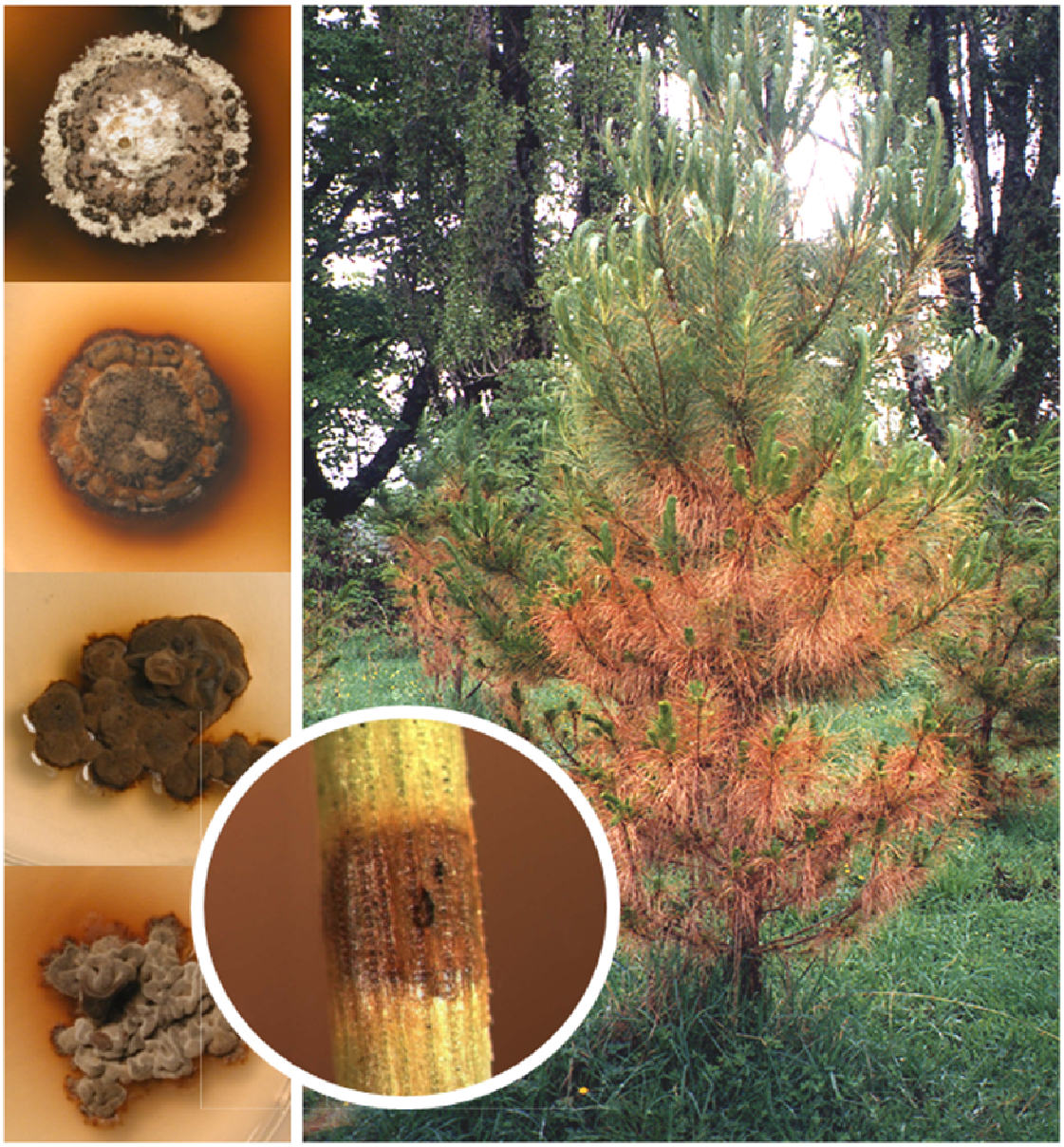
Chapter 1



Biological invasions: A pine pathogen perspective with special reference to Dothistroma needle blight

Literature Review

1.0 INTRODUCTION

Biological invasions and their potential impact on the environment have been recognised and studied for many years (Elton 1958, Richardson & Pyšek 2008, Lockwood *et al.* 2008). However, during the course of the last two decades, there has been a surge of interest, and number of publications, on this topic (Figure 1). Entire journals are now dedicated to this area of science and books have been written relating to biological invasions, invasion biology, alien invasives and emerging pests and diseases etc. (Table 1). The central theme of this recent focus is the introduction of non-native species into new environments and the subsequent impact that they have had on native species, communities and ecosystems.

