CHAPTER 1

THE MOVEMENT OF PLANT PATHOGENS ON A WORLDWIDE SCALE, THE IMPACT AND THREATS THEY POSE

INTRODUCTION

Since early times bacterial, fungal and viral pathogens have moved from their native ranges to "new" areas where they have, in many cases, become established. These pathogens are known as exotic, invasive or non-indigenous organisms. In order to discuss the movement of these pathogens, as well as the impact and threat they pose, one needs to clearly define the three above-mentioned terms.

There is no widely accepted definition for the word "non-indigenous", except that it is "the opposite of indigenous" (Flower & Flower 1969). Therefore, to define non-indigenous, one needs first to define "indigenous". Flower and Flower (1969) define "indigenous" as native or naturally belonging in an area. They further define "native" as "inborn, innate, derived from ones country or found in pure or un-combined state". Allen & Humble (2002) define a non-indigenous organism as one that occurs outside its natural ecosystem, therefore, not referring to movement between continents or countries, but between ecosystems. For this review, I will use "non-indigenous" to refer to organisms that did not originate in the specific area in which they are currently found.

The word "exotic" is generally used as a synonym for non-indigenous (Bogus *et al.* 1968). It, furthermore, describes an organism as possessing characteristics foreign to an area, with the organism not being naturalized. Bogus *et al.* (1968), however, do not define "naturalized". In a biological context, this could imply that it is not yet established, or not yet in a state of equilibrium. Flower & Flower (1969) clearly define "exotic" as "introduced from abroad". Ilson *et al.* (1984) also define exotic organisms as those from another part of the world, possessing "the charm of the unfamiliar". For this review, I use non-indigenous to refer to organisms that are introduced into an area where they did not previously occur, not necessarily from abroad or from another part of the world, but from another geographic area.

Another term often used when discussing the movement of pathogens is "invasive". This generally describes the means by which an organism has established itself and how conspicuous the result of the establishment has been. Ilson *et al.* (1984) define "invasive" as the tendency to spread or invade and use "aggression" to describe

invasiveness. Flower & Flower (1969) rather use the word "encroach" to define "invasive". "Encroach" is further defined as "intruding" (Flower & Flower 1969). In essence, "invasive" describes the intrusion of an organism from one area to another with aggression. "Intruding" implies the unwanted presence of the organism. This is also the context in which "invasive" will be used in this review.

In this review, I consider the world-wide movement of pathogens, especially those of trees. This is clearly a very broad subject that has been the topic of many previous reviews. It is impossible to treat even a small number of the diseases that have been studied and I thus make extensive use of examples. These examples are mainly those of tree pathogens, but examples from agriculture are also used in some cases. My aim is to provide a broad background to the subject of how pathogens spread and to consider prospects for reducing outbreaks of new diseases caused by non-native pathogens in the future. This review also serves as a foundation for a series of studies presented in this thesis. These studies focus to some extent on alternative hosts of some important stem canker pathogens of *Eucalyptus* spp., in areas where these trees are grown as exotics in plantations.

MEANS BY WHICH PLANT PATHOGENS SPREAD

It is reasonably well established that non-indigenous, exotic or invasive organisms are introduced from geographical areas other than those in which they are native. The means by which they spread or move deserves discussion. This is a crucial aspect of the review as it provides an understanding of not only how organisms move from one geographical area to another, but also provides an insight into preventing or restricting the movement of pathogens. Care must be taken not to confuse the natural expansion of a pathogen's range with spread or intrusion of the pathogen. Expansion is a natural process forming part of the normal dispersal of pathogens (Brown & Hovmøller 2002).

Agriculture mostly relies on a small fraction of many thousands of plant species world-wide, resulting in large areas of monoculture and low genetic diversity. Risk of global spread of plant pathogens has increased due to this limited genetic variation of crops, brought about by breeding programs and the increased use of clonal germplasm. Wheat, maize, coffee and banana immediately come to mind as examples of single

genotype crops covering vast areas in many countries. Coffee and bananas are two extreme examples where large areas are planted with single clones or cultivars (Stover & Simmonds 1987). As most of these crops and other plant material have been moved around the world, pathogens and other organisms have inadvertently moved with them (Mathys & Baker 1980).

The movement of pathogens can be divided into two main categories. These are long and short distance forms of dispersal. Long distance dispersal occurs in one step and is usually unnoticed at first (Nagarajan & Singh 1990, Agrios 1997). Examples of pathogens dispersed in this manner are the spread of the Dutch elm pathogens *Ophiostoma ulmi* (Buis.) Nannf. and *O. novo-ulmi* Brasier from Asia to Europe and the United States (Brasier 2001) and the wheat rust pathogen, *Puccinia graminis* Pers., from Mexico to Canada (Viljanen-Rollinson & Cromey 2002). Some biotrophic fungi such as rusts, powdery mildews and downy mildew pathogens are specially adapted for aerial dispersal through the production of extremely high numbers of dry spores. This adaptation is also essential for the survival of some pathogens that depend on living plant material. Long distance dispersal, therefore, enables the pathogen to move from an area with no living host to an area with abundant host tissue. Short distance spread of pathogens tends to occur more gradually than long distance spread (Brown & Hovmøller 2002). It facilitates the spread of a pathogen within an area but does not, as in the case of long distance dispersal, support the survival of certain species.

Wind dispersal

Wind dispersal of fungal pathogens takes place over both short and long distances. Long distance dispersal in the air is an important survival strategy, enabling pathogen populations to colonize new areas. Most rust pathogens, for example, are dispersed by wind (Wilkinson & Spiers 1976, McKenzie 1998, Viljanen-Rollinson & Cromey 2002). It has thus been shown that sugar cane rust was almost certainly introduced into the Dominican Republic from Cameroon in West Africa through the transport of uredinio spores by cyclonic winds in 1978 (Brown & Hovøller 2002). Another example of a rust pathogen that has been dispersed by the wind from one area to another is coffee rust (*Hemileia vastatrix* Berk. & Br.) that was spread from Angola to Brazil (Carefoot &

Sprott 1967, Bowden, Gregory & Johnson 1971), presumably by wind currents. It is also suggested that *Phytophthora infestans* (Mont.) De Bary., the causal agent of potato blight, spread from Ireland to Russia by means of cyclonic winds (Carefoot & Sprott 1967).

