGTGTAGT	
Pyhtium aphanidermatum	AJ233438	TCAAACTTGC	CTTTCTTT–T	TCTGTGTAGT	
					420
CMW 13800		CGGTGGA–GG	AGATGT–CAG	ATGTGAAGTG	
CMW 13816		CGGTGGA–GG	AGATGT–CAG	ATGTGAAGTG	
CMW 13804		CGGTGGATGG	AGGTGC–CAG	ACGTGAGGTG	
P. cinnamomi	AF266764	CGGTGGATGG	AGGTGC–CAG	ACGTGAGGTG	
P. cryptogea	AF266796	CGGTGGATGG	GGACGCGCAG	ATGTGAAGTG	
P. infestans	AF266779	CGGTGG–AGG	AGATGC–CAG	ATGTGAAGTG	
P. nicotianae	AF266776	CGGTGGA–GG	AGATGT–CAG	ATGTGAAGTG	
P. palmivora	AF266780	CGGTGG–TGG	ATGTGC–CAG	ATGTGAAGTG	
Pyhtium aphanidermatum	AJ233438	CAG–GGAGAG	AGATGGCAGA	ATGTGAGGTG	
					450
CMW 13800		TCTTGC–GAT	TGGTCTTC—	—GGACCGGC	
CMW 13816		TCTTGC–GAT	TGGTCTTC—	—GGACCGGC	
CMW 13804		TCTTGC–GGG	CGGTCTTC—	—GGACTGGC	
P. cinnamomi	AF266764	TCTTGC–GGG	CGGTCTTC—	—GGACTGGC	
P. cryptogea	AF266796	TCTTGC–GGC	TGGTCTTC—	—GGTCCGGC	
P. infestans	AF266779	TCTTGC–GGT	TGGTTTCC—	—GGACCGAC	
P. nicotianae	AF266776	TCTTGC–GAT	TGGTCTTC—	—GGACCGGC	
P. palmivora	AF266780	TCTTGC–GGC	TGGTCTTC—	—GGATCGGC	
Pyhtium aphanidermatum	AJ233438	TCTCGCTGGC	TCCCTTTTCG	GAGGAGAAGA	
					480
CMW 13800		TGCGAGTCCT	TTTAAATGTA	C–TAAACTGA	
CMW 13816		TGCGAGTCCT	TTTAAATGTA	C–TAAACTGA	
CMW 13804		TGTGAGTCCC	TTGAAATGTA	C–TGAACTGT	
P. cinnamomi	AF266764	TGTGAGTCCC	TTGAAATGTA	C–TGAACTGT	
P. cryptogea	AF266796	TGCGAGTCCT	TTGAAATGTA	C–TACACTGT	
P. infestans	AF266779	TGCGAGTCCT	TTGAAATGTA	C–TAAACTGT	
P. nicotianae	AF266776	TGCGAGTCCT	TTTAAATGTA	C–TAAACTGA	
P. palmivora	AF266780	TGTGAGTCCT	TTGAAATGTA	C–TGAACTGT	
Pyhtium aphanidermatum	AJ233438	CGCGAGTCCC	TTTAAATGTA	CGTTCGCTCT	
					510
CMW 13800		ACTTCTCTTT	GCTCGAAAAG	TGGTGGCGTT	
CMW 13816		ACTTCTCTTT	GCTCGAAAAG	TGGTGGCGTT	
CMW 13804		ACTTCTCTTT	GCTCGAAAAG	CG–TGACGTT	
P. cinnamomi	AF266764	ACTTCTCTTT	GCTCGAAAAG	CG–TGACGTT	
P. cryptogea	AF266796	ACTTCTCTTT	GCTCGAAAAG	CG–TGACGTT	
P. infestans	AF266779	ACTTCTCTTT	GCTCCAAAAG	TGGTGGCATT	
P. nicotianae	AF266776	ACTTCTCTTT	GCTCGAAAAG	TGGTGGCGTT	
P. palmivora	AF266780	ACTTCTCTTT	GCTCCAAAAG	CG–TGGCGTT	
Pyhtium aphanidermatum	AJ233438	TTCTTGTGTC	TAAGATGAAG	TG–TGAT–TC	
					540
CMW 13800		GCTGGTTGTG	AAGG–CTGCT	ATTGTGGCAA	
CMW 13816		GCTGGTTGTG	AAGG–CTGCT	ATTGTGGCAA	
CMW 13804		GCTGGTTGTG	GAGG–CTGCC	TGTATGGCCA	
P. cinnamomi	AF266764	GCTGGTTGTG	GAGG–CTGCC	TGTATGGCCA	
P. cryptogea	AF266796	GCTGGTTGTG	GAGG–CTGCC	TGTGTGGCAT	
P. infestans	AF266779	GCTGGTTGTG	GACG–CTGCT	ATTGTAGCGA	
P. nicotianae	AF266776	GCTGGTTGTG	AAGG–CTGCT	ATTGTGGCAA	
P. palmivora	AF266780	GCTGATTGTG	GAGG–CTGCT	TGCGTAGCCA	
Pyhtium aphanidermatum	AJ233438	TCGAATCGCG	GTGATCTGTT	TGGATCGCTT	

				570
CMW 13800		ATT–GGCGAC	TGGTTTGTCT	GCTGCGGCA–
CMW 13816		ATT–GGCGAC	TGGTTTGTCT	GCTGCGGCA–
CMW 13804		GTC–GGCGAC	CGGTTTGTCT	GCTGCGGCGT
P. cinnamomi	AF266764	GTC–GGCGAC	CGGTTTGTCT	GCTGCGGCGT
P. cryptogea	AF266796	GTC–GGCGAC	CGGTTTGTCT	GCTGCGGCGT
P. infestans	AF266779	GTT–GGCGAC	CGGTTTGTCT	GCTGCGGCGT
P. nicotianae	AF266776	ATT–GGCGAC	TGGTTTGTCT	GCTGCGGCA–
P. palmivora	AF266780	GTCTGGCGAC	CAGTTTGTCT	GCTGTGGCGT
Pyhtium aphanidermatum	AJ233438	TGC–GCATTT	GGGCGACTTC	GGTTAGGACA

				600
CMW 13800		TTAATGGAAG	AGTGTTCGAT	TCGTGGTATG
CMW 13816		TTAATGGAAG	AGTGTTCGAT	TCGTGGTATG
CMW 13804		TTAATGGAGG	AGTGTCCGAT	TCGCGGTATG
P. cinnamomi	AF266764	TTAATGGAGG	AGTGTCCGAT	TCGCGGTATG
P. cryptogea	AF266796	TTAATGGAGG	AGTGTTCGAT	TCGCGGTATG
P. infestans	AF266779	T–AATGGAGA	AATGCTCGAT	TCGTGGTATG
P. nicotianae	AF266776	TTAATGGAAG	AGTGTTCGAT	TCGTGGTATG
P. palmivora	AF266780	T–AATGGAGG	AGTGTTCGAT	TCGCGGTATG
Pyhtium aphanidermatum	AJ233438	TTAAAGGAAG	CAACCTCTAT	TGGCGGTATG

				630
CMW 13800		GTTGGCTTCG	GCTGAACAA–	TGCACTTATT
CMW 13816		GTTGGCTTCG	GCTGAACAA–	TGCACTTATT
CMW 13804		GTTGGCTCCG	GCTGAACAA–	AGCGCTTATT
P. cinnamomi	AF266764	GTTGGCTCCG	GCTGAACAA–	AGCGCTTATT
P. cryptogea	AF266796	GTTGGCTTCG	GCTGAACA—	GACGCTTATT
P. infestans	AF266779	GTTGCCTTCG	GCTGAACAA–	TGCGCTTATT
P. nicotianae	AF266776	GTTGGCTTCG	GCTGAACAA–	TGCACTTATT
P. palmivora	AF266780	GTTGGCTTCG	GCTGAACAG–	A–CGCTTATT
Pyhtium aphanidermatum	AJ233438	TTAGGCTTCG	GCCCGACGTT	GCAGCTGACA

				660
CMW 13800		GGACGTTTTT	CC–TGCTGTG	GCGTGATGGA
CMW 13816		GGACGTTTTT	CC–TGCTGTG	GCGTGATGGA
CMW 13804		GGATGTTCCT	CCCTGCTGTG	GCGGTACGGA
P. cinnamomi	AF266764	GGATGTTCCT	CCCTGCTGTG	GCGGTACGGA
P. cryptogea	AF266796	GGGTGCTTTT	CC–TGCTGTG	GCTGGATGGA
P. infestans	AF266779	GGGTGATTTT	CCT–GCTGTG	GCGTGATGGA
P. nicotianae	AF266776	GGACGTTTTT	CC–TGCTGTG	GCGTGATGGA
P. palmivora	AF266780	GAATATTTCT	TCA–GCTGTG	GTGGTATG–A
Pyhtium aphanidermatum	AJ233438	GAGTGTGGTT	—TTCTGTT	CTTTCCTTGA

				690
CMW 13800		CTGGTGAACC	ATAGCTCGGT	—GGCTTGGC
CMW 13816		CTGGTGAACC	ATAGCTCGGT	—GGCTTGGC
CMW 13804		TCGGTGAACC	GTAGCTGTGC	TAGGCTTGGC
P. cinnamomi	AF266764	TCGGTGAACC	GTAGCTGTGC	TAGGCTTGGC
P. cryptogea	AF266796	CTGGTGAACC	GTAGCTGTGC	TAGGCTTGGC
P. infestans	AF266779	CTGGTGAACC	ATGGCTCT—	TTAGCTTGGC
P. nicotianae	AF266776	CTGGTGAACC	ATAGCTCGGT	—GGCTTGGC
P. palmivora	AF266780	TTGGTGAACC	GTAGCTATG–	TGAGCTTGGC
Pyhtium aphanidermatum	AJ233438	—GGTGTACC	TGAATTGTGT	GAGGCAATG–

				720
CMW 13800		TTTTGAATTG	GCTTTGCTGT	TGCGAAGTAG
CMW 13816		TTTTGAATTG	GCTTTGCTGT	TGCGAAGTAG
CMW 13804		GTTTGAACCG	GCGGTGTTGT	TGCGAAGTAG
P. cinnamomi	AF266764	GTTTGAACCG	GCGGTGTTGT	TGCGAAGTAG
P. cryptogea	AF266796	GTTTGAACCG	GCGGTG–TGG	TGCGAAGTAG
P. infestans	AF266779	ATTTGAATCG	GCTTTGCTGT	TGCGAAGTAG
P. nicotianae	AF266776	TTTTGAATTG	GCTTTGCTGT	TGCGAAGTAG
P. palmivora	AF266780	TTTTGAATTG	GCTTTGCTGT	TGCGAAGTAG
Pyhtium aphanidermatum	AJ233438	GTCTGGGCAA	ATGGT—TGC	TGTGTAGTAG

				750
CMW 13800		GGTGG——	————	————C –
CMW 13816		GGTGG——	————	————C –
CMW 13804		GGTGG—CGG	CTTC—GGCT	GTCGAGGGTC
P. cinnamomi	AF266764	GGTGG——	————	– ————C
P. cryptogea	AF266796	GGTGT—CTG	TTCT—GGCG	——– ——TA
P. infestans	AF266779	AGTGG——	————	– ————C
P. nicotianae	AF266776	GGTGG——	————	—– ——C –
P. palmivora	AF266780	AGTGG——	————	– ————C
Pyhtium aphanidermatum	AJ233438	GGTTTTGCTG	CTCTTGGACG	CCCTGTTTTC

				780
CMW 13800		AGCTTCGGTT	GTCGAGGGTC	GATCCATTTG
CMW 13816		AGCTTCGGTT	GTCGAGGGTC	GATCCATTTG
CMW 13804		– GATCCATTT	GGGAACTCTG	TGTCTCTCCG
P. cinnamomi	AF266764	GGCTTCGGCT	GTCGAGGGTC	GATCCATTTG
P. cryptogea	AF266796	AGCTGGGGTG	GACGAGGGTC	GATCCATTTG
P. infestans	AF266779	GGCTTCGGCT	GCCGAGGGTC	GATCCATTTG
P. nicotianae	AF266776	AGCTTCGGTT	GTCGAGGGTC	GATCCATTTG
P. palmivora	AF266780	GGCTTCGGCT	GTCGAGGGTC	GATCCATTTG
Pyhtium aphanidermatum	AJ233438	GGATAGGGTA	AAGGAGGCAA	CACCAATTTG

				810
CMW 13800		GGA–ACTTAA	TGTGTACTTC	GGTATGCATC
CMW 13816		GGA–ACTTAA	TGTGTACTTC	GGTATGCATC
CMW 13804		GCGCACTT—	–GTGTGCTTG	TGGTGGCATC
P. cinnamomi	AF266764	GGA–ACTCTG	TGTCT–CTCC	GGCGCACTTG
P. cryptogea	AF266796	GGAAACGTTG	TGTGCGCTTC	GGCGCGCATC
P. infestans	AF266779	GGA–AATGT–	TGTGTACTTC	GGTATGCATC
P. nicotianae	AF266776	GGA–ACTTAA	TGTGTACTTC	GGTATGCATC
P. palmivora	AF266780	GGA–ACT–TG	TGTATGCTTC	GGCATGCATC
Pyhtium aphanidermatum	AJ233438	GGACTGTTTG	CAATTTATTG	TGAACAACTT

		814
CMW 13800		TCAA
CMW 13816		TCAA
CMW 13804		TCAA
P. cinnamomi	AF266764	TGTG
P. cryptogea	AF266796	TCAA
P. infestans	AF266779	TCAA
P. nicotianae	AF266776	TCAA
P. palmivora	AF266780	TCAA
Pyhtium aphanidermatum	AJ233438	TCTA

Alignment 4. The combined ITS1, 5.8s subunit and ITS2 regions of the genomic RNA gene DNA sequence of the *Pythium* isolates obtained from the *Eucalyptus* hedge plants.