In addition to the proliferation of literature, many committees have been established to deliberate on issues pertaining to biological invasions. Websites with general information and databases have been constructed with the intention of increasing public awareness and establishing the ecological and economic impact that invasive species have on environments and economies (Table 1). For example, the newly established DAISIE (Delivering Alien Invasive Species Inventories for Europe: www.europe-aliens.org), estimates that over 11,000 alien invasives have been introduced into Europe. Over 50,000 aliens are reportedly established in the United States causing an estimated economic loss of U.S. $125 billion per year (Pimentel *et al.* 2000). A global estimate of the damage caused by invasive species amounts to £1.5 trillion per year; approximately 5 % of the global GDP (CABI: http://www.cabi.org/datapage.asp?iDocID=173).

Invasive or alien species are defined as plants, animals or micro-organisms (fungi, bacteria, viruses) that are present in a new environment (Drake *et al.* 1989). They include competitors, predators, pathogens and parasites and are present in almost all native ecosystems. Generally, invasive species have a profound, often irreversible, impact on agriculture, recreation, and natural resources and they thus pose a serious threat to global biodiversity, food security, habitat quality, ecosystem functionality and economic development (Sakai *et al.* 2001, Allendorf & Lundquist 2003). The key factors associated with the increase in biological invasions during the last half century have been the growth of globalization through commercial trade and travel, resulting in an escalating introduction of organisms into new environments, either intentionally or accidentally (Mack & Lonsdale 2001, Rossman 2001, Wingfield *et al.* 2001, Brasier 2008). In addition, climate change is believed to have an

additive effect to the impact of invasives (Ayres & Lombardero 2000, Lebarbenchon *et al.* 2008).

Various life history traits and characteristics of organisms contribute strongly to the invasiveness of species. These characteristics have been very well studied in plants and animals but less so in terms of fungi and other micro-organisms (Desprez-Loustau *et al.* 2007). Invasive traits in plants, for example, include their ability to become "weedy" due to early germination with rapid growth from seedling to sexual maturity, the potential to reproduce both sexually and asexually, high tolerance to environmental heterogeneity and adaptation to environmental stress (Heywood 1989, Rejmanek & Richardson 1996). For micro-organisms, effective survival and dispersal mechanisms would be important life-history traits, as well as their ability to infect hosts and reproduce rapidly. For example, some fungi produce clamydospores that are resistant to environmental stresses and that provide an important survival strategy for the pathogen, especially during movement or transportation (Desprez-Loustau *et al.* 2007). Likewise, a common factor favouring spread is the fact that fungi produce large numbers of spores that are easily dispersed by wind and water (Agrios 1997).

Although the ecology of invasives has been well studied and documented (Lockwood *et al.* 2008, Richardson & Pyšek 2008), the genetic and evolutionary factors contributing to biological invasions are increasingly being seen to play an even more important role in understanding these invasions (Kirkpatrick & Barton 1997, Barrett *et al.* 2008). Understanding the population biology of a species makes it possible to predict aspects related to the invasive potential of the species (Allendorf & Lundquist 2003, Richardson *et al.* 2005). For example, introduced species can encounter novel stresses and selection pressures to which they might be poorly adapted (Gilbert 2002, Prentis *et al.* 2008). They might, however, be able to overcome these selection pressures by some form of adaptation or evolution due to increased genetic diversity or evolution in quantitative traits, giving them the ability to then become invasive. Studying potentially invasive species at a population level can provide an indication of the genetic factors that predisposes a species to adapt and become invasive (McDonald & Linde 2002). Thus, studies of population structure, genetic diversity, geographic patterns, range expansion, lag times and the potential for rapid evolution of invasive organisms may offer a unique understanding of the dynamics involved in the colonisation and spread of invasive species (Sakai *et al.* 2001, Prentis *et al.* 2008). This knowledge could also contribute to the management of these species by providing insight into

the stage of the invasion process that an organism has reached. This in turn could highlight whether quarantine measures are likely to be effective or whether other actions such as eradication, would be more practical (Allendorf & Lundquist 2003). Therefore, it is important to combine both the knowledge of ecology and the population genetics of organisms when attempting to predict invasions or control the invasives that cause them.