Spread of plant pathogens on germplasm

An increasing number of plant pathogens apparently spread both long and short distances through the movement of infected plant material (Gibson, Christensen & Munga 1964, Gilmour 1967, Edwards & Walker 1978, Walker 1987, Wingfield *et al.* 2001a). Humans have moved plant material since the early days of sea travel and global exploration. Between 1764 and 1775 Benjamin Franklin, for example, sent plant material from his post in England to America. Large numbers of agricultural crops have been moved in similar fashion between countries to be established as exotics. Between 45% and 86% of various categories of pests introduced into California and the United States in recent decades, have entered that country on horticultural imports (Annonymous 1993, Palm 2001). It has, for example, been reported that the initial introduction of potato late blight into Europe from Mexico was on infected tubers in the 1840`s (Bourke 1964, Fry *et al.* 1993, Palm 2001).

Forest pathogens have been imported into countries on plant products such as timber, seeds and nursery stock (Wingfield *et al.* 2001a). Examples of such introductions are white pine blister rust (*Cronatium ribicola* J.C. Fisch.) introduced into Canada in 1910 on infected nursery stock (Allen & Humble 2002) and *Dothistroma septospora* Hulbary. introduced into various southern hemisphere countries, presumably on infected planting stock, seeds and cones (Gibson 1979, Millar & Minter 1980, Roux 1984, Wingfield *et al.* 2001a). A number of pathogens have also been hypothesised to have been introduced into countries on *Eucalyptus* spp. imported for timber and pulp production. These, for example, are thought to include species of *Mycosphaerella* Johanson (Park & Keane 1982, Crous & Wingfield 1996, Crous 1998), *Phaeophloeospora* (Hansf.) Walker. (Heather 1967, Chimpompha 1987, Crous, Knox-Davis & Wingfield 1989, Crous, Ferreira & Sutton 1997) and *Aulographina eucalypti* (Cooke & Massee) Arx & E. Müll. (Wall & Keane 1984).

Spread of plant pathogens on seed

Many pathogens are thought to have entered countries through the trade in seeds (Noble 1979, Richardson 1979, Walker 1987). *Claviceps africana* Freder, Mantle & De Milliano was introduced from Africa and Asia into Brazil (1995) and Australia (1996) on infected seeds (Bandyopadhyay *et al.* 1998), while urediniospores of *Puccinia antirrhini* Dietel & Holway. were introduced into Australia (1952) in seed lots (Walker 1987). Rees (1981) reported that he recovered 155 fungal species, of which 27 were plant pathogens, from tropical pine seeds. Seed borne pathogens that could cause flower, cone and seed losses, have also been reported to lead to 98% infection in a given *Pinus elliottii* Engelm. seed batch (Anderson, Belcher & Miller 1984).

One of the best forestry examples of the introduction of a plant pathogen into another country on seed is that of *Diplodia pinea* (Desm.) J. Kickx. [Synonym: *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton] (De Wet *et al.* 2003). This fungus is an endophyte in all *Pinus* spp. (Smith *et al.* 1996) and is found wherever pines grow (Gibson 1979). *Diplodia pinea* is an opportunistic pathogen and disease symptoms are expressed when the host is subjected to unfavourable growing conditions and stress (Swart, Wingfield & Knox-Davis 1987, Zwolinski, Swart & Wingfield 1990). As all pine plantations in the Southern Hemisphere are exotic, *D. pinea* is hypothesised to have been introduced from the native regions of pine species into the Southern Hemisphere in the early development of exotic pine forestry in these regions (Gibson 1979). For the establishment of pine forestry in South Africa, Australia and New Zealand, seed stock was imported from Europe, Japan and California (Brown 1883, Fieldings 1975, Poynton 1977, Eldridge & Simpson 1987, Lavery 1986, Shepherd 1990 a,b, Burgess & Wingfield 2002). Seed was also moved between South Africa, Australia and New Zealand (Poynton 1977). Because phytosanitary and certification certificates were not required for trade in seed prior to the 1950's the movement of seeds and, therefore, *D. pinea,* took place unknowingly and without control (Burgess & Wingfield 2001).

Spread of plant pathogens on alternate and alternative hosts

Economically important, as well as non-commercial alternative and alternate hosts have played a significant role in the world-wide distribution of plant pathogens. *Puccinia graminis* Pers. Pers. (wheat stem rust), introduced into America in 1660 on Barberry (Yarwood 1983) is one of the first reported cases where an alternate host has resulted in the entrance of a pathogen into a new country. Pine needle rust caused by *Coleosporium* spp. is a threat to many countries producing pine timber, and could be carried on a number of ornamentals in the *Asteraceae* family, including *Aster, Callistephus* and *Solidago* spp. (Walker 1987). The introduction of plant pathogens on economically unimportant crops is not only difficult to track due to their lack of importance, but also complicated. Due to the relative unimportance of these hosts, the control and screening of these plants would not be sufficiently thorough to prevent spread. Alternative hosts are also important sources of introductions of plant pathogens. An example of how a plant pathogen was introduced into a country on an alterantive host is *P. infestans*. It is hypothesised that *P. infestans* was introduced into Ireland on infected tomato (Fry & Goodwin 1997). The spread of plant pathogens on these hosts are, however, easier to track due to the screening of these hosts themselves.

Spread of pathogens by insects

A number of cases have been reported where insects have carried plant pathogens, not only on a small spatial scale, but also beyond country borders. For example, a large number of the Ophiostomatoid fungi are known to be vectored by insects (Upadhyay 1981, Solheim 1986, Brasier 2001, Jacobs & Wingfield 2001, Rossman 2001, Wingfield *et al.* 2001b).