					30
CMW 13819		CCACACC– AA	AAAAA – CTTT	CCACGTGAA–	
CMW 13822		CCACACC– TA	AAAAA – CTTT	CCACGTGAA–	
CMW 13805		CCACACC– AT	AAAA — CTTT	CCACGTGAA–	
CMW 13796		CCACACC– TA	AAAAA – CTTT	CCACGTGAA–	
CMW 13820		CCACACC– AA	AAAAA – CTTT	CCACGTGAA–	
CMW 13807		CCACACCAAA	AAAA — CTTT	CCACGTGAA–	
CMW 13808		CCACACCTAA	AAACATCTTT	CCACGTGAA–	
CMW 13815		CCACACC – AT	AAAAA – CTTT	CCACGTGAA–	
CMW 13817		CCACACCTAA	AAACATCTTT	CCACGTGAA–	
CMW 13810		CCACACC— A	TAAAAACTTT	CCACGTGAA–	
P. aphanidermatum	AJ233438	CCACACC– AT	AAAAA – CTTT	CCACGTGAA–	
P. deliense	AJ233442	CCACACC– AT	AAAA — CTTT	CCACGTGAA–	
P. dissotocum	AJ233443	CCACACC– AA	AAAAA – CTTT	CCACGTGAA–	
P. helicoides	AB108061	CCACACCTAA	AAACATCTTT	CCACGTGAA–	
P. intermedium	AJ233447	CCACACC– TA	AAAAA – CTTT	CCACGTGAA–	
P. irregulare	AJ233448	CCACACCTAA	AAAAA – CTTT	CCACGTGAA–	
P. ultimum	AB108064	CCACACT– TT	AAAAAACTGT	CCACGTGAA–	
P. vexans	AJ233462	CCACACC– AA	AAAAA – CTTT	CCACGTGAA–	
Phytophthora cinnamomi	AF266764	CTGCTCTGGG	CGGCGGTTGT	CGATGTCAAA	

					60
CMW 13819		——CCGTT	–TTGTG–CGT	TTTGTGCTTG	
CMW 13822		——CTGTC	ATTATT–TGT	TGTGCGCTCT	
CMW 13805		——CCGTT	–GAAAT–CAT	GTTCTG——	
CMW 13796		——CTGTC	ATTATT–TGT	TGTGCGCTCT	
CMW 13820		——CCGTT	–GTAAC–TAT	GTTCTG——	
CMW 13807		——CCGTT	GTAACTATGT	TCTGTGCTCT	
CMW 13808		——CCGTT	TGTGACATGG	T–TGGGCTTG	
CMW 13815		——CCGTT	G—AAATCAT	GTTCTGTG—	
CMW 13817		——CCGTT	T—GTGACAT	GGTTGGGC—	
CMW 13810		——CCGTT	G—AAATCAT	GTTCTGTG—	
P. aphanidermatum	AJ233438	——CCGTT	G—AAATCAT	GTTCTGTG—	
P. deliense	AJ233442	——CCGTT	–GAAAT–CAT	GTTCTG——	
P. dissotocum	AJ233443	——CCGTT	GTAACTATGT	TCTGTGCTCT	
P. helicoides	AB108061	——CCGTT	—TGTGACAT	GGTTGGGC—	
P. intermedium	AJ233447	——CTGTC	ATTATT–TGT	TGTGCGCTCT	
P. irregulare	AJ233448	——CTGTC	GTTATT–TGT	TGTGTGTGTG	
P. ultimum	AB108064	——CTGTA	AGCAAGTCTA	GCGCTGTGAC	
P. vexans	AJ233462	——CCGTT	–TTGTG–CGT	TTTGTGCTTG	
Phytophthora cinnamomi	AF266764	GTCGACGGTT	GCTGTTGCGT	GGGGGGCC—	

					90
CMW 13819		TTGTTTTTCG	ATCTGCTCTC	TGTGCCT——	
CMW 13822		CTGCGGTGTC	GGTGGCGTCT	GTTGGCTGTA	
CMW 13805		——————	——TGCTCT–	———CT——	
CMW 13796		CTGCGGTGTC	GGTGGCGTCT	GTTGGCTGTA	
CMW 13820		——————	——TGCTCT–	———CTT—	
CMW 13807		CTTC———	——————	——————	
CMW 13808		TGCG———	——————	——————	
CMW 13815		——————	CTCTCTTT—	——————	
CMW 13817		——————	TTGTGCGT—	——————	
CMW 13810		——————	CTCTCTTT—	——————	
P. aphanidermatum	AJ233438	——————	CTCTCTTT—	——————	
P. deliense	AJ233442	——————	——TGCTCT–	———CT——	
P. dissotocum	AJ233443	CTTC———	——————	——————	
P. helicoides	AB108061	——————	TTGT———	——————	
P. intermedium	AJ233447	CTGCGGTGTC	GGTGGCGTCT	GTTGGCTGTA	
P. irregulare	AJ233448	CGTGTTGGTA	GCATGCGTGT	TTGCTTACGC	
P. ultimum	AB108064	TGAGCTGGTG	TTTTCATTTT	TGGACACTGG	
P. vexans	AJ233462	TTGTTTTTCG	ATCTGCTCTC	TGTGCCT——	
Phytophthora cinnamomi	AF266764	——————	CTATCACT—	——————	

120

CMW 13819		TTCGGGTGT–	G——GAGTGT	GGGGAAGAAA
CMW 13822		TTTGATACT–	GCTGGCGGGT	GCGAGCCGGA
CMW 13805		CTCGGGAG—	——————	——————
CMW 13796		TTTGATACT–	GCTGGCGGGT	GCGAGCCGGA
CMW 13820		CTCGGAGAG–	——————	——————
CMW 13807		–TCGGAGAG–	——————	——————
CMW 13808		–TTTTCTCT–	——————	——————
CMW 13815		—CGGGAG—	——————	——————
CMW 13817		—TTTCTC—	——————	——————
CMW 13810		—CGGGAG—	——————	——————
P. aphanidermatum	AJ233438	—CGGGAG—	——————	——————
P. deliense	AJ233442	CTCGGGAG—	——————	——————
P. dissotocum	AJ233443	——————	——————	——TCGGA
P. helicoides	AB108061	–GCGTTTTC–	——————	——————
P. intermedium	AJ233447	TTTGATACT–	GCTGGCGGGT	GCGAGCCGGA
P. irregulare	AJ233448	TTTGGGGGTT	GCGAGTGTGT	GTGTTGTCGG
P. ultimum	AB108064	AACGGGAGT–	——————	——————
P. vexans	AJ233462	TTCGGGTGT–	G——GAGTGT	GGGGAAGAAA
Phytophthora cinnamomi	AF266764	GGCGAGCGT–	——————	——————

150

CMW 13819		GCTCCAGGCT	AAACGAAGGC	–TGCGAGTTT
CMW 13822		TGCAGAGGCT	GAACGAAGGT	–CG—AGTTG
CMW 13805		———GGCT	GAACGAAGGT	–GGGCTGCTT
CMW 13796		TGCAGAGGCT	GAACGAAGGT	–CG—AGTTG
CMW 13820		———AGCT	GAACGAAGGT	–GGGCTGCTT
CMW 13807		———AGCT	GAACGAAGGT	–GGGCTGCTT
CMW 13808		———CTCT	TT–TGTAGGG	–GGGATGCGT
CMW 13815		———GGCT	GAACGAAGGT	GGG–CTGCTT
CMW 13817		———TCTC	TTTTGTAGGG	GGG–ATGCGT
CMW 13810		———GGCT	GAACGAAGGT	GGG–CTGCTT
P. aphanidermatum	AJ233438	———GGCT	GAACGAAGGT	GGG–CTGCTT
P. deliense	AJ233442	———GGCT	GAACGAAGGT	–GGGCTGCTT
P. dissotocum	AJ233443	—GAGAGCT	GAACGAAGGT	–GGGCTGCTT
P. helicoides	AB108061	——TCTCT	CTTTTGTAGG	GGGGATGCGT
P. intermedium	AJ233447	TGCAGAGGCT	GAACGAAGGT	CGAGTTGCTT
P. irregulare	AJ233448	TGCGCAGACT	GAACGAAGGT	–CGTGTGTTG
P. ultimum	AB108064	——CAGCA	GGACGAAGGT	TGGTCTGTTG
P. vexans	AJ233462	GCTCCAGGCT	AAACGAAGGC	–TGCGAGTTT
Phytophthora cinnamomi	AF266764	———TTGGG	TCCCCCTCGG	GGGAAC——

180

CMW 13819		CGTGCTTG—	——————	——————
CMW 13822		CTTTGCTCT–	——————	——————
CMW 13805		AATTGTGGT–	——————	——————
CMW 13796		CTTTGCTCT–	——————	——————
CMW 13820		AATTGTAGT–	——————	——————
CMW 13807		AATTGTAGT–	——————	——————
CMW 13808		GCGAGCTAT–	——————	——————
CMW 13815		AATTGTAGTC	T—————	——————
CMW 13817		——GCGAGC	T—————	——————
CMW 13810		AATTGTAGTC	T—————	——————
P. aphanidermatum	AJ233438	AATTGTAGTC	T—————	——————
P. deliense	AJ233442	AATTGTGGT–	——————	——————
P. dissotocum	AJ233443	AATTGTAGT–	——————	——————
P. helicoides	AB108061	——GCGAGC	T—————	——————
P. intermedium	AJ233447	——TGCTCT–	——————	——————
P. irregulare	AJ233448	CTGTGTGCCT	GCTGCACTGC	TGACTTTGCA
P. ultimum	AB108064	TAATGCAAGT	TATGATGGAC	——————
P. vexans	AJ233462	CGTGCTTG—	——————	——————
Phytophthora cinnamomi	AF266764	———TGAGC	T—————	——————

				210
CMW 13819		——————	——————	——————
CMW 13822		——————	——————	——————
CMW 13805		——————	——————	——————
CMW 13796		——————	——————	——————
CMW 13820		——————	——————	——————
CMW 13807		——————	——————	——————
CMW 13808		——————	——————	——————
CMW 13815		——————	——————	——————
CMW 13817		——————	——————	——————
CMW 13810		——————	——————	——————
P. aphanidermatum	AJ233438	——————	——————	——————
P. deliense	AJ233442	——————	——————	——————
P. dissotocum	AJ233443	——————	——————	——————
P. helicoides	AB108061	——————	——————	——————
P. intermedium	AJ233447	——————	——————	——————
P. irregulare	AJ233448	TTGATTTGCA	TGGTGTTGGC	GGAGCGGCGG
P. ultimum	AB108064	——————	——————	——————
P. vexans	AJ233462	——————	——————	——————
Phytophthora cinnamomi	AF266764	——————	——————	——————

				240
CMW 13819		——————	———CGGCC	GATTTATTCT
CMW 13822		——————	———CGGCT	GACTTATT–T
CMW 13805		——————	———CTGCC	GATGTATT–T
CMW 13796		——————	———CGGCT	GACTTATT–T
CMW 13820		——————	———CTGCC	GATGTACT—
CMW 13807		——————	———CTGCC	GATGTACT—
CMW 13808		——————	———CTGTA	AAC—TT—
CMW 13815		——————	————GCC	GATGTATTTT
CMW 13817		——————	————ATC	TGTAAACTTG
CMW 13810		——————	————GCC	GATGTATTTT
P. aphanidermatum	AJ233438	——————	————GCC	GATGTATTTT
P. deliense	AJ233442	——————	———CTGCC	GATGTATT–T
P. dissotocum	AJ233443	——————	———CTGCC	GATGTACT–T
P. helicoides	AB108061	——————	————ATC	TGTAAACTTG
P. intermedium	AJ233447	——————	———CGGCT	GACTTATT–T
P. irregulare	AJ233448	GTGCTGTTGC	ATGCGCGGCT	GACCTATTTT
P. ultimum	AB108064	——————	———TAGCT	GATGAACTTT
P. vexans	AJ233462	——————	———CGGCC	GATTTATTCT
Phytophthora cinnamomi	AF266764	——————	————AGT	AGCCTCTCTT