In comparison to their plant and animal counterparts, plant pathogens have not been widely studied as invasives. This is surprising given the fact that their impact has been felt for many centuries. Indeed, the rust God Robigo, who was created by the Romans a few hundred years B.C., was offered sacrifices of red dogs and sheep during the special spring holiday known as Robigalia in order to prevent infection of their grain crops by rusts (Agrios 1997).

Perhaps the two best known biological invasions caused by plant pathogens, and which have had huge impacts, are those of late blight of potatoes and chestnut blight. Late blight, caused by *Phytophthora infestans*, destroyed the entire potato crop in Ireland in 1845 leading to a large-scale famine that resulted in the deaths of hundreds of thousands of people and prompted over 1.5 million people to emigrate from Ireland to the United States (Large 1940, Bourke 1964). This epidemic was caused by a single asexually reproducing strain of *P. infestans* that was introduced from Mexico via the United States (Fry *et al.* 1993, Goodwin 1997). Similarly, *Cryphonectria parasitica* was introduced into North America from Asia around 1900 and destroyed almost all of the American chestnut throughout its native range (Elton 1958, Hepting 1974, Anagnostakis 1987).

Since the first alien invasive plant pathogens were described, there has been an exponential increase of invasive plant pathogens reported globally (Brasier 2008). In France for example, it has been documented that the rate of introduction of invasive fungi has included, on average, two species per year since the 1970's, compared to 0.5 species per year before 1930 (Desprez-Loustau 2009). The disturbing realisation that less than 10 % of the world fungi are known (Hawksworth 2001) is of particular concern, considering the potential that some of these organisms might have to cause major diseases.

The invasion process resulting from non-native species being introduced into a new area includes a sequence of events that can be divided into several definitive stages (Figure 2): These are (a) The introduction or arrival into a new habitat; (b) Initial colonisation and survival; (c) Establishment and (d) Subsequent dispersal or spread of the pathogen. During all

of these stages, there is great potential for genetic change to occur. Studying population genetic parameters such as genetic drift, gene flow, hybridisation, natural selection and adaptation could be useful in predicting whether or not a non-native or introduced species will have the potential to ultimately become invasive (Sakai *et al.* 2001, Allendorf & Lundquist 2003).

This review is arranged in three main sections. The first section provides a discussion of the different stages of invasiveness, particularly in the case of fungal plant pathogens. For each of these stages, a few examples are given where population genetic studies have provided a better understanding of the biology of the plant pathogen and aspects related to the components of its invasiveness (Table 2). There are different categories of invasiveness related to whether the host or pathogen is native or introduced and vice-versa (Figure 2). In the second section, these situations are described, specifically using examples of pine species and their fungal pathogens. Fungal pathogens chosen to illustrate these situations are either well known or those that have been particularly damaging. The third section of this review presents a case study based on the pine pathogen *Dothistroma septosporum,* which is known to be invasive in many parts of the world.

2.0 STAGES IN THE FUNGAL PLANT PATHOGEN INVASION PROCESS

2.1 Introduction of the pathogen

The first stage in the invasion process is marked by the arrival of the pathogen in a new environment. This occurs either through short distance dispersal, discussed later in this review, or long distance movement (Brown & Hovmøller 2002, Stukenbrock *et al.* 2006). Long distance transport of invasive species is usually considered to be a consequence of human activities, either directly or indirectly, and resulting from trade and travel (Mack & Lonsdale 2001, Rossman 2001, Wingfield *et al.* 2001, Brasier 2008, Sakai *et al.* 2001).

For many centuries, plants and plant parts have been moved around for agricultural, forestry and ornamental purposes, usually without quarantine to guard against the movement of pathogenic organisms (Burgess & Wingfield 2001). Thus, numerous pathogens associated with the plants or plant parts have been moved extensively. The pathogens easily avoid detection, in part due to their microscopic nature. Many also exist as symptom-less

endophytes within the plants (Schoeneweiss 1983, Stanosz *et al.* 1997), and in other cases infections are latent with symptoms only developing later (Bassett & Fenn 1984, Desprez-Loustau *et al.* 2007).