The best known example of a tree pathogen that has spread by means of insects is that of the Dutch elm disease fungi (Brasier 2001). The Dutch elm disease pathogens are transmitted by bark beetles in the genus *Scolytus* (Brasier 2001) and are devastating to susceptible elm trees. Currently, there are two fungal species responsible for Dutch elm disease, namely *O. ulmi* and *O. novo-ulmi* and they have been responsible for two separate pandemics in the history of the disease. The first Dutch elm disease pandemic, caused by *O. ulmi*, began in northwest Europe around 1910 (Brasier 2001). The disease

spread eastwards forming a number of epidemic fronts across Europe and into southwest Asia (Brasier & Kirk 2001). In 1927 it was introduced into the United Kingdom and North America and by 1930 reached central Asia (Peace 1960, Brasier 1990). By the 1940's the severity of the epidemic declined after approximately 40% loss of elms was recorded in most European countries (Peace 1960).

The second Dutch elm disease pandemic was caused by *O. novo-ulmi*. Although this pandemic was only noticed in the 1970's in Britain and the neighbouring regions of Europe, it is believed that the pandemic had actually begun in the 1940's at two different locations. Each of these locations was the source of two different forms of the fungus. The first region was the Maldova-Ukraine region in eastern Europe (EAN form) and the second the southern Great Lakes area in North America (NAN form) (Brasier 1990). The EAN form moved westward across Europe reaching the Netherlands by the 1970's. The pandemic then spread to Southwest Asia (Brasier 2001). The NAN form spread across the North American continent and was present from the west to the east coast by the 1980's (Brasier 2001). The NAN form was also introduced into Britian from Canada during the 1960's (Brasier & Gibbs 1973). The second pandemic was more severe than the first. This was attributed to a more virulent form of the fungus and the late detection of the introduction due to mis-identification of the pathogen and a resulting lack in efficient quarantine (Brasier 2001). This pandemic lead to the mortality of most mature elms in Europe and approximately 30 million elms in the United Kingdom. In Northern America, the impact has been catastrophic and resulted in the death of hundreds of millions of elms (Brasier 2001).

A more recent example of the role that insects have played in the distribution of forest pathogens is from Canada. In 1998, the mortality of *Picea rubens* Sarg. (red spruce) trees was noted in the Point Pleasant Park, Halifax, Nova Scotia (Jacobs *et al.* 2003). This raised concern amongst local politicians, residents, foresters and environmentalists. Investigations into the death of the trees revealed that the causal insect was the bark beetle *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambicidae). This beetle is exotic in Canada and native in northern and central Europe and Siberia (Smith & Hurley 2000). In Europe, *T. fuscum* is considered as one of the most economically important longhorn beetles on conifers (Hellrigl 1974) attacking stressed and recently dead trees (Schimitschek 1929, Hellrigl 1974). It also has a close association with *Ophiostoma*

spp., which result in blue staining of timber. After investigation of the dying trees in Canada, *Ophiostoma tertropii* Math. was commonly found in trees attacked by *T. fuscum* and was the most frequently isolated fungus from this source (Jacobs *et al.* 2003). *Ophiostoma tetropii* was, however, not isolated from *T. cinnamopterum* Kirby., the most common *Tetropium* sp. found in Canada. Jacobs *et al.* (2003) also found that the *O. tetropii* isolated from the dead trees and the galleries of *T. fuscum*, was morphologically and phylogenetically identical to the fungus associated with the same beetle in Europe. This indicated that *O. tetropii* was moved into the country from its centre of origin in Europe on the bark beetle *T. fuscum*.

Spread of plant pathogens assisted by humans

A large amount of plant material is moved around the world, but this is not nearly as great as the number of humans that commute around the world for business and pleasure. Movement of people certainly facilitates the movement of plant pathogens (Allen & Humble 2002). Humans have surpassed natural forces as the main global dispersers of vascular plants (Mack & Lonsdale 2001, Palm 2001). Although humans do not spread diseases knowingly, this happens inadvertently. This occurs through the movement of seeds, souvenirs made of plant material, the movement of ornamental plants, on baggage and even on clothes. Wellings, McIntosh & Walker (1987) reported that wheat yellow rust (*Puccinia striiformis* Westend.) was introduced into Australia from Europe on clothes. During a period of four weeks of sampling clothes and baggage at Wellington airport, it was estimated that 198 270 000 viable rust urediospores were brought into New Zealand (Sheridan 1989). It has also been shown that the *Eucalyptus* rust pathogen, *P. psidii* Winter can be isolated from clothes, camera bags and footwear of researchers working on this important forestry pathogen (Langrell *et al.* 2003). These are only two examples of the impact that humans are having on the spread of plant pathogens.

Spread of plant pathogens by inconspicuous means

There are many less prominent and inconspicuous means by which pathogens move around the world. These include soil (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a), wooden products (Walker 1987) and even

packaging material (Campbell 2001). One forestry example is provided by *Rhizina undulata* Fr., one of the best-known soil-borne pathogens of *Pinus* spp. (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a). It has been hypothesised that *R. undulata* was introduced into South Africa with soil from the Boreal region (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a) when pine plantations were established in the country. Pathogens have also been moved around the world on unprocessed lumber, logs, wood packaging, dunnage and wood chips (Campbell 2001).