				270
CMW 13819		TT——CAAAC	CCATACATTA	AA—CACTGA
CMW 13822		TT——CAAAC	CCA–ATACCC	AACTTACTGA
CMW 13805		TT——CAAAC	CCATTTACCT	AA—TACTGA
CMW 13796		TT——CAAAC	CCA–ATACCC	AACTTACTGA
CMW 13820		TT——TAAAC	CCATTAAACT	AA—TACTGA
CMW 13807		TT——TAAAC	CCATTAAACT	—AATACTGA
CMW 13808		GT——CAAAC	CCATTCTTTT	TGATAACTGA
CMW 13815		T———CAAAC	CCATT–TACC	TAATA–CTGA
CMW 13817		T———CAAAC	CCATTCTTTT	TGATAACTGA
CMW 13810		T———CAAAC	CCATT–TACC	TAATA–CTGA
P. aphanidermatum	AJ233438	T———CAAAC	CCATT–TACC	TAATA–CTGA
P. deliense	AJ233442	TT——CAAAC	CCATTTACCT	AA—TACTGA
P. dissotocum	AJ233443	TT———AAAC	CCATTAAACT	AA—TACTGA
P. helicoides	AB108061	T———CAAAC	CCATTCTTTT	TGATAACTGA
P. intermedium	AJ233447	TT——CAAAC	CCAATACCCA	AC—TTACTG
P. irregulare	AJ233448	TT——TCAAA	CCCCATACCT	AAATGACTGA
P. ultimum	AB108064	TGTTTTTAAA	CCCTT–ACCT	AAATA–CTGA
P. vexans	AJ233462	TT——CAAAC	CCATACATTA	AA—CACTGA
Phytophthora cinnamomi	AF266764	T———TAAAC	CCATCCTGT–	—AATACTGA

				300
CMW 13819		AGTATACTGT	GAGGACGAAA	GTCCTTGCTT
CMW 13822		T–TATACTGT	GAGAACGAAA	GTTCTTGCTT
CMW 13805		TCTATACTCC	AAAAACGAAA	GTTTCTGGTT
CMW 13796		T–TATACTGT	GAGAACGAAA	GTTCTTGCTT
CMW 13820		ACTATACTCC	GAAAACGAAA	GTCTTTGGTT
CMW 13807		ACTATACTCC	GAAAACGAAA	GTCTTTGGTT
CMW 13808		AACATACTGT	GGGGACGAAA	GTCTCTGCTT
CMW 13815		TCTATACTCC	AAAAACGAAA	GTTTATGGTT
CMW 13817		AACATACTGT	GGGGACGAAA	GTCTCTGCTT
CMW 13810		TCTATACTCC	AAAAACGAAA	GTTTATGGTT
P. aphanidermatum	AJ233438	TCTATACTCC	AAAAACGAAA	GTTTATGGTT
P. deliense	AJ233442	TCTATACTCC	AAAAACGAAA	GTTTCTGGTT
P. dissotocum	AJ233443	ACTATACTCC	GAAAACGAAA	GTCTTTGGTT
P. helicoides	AB108061	AACATACTGT	GGGGACGAAA	GTCTCTGCTT
P. intermedium	AJ233447	ATTATACTGT	GAGAACGAAA	GTTCTTGCTT
P. irregulare	AJ233448	T–TATACTGT	GAGAACGAAA	GTTCTTGCTT
P. ultimum	AB108064	TTTATACTGT	GGGGACGAAA	GTCCTTGCTT
P. vexans	AJ233462	AGTATACTGT	GAGGACGAAA	GTCCTTGCTT
Phytophthora cinnamomi	AF266764	A–CATACTGT	GGGGACGAAA	GTCTCTGCTT

				330
CMW 13819		TGAACTAGAT	AGCAACTTTC	AGCAGTGGAT
CMW 13822		TTAACTAGAT	AACAACTTTC	AGCAGTGGAT
CMW 13805		TTAATCC–AT	AACAACTTTC	AGCAGTGGAT
CMW 13796		TTAACTAGAT	AACAACTTTC	AGCAGTGGAT
CMW 13820		TTAATCA–AT	AACAACTTTC	AGCAGTGGAT
CMW 13807		TTAATCA–AT	AACAACTTTC	AGCAGTGGAT
CMW 13808		TGAACTAGAT	AGCAACTTTC	AGCAGTGGAT
CMW 13815		TTAA–TCTAT	AACAACTTTC	AGCAGTGGAT
CMW 13817		TGAACTAGAT	AGCAACTTTC	AGCAGTGGAT
CMW 13810		TTAA–TCTAT	AACAACTTTC	AGCAGTGGAT
P. aphanidermatum	AJ233438	TTAA–TCTAT	AACAACTTTC	AGCAGTGGAT
P. deliense	AJ233442	TTAATCC–AT	AACAACTTTC	AGCAGTGGAT
P. dissotocum	AJ233443	TTAATCA–AT	AACAACTTTC	AGCAGTGGAT
P. helicoides	AB108061	TGAACTAGAT	AGCAACTTTC	AGCAGTGGAT
P. intermedium	AJ233447	TTAACTAGAT	AACAACTTTC	AGCAGTGGAT
P. irregulare	AJ233448	TTAACTAGAT	AACAACTTTC	AGCAGTGGAT
P. ultimum	AB108064	TTAC–TAGAT	AACAACTTTC	AGCAGTGGAT
P. vexans	AJ233462	TGAACTAGAT	AGCAACTTTC	AGCAGTGGAT
Phytophthora cinnamomi	AF266764	TTAACTAGAT	AGCAACTTTC	AGCAGTGGAT

				360
CMW 13819		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13822		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13805		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13796		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13820		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13807		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13808		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13815		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13817		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
CMW 13810		GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. aphanidermatum	AJ233438	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. deliense	AJ233442	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. dissotocum	AJ233443	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. helicoides	AB108061	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. intermedium	AJ233447	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. irregulare	AJ233448	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. ultimum	AB108064	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
P. vexans	AJ233462	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT
Phytophthora cinnamomi	AF266764	GTCTAGGCTC	GCACATCGAT	GAAGAACGCT

				390
CMW 13819		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13822		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13805		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13796		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13820		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13807		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13808		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13815		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13817		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
CMW 13810		GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. aphanidermatum	AJ233438	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. deliense	AJ233442	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. dissotocum	AJ233443	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. helicoides	AB108061	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. intermedium	AJ233447	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. irregulare	AJ233448	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. ultimum	AB108064	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
P. vexans	AJ233462	GCGAACTGCG	ATACGTAATG	CGAATTGCAG
Phytophthora cinnamomi	AF266764	GCGAACTGCG	ATACGTAATG	CGAATTGCAG

				420
CMW 13819		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13822		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13805		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13796		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13820		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13807		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13808		GATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13815		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13817		GATTCAGTGA	GTCATCGAAA	TTTTGAACGC
CMW 13810		AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. aphanidermatum	AJ233438	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. deliense	AJ233442	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. dissotocum	AJ233443	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. helicoides	AB108061	GATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. intermedium	AJ233447	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. irregulare	AJ233448	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. ultimum	AB108064	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
P. vexans	AJ233462	AATTCAGTGA	GTCATCGAAA	TTTTGAACGC
Phytophthora cinnamomi	AF266764	GATTCAGTGA	GTCATCGAAA	TTTGGAACGC

				450
CMW 13819		ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13822		ATATTGCACT	TCCGGGTTAT	GCCTGGAAGT
CMW 13805		ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13796		ATATTGCACT	TCCGGGTTAT	GCCTGGAAGT
CMW 13820		ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13807		ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13808		ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13815		ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13817		ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
CMW 13810		ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. aphanidermatum	AJ233438	ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. deliense	AJ233442	ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. dissotocum	AJ233443	ACATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. helicoides	AB108061	ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. intermedium	AJ233447	ATATTGCACT	TCCGGGTTAT	GCCTGGAAGT
P. irregulare	AJ233448	ATATTGCACT	TCCGGGTTAT	GCCTGGAAGT
P. ultimum	AB108064	ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
P. vexans	AJ233462	ATATTGCACT	TTCGGGTTAT	GCCTGGAAGT
Phytophthora cinnamomi	AF266764	ATATTGCACT	TCCGGGTTAG	TCCTGGGAGT

				480
CMW 13819		ATGTCTGTAT	CAGTGTCCGT	ACCTCAACCT
CMW 13822		ATGTCTGTAT	CAGTGTCCGT	AAATCAACCT
CMW 13805		ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
CMW 13796		ATGTCTGTAT	CAGTGTCCGT	AAATCAACCT
CMW 13820		ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
CMW 13807		ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
CMW 13808		ATGTCTGTAT	CAGTGTCCGT	ACACTAAACT
CMW 13815		ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
CMW 13817		ATGTCTGTAT	CAGTGTCCGT	ACACTAAACT
CMW 13810		ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
P. aphanidermatum	AJ233438	ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
P. deliense	AJ233442	ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
P. dissotocum	AJ233443	ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT
P. helicoides	AB108061	ATGTCTGTAT	CAGTGTCCGT	ACACTAAACT
P. intermedium	AJ233447	ATGTCTGTAT	CAGTGTCCGT	AAATCAACCT
P. irregulare	AJ233448	ATGTCTGTAT	CAGTGTCCGT	AAATCAAACT
P. ultimum	AB108064	ATGTCTGTAT	CAGAGTCCGT	AAATCAAACT
P. vexans	AJ233462	ATGTCTGTAT	CAGTGTCCGT	ACCTCAACCT
Phytophthora cinnamomi	AF266764	ATGCCTGTAT	CAGTGTCCGT	ACATCAAACT

				510
CMW 13819		TGCCTTTCTT	T–TCCTGTGT	AGTCAG——
CMW 13822		TGCCTTTCTT	CCTTCTGTGT	AGTCAG——
CMW 13805		TGCCTTTCTT	T–TTCTGTGT	AGTCAG——
CMW 13796		TGCCTTTCTT	CCTTCTGTGT	AGTCAG——
CMW 13820		TGCCTTTCTT	T–TTTTGTGT	AGTCAA——
CMW 13807		TGCCTTTCTT	T–TTTTGTGT	AGTCAA——
CMW 13808		TGCCTCCTTT	G–CGTCGTGT	AGTCGTCGCG
CMW 13815		TGCCTTTCTT	TTTCT–GTGT	AGTCAG——
CMW 13817		TGCCTCCTTT	GCGTC–GTGT	AGTCGTCGCG
CMW 13810		TGCCTTTCTT	TTTCT–GTGT	AGTCAG——
P. aphanidermatum	AJ233438	TGCCTTTCTT	TTTCT–GTGT	AGTCAG——
P. deliense	AJ233442	TGCCTTTCTT	T–TTCTGTGT	AGTCAG——
P. dissotocum	AJ233443	TGCCTTTCTT	T–TTTTGTGT	AGTCAA——
P. helicoides	AB108061	TGCCTCCTTT	GCGTC–GTGT	AGTCGTCGCG
P. intermedium	AJ233447	TGCCTTTCTT	CCTTCTGTGT	AGTCAG——
P. irregulare	AJ233448	TGCGTTTCTT	CCTTCCGTGT	AGTCGG——
P. ultimum	AB108064	TGCCTTTCTT	TTTCT–GTGT	AGTCAG——
P. vexans	AJ233462	TGCCTTTCTT	T–TCCTGTGT	AGTCAG——
Phytophthora cinnamomi	AF266764	TGGCTCTCTT	CCTTCCGTGT	AGTCGGTG–G