In some rusts, latency can last up to two years before symptoms develop (Diekmann *et al.* 2002). *Cronartium ribicola*, for example, is a fungal stem rust pathogen that was introduced into Western North America from Europe with shipments of eastern white pine seedlings, grown in France in 1910 (Allen & Humble 2002). In another pine example, *Diplodia pinea* (syn. *Sphaeropsis sapinea*), an endophyte and latent pathogen of pines (Smith *et al.* 1996, Flowers *et al.* 2003), was probably introduced into the Southern Hemisphere along with its host (Burgess *et al.* 2001b). Likewise, the grape pathogen *Plasmopara viticola*, a downy mildew oomycete, was introduced into Europe in 1878 with pylloxera-resistant wild American rootstocks from North America (Gobbin *et al.* 2006, Hesler 2008).

Introductions of plant pathogens are not always accidental. Fungi are for example introduced for biological control purposes (Kok *et al.* 2000, Desprez-Loustau *et al.* 2007, Goettel 1995). Likewise, beneficial mycorrhizal species have been widely moved to facilitate the establishment of commercial forestry plantations (Richardson 1998).

Long distance dispersal of pathogens can occur naturally by means of wind blown spores. This means of dispersal occurs in some fungi such as rusts that can spread on a continental and global scale (Brown & Hovmøller 2002). Aylor (2003), for example, calculated that spores of the oomycete *Peronospora tabacina* (tobacco blue mould disease) can move on average between 9 and 18 km per day via wind currents and that it has the capacity to cover distances of 500 – 1000 km. Similarly, the urediniospores of the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* can move between 16 and 76 km per day and could spread anywhere between 1500 – 2500 km via wind currents in the upper atmosphere. In one striking case, distinct pathotypes of *Puccinia graminis* f.sp. *tritici* were shown to have migrated from Southern Africa, across the Indian Ocean, to Australia in a single flight (Burdon *et al.* 1982). Meteorological data captured with high-altitude balloons showed that at this time, circular patterns were responsible for this long-distance dispersal. These amazingly effective dispersal mechanisms clearly illustrate a substantial threat to agriculture and forestry because the nature of the spread is natural, substantially complicating disease prediction and control.

The origin or source population of invasive pathogens can be elucidated by studying the levels of diversity between pathogen populations, co-evolution between host and pathogen, and by determining the historical source and sink patterns of migration amongst populations (Sakai *et al.* 2001, McDonald & Linde 2002, Burdon *et al.* 2006, Desprez-Loustau *et al.* 2007). For example, the highest levels of genetic variability observed in Central Asian isolates of the apple scab pathogen, V*enturia inaequalis,* showed that the geographic origin of the pathogen is the same as that of the host (Gladieux *et al.* 2008). Similarly, North America is considered to be the centre of origin for *Plasmopara viticola* given that American grape varieties show resistance to the pathogen (Gobbin *et al.* 2006). Banke *et al.* (2004), used phylogeographical studies to show that the origin of the wheat pathogen, *Mycosphaerella graminicola* was in the Middle East, which is considered to be the centre of origin of wheat (Harlan 1971). This was based on high sequence diversity found in these populations. Low levels of sequence divergence were found in the "new world" (North and South America and Australia) populations and thus suggested that recent migrations of this pathogen into these regions occurred as a direct consequence of the international wheat trade (Banke & McDonald 2005).

2.2 Host colonisation

After a pathogen has entered a new environment, the invasion process proceeds with a susceptible host plant being successfully infected (Sakai *et al.* 2001). Several genetic indicators can be used to distinguish between populations in their initial stages of colonisation and those that are already established.

2.2.1 Reduced genetic diversity

The main characteristic of a population that has successfully colonised its host plant is found in its reduced genetic diversity (Sakai *et al.* 2001). This generally arises from founder effects or genetic bottlenecks (McDonald & Linde 2002). Thus, a limited number of organisms are able to establish themselves and the genetic diversity of the invasive population is typically lower than that of the source population (Nei *et al.* 1975, Hartl & Clark 1989).

Founder effects result in the presence of only a few alleles of