The wood wasp, *Sirex noctilio* F., is a vector for *Amylosterium areolatum* (Chaillet) Boidin and together these two organisms have the potential to cause serious damage and mortality to conifer species including *Pinus*, *Abies*, *Picea* and *Pseudotsuga* (Spradberry & Kirk 1978, 1981). In the Northern Hemisphere, where the wood wasp is native, a natural balance exists between the wasp, its symbiotic fungus and their tree hosts, to such an extent that they are considered secondary invaders (Hall 1978, Spradberry & Kirk 1978). The *S. noctilio/A. areolatum* complex was introduced into New Zealand in 1900 (Gourley 1951, Gilmour 1965, Chou 1991), Australia in 1961 (Gaurt 1970, Talbot 1977, Neumann and Minko 1981, Madden 1988) and South Africa in 1994 (Baxter, Rong & Schutte 1995, Tribe 1995, Wingfield *et al.* 2002) most likely within conifer packaging. In these Southern Hemisphere countries, the complex causes severe damage to non-indigenous *Pinus* plantations (Neuman & Marks 1990, Chou 1991, Bedding 1995) and is considered a primary problem of great economic concern (Slippers *et al.* 2000).

IMPACT OF NON-INDIGENOUS PATHOGENS

In order to appreciate the importance of the world-wide movement of plant pathogens it is necessary to consider the recorded impacts that some of these introductions have had. The impact of pathogens can be divided into three main groups, namely social, economic and environmental. In each of these categories at least one example is provided.

Economic impacts

Major losses have been recorded in plantation forestry world-wide. For example, Zwolinski *et al.* (1990) calculated the annual loss due to *D. pinea* infection of hail stressed *Pinus* spp. in South Africa at R5000-00/ha. Wright & Marks (1970) and Currie & Toes (1978) calculated the increment and potential merchantable volume loss of *Pinus* spp. for Australia and New Zealand due to *D. pinea* at 40% and 60% respectively. Millions of dollars of losses to *P. radiata* in New Zealand, Australia, Chile and Kenya (Wingfield *et al.* 2001a) are associated with crop or yield loss, decrease in quality of product and cost of control as a result of *D. septospora* infection.

Approximately 65% of losses in United States crops and forests have been attributed to introduced pathogens (Madden 2001). Infected seeds can result in the failure of germination, the death of entire seedling beds or lead to field infection after transplanting where additional losses could occur (Anderson *et al.* 1984). Yield losses due to rust diseases of cereals have been estimated at 10% of world production with losses of up to 70% for certain crops (Viljanen-Rollinson & Cromey 2002). Palm (2001) estimated the annual loss due to such introductions at approximately US$30 million. The eradication of Citrus canker in the United States, for example, cost a total of US$200 million (Madden 2001). These losses do not include the losses due to trade restrictions, embargoes (Allen & Humble 2002) or breeding programs to resolve the disease problems.

Environmental impacts

It is difficult to estimate the impact of exotic pests on the environment. It is, however, clear that these invaders harm the environment and influence the ecological balance of invaded areas (Vitousek *et al.* 1966, Walker 1987, Old & Dudzinski 1998, Palm 1999, Allen & Humble 2002). Approximately 49% of all imperilled plant species in the United States are threatened in part by alien plant pathogens (Wilcove *et al.* 1998). These alien species can change the community and forest composition of an area (Keever 1953, Mackey & Sivec 1973).

Drastic changes can combine to form debilitating chain reactions altering environmental conditions even on a micro spatial scale. Pathogens affecting the flora could have a severe impact on the fauna by altering animal habitats and food stocks (Franklin, Shugart & Harmon 1987) and in that way animal and bird diversity (Mlot 1991). Blister rust on *Pinus albicaulis* Engelm. has, for example, affected grizzly bear and nuthatch populations (Byler, Marsden & Hagle 1990, Allen & Humble 2002). These animals feed on pine nuts, therefore, a reduction in the flora population severely impacted the fauna.

Possibly the most comprehensively researched impact of invasive pathogens on indigenous vegetation and ecosystems is that asociated with *Cryphonectria parasitica* (Murr.) Barr. on the American chestnut *Castanea dentata* (Marsh.) Borkh. The American chestnut was once a dominant canopy species in the hardwood forests of the eastern United States (Anagnostakis 1987). The fruit produced by the tree was an important food source for wildlife, domestic livestock and enjoyed by humans (Anagnostakis 1987).

Chestnut blight was first noticed by Merkel in 1905 (Anagnostakis 1987) on ornamentals in the New York Zoological Gardens. *Cryphonectria parasitica* soon spread from New York and an epidemic began, which could not be stopped even though drastic chemical control was attempted (Murrill 1906). Chestnut blight progressed rapidly throughout the entire native range of chestnut trees. By 1938 the epidemic had moved abroad to Italy (Woodruf 1946) and France (Anagnostakis 1987) infecting European chestnut trees (*Castanea sativa* Mill.). Subsequent studies indicated that the fungus was spread by insects, birds, animals and possibly wind (Anagnostakis 1987). The epidemic led to the devastation of the North American chestnut tree (Anagnostakis 1987, Shearer & Tippett 1989, Castello, Leopold & Smallidge 1995, Hubbes 1999, Palm 2001). Within 50 years this, once dominant forest tree, was reduced to a multiple stemmed, low shrub (Anagnostakis & Hillman 1992). The introduction of the Japanese chestnut tree into the United States, which is resistant to infection by *C. parasitica,* has been implicated as the source of the destructive pathogen (Anagnostakis 1987, Palm 2001). The American landscape has been changed forever by chestnut blight with many possible impacts that remain unknown or poorly defined.

Social impacts

One of the best-recorded examples of the impact of a forestry pathogen on a social scale is the blight of chestnut trees caused by *C. parasitica* (Anagnostakis 1987). The wood of the chestnut tree is extremely resistant to decay due to the high levels of tannins present in the wood and bark. The high value timber was used in construction, woodwork, furniture, fencing, barrel staves and musical instruments (Gibson 1913). The fruits of the American chestnut were an important food source for wildlife, domestic livestock and humans (Anagnostakis 1987). The die-back of this native tree species due to chestnut blight led to a productivity decrease of between 15-20 % (Boyce 1961). The tannin industry, on which a number of communities largely depended, was brought to a halt (Boyce 1961). The industry managed to continue by utilizing dead trees, but the end was inevitable and thousands of people were left unemployed when the once dominant hardwood tree had disappeared from the forests.