				540
CMW 13819		—GAGAGGAA	ACGAGCAGAC	TTGAAGTGTC
CMW 13822		—TGGAGGAT	GTGGC–AGAC	GTGAAGTGTC
CMW 13805		—GGAGAGAG	ATGGCAGAAT	GTGAGGTGTC
CMW 13796		—TGGAGGAT	GTGGC–AGAC	GTGAAGTGTC
CMW 13820		—GAAGAGAG	ATGGCAGACT	GTGAGGTGTC
CMW 13807		—GAAGAGAG	ATGGCAGACT	GTGAGGTGTC
CMW 13808		TTGGAAATTT	GTGGCAGA–T	GTGAGGTGTC
CMW 13815		—GGAGAGAG	ATGGCAGAAT	GTGAGGTGTC
CMW 13817		TTGGAAATTT	GTGGCAGA–T	GTGAGGTGTC
CMW 13810		—GGAGAGAG	ATGGCAGAAT	GTGAGGTGTC
P. aphanidermatum	AJ233438	—GGAGAGAG	ATGGCAGAAT	GTGAGGTGTC
P. deliense	AJ233442	—GGAGAGAG	ATGGCAGAAT	GTGAGGTGTC
P. dissotocum	AJ233443	—GAAGAGAG	ATGGCAGACT	GTGAGGTGTC
P. helicoides	AB108061	TTGGAAATTT	GTGGCAGA–T	GTGAGGTGTC
P. intermedium	AJ233447	—TGGAGGAT	GTGGCAGAC–	GTGAAGTGTC
P. irregulare	AJ233448	—TGGAGGAG	AGTTGCAGAT	GTGAAGTGTC
P. ultimum	AB108064	—GGATGGAA	TGTGCAGA–T	GTGAAGTGTC
P. vexans	AJ233462	—GAGAGGAA	ACGAGCAGAC	TTGAAGTGTC
Phytophthora cinnamomi	AF266764	ATGGAG——	GTGCCAGA–C	GTGAGGTGTC

				570
CMW 13819		TCGCCT--GT	TGGTTTT---	---CGATTGT
CMW 13822		TCGCTGTGGC	TGGTTTTTGG	--TCGTTTCG
CMW 13805		TCG------T	TGACTCC---	---CTTTTCG
CMW 13796		TCGCTGTGGC	TGGTTTTTGG	--TCGTTTCG
CMW 13820		TCG------C	TGACTCC---	---CTCTTCG
CMW 13807		TCG------C	TGACTCC---	---CTCTTCG
CMW 13808		TTG------T	TTGCTGT---	---GTCTTTG
CMW 13815		TCGC------	TGGCTCC---	---CTTTTCG
CMW 13817		TTGT------	TTGCTGT---	---GTCTTTG
CMW 13810		TCGC------	TGGCTCC---	---CTTTTCG
P. aphanidermatum	AJ233438	TCGC------	TGGCTCC---	---CTTTTCG
P. deliense	AJ233442	TCG------T	TGACTCC---	---CTTTTCG
P. dissotocum	AJ233443	TCGCTGACTC	C---------	---CTCTTCG
P. helicoides	AB108061	TTGT------	TTGCTGT---	---GTCTTTG
P. intermedium	AJ233447	TCGCTGTGGC	TGGTTTTTGG	--TCGTTTCG
P. irregulare	AJ233448	TCGCTGTGGT	TGGTGTTTG-	--TTGTTTGC
P. ultimum	AB108064	TCGCA-----	TGGTTGCGTT	CGTTTTTTCG
P. vexans	AJ233462	TCGCCT--GT	TGGTTTT---	---CGATTGT
Phytophthora cinnamomi	AF266764	TTGC------	GGGCGGT---	---CTTCGGA

				600
CMW 13819		GTAAATACGA	CTGG--GTGA	GTCCTTTTAA
CMW 13822		GCTATGAATA	CAGTTTGCGA	GTCCTTTTAA
CMW 13805		GAGGAGAAGA	C-----GCGA	GTCCCTTTAA
CMW 13796		GCTATGAATA	CAGTTTGCGA	GTCCTTTTAA
CMW 13820		GAGGAGAAGA	C-----GCGA	GTCCCTTTAA
CMW 13807		GAGGAGAAG-	AC----GCGA	GTCCCTTTAA
CMW 13808		TTGATGCGGC	GG----GCAA	GTCCCTTGAA
CMW 13815		GAGGAGAAGA	CG-----CGA	GTCCCTTTAA
CMW 13817		TTGATGCGGC	GG----GCAA	GTCCCTTGAA
CMW 13810		GAGGAGAAG-	AC----GCGA	GTCCCTTTAA
P. aphanidermatum	AJ233438	GAGGAGAAGA	CG-----CGA	GTCCCTTTAA
P. deliense	AJ233442	GAGGAGAAGA	C-----GCGA	GTCCCTTTAA
P. dissotocum	AJ233443	GAGGAGAAGA	C-----GCGA	GTCCCTTTAA
P. helicoides	AB108061	TTGATGCGGC	GG----GCAA	GTCCCTTGAA
P. intermedium	AJ233447	GCTATGAATA	CAGTTTGCGA	GTCCTTTTAA
P. irregulare	AJ233448	AATGAATGCA	CAGCTTGCGA	GTCCTTTTAA
P. ultimum	AB108064	ATCGAGAATC	TG----TCGA	GTCCTTTTAA
P. vexans	AJ233462	GTAAATACGA	CTGG--GTGA	GTCCTTTTAA
Phytophthora cinnamomi	AF266764	CTGGCT----	------GTGA	GTCCCTTGAA

				630
CMW 13819		A-----TGGA	CA---CGATC	TTTCTGCTGT
CMW 13822		A-----TGGA	CACGACTTTC	TCTTTTTTGT
CMW 13805		A-----TGTA	CGTT-CGCTC	TTTCTTGTGT
CMW 13796		A-----TGGA	CACGACTTTC	TCTTTTTTGT
CMW 13820		A-----TGTA	CGTT-CGCTC	TTTCTTGTGT
CMW 13807		A-----TGTA	CGTT-CGCTC	TTTCTTGTGT
CMW 13808		A-----GTCG	GACG-CGTAT	CTTTGCGTGC
CMW 13815		A-----TGTA	C-GTTCGCTC	TTTCTTGTGT
CMW 13817		A-----GTCG	G-ACGCGTAT	CTTTGCGTGC
CMW 13810		A-----TGTA	C-GTTCGCTC	TTTCTTGTGT
P. aphanidermatum	AJ233438	A-----TGTA	C-GTTCGCTC	TTTCTTGTGT
P. deliense	AJ233442	A-----TGTA	CGTT-CGCTC	TTTCTTGTGT
P. dissotocum	AJ233443	A-----TGTA	CGTT-CGCTC	TTTCTTGTGT
P. helicoides	AB108061	A---GTCGGA	C---GCGTAT	CTTTGCGTGC
P. intermedium	AJ233447	A-----TGGA	CACGACTTTC	TCTTTTTTGT
P. irregulare	AJ233448	A-----TGGA	CACGACTTTC	TCTTTTTTGT
P. ultimum	AB108064	A-----TGGA	C-A--CGGTC	TTTTCTATGG
P. vexans	AJ233462	A-----TGGA	CA---CGATC	TTTCTGCTGT
Phytophthora cinnamomi	AF266764	ATGTACTGAA	CTGTACTTCT	CTTTGCTCGA

				660
CMW 13819		TTTCTACGGA	G–TGTGGCGC	TCGAAAGGCG
CMW 13822		TT–CTGCGAG	G–TGCTGTGC	TCGAA–CGCG
CMW 13805		CTAAGATGAA	G–TGTGATTC	TCGAATCGCA
CMW 13796		TT–CTGCGAG	G–TGCTGTGC	TCGAA–CGCG
CMW 13820		TTAAGATGAA	G–TGTGACTT	TCGAA–CGCA
CMW 13807		TTAAGATGAA	G–TGTGACTT	TCGAA–CGCA
CMW 13808		GTTGGGTGCC	G–GTGGGCTG	TGGGA–CGCG
CMW 13815		CTAAGATGAA	G–TGTGATTC	TCGAATCGCG
CMW 13817		GTTGGGTGCC	G—GTGGGCT	GTGGGACGCG
CMW 13810		CTAAGATGAA	G–TGTGATTC	TCGAATCGCG
P. aphanidermatum	AJ233438	CTAAGATGAA	G–TGTGATTC	TCGAATCGCG
P. deliense	AJ233442	CTAAGATGAA	G–TGTGATTC	TCGAATCGCA
P. dissotocum	AJ233443	TTAAGATGAA	G–TGTGACTT	TCGAA–CGCA
P. helicoides	AB108061	GTTGGGTGCC	GGTG—GGCT	GTGGGACGCG
P. intermedium	AJ233447	TTCTG–CGAG	G–TGCTGTGC	TCGAA–CGCG
P. irregulare	AJ233448	AT–GTGCGCG	G–TGCTGTGC	GTGAA–CGCG
P. ultimum	AB108064	TTTCTATGAA	G–TGTAATGG	TTGGAAGGCA
P. vexans	AJ233462	TTTCTACGGA	G–TGTGGCGC	TCGAAAGGCG
Phytophthora cinnamomi	AF266764	AAAGCGTGAC	GTTGCTGGTT	GTGGAGGCTG

				690
CMW 13819		GCGGTTTCCT	TCGGGATCGC	TCGCAGTCGG
CMW 13822		GTGGTTTTC–	——GGATCGC	TCGCGGC–TG
CMW 13805		GTGATCTGTT	T—GGATCGC	TTTGCGCATT
CMW 13796		GTGGTTTTC–	——GGATCGC	TCGCGGC–TG
CMW 13820		GTGATCTGTT	T—GGATCGC	TTTGCTCGAG
CMW 13807		GTGATCTGTT	T—GGATCGC	TTTGCTCGAG
CMW 13808		——TCTGTT	——GACGAG	TCT———
CMW 13815		GTGATCTGTT	T—GGATCGC	TTTGCGCATT
CMW 13817		——TCTGTT	G—A———	——CGAGTC
CMW 13810		GTGATCTGTT	T—GGATCGC	TTTGCGCATT
P. aphanidermatum	AJ233438	GTGATCTGTT	T—GGATCGC	TTTGCGCATT
P. deliense	AJ233442	GTGATCTGTT	T—GGATCGC	TTTGCGCATT
P. dissotocum	AJ233443	GTGATCTGTT	T—GGATCGC	TTTGCTCGAG
P. helicoides	AB108061	——TCTGT–	T—GACGAGT	CTGGCGACC–
P. intermedium	AJ233447	GTGGTTT—T	C—GGATCGC	TC–GCGGCTG
P. irregulare	AJ233448	GTGGTTTTC–	——GGATCGC	TCGCGGC–TG
P. ultimum	AB108064	GTGATTT—T	C—GGATTGC	TG–GCGGCTT
P. vexans	AJ233462	GCGGTTTCCT	TCGGGATCGC	TCGCAGTCGG
Phytophthora cinnamomi	AF266764	——CCTGTA	T—GGCCAGT	C–GGCGACC–

				720
CMW 13819		ACGGCGAC–T	TTGGCGAATA	CAT–ATGGGA
CMW 13822		TTGGCGAC–T	TCGGTGAATG	CATTATGGAG
CMW 13805		TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
CMW 13796		TTGGCGAC–T	TCGGTGAATG	CATTATGGAG
CMW 13820		TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
CMW 13807		TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
CMW 13808		—GGCGACCT	TTGGTGCGTG	CATGCTTGG–
CMW 13815		TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
CMW 13817		TGGCGACC–T	TTGGTGCGTG	CATGCTTGG–
CMW 13810		TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
P. aphanidermatum	AJ233438	TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
P. deliense	AJ233442	TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
P. dissotocum	AJ233443	TGGGCGAC–T	TCGGTTAGGA	CATTAAAGGA
P. helicoides	AB108061	—————T	TTGGTGCG—	TGCATGCTTG
P. intermedium	AJ233447	TTGGCGAC–T	TCGGTGAATG	CATTATGGAG
P. irregulare	AJ233448	TCGGCGAC–T	TCGGTGAATG	CATAATGGAG
P. ultimum	AB108064	TTGGCGAC–T	TCGGTATGAA	CGT—ATGGA
P. vexans	AJ233462	ACGGCGAC–T	TTGGCGAATA	CAT–ATGGGA
Phytophthora cinnamomi	AF266764	—GGTTTG–T	CTGCTGCGG–	CGTTTAATGG

750

CMW 13819		AGCAGACTCG	–ACTCGCGGT	ACGTTAGGTG
CMW 13822		TG–GACCTCG	–ATTCGCGGT	ATGTTGGGC–
CMW 13805		AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
CMW 13796		TG–GACCTCG	–ATTCGCGGT	ATGTTGGGC–
CMW 13820		AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
CMW 13807		AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
CMW 13808		–GCACTGTGT	–ATTGGCGGT	ATGTTAGGC–
CMW 13815		AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
CMW 13817		–GCACTGTGT	–ATTGGCGGT	ATGTTAGGC–
CMW 13810		AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
P. aphanidermatum	AJ233438	AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
P. deliense	AJ233442	AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
P. dissotocum	AJ233443	AGCAACCTCT	–ATTGGCGGT	ATGTTAGGC–
P. helicoides	AB108061	GGCACTGTGT	–ATTGGCGGT	ATGTTAGGC–
P. intermedium	AJ233447	TG–GACCTCG	–ATTCGCGGT	ATGTTGGGC–
P. irregulare	AJ233448	TG–GACCTCG	–ATTCGCGGT	ATGTTGGGC–
P. ultimum	AB108064	GACTAGCTCA	–ATTCGTGGT	ATGTTAGGC–
P. vexans	AJ233462	AGCAGACTCG	–ACTCGCGGT	ACGTTAGGTG
Phytophthora cinnamomi	AF266764	AGGAGTGTCC	GATTCGCGGT	ATGGTTGGC–

780

CMW 13819		TGTTGCTTTT	GCGGCGTGCT	GAACAATGTT
CMW 13822		———————TTC	G——————GCT	GGACAATGTT
CMW 13805		———————TTC	G——————GCC	CGACGTTGCA
CMW 13796		———————TTC	G——————GCT	GGACAATGTT
CMW 13820		———————TTC	G——————GCC	CGACTTTGCA
CMW 13807		———————TTC	G——————GCC	CGACTTTGCA
CMW 13808		———————TGC	G——————TTC	GCGCGGCTTT
CMW 13815		———————TTC	G——————GCC	CGACGTTGCA
CMW 13817		———————TGC	G——————TTC	G—————CGCG
CMW 13810		———————TTC	G——————GCC	CGACGTTGCA
P. aphanidermatum	AJ233438	———————TTC	G——————GCC	CGACGTTGCA
P. deliense	AJ233442	———————TTC	G——————GCC	CGACGTTGCA
P. dissotocum	AJ233443	———————TTC	G——————GCC	CGACTTTGCA
P. helicoides	AB108061	———————TGC	G——————TTC	G—————CGCG
P. intermedium	AJ233447	———————TTC	G——————GCT	GGACAATGTT
P. irregulare	AJ233448	———————TTC	G——————GCT	GGACAATGTT
P. ultimum	AB108064	———————TTC	G——————GCT	CGACAATGTT
P. vexans	AJ233462	TGTTGCTTTT	GCGGCGTGCT	GAACAATGTT
Phytophthora cinnamomi	AF266764	———————TCC	G——————GCT	G—————————

810

CMW 13819		GCGTGTTGTG	GTCTTGTTTC	CTGTGTTCGC
CMW 13822		GCTTATTGTG	TGTTTGTT——	CCGCGTTCGC
CMW 13805		GC–TGACGGA	GTGTGGTTTT	CTGTTCTTTC
CMW 13796		GCTTATTGTG	TGTTTGTT——	CCGCGTTCGC
CMW 13820		GCTGACTGGA	G—TTGTTTT	CTGTTCTTTC
CMW 13807		GCTGACTGGA	G—TTGTTTT	CTGTTCTTTC
CMW 13808		GACAA–TGCA	G—CTGATGC	GTGTGTTTGG
CMW 13815		GC–TGACAGA	GTGTGGTTTT	CTGTTCTTTC
CMW 13817		GC–TTTGACA	ATGCAGCTGA	TGCGTGTGT–
CMW 13810		GC–TGACAGA	GTGTGGTTTT	CTGTTCTTTC
P. aphanidermatum	AJ233438	GC–TGACAGA	GTGTGGTTTT	CTGTTCTTTC
P. deliense	AJ233442	GC–TGACGGA	GTGTGGTTTT	CTGTTCTTTC
P. dissotocum	AJ233443	GCTGACTGGA	GTTGTTTT——	CTGTTCTTTC
P. helicoides	AB108061	GC–TTTGACA	ATGCAGCTGA	TGCGTGTGTT
P. intermedium	AJ233447	GCTTATTGTG	TGTTTGTT——	CCGCGTTCGC
P. irregulare	AJ233448	GCTTATTGTG	TGTCTGTT——	CCGTGTTCGC
P. ultimum	AB108064	GCGTAATTGT	GTGTGGTCTT	–TGTTTGTGC
P. vexans	AJ233462	GCGTGTTGTG	GTCTTGTTTC	CTGTGTTCGC
Phytophthora cinnamomi	AF266764	——————AACA	AAGCGCTTAT	TGGATGTTCC

				840	
CMW 13819		TTCGAGGTGT	ACTGTCTAAT	GGCTG–TGGG	
CMW 13822		CTTGAGGTGT	ACTTTCTGCT	GTGTGCTTGA	
CMW 13805		CTTGAGGTGT	AC——CTGAT	TTGTG–TGAG	
CMW 13796		CTTGAGGTGT	ACTTTCTGCT	GTGTGCTTGA	
CMW 13820		CTTGAGGTGT	AC——CTGTC	TTGTG–TGAG	
CMW 13807		CTTGAGGTGT	AC——CTGTC	TTGTG–TGAG	
CMW 13808		GCTGTGGTGC	——————	–TGTA–TGGG	
CMW 13815		CTTGAGGTGT	AC——CTGAA	TTGTG–TGAG	
CMW 13817		–TTGGGCTGT	G————GTG	CTGTA–TGGG	
CMW 13810		CTTGAGGTGT	AC——CTGAA	TTGTG–TGAG	
P. aphanidermatum	AJ233438	CTTGAGGTGT	AC——CTGAA	TTGTG–TGAG	
P. deliense	AJ233442	CTTGAGGTGT	AC——CTGAT	TTGTG–TGAG	
P. dissotocum	AJ233443	CTTGAGGTGT	AC——CTGTC	TTGTG–TGAG	
P. helicoides	AB108061	TGG—GCTGT	GG——TGCTG	T——A–TGGG	
P. intermedium	AJ233447	CTTGAGGTGT	ACTTTCTGCT	GTGTGCTTGA	
P. irregulare	AJ233448	CTTGAGGTGT	ACTGATGGCT	GTGGGATTGA	
P. ultimum	AB108064	CTTGAGGTGT	AC——TAGAG	GT–TG–TCGG	
P. vexans	AJ233462	TTCGAGGTGT	ACTGTCTAAT	GGCTG–TGGG	
Phytophthora cinnamomi	AF266764	TCCCTGCTGT	GG——CGGTA	CGG–A–TCGG	

			851
CMW 13819		TTTCGAACCT	G
CMW 13822		ACTGGGATCT	G
CMW 13805		GCAATGGTCT	G
CMW 13796		ACTGGGATCT	G
CMW 13820		GCAATGGTCT	G
CMW 13807		GCAATGGTCT	G
CMW 13808		TGAACCGGAT	G
CMW 13815		GCAATGGTCT	G
CMW 13817		TGAACCGGAT	G
CMW 13810		GCAATGGTCT	G
P. aphanidermatum	AJ233438	GCAATGGTCT	G
P. deliense	AJ233442	GCAATGGTCT	G
P. dissotocum	AJ233443	GCAATGGTCT	G
P. helicoides	AB108061	TGAACCGGAT	G
P. intermedium	AJ233447	ACTGGGATCT	G
P. irregulare	AJ233448	ACTGGTTACT	G
P. ultimum	AB108064	TT—TGAACC	G
P. vexans	AJ233462	TTTCGAACCT	G
Phytophthora cinnamomi	AF266764	TGAACCGTAG	G

Chapter 3.

Alignment 5. 5' end of the β - tubulin gene DNA sequence alignment of selected *Cylindrocladium* isolates from the survey *Eucalyptus* hybrid cuttings.

					30
CMW8734		GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
CMW9108		GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
CMW9190		GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
CMW9109		GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
CMW9189		GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium pauciramosum	AY162320	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium pauciramosum	AF449448	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium insulare	AF44951	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium insulare	AF44950	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium colhounii	AF232855	GCTGCCCCTG	ATTCTACCCC	GCCGACCCGG	
Cylindrocladium colhounii	AF232854	GCTGCCCCTG	ATTCTACCCC	GCCGACCCGG	
Cylindrocladium gracile	AF333405	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium gracile	AF333404	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium clavatum	AF232850	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium candelabrum	AF210858	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium candelabrum	AF210857	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium scoparium	AF210875	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium scoparium	AF210874	GCTGCCCCTG	ATTCTACCCC	GCCGCCCCGG	
Cylindrocladium ilicicola	AF333413	GCTGCCCCTG	AGCGTACCCC	GCCGCCCCGG	
Cylindrocladium ilicicola	AF333412	GCTGCCCCTG	AGCGTACCCC	GCCGCCCCGG	
Cylindrocladiella infestans	AF320190	GCTGCCCCTN	ATTCTACCCC	GCCGAATCGT	
					60
CMW8734		TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
CMW9108		TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
CMW9190		TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
CMW9109		TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
CMW9189		TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium pauciramosum	AY162320	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium pauciramosum	AF449448	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium insulare	AF44951	TTTCCACC - -	- - ACCTCG - -	- ACAACAAAG	
Cylindrocladium insulare	AF44950	TTTCCACC - -	- - ACCTCGAC	GACAACAAAG	
Cylindrocladium colhounii	AF232855	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium colhounii	AF232854	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium gracile	AF333405	TTTCCACC - -	- - GCTTCGAC	GACAA - AAAG	
Cylindrocladium gracile	AF333404	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium clavatum	AF232850	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium candelabrum	AF210858	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium candelabrum	AF210857	TTTCCACC - -	- - GCTTCGAC	GACAACAAAG	
Cylindrocladium scoparium	AF210875	TTTCCACC - -	- - ACATCGAC	GACAACAAAG	
Cylindrocladium scoparium	AF210874	TTTCCACC - -	- - ACATCGAC	GACAACAAAG	
Cylindrocladium ilicicola	AF333413	TTTCCACC - -	- - GCTTCGAC	AACAACAAAG	
Cylindrocladium ilicicola	AF333412	TTTCCACC - -	- - GCTTCGAC	AACAACAAAG	
Cylindrocladiella infestans	AF320190	TTTCCACCCA	CCGCCTCGAC	AACAACAAAG	

					90
CMW8734		CCGCAGCCTC	ACGATCATAA	CGAGATATCA	
CMW9108		CCGCAGCCTC	ACGATCATAA	CGAGATATCA	
CMW9190		CCGCAGCCTC	ACGATCATGA	CGAGATATCA	
CMW9109		CCGCAGCCTC	ACGATCATAA	CGAGATATCA	
CMW9189		CCGCAGCCTC	ACGATCATGA	CGAGATATCA	
Cylindrocladium pauciramosum	AY162320	CCGCAGCCTC	ACGATCATAA	CGAGATATCA	
Cylindrocladium pauciramosum	AF449448	CCGCAGCCTC	ACGATCATAA	CGAGATATCA	
Cylindrocladium insulare	AF44951	CCGCAGCCTC	ACGATCATGA	TGTGATATCA	
Cylindrocladium insulare	AF44950	CCGCAGCCTC	ACGATCATGA	TGTGATATCA	
Cylindrocladium colhounii	AF232855	CCGCAGCCTC	ACGAACATGG	CGAGATATCG	
Cylindrocladium colhounii	AF232854	CCGCAGCCTC	ACGAGCATGA	CGAGATATCG	
Cylindrocladium gracile	AF333405	CCGCAGCCTC	ACGAACATGG	CGAGATATCA	
Cylindrocladium gracile	AF333404	CCGCAGCCTC	ACGAACATGG	CGAGATATCA	
Cylindrocladium clavatum	AF232850	CCGCAGCCTC	ACGAGCATGG	CGAGATATCA	
Cylindrocladium candelabrum	AF210858	CCGCAGCCTC	ACGATCATGA	CGAGATATCA	
Cylindrocladium candelabrum	AF210857	CCGCAGCCTC	ACGATCATGA	CGAGATATCA	
Cylindrocladium scoparium	AF210875	CCGCAGCCTC	ACGAACATGA	TGTGATATCA	
Cylindrocladium scoparium	AF210874	CCGCAGCCTC	ACGAACATGA	TGTGATATCA	
Cylindrocladium ilicicola	AF333413	CTGGACGACC	CCAAGCACGA	TGTGATATCG	
Cylindrocladium ilicicola	AF333412	CTGGACGACC	CCAAGCACGA	TGTGATATCG	
Cylindrocladiella infestans	AF320190	CTCGCGATGC	CCACCCACAT	CGTGATATCT	

					120
CMW8734		GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
CMW9108		GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
CMW9190		GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
CMW9109		GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
CMW9189		GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium pauciramosum	AY162320	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium pauciramosum	AF449448	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium insulare	AF44951	GA - - ACAAGA	TT - GCTAACC	ATGTGCTTCT	
Cylindrocladium insulare	AF44950	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium colhounii	AF232855	GA - - ACAAGA	TTTGCTGACC	ATGTGCTTCT	
Cylindrocladium colhounii	AF232854	GA - - ACAAGA	TTTGCTGACC	ATGTGCTTCT	
Cylindrocladium gracile	AF333405	GA - - ACAAGA	TA - ACNAACC	ATGTGCTTCT	
Cylindrocladium gracile	AF333404	GA - - ACAAGA	TT - GCTAACC	ATGTGCTTCT	
Cylindrocladium clavatum	AF232850	GA - - ACAAGA	TT - GCTAACC	ATGTGCTTCT	
Cylindrocladium candelabrum	AF210858	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium candelabrum	AF210857	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium scoparium	AF210875	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium scoparium	AF210874	GA - - ACAAGA	TT - GCTAACC	GTGTGCTTCT	
Cylindrocladium ilicicola	AF333413	GAGGACAAGG	TT - GCTGACT	AT - - - - TTCT	
Cylindrocladium ilicicola	AF333412	GAGGACAAGG	TT - GCTGACT	AT - TATTTCT	
Cylindrocladiella infestans	AF320190	GA - - AGACAA	TG - GCTAATT	TTGTG - TGTT	