There are many social impacts of pathogens on agriculture, including that associated with wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. This pathogen has caused a scarcity of wheat in Europe and the United States. In Europe this resulted in the use of rye and in the U.S.A. corn as alternatives (Palm 2001). A more severe impact is that of ergot of rye caused by *Claviceps purpurea* (Fr.) Tulasne in France, which lead to the death of 40 000 people due to toxins produced by the fungus (Waterford & White 1982).

The most severe impact that an introduced pathogen has had on society is that associated with *P. infestans*, the cause of the Irish potato famine (Large 1940, Waterford & White 1982, Palm 2001). The first outbreak of the disease was in 1845 in Ireland (Large 1940, Carefoot & Sprott 1967, Waterford & White 1982, Palm 2001). The disease resulted in shortages of potatoes, the staple diet of the Irish people. This resulted in the death of large numbers of people and the neglect of education when children were kept out of school to forage for food (Carefoot & Sprott 1967).

The first death by starvation, as a result of the potato famine was recorded in August 1846 (Carefoot & Sprott 1867). During the period 1846 to 1866, two million people emigrated and 1 million died as a result of the famine (Large 1940, Waterford & White

1982, Palm 2001). Many more died in quarantine camps in America and England due to diseases associated with poor living conditions. *Phytophthora infestans* soon spread to European countries by means of wind (Carefoot & Sprott 1967). In 1848, hunger lead to a revolution in Europe subsequantly resulting in political changes. In 1872, blight continued to destroy millions of potato plants and tubers in England and Ireland. In 1879 crop losses amounted to 6 million pounds Sterling (Carefoot & Sprott 1967) and in 1916 late blight lead to the death of seven hundred thousand Germans (Carefoot & Sprott 1967). The disease was only brought under control in 1950 when Dr. Lindley produced a number of resistant potato varieties (Carefoot & Sprott 1967, Agrios 1997).

MEANS TO COMBAT THE SPREAD OF PLANT PATHOGENS

Quarantine measures

In order to combat the impact of invasive plant pathogens, strict control measures are needed. Since 1660, quarantine measures have been used to successfully limit the worldwide movement of pathogens. The first form of quarantine was in France when the movement of barberry, the alternate host of the wheat rust pathogen *P. graminis* (Yarwood 1983), was prevented to stop the spread of the pathogen (Palm 1999). The first plant quarantine regulations were established in Europe in 1870 after the introduction of several pests such as grapevine downy mildew [*Plasmopara viticola* (Berk. and M.A. Curtis) Berl. & De Toni.] on grapes from the United States of America into France (Palm 2001).

In 1873, Germany and the United Kingdom established a quarantine programme to prevent the introduction of Colorado potato beetle from the U.S.A. (Waterford & White 1982). The first federal plant quarantine law was established in the U.S.A. in 1912 (Waterford & White 1982). This law was initiated due to a donation of 2000 Japanese cherry trees from Tokyo to the U.S.A. These trees had to be destroyed after it was found that they were infected with an array of pests and diseases.

Since the establishment of the first Federal Plant Quarantine Act in the U.S.A. in 1912, quarantine has played a significant role in the fight against pathogen spread (Walker 1987, Palm 1999, Burgess & Wingfield 2001). This is true both in forestry and in

agriculture. The basis of quarantine is to screen imported commodities for possible pests, thereby preventing unwanted introductions. This is most strongly imposed at ports of entry into countries such as at border posts, harbours and airports.

It is well known that most introductions occur near shipping ports (Allen & Humble 2002). Screening imported commodities for plant pathogens is, however, difficult due to the quantity of entries into a country. The nature of import commodities sampled, methods and intensity of sampling, and the capacity to identify samples (e.g. insects, larvae, fruits, fungal spores and vegetative tissue) makes quarantine extremely difficult (Allen & Humble 2002).

The incubation period of non-indigenous species entering a country could be a complicating factor in the screening of non-indigenous species. Some species could enter a country on material without any screening system detecting them, due to long incubation periods of the organisms. While fungi, nematodes and bacteria can generally be isolated and identified from plant samples entering a country, some insects may need an incubation period before the adults exit the samples. This is quite frequent on, for example, dry timber such as packaging crates. Such insects could also vector pathogens (Gourley 1951, Gilmour 1965, Gaurt 1970, Talbot 1977, Neumann & Minko 1981, Walker 1987, Madden 1988, Chou 1991, Tribe 1995, Baxter *et al.* 1995, Wingfield *et al.* 2002). An example is provided where *Picea abies* (L.) Karsten bolts were used to brace large granite blocks inside shipping containers. More than 2500 adult insects representing more than 40 species of bark beetles, wood borers, and their associated parasitoids, nematodes and blue stain fungi (Allen & Humble 2002) were isolated from these bolts after an incubation period.

For quarantine to be successful it is important to know what pathogens are present in the countries of origin of the plants and which of these organisms might pose a threat to the importing country. Plant pathogen risk assessments form the basis for gathering such information. In this way, the likelihood that an organism will arrive, survive and thrive in a new habitat, can be determined. Pathogen risk assessments enable quarantine officials to focus on the most important plant pathogens (Palm 2001).

There are a number of factors that complicate crucial steps in the quarantine process. A major limiting factor in effective quarantine is the limited knowledge pertaining to fungi. Hawksworth (1991) estimated that only 5% of the worlds 1 500 000 fungi are known, with a mere 1% of these fungi held in culture collections. Campbell (2001) supported this view and estimated the percentage of named fungi to be less than 5% of the total. Walker (1987) stated that the huge number of taxonomic errors that exist, have complicated quarantine even more. This leaves a major gap in the protection system. In the past, focus has been placed on species identifications and this is an area that remains poorly studied.