					150
CMW8734		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW9108		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW9190		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW9109		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW9189		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium pauciramosum	AY162320	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium pauciramosum	AF449448	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium insulare	AF44951	TTCTCGATTG	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium insulare	AF44950	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium colhounii	AF232855	TTCTCAACTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium colhounii	AF232854	TTTTCAATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium gracile	AF333405	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium gracile	AF333404	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium clavatum	AF232850	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium candelabrum	AF210858	TTTTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium candelabrum	AF210857	TTTTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium scoparium	AF210875	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium scoparium	AF210874	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium ilicicola	AF333413	TTCTCAATTC	TAGGTCCACC	TCCAGACCGG	
Cylindrocladium ilicicola	AF333412	TTCTCAATTC	TAGGTCCACC	TCCAGACCGG	
Cylindrocladiella infestans	AF320190	TCTGCGAATA	TAGGTCCACC	TCCAGACCGG	

					180
CMW8734		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW9108		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW9190		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW9109		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW9189		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
Cylindrocladium pauciramosum	AY162320	TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
Cylindrocladium pauciramosum	AF449448	TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
Cylindrocladium insulare	AF44951	TCAGTGCGTA	AGTGCT - - CT	TCAGCTCCAA	
Cylindrocladium insulare	AF44950	TCAGTGCGTA	AGTGCTCTTC	- - AACTCCAA	
Cylindrocladium colhounii	AF232855	TCAGTGCGTA	AGTGCTCTTC	TCAACTCCGA	
Cylindrocladium colhounii	AF232854	CCAGTGCGTA	AGTGCTCTTC	TCAACTCCAA	
Cylindrocladium gracile	AF333405	TCAGTGCGTA	AGTGATATTC	TCAACTCCAA	
Cylindrocladium gracile	AF333404	TCAGTGCGTA	AGTGATATTC	TCAACTCCAA	
Cylindrocladium clavatum	AF232850	TCAGTGCGTA	AGTGATATTC	TCAACTCCAA	
Cylindrocladium candelabrum	AF210858	TCAGTGCGTA	AGTACCCTTC	TCAACTCCGA	
Cylindrocladium candelabrum	AF210857	TCAGTGCGTA	AGTACCCTTC	TCAACTCCGA	
Cylindrocladium scoparium	AF210875	TCAGTGCGTA	AGTGCTCTTC	- - AACTCCAA	
Cylindrocladium scoparium	AF210874	TCAGTGCGTA	AGTGCTCTTC	- - AACTCCAA	
Cylindrocladium ilicicola	AF333413	TCAGTGCGTA	AGTGATCATT	CCAGCTTCCA	
Cylindrocladium ilicicola	AF333412	TCAGTGCGTA	AGTGATCATT	CCAGCTTCCA	
Cylindrocladiella infestans	AF320190	TCAGTGCGTA	AGTACATTCC	TCA - CCTCGA	
					210
CMW8734		CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
CMW9108		CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
CMW9190		CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
CMW9109		CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
CMW9189		CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
Cylindrocladium pauciramosum	AY162320	CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
Cylindrocladium pauciramosum	AF449448	CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
Cylindrocladium insulare	AF44951	CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
Cylindrocladium insulare	AF44950	CAAAATTCTC	ACGACGAGAT	TCACTGACAG	
Cylindrocladium colhounii	AF232855	CAATATTATC	ACGGCGAGGT	TTACTGACAG	
Cylindrocladium colhounii	AF232854	CAATATTATC	ACGA - - - GAT	TTACTGACAT	
Cylindrocladium gracile	AF333405	CAAAATTCTC	ACGATGAGAT	TCACTGACAT	
Cylindrocladium gracile	AF333404	CAAAATTCTC	ACGATGAGAT	TCACTGACAT	
Cylindrocladium clavatum	AF232850	CAAAATTCTC	ACGATGAGAT	TCACTGACAT	
Cylindrocladium candelabrum	AF210858	CCAAATTCTC	ACGATGAGAT	TCACTGACAG	
Cylindrocladium candelabrum	AF210857	CCAAATTCTC	ACGATGAGAT	TCACTGACAG	
Cylindrocladium scoparium	AF210875	CAAAATTCTC	ACGATGAGAT	TCACTGACAG	
Cylindrocladium scoparium	AF210874	CAAAATTCTC	ACGATGAGAT	TCACTGACAG	
Cylindrocladium ilicicola	AF333413	- AAAACTGC -	- CCTGGGGAT	TCACTAACAT	
Cylindrocladium ilicicola	AF333412	- AAAACTGCC	TTGA - - GGAT	TCACTAACAT	
Cylindrocladiella infestans	AF320190	CAAGCTT - - -	- CGTCAACGG	CTGCTAACGG	
					240
CMW8734		T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
CMW9108		T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
CMW9190		T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
CMW9109		T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
CMW9189		T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium pauciramosum	AY162320	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium pauciramosum	AF449448	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium insulare	AF44951	T - TATCGACA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium insulare	AF44950	T - TATCGACA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium colhounii	AF232855	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium colhounii	AF232854	A - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium gracile	AF333405	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium gracile	AF333404	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium clavatum	AF232850	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium candelabrum	AF210858	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium candelabrum	AF210857	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium scoparium	AF210875	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium scoparium	AF210874	T - TGTCGATA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium ilicicola	AF333413	T - GGCGATCA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladium ilicicola	AF333412	T - GGCGATCA	GGGTAACCAA	ATTGGTGCTG	
Cylindrocladiella infestans	AF320190	TGTCTCGATA	GGGTAACCAA	ATTGGTGCTG	

					270
CMW8734		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
CMW9108		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
CMW9190		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
CMW9109		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
CMW9189		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium pauciramosum	AY162320	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium pauciramosum	AF449448	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium insulare	AF44951	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium insulare	AF44950	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium colhounii	AF232855	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium colhounii	AF232854	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium gracile	AF333405	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium gracile	AF333404	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium clavatum	AF232850	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium candelabrum	AF210858	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium candelabrum	AF210857	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium scoparium	AF210875	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium scoparium	AF210874	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG	
Cylindrocladium ilicicola	AF333413	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladium ilicicola	AF333412	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	
Cylindrocladiella infestans	AF320190	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG	

					300
CMW8734		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW9108		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW9190		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW9109		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW9189		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium pauciramosum	AY162320	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium pauciramosum	AF449448	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium insulare	AF44951	GTCTCGACAG	CAATGGTGTC	TACACTGGTA	
Cylindrocladium insulare	AF44950	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
Cylindrocladium colhounii	AF232855	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
Cylindrocladium colhounii	AF232854	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium gracile	AF333405	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium gracile	AF333404	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium clavatum	AF232850	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium candelabrum	AF210858	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium candelabrum	AF210857	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium scoparium	AF210875	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium scoparium	AF210874	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
Cylindrocladium ilicicola	AF333413	GTCTCGACAG	CAATGGTGTC	TACAACGGTA	
Cylindrocladium ilicicola	AF333412	GTCTCGACAG	CAATGGTGTC	TACAACGGTA	
Cylindrocladiella infestans	AF320190	GTCTCGACAG	CAATGGTGTC	TACAACGGCA	

					330
CMW8734		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW9108		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW9190		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW9109		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW9189		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium pauciramosum	AY162320	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium pauciramosum	AF449448	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium insulare	AF44951	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium insulare	AF44950	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium colhounii	AF232855	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium colhounii	AF232854	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium gracile	AF333405	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium gracile	AF333404	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium clavatum	AF232850	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium candelabrum	AF210858	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium candelabrum	AF210857	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium scoparium	AF210875	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium scoparium	AF210874	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladium ilicicola	AF333413	CCTCCGACCT	CCAGTTGGAG	CGCATGAACG	
Cylindrocladium ilicicola	AF333412	CCTCCGACCT	CCAGTTGGAG	CGCATGAACG	
Cylindrocladiella infestans	AF320190	GCTCTGAGCT	CCAGCTCGAG	CGCATGAGCG	

				360
CMW8734		TCTACTTCAA	CGAGGTATGT	GAAAACCACT
CMW9108		TCTACTTCAA	CGAGGTATGT	GAAAACCACT
CMW9190		TCTACTTCAA	CGAGGTATGT	GAAAACCACT
CMW9109		TCTACTTCAA	CGAGGTATGT	GAAAACCACT
CMW9189		TCTACTTCAA	CGAGGTATGT	GAAAACCACT
Cylindrocladium pauciramosum	AY162320	TCTACTTCAA	CGAGGTATGT	GAAAACCACT
Cylindrocladium pauciramosum	AF449448	TCTACTTCAA	CGAGGTATGT	GAAAACCACT
Cylindrocladium insulare	AF44951	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium insulare	AF44950	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium colhounii	AF232855	TCTACTTCAA	CGAGGTATGT	GAAACCCATC
Cylindrocladium colhounii	AF232854	TCTACTTCAA	CGAGGTATGT	GAAAGCTGCT
Cylindrocladium gracile	AF333405	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium gracile	AF333404	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium clavatum	AF232850	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium candelabrum	AF210858	TCTACTTCAA	CGAGGTATGT	GAAAACCACT
Cylindrocladium candelabrum	AF210857	TCTACTTCAA	CGAGGTATGT	GAAAACCACT
Cylindrocladium scoparium	AF210875	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium scoparium	AF210874	TCTACTTCAA	CGAGGTATGT	GAAAACCACG
Cylindrocladium ilicicola	AF333413	TCTACTTCAA	CGAGGTATGT	GGTTA - - AAA
Cylindrocladium ilicicola	AF333412	TCTACTTCAA	CGAGGTATGT	GGTTA - - AAA
Cylindrocladiella infestans	AF320190	TCTACTTCAA	CGAGGTACGT	GACTATGGCA

				390
CMW8734		CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
CMW9108		CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
CMW9190		CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
CMW9109		CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
CMW9189		CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
Cylindrocladium pauciramosum	AY162320	CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
Cylindrocladium pauciramosum	AF449448	CGAAGCACTC	CCTTGACCGA	GAAGC - - ACA
Cylindrocladium insulare	AF44951	CGGTGTACTC	ACTCG - CCGA	TAGGC - - ACA
Cylindrocladium insulare	AF44950	CGGTGTACTC	ACTCG - CCGA	GAGGC - - ACA
Cylindrocladium colhounii	AF232855	TGATCTGACC	CCTTCGTCGA	TAGGCA - CAA
Cylindrocladium colhounii	AF232854	TGATCTGCCC	CTATG - TCGA	GAGAC - - GCA
Cylindrocladium gracile	AF333405	CGGAGCACTC	CCTTGATCGA	GAAGC - - ACA
Cylindrocladium gracile	AF333404	CGGAGCACTC	CCTTGATCGA	GAAGC - - ACA
Cylindrocladium clavatum	AF232850	CGGAGCACTC	CCTTGATCGA	GAAGC - - ACA
Cylindrocladium candelabrum	AF210858	CGAAGCACTC	CCTTGATCGA	GAAGC - - ACA
Cylindrocladium candelabrum	AF210857	CGAAGCACTC	CCTTGATCGA	GAAGC - - ACA
Cylindrocladium scoparium	AF210875	CGGTGTACTC	ACACG-CCGA	GAGGC - - ACA
Cylindrocladium scoparium	AF210874	CGGTGTACTC	ACACG-CCGA	GAGGC - - ACA
Cylindrocladium ilicicola	AF333413	CAAAACGCTC	GCCTGGTCGA	AAAACTTGTG
Cylindrocladium ilicicola	AF333412	TAAAACGCTC	GCCTGGTCGA	GAAAC - - TTG
Cylindrocladiella infestans	AF320190	CTCA - CATTT	GCTACACTGT	GAAAT - - CAG