In terms of identification, effective quarantine needs to delve more deeply than species level identifications. In this regard, pathogens need to be viewed as components of populations (Wingfield 2003). In pathogens capable of sexual reproduction, newly introduced genotypes crossing with existing genotypes or the hybridisation of closely related species would allow for greater gene diversity and a greater risk of pathogens overcoming tree resistance.

A number of general and more civilian measures could be taken to improve quarantine and the control of invading organisms. A cornerstone of this strategy would be the quality of staff at airports, border posts and ports (Wylie & Peters 1987). Public awareness should also be increased and could lift the burden on officials and personnel in areas where quarantine is enforced (Wylie & Peters 1987). Once an invasive organism has been noted rapid response and action is crucial. Delayed control could lead to huge financial losses and serious environmental impacts. For example, delayed control of *D. septospora* in Australia, brought about by bad weather conditions, is believed to have resulted in the subsequent spread of the disease (Eldridge & Simpson 1987).

There are approximately six examples of fungal species hybridisation (Brasier & Mehrota 1995). An excellent example is found in the case of Dutch elm disease. Limited sexual recombination between *O. ulmi* and *O. novo-ulmi* has been shown (Brasier 2001). Although it was believed that the progeny would not be able to survive in nature due to low fitness (Kile & Brasier 1990), recent research has indicated that *O. ulmi* - *O. novo-ulmi* hybrids exist in nature, but disappear when in competition with the

parent species (Brasier *et al.* 1998). Despite the low fitness of these hybrids, they could provide a "genetic bridge" allowing gene flow from one species to another possibly leading to new, more aggressive forms of the fungus (Brasier 2001). Hybridisation has also been shown between the North American (NAN) and Eurasian (EAN) races of *O. novo-ulmi* (Brasier 2001). These "new" hybridised forms of the fungus have been found to replace the pure strains in nature (Brasier 2001) and could represent a higher level of fitness.

Another example of a forest pathogen that has been considered from a population perspective is *Diplodia pinea*. This pathogen is a cosmopolitan endophyte on *Pinus* spp. and, therefore, little attention has been placed on quarantine to prevent its spread. Burgess and Wingfield (2002), however, stressed the importance of viewing the pathogen as part of a population. They reported on the impact that multiple introductions of the fungus could have on control in the sense that the introduction of multiple or new genotypes could also reduce the success of programmes aimed at breeding resistance. De Wet *et al.* (2000) also reported differences in aggressiveness in the various morphotypes of *D. pinea*, once again indicating that the presence of a fungus does not mean that quarantine is not necessary.

Molecular detection methods and diagnostic tools

In order to optimise quarantine measures, it is becoming increasingly necessary to develop detection methodologies and diagnostic tools designed to identify a broad range of organisms. Traditional diagnostic methods for identifying fungi need to be expanded and optimised using DNA-based techniques that are becoming increasingly available. These tools could provide greater diagnostic capacity with higher resolution and greater precision. Together with traditional morphology-based approaches, these precautions should provide an adequate means to identify, detect and prevent the introduction of pests and diseases into a country (Seifert, Wingfield & Wingfield 1995).

These identification tools could be grouped under the collective term "systematics". Systematics is the science of discovering, organizing and interpreting biodiversity. It increases knowledge of the biodiversity and population structure of fungi, their biology, ecology, physiology and pathology. Risk assessment and decisions pertaining to

quarantine will logically be substantially enhanced by increased knowledge of pathogens (Walker 1987, Palm 2001).

An example where systematics has promoted the control of a pathogen has been in the case of Karnal bunt caused by *Tilletia indica* Mitra. This smut fungus is difficult to distinguish from the related but non-pathogenic *T. horrida* Takah. *Tilletia indica* has posed a threat to wheat producing countries world-wide and quarantine measures were initiated to prevent the spread of Karnal bunt. Due to international quarantine regulations, the entire U.S.A. export of wheat was threatened after the discovery of Karnal bunt in that country (Yakema *et al.* 1996). However, with information obtained by means of molecular systematics it was determined that it was actually the closely related *T. horrida* that had been introduced into the USA. Trade restrictions limiting imports from the U.S.A. were thus lifted and the U.S.A. was able to maintain most of the US$5 billion wheat export market in 1996 and 1997 (Palm 1999).

Molecular systematics has been crucially important in dealing with regulations relating to many other pathogens. An example is the confusion regarding the identity of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletorichum acutatum* J.H. Simmonds. Despite similar morphological characteristics, research has shown that taxa within the *Colletotrichum* complex differ in biological characteristics and virulence (Palm 1999). Anthracnose of lupins (*Lupinus* spp.) is attributed to *C. gloeosporioides* (Palm 1999). Because the taxon was considered to be widely distributed, the movement of infected lupin seeds was not restricted. Anthracnose of lupins, however, suddenly became a restricting factor in lupin production in in the U.S.A. (Paulitz, Atlin & Gray 1995) and Australia (Sweetingham *et al.* 1995) when above average losses ascribed to *C. gloeosporioides* were experienced. Based on rDNA sequence data, the causal agent was later identified as *C. acutatum* (Sreenivasaprasad, Mills & Brown 1994), which based on morphological characteristics is easily confused with *C. gloeosprioides*. This error in identification of *C. acutatum* was corrected based on DNA sequence data and *C. acutatum* was elevated to quarantine status. This enabled quarantine to be streamlined and increased in effectiveness. This illustrates the value and growing importance of modern diagnostic techniques to identify pathogens.

DETERMINING THE ORIGIN OF INTRODUCED PLANT PATHOGENS

A crucial link in the management of plant diseases and the enforcement of quarantine legislation is a comprehensive knowledge pertaining to the origin of pathogens. Once the origin of a pathogen is known, risk assessment is improved and centres can be identified for possible research into control measures (Linde, Zhan & McDonald 2002). Linde *et al.* (2002) stated that the centre of origin of a pathogen is the most likely area to prospect for resistance genes, because these are the locations where co-evolution is most likely to have increased the frequency of resistance alleles in natural host populations. Thus, knowledge of the area of origin of a pathogen will greatly focus quarantine measures. Numerous techniques have been considered to determine the origin of a pathogen. Some are based on historical events, others on wind patterns and more recently molecular techniques have provided more direct results with higher resolution (Taylor *et al.* 1999).