				420
CMW8734		AGCCAACTCA	CACCATCATG	TAGGCTTCCG
CMW9108		AGCCAACTCA	CACCATCATG	TAGGCTTCCG
CMW9190		AGCCAACTCA	CACCATCATG	TAGGCTTCCG
CMW9109		AGCCAACTCA	CACCATCATG	TAGGCTTCCG
CMW9189		AGCCAACTCA	CACCATCATG	TAGGCTTCCG
Cylindrocladium pauciramosum	AY162320	AGCCAACTCA	CACCATCATG	TAGGCTTCCG
Cylindrocladium pauciramosum	AF449448	AGCCAACTCA	CACCATCATG	TAGGCTTCCG
Cylindrocladium insulare	AF44951	AGCAAACT - -	GAC - ACCATG	TAGGCTTCTG
Cylindrocladium insulare	AF44950	AGCAAACTGA	CACC - - - ATG	TAGGCTTCTG
Cylindrocladium colhounii	AF232855	AGTAAACTGA	CACG - CTATG	TAGGCTTCCG
Cylindrocladium colhounii	AF232854	ACTAAACTGA	CACCAT - - - G	TAGGCCTCCG
Cylindrocladium gracile	AF333405	AGCAAACTCA	CACA - CCATG	TAGGCTTCCG
Cylindrocladium gracile	AF333404	AGCAAACTCA	CAC - ACCACG	TAGGCTTCCG
Cylindrocladium clavatum	AF232850	AGCAAACTCA	CAC - ACCATG	TAGGCTTCCG
Cylindrocladium candelabrum	AF210858	ATCCGACTCA	CACCATCATG	TAGGCTTCCG
Cylindrocladium candelabrum	AF210857	ATCCGACTCA	CACCATCATG	TAGGCTTCCG
Cylindrocladium scoparium	AF210875	AGCAAACTGA	CAC - - - CATG	TAGGCTTCCG
Cylindrocladium scoparium	AF210874	AGCAAACTGA	CAC - - - CATG	TAGGCTTCCG
Cylindrocladium ilicicola	AF333413	CACAAACTCA	CACG - - - ATA	CAGGCTTCCG
Cylindrocladium ilicicola	AF333412	TGCCCCAACT	CAC - ACAATA	CAGGCTTTCG
Cylindrocladiella infestans	AF320190	AATGTACTCA	CGCT - - - CCG	TAGGCTTCTG

				450
CMW8734		GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
CMW9108		GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
CMW9190		GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
CMW9109		GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
CMW9189		GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium pauciramosum	AY162320	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium pauciramosum	AF449448	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium insulare	AF44951	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium insulare	AF44950	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium colhounii	AF232855	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium colhounii	AF232854	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium gracile	AF333405	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium gracile	AF333404	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium clavatum	AF232850	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium candelabrum	AF210858	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium candelabrum	AF210857	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium scoparium	AF210875	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium scoparium	AF210874	GCAACAAGTT	CGTTCCTCGC	GCTGTCCTCG
Cylindrocladium ilicicola	AF333413	GCAACAAGTA	TGTCCCTCGA	GCTGTCCTCG
Cylindrocladium ilicicola	AF333412	GCAACAACTA	TGTCCCTCGC	GCTGTCCTCG
Cylindrocladiella infestans	AF320190	GCAACAAGTA	TGTCCCTCGC	GCCGTCCTCG

				480
CMW8734		TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
CMW9108		TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
CMW9190		TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
CMW9109		TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
CMW9189		TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
Cylindrocladium pauciramosum	AY162320	TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
Cylindrocladium pauciramosum	AF449448	TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
Cylindrocladium insulare	AF44951	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium insulare	AF44950	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium colhounii	AF232855	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium colhounii	AF232854	TCG - TCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium gracile	AF333405	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium gracile	AF333404	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium clavatum	AF232850	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium candelabrum	AF210858	TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
Cylindrocladium candelabrum	AF210857	TCGATCTTGA	GCCCGGTACC	ATGGACGCCG
Cylindrocladium scoparium	AF210875	TCGATCTCGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium scoparium	AF210874	TCGATCTCGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium ilicicola	AF333413	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG
Cylindrocladium ilicicola	AF333412	TCGATCTTGA	GCCTGGTACC	ATGGATGCCG
Cylindrocladiella infestans	AF320190	TCGATCTTGA	GCCCGGTACC	ATGGATGCCG

			495
CMW8734		TCCGTGCCGG	TCCTT
CMW9108		TCCGTGCCGG	TCCTT
CMW9190		TCCGTGCCGG	TCCTT
CMW9109		TCCGTGCCGG	TCCTT
CMW9189		TCCGTGCCGG	TCCTT
Cylindrocladium pauciramosum	AY162320	TCCGTGCCGG	TCCTT
Cylindrocladium pauciramosum	AF449448	TCCGTGCCGG	TCCTT
Cylindrocladium insulare	AF44951	TCCGTGCCGG	TCCTT
Cylindrocladium insulare	AF44950	TCCGTGCCGG	TCCTT
Cylindrocladium colhounii	AF232855	TCCGTGCCGG	TCCTT
Cylindrocladium colhounii	AF232854	TCCGTGCCGG	TCCTT
Cylindrocladium gracile	AF333405	TCCGTGCCGG	TCCTT
Cylindrocladium gracile	AF333404	TCCGTGCCGG	TCCTT
Cylindrocladium clavatum	AF232850	TCCGTGCCGG	TCCTT
Cylindrocladium candelabrum	AF210858	TCCGTGCCGG	TCCTT
Cylindrocladium candelabrum	AF210857	TCCGTGCCGG	TCCTT
Cylindrocladium scoparium	AF210875	TCCGTGCCGG	TCCTT
Cylindrocladium scoparium	AF210874	TCCGTGCCGG	TCCTT
Cylindrocladium ilicicola	AF333413	TCCGTGCCGG	TCCTT
Cylindrocladium ilicicola	AF333412	TCCGTGCCGG	TCCTT
Cylindrocladiella infestans	AF320190	TCCGTGCCGG	TCCTT

Chapter 4.

Alignment 6. 5' end of the β - tubulin gene DNA sequence alignment of selected *Cylindrocladium* isolates from *Acacia mearnsii* seedlings and other *Cylindrocladium* species.

				30
CMW 9156		GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 9159		GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 9164		GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 9169		GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
CMW 9171		GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. pauciramosum	AY162320	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. pauciramosum	AY449448	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. candelabrum	AF210858	GCTTGCCCCT	GATTCTACCC	CGCCGCCCCG
C. candelabrum	AF210857	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. quinqueseptatum	AF232869	GCT–GCCCCT	GATTCTACCC	CGCCGACCCG
C. quinqueseptatum	AF232870	GCT–GCCCCT	GATTCTACCC	CGCCGACCCG
C. theae	AF232861	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. theae	AF232862	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
C. reteaudii	AF 389846	GCT–GCCCCT	GATTCTACCC	CGCCGACCCG
C. reteaudii	AF389847	GCT–GCCCCT	GATTCTACCC	CGCCGACCCG
C. scoparium	AF210874	GCTTGCCCCT	GATTCTACCC	CGCCGCCCCG
C. scoparium	AF210875	GCT–GCCCCT	GATTCTACCC	CGCCGCCCCG
Cylindrocladiella infestans	AF320190	GCT–GCCCCT	– ATTCTATCC	– GCCGAATCG

				60
CMW 9156		GTTTCCACC–	—— GCTTCGA	CGACAACAAA
CMW 9159		GTTTCCACC–	—— GCTTCGA	CGACAACAAA
CMW 9164		GTTTCCACC–	—— GCTTCGA	CGACAACAAA
CMW 9169		GTTTCCACC–	—— GCTTCGA	CGACAACAAA
CMW 9171		GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. pauciramosum	AY162320	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. pauciramosum	AY449448	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. candelabrum	AF210858	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. candelabrum	AF210857	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. quinqueseptatum	AF232869	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. quinqueseptatum	AF232870	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. theae	AF232861	GTTTCCACC–	—— GCCTCGA	TGGCAGCGAA
C. theae	AF232862	GTTTCCACC–	—— GCCTCGA	TGGCAGCGAA
C. reteaudii	AF 389846	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. reteaudii	AF389847	GTTTCCACC–	—— GCTTCGA	CGACAACAAA
C. scoparium	AF210874	GTTTCCACC–	—— ACATCGA	CGAAAACAAA
C. scoparium	AF210875	GTTTCCACC–	—— ACATCGA	CGAAAACAAA
Cylindrocladiella infestans	AF320190	TTTCCCACCC	ACCGCCTCGA	CAACAACAAA

				90
CMW 9156		GCCGCAGCCT	CACGATCATA	A– –CGAGATA
CMW 9159		GCCGCAGCCT	CACGATCATA	A– –CGAGATA
CMW 9164		GCCGCAGCCT	CACGATCATA	A– –CGAGATA
CMW 9169		GCCGCAGCCT	CACGATCATA	A– –CGAGATA
CMW 9171		GCCGCAGCCT	CACGATCATA	A– –CGAGATA
C. pauciramosum	AY162320	GCCGCAGCCT	CACGATCATA	A– –CGAGATA
C. pauciramosum	AY449448	GCCGCAGCCT	CACGATCATA	A– –CGAGATA
C. candelabrum	AF210858	GCCGCAGCCT	CACGATCATG	A– –CGAGATA
C. candelabrum	AF210857	GCCGCAGCCT	CACGATCATG	A– –CGAGATA
C. quinqueseptatum	AF232869	GCCGCAACAT	CATGAACAAG	A– –CGAGATA
C. quinqueseptatum	AF232870	GCCGCAACAT	CATGAACAAG	A– –CGAGATA
C. theae	AF232861	GCCGCATCCT	CATGAACAAA	AGACGAGGCA
C. theae	AF232862	GCCGCATCCT	CATGAACAAA	AGACGAGGCA
C. reteaudii	AF 389846	GCCGCAACAT	CATGAACAA–	– GACGAGATA
C. reteaudii	AF389847	GCCGCAACAT	CATGAACAA–	– GACGAGATA
C. scoparium	AF210874	GCCGCAGCCT	CACGAACAT–	– GATGTGATA
C. scoparium	AF210875	GCCGCAGCCT	CACGAACAT–	– GATGTGATA
Cylindrocladiella infestans	AF320190	GCTCGCGATG	CCCACCCACA	T– –CGTGATA

					120
CMW 9156		TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
CMW 9159		TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
CMW 9164		TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
CMW 9169		TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
CMW 9171		TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. pauciramosum	AY162320	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. pauciramosum	AY449448	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. candelabrum	AF210858	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. candelabrum	AF210857	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. quinqueseptatum	AF232869	TCGGAACAAG	ATTGCTAACC	ATGTGCTTCT	
C. quinqueseptatum	AF232870	TCGGAACAAG	ATTGCTAACC	ATGTGCTTCT	
C. theae	AF232861	TCAGAACCAG	ATTGCTGACC	ATGTCCTTTT	
C. theae	AF232862	TCAGAACCAG	ATTGCTGACC	ATGTCCTTTT	
C. reteaudii	AF 389846	TCGGAACAAG	ATTGCTAACC	ATGTGCTTCT	
C. reteaudii	AF389847	TCGGAACAAG	ATTGCTAACC	ATGTGCTTCT	
C. scoparium	AF210874	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
C. scoparium	AF210875	TCAGAACAAG	ATTGCTAACC	GTGTGCTTCT	
Cylindrocladiella infestans	AF320190	TCTGAAGACA	ATGGCTAATT	TTGTGTGTTT	

					150
CMW 9156		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW 9159		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW 9164		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW 9169		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
CMW 9171		TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. pauciramosum	AY162320	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. pauciramosum	AY449448	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. candelabrum	AF210858	TTTTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. candelabrum	AF210857	TTTTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. quinqueseptatum	AF232869	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. quinqueseptatum	AF232870	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. theae	AF232861	CTCTGAATTA	TAGGTCCACC	TCCAGACCGG	
C. theae	AF232862	CTCTGAATTA	TAGGTCCACC	TCCAGACCGG	
C. reteaudii	AF 389846	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. reteaudii	AF389847	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. scoparium	AF210874	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
C. scoparium	AF210875	TTCTCGATTA	TAGGTCCACC	TCCAGACCGG	
Cylindrocladiella infestans	AF320190	CTG–CGAATA	TAGGTCCACC	TCCAGACCGG	