The study of biological patterns and historical records

Purdy, Krupa & Dean (1985) studied cyclonic wind patterns and were able to determine that the origin of *Puccinia melanocephala* Syd. & Syd. infection of sugarcane in the Dominican Republic, was from West Africa. They were also able to identify 1978 as the year of introduction. There are many other examples of studies involving wind patterns to determine the origin of pathogens such as *P. graminis* Pers. f. sp. *tritici* Eriks. & L. Henn. that was introduced into Canada from Mexico (Viljanen-Rollinson & Cromey 2002), *H. vastatrix* that was introduced from Angola to Brazil (Bowden *et al.* 1971) and a number of other rust species (Wilkinson & Spiers 1976, McKenzie 1998, Viljanen-Rollinson & Cromey 2002).

The use of molecular techniques

A number of hypotheses regarding the origin of pathogens and other fungi have been based on the comparison of genetic diversity and the knowledge of genetic structure of pathogen populations (Anagnostakis 1977, Anagnostakis 1982, Anagnostakis, Hau & Kranz 1986, Coates 1988, Perkins 1991, Morelet, Lipari & Powell 1992, Leslie 1993, Van Heerden *et al.* 1997, Van Zyl *et al.* 1998, Van Heerden & Wingfield 2001). Such

knowledge has provided insight into the life history of pathogens, including the evolutionary processes that shape pathogen populations in agro-ecosystems (Linde *et al.* 2002). A number of the hypotheses have been formulated to show that populations with high genotypic diversity are more likely to be native to an area than pathogen populations with a low diversity (Anagnostakis *et al.* 1986, Redlin 1991, McDonald & McDermotte 1993).

One of the most common methods that has been used to conduct population studies has been based on vegetative compatibility groups (VCG) (Anagnostakis *et al.* 1986). Using this technique, along with the study of historical events, Burgess & Wingfield (2002) were able to determine the origin and time that *D. pinea* was introduced into South Africa, New Zealand and Australia.

With the growing availability of DNA-based methods, a number of techniques based on genotypic rather than phenotypic characteristics have been developed to study populations. These techniques not only makes it possible to calculate genotypic diversity in a fungal population, but also provide an insight into the genetic structure of populations. This is achieved by determining gene flow (Slatkin 1987, Slatkin & Barton 1989, Boeger, Chen & McDonald 1993), mode of reproduction, gametic disequilibrium and population differentiation (Agapow & Austin 2001). To examine fungal populations at such a fine spatial scale requires the use of genetic markers that can discriminate among individuals in a population, which are usually morphologically identical (McDonald & Martinez 1990). A number of methods based on these principles have been developed and used and these will impact positively on quarantine.

The first DNA markers to be used for fungal evolutionary studies were restriction fragment length polymorphisms (RFLPs). This technique has been used with great success in numerous studies (Jeffereys, Wilson & Thein 1985a, b, Taylor 1986, Perkins & Turner 1988, McDonald *et al.* 1989, Leuchtman & Clay 1989, Gilbert *et al.* 1990, McDonald & Martinez 1990, Smith *et al.* 1990) and continues to be widely used in population genetic studies (Taylor *et al.* 1999). This approach is advantageous as it is highly reproducible and co-dominant (Hantula, Dusabenyagasani & Hamelin 1996).

The analysis of randomly amplified polymorphic DNA (RAPD) is based on random priming of DNA with short primers (Welsh & McClelland 1990, Williams *et al.* 1990). This technique is similar to RFLP analysis in that it assays DNA sequence variation in short regions. Instead of analysing restriction endonuclease recognition sequences it focuses on polymerase chain reaction (PCR) priming regions (Williams *et al.* 1990) and has been particularly popular for the study of fungal population genetics (Hantula *et al.* 1996). The disadvantage of RAPD markers is that they are not co-dominant.

Amplified fragment polymorphism (AFLP) analysis is a PCR based assay that selectively amplifies DNA fragments (Maughan *et al.* 1996, Rosendahl & Taylor 1997). DNA is digested with two endonucleases and site-specific adapters are then ligated to the DNA fragments. Primers complementary to the adapters and to the restriction sites are designed with selective nucleotides added to the 3′ ends of the primers (Steinger *et al.* 2002). Thus, only DNA fragments with nucleotides flanking the restriction sites that match the selective nucleotides of the primers are amplified during PCR (Maughan *et al.* 1996, Mueller, Lipari & Milgroom 1996). Resolution of the resulting DNA fragments on standard sequencing gels allows for the detection of AFLP`s. Similar to RAPD analysis, AFLP assay requires no prior sequencing knowledge, but can detect a much greater number of loci than those detected by RAPD analysis. Therefore, one could screen thousands of samples or independent genetic loci (Young *et al.* 1988, Maughan *et al.* 1996, Mueller *et al.* 1996).