					180
CMW 9156		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW 9159		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW 9164		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW 9169		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
CMW 9171		TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
C. pauciramosum	AY162320	TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
C. pauciramosum	AY449448	TCAGTGCGTA	AGTACTCTTC	TCAACTCCAA	
C. candelabrum	AF210858	TCAGTGCGTA	AGTACCCTTC	TCAACTCCAA	
C. candelabrum	AF210857	TCAGTGCGTA	AGTACCCTTC	TCAACTCCAA	
C. quinqueseptatum	AF232869	TCAGTGCGTA	AGTGCTCCTC	TCGATTCCAA	
C. quinqueseptatum	AF232870	TCAGTGCGTA	AGTGCTCCTC	TCGATTCCAA	
C. theae	AF232861	TCAGTGCGTA	AGTGCTCTTC	TCAACTCCAA	
C. theae	AF232862	TCAGTGCGTA	AGTGCTCTTC	TCAACTCCAA	
C. reteaudii	AF 389846	TCAGTGCGTA	AGTGCTCCTC	TCGATTCCAA	
C. reteaudii	AF389847	TCAGTGCGTA	AGTGCTCCTC	TCGATTCCAA	
C. scoparium	AF210874	TCAGTGCGTA	AGTGCTCTT–	– CAACTCCAA	
C. scoparium	AF210875	TCAGTGCGTA	AGTGCTCTT–	– CAACTCCAA	
Cylindrocladiella infestans	AF320190	TCAGTGCGTA	AGTACATTCC	TC– ACCTCGA	

				210
CMW 9156		CAAAATTCTC	ACGACGAGAT	TCACTGACAG
CMW 9159		CAAAATTCTC	ACGACGAGAT	TCACTGACAG
CMW 9164		CAAAATTCTC	ACGACGAGAT	TCACTGACAG
CMW 9169		CAAAATTCTC	ACGACGAGAT	TCACTGACAG
CMW 9171		CAAAATTCTC	ACGACGAGAT	TCACTGACAG
C. pauciramosum	AY162320	CAAAATTCTC	ACGACGAGAT	TCACTGACAG
C. pauciramosum	AY449448	CAAAATTCTC	ACGACGAGAT	TCACTGACAG
C. candelabrum	AF210858	CCAAATTCTC	ACGACGAGAT	TCACTGACAG
C. candelabrum	AF210857	CCAAATTCTC	ACGACGAGAT	TCACTGACAG
C. quinqueseptatum	AF232869	ATATGTTCTC	ATGACAAGAT	TCACTGACAG
C. quinqueseptatum	AF232870	ATATGTTCTC	ATGACAAGAT	TCACTGACAG
C. theae	AF232861	CAAAATTCTC	ACGACGGGAT	TCGCTGACAC
C. theae	AF232862	CAAAATTCTC	ACGACGGGAT	TCGCTGACAC
C. reteaudii	AF 389846	ATATGTTCTC	ATGACAAGAT	TCACTGACAG
C. reteaudii	AF389847	ATATGTTCTC	ATGACAAGAT	TCACTGACAG
C. scoparium	AF210874	CAAAATTCTC	ACGACCGGAT	TCACTGACAG
C. scoparium	AF210875	CAAAATTCTC	ACGACCGGAT	TCACTGACAG
Cylindrocladiella infestans	AF320190	CAAGCTTC– –	– – GTCAACGG	CTGCTAACGG

				240
CMW 9156		T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
CMW 9159		T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
CMW 9164		T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
CMW 9169		T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
CMW 9171		T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. pauciramosum	AY162320	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. pauciramosum	AY449448	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. candelabrum	AF210858	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. candelabrum	AF210857	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. quinqueseptatum	AF232869	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. quinqueseptatum	AF232870	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. theae	AF232861	T– CGCGGATA	GGGTAACCAA	ATCGGTGCTG
C. theae	AF232862	T– CGCGGATA	GGGTAACCAA	ATCGGTGCTG
C. reteaudii	AF 389846	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. reteaudii	AF389847	T– TGTCGATA	GGGTAACCAA	ATTGGTGCTG
C. scoparium	AF210874	T– TATCGACA	GGGTAACCAA	ATTGGTGCTG
C. scoparium	AF210875	T– TATCGACA	GGGTAACCAA	ATTGGTGCTG
Cylindrocladiella infestans	AF320190	TGTCTCGATA	GGGTAACCAA	ATTGGTGCTG

				270
CMW 9156		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
CMW 9159		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
CMW 9164		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
CMW 9169		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
CMW 9171		CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. pauciramosum	AY162320	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. pauciramosum	AY449448	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. candelabrum	AF210858	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG
C. candelabrum	AF210857	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG
C. quinqueseptatum	AF232869	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG
C. quinqueseptatum	AF232870	CTTTTTGGCA	GACCATCTCT	GGCGAGCACG
C. theae	AF232861	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. theae	AF232862	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. reteaudii	AF 389846	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG
C. reteaudii	AF389847	CTTTTTGGCA	GACCATCTCT	GGCGAGCACG
C. scoparium	AF210874	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
C. scoparium	AF210875	CTTTCTGGCA	GACCATTTCT	GGCGAGCACG
Cylindrocladiella infestans	AF320190	CTTTCTGGCA	GACCATCTCT	GGCGAGCACG

					300
CMW 9156		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW 9159		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW 9164		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW 9169		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
CMW 9171		GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
C. pauciramosum	AY162320	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
C. pauciramosum	AY449448	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
C. candelabrum	AF210858	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
C. candelabrum	AF210857	GTCTCGACAG	CAATGGTGTC	TACGCCGGTA	
C. quinqueseptatum	AF232869	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. quinqueseptatum	AF232870	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. theae	AF232861	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. theae	AF232862	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. reteaudii	AF 389846	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. reteaudii	AF389847	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. scoparium	AF210874	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
C. scoparium	AF210875	GTCTCGACAG	CAATGGTGTC	TACGCTGGTA	
Cylindrocladiella infestans	AF320190	GTCTCGACAG	CAATGGTGTC	TACAACGGCA	

					330
CMW 9156		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW 9159		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW 9164		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW 9169		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
CMW 9171		CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. pauciramosum	AY162320	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. pauciramosum	AY449448	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. candelabrum	AF210858	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. candelabrum	AF210857	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. quinqueseptatum	AF232869	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. quinqueseptatum	AF232870	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. theae	AF232861	CTTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. theae	AF232862	CTTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. reteaudii	AF 389846	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. reteaudii	AF389847	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. scoparium	AF210874	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
C. scoparium	AF210875	CCTCCGAGCT	CCAGCTCGAG	CGTATGAACG	
Cylindrocladiella infestans	AF320190	GCTCTGAGCT	CCAGCTCGAG	CGTATGAACG	

					360
CMW 9156		TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
CMW 9159		TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
CMW 9164		TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
CMW 9169		TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
CMW 9171		TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
C. pauciramosum	AY162320	TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
C. pauciramosum	AY449448	TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
C. candelabrum	AF210858	TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
C. candelabrum	AF210857	TCTACTTCAA	CGAGGTATGT	GAAAACCACT	
C. quinqueseptatum	AF232869	TCTACTTCAA	CGAGGTATGC	GAAAAACCAT	
C. quinqueseptatum	AF232870	TCTACTTCAA	CGAGGTATGC	GAAAAACCAT	
C. theae	AF232861	TCTACTTCAA	CGAGGTATGT	GAAAAAAGCA	
C. theae	AF232862	TCTACTTCAA	CGAGGTATGT	GAAAAAAGCA	
C. reteaudii	AF 389846	TCTACTTCAA	CGAGGTATGC	GAAAAACCAT	
C. reteaudii	AF389847	TCTACTTCAA	CGAGGTATGC	GAAAAACCAT	
C. scoparium	AF210874	TCTACTTCAA	CGAGGTATGT	GAAAA–CCAC	
C. scoparium	AF210875	TCTACTTCCA	CGAGGTATGT	GAAAA–CCAC	
Cylindrocladiella infestans	AF320190	TCTACTTCAA	CGAGGTACGT	GACTATGGCA	

390

CMW 9156		CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
CMW 9159		CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
CMW 9164		CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
CMW 9169		CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
CMW 9171		CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
C. pauciramosum	AY162320	CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
C. pauciramosum	AY449448	CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
C. candelabrum	AF210858	CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
C. candelabrum	AF210857	CGAAGCACTC	CCTT –GACCG	AGAAGCACAA
C. quinqueseptatum	AF232869	GCCTGCGCTC	GCTTTGTCGA	AAAAGCACAA
C. quinqueseptatum	AF232870	GCCTGCGCTC	GCTTTGTCGA	AAAAGCACAA
C. theae	AF232861	CGCACA –GTT	GTG–AAC– –G	CGAAGGACA–
C. theae	AF232862	CGCACA –GTT	GTGTAAC– –G	CGAAGGACA–
C. reteaudii	AF 389846	GCCTGCGCTC	GCTTTGTCGA	AAAAGCACAA
C. reteaudii	AF389847	GCCTGCGCTC	GCTTTGTCGA	AAAAGCACAA
C. scoparium	AF210874	GCGGTGTACT	CACACG–CCG	AGAGGCACAA
C. scoparium	AF210875	GCGGTGTTCT	CACACG–CCG	AGAGGCACAA
Cylindrocladiella infestans	AF320190	CTCA – CATTT	GCTA– CACTG	TGAAATCAGA

420

CMW 9156		GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
CMW 9159		GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
CMW 9164		GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
CMW 9169		GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
CMW 9171		GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
C. pauciramosum	AY162320	GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
C. pauciramosum	AY449448	GCCAACTCAC	ACCATCATGT	AGGCTTCCGG
C. candelabrum	AF210858	TCCGACTCAC	ACCATCATGT	AGGCTTCCGG
C. candelabrum	AF210857	TCCGACTCAC	ACCATCATGT	AGGCTTCCGG
C. quinqueseptatum	AF232869	GCAAACTGAC	AC–ACCATGT	AGGCTTCCGG
C. quinqueseptatum	AF232870	GCAAACTGAC	AC–ACCATGT	AGGCTTCCGG
C. theae	AF232861	GCCAACTCAC	ACCA– – –TGT	AGGCTTCCGG
C. theae	AF232862	GCCAACTCAC	ACCA– – –TGT	AGGCTTCTGG
C. reteaudii	AF 389846	GCAAACTGAC	AC–ACCATGT	AGGCTTCTGG
C. reteaudii	AF389847	GCAAACTGAC	AC–ACCATGT	AGGCTTCCGG
C. scoparium	AF210874	GCAAACTGAC	ACCAT– – –GT	AGGCTTCCGG
C. scoparium	AF210875	GCAAACTGAC	ACCAT– – –GT	AGGCTTCTGG
Cylindrocladiella infestans	AF320190	ATGTACTCAC	GC– – –TCCGT	AGGCTTCTGG

450

CMW 9156		CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
CMW 9159		CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
CMW 9164		CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
CMW 9169		CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
CMW 9171		CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. pauciramosum	AY162320	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. pauciramosum	AY449448	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. candelabrum	AF210858	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. candelabrum	AF210857	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. quinqueseptatum	AF232869	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. quinqueseptatum	AF232870	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. theae	AF232861	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. theae	AF232862	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. reteaudii	AF 389846	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. reteaudii	AF389847	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. scoparium	AF210874	CAACAAGTTC	GTTCCTCGCG	CTGTCCTCGT
C. scoparium	AF210875	CAACAAGTTC	CGTCCTTGTG	CTGTCCTCGT
Cylindrocladiella infestans	AF320190	CAACAAGTAT	GTTCCTCGCG	CCGTCCTCGT

					480
CMW 9156		CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
CMW 9159		CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
CMW 9164		CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
CMW 9169		CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
CMW 9171		CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
C. pauciramosum	AY162320	CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
C. pauciramosum	AY449448	CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
C. candelabrum	AF210858	CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
C. candelabrum	AF210857	CGATCTTGAG	CCCGGTACCA	TGGACGCCGT	
C. quinqueseptatum	AF232869	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. quinqueseptatum	AF232870	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. theae	AF232861	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. theae	AF232862	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. reteaudii	AF 389846	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. reteaudii	AF389847	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
C. scoparium	AF210874	CGATCTCGAG	CCCGGTACCA	TGGATGCCGT	
C. scoparium	AF210875	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	
Cylindrocladiella infestans	AF320190	CGATCTTGAG	CCCGGTACCA	TGGATGCCGT	

				507
CMW 9156		CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
CMW 9159		CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
CMW 9164		CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
CMW 9169		CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
CMW 9171		CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. pauciramosum	AY162320	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. pauciramosum	AY449448	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. candelabrum	AF210858	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. candelabrum	AF210857	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. quinqueseptatum	AF232869	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. quinqueseptatum	AF232870	CCGTGCCGGT	CCTTTCGGTC	AGCTTTT
C. theae	AF232861	CCGTGCCGGT	CCTTTCGGTC	AGCTTTT
C. theae	AF232862	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. reteaudii	AF 389846	TCGTGCCGGT	CCTTTCTGGT	AGCTCTT
C. reteaudii	AF389847	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. scoparium	AF210874	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
C. scoparium	AF210875	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT
Cylindrocladiella infestans	AF320190	CCGTGCCGGT	CCTTTCGGTC	AGCTCTT