The amplification of microsatellites using flanking primers, followed by analysis of length polymorphisms within each locus is a more efficient and accurate technique in determining genetic structure of a population (Hantula *et al.* 1996). This technique has been used to reflect intra-specific relationships in fungal pathogen populations and genetic diagnostics as reported by Moon, Tapper & Scott (1999), Queller, Stassmann & Hughes (1993), Rafalski & Tingey (1993), Longato & Bonfante (1997), Neu *et al.* (1999), Lynn & Heun (1993) and Barnes *et al.* (2001). These markers enable the detection and characterisation of multiple alleles at a specific locus. A number of studies have used microsatellite markers to study population structure, genetic diversity and related questions (Queller *et al.* 1993). In recent years this approach has also been used successfully to study a number of tree pathogens (O'Donnell, Cigelnik & Nirenberg, 1998, Barnes *et al.* 2001, Carbone & Kohn 2001)

POSSIBLE THREAT OF PATHOGENS FROM NATIVE HOSTS TO NON-INDIGENOUS PLANTATION SPECIES

There are numerous examples where pathogens have moved from hosts native to a country onto exotic forestry species, impacting negatively on the non-indigenous plantation species. One of most interesting examples is that of *Eucalyptus* rust caused by *P. psidii*. This is the only rust able to infect the Myrtaceae and it is not known in the native range of *Eucalyptus* spp. (Coutinho *et al.* 1998). The pathogen was first reported from *Psidium pomiferum* L. in 1884 from its native habitat, Brazil (Winter 1884). In 1973, a serious outbreak of *P. psidii* on *Eucalyptus* was reported from Brazil where it led to excessive losses in nurseries and young plantations (Ferreira 1981). This fungus poses a huge threat to *Eucalyptus* forestry world-wide and also to species in the Myrtaceae in countries such as Australia where this family of plants shows a great diversity. The occurrence of the pathogen on *Callistemon speciosus* (Sims) CD. in South America (Ferreira 1989), another Australian native, has highlighted the threat *P. psidii* could pose to a number of native myrtaceous species in Australia. The cross infection of *P. psidii* from a native host to an introduced species is a good example of the unfortunate consequence of exposing a non-indigenous plant species to a native pathogen.

The canker pathogen *Cryphonectria cubensis* (Bruner) Hodges. was first reported on *Eucalyptus* in Cuba (Bruner 1917). This was followed by reports on a wide range of *Eucalyptus* spp. world-wide, including those from Northern Australia (Davison & Coates 1991), Asia (Florence, Sharma & Mohanan 1986), South and Central America (Hodges, Geary & Cordell 1979, Hodges *et al.* 1986) and Africa (Gibson 1980, Wingfield, Swart & Abear 1989). *Cryphonectria cubensis* has also been reported as an opportunistic pathogen causing die-back of *Syzygium aromaticum* (L.) Merr. & Perry (clove) in Brazil and Indonesia (Hodges *et al.* 1986). The pathogen was found to be more aggressive on *Eucalyptus* than on *S. aromaticum* (Hodges *et al.* 1986). Hodges *et al.* (1986) stated that this *S. aromaticum/Eucalyptus* connection was somewhat analogous to the situation with the chestnut blight fungus that posed no threat to its native host in its centre of origin (Asia) but had a devastating effect on the European and American chestnuts. This hypothesis led the researchers to believe that the origin

of *C. cubensis* is Indonesia, with *S. aromaticum* its native host and that it had cross-infected to *Eucalyptus*.

Until 2001, *C. cubensis* was known only on genera in the Myrtaceae. However, in 2001 the fungus was reported on native *Tibouchina urvilleana* (CD.) Long. and *T. lepidota* Baill. from Colombia, where the pathogen also occurs on *Eucalyptus* spp. (Wingfield *et al.* 2001b). These two species reside in the Melastomataceae, a family recently shown to be closely related to the Myrtaceae. Both families reside in the Myrtales (Conti *et al.* 1997). In the study by Wingfield *et al.* (2001b) sufficient evidence could, however, not be gathered to conclusively state whether the fungus had moved from the native *Tibouchina* spp. to the non-indigenous *Eucalyptus* spp.

In 2002, after the first report of *C. cubensis* on *Tibouchina* spp. from South Africa (Myburg *et al.* 2002a), Myburg *et al.* (2002b) reported that the South African fungus is distinct from the fungus with the same name, occurring in the rest of the world, based on ITS and β-tubulin and Histone *H3* gene sequences. This raises the question as to the origin of the South African fungus and the possibility of it having a native host in Africa. A number of native Myrtaceae occur in South Africa including *Syzygium cordatum* Hachst. and other *Syzygium* spp., *Eugenia* spp. and *Metrosideros angustifolius* (L.) J.E.Sm. These trees grow in close proxomity to *Eucalyptus* plantations and any of these native African Myrtales could represent the indigenous hosts of South African *C. cubensis*

CONCLUSIONS

The huge threat and potential impact of invasive plant pathogens, make it clear that all possible means should be exercised to combat new introductions. With changes in global trade patterns new introductions of pathogens are likely to continue to occur and most probably to increase in number. If increased efforts are not made to curb these introductions, the world stands to experience new and devastating disease problems equivalent in scale to late blight of potato in Ireland, the Chestnut blight in the United States and others.

In order to achieve reduced movement of pathogens, substantially increased knowledge of pathogens will become increasingly crucial. Means of spread, techniques for identification and detection, and areas of origin of high-risk pathogens must be determined. In this regard, new technologies are providing improved methods that are likely to reduce risks. However, these increased opportunities are counterbalanced by increased trade and movement of people and products, which clearly increase risks.

In developing strategies to reduce the movement of alien pathogens, it is important to consider trends and experiences of the past and to learn from previous oversights. Similarly, knowledge of successes can be used to improve current control strategies. As the problem of invasive species is cosmopolitan and relevant globally, it is clear that co-operative ventures between countries will be essential. Invasive species do not recognise political borders and, therefore, humans should adopt the same view. Only through co-operation between all parties involved, together with research, training and education will it be possible to combat the spread and reduce the threat imposed by invasive species.

In the future, I predict that we will increasingly have to deal not only with pathogens introduced into new environments, but also with pathogens moving from native to exotic hosts. These "new" pathogens will also threaten trees in their areas of origin. In the research chapters of this thesis, I deal with alternative hosts of some important canker pathogens of *Eucalyptus* spp. Some of these pathogens clearly threaten *Eucalyptus* spp. and perhaps other related trees, in their areas of origin. Hopefully these studies will contribute, at least in part, to reducing threats that have perhaps not previously been recognised.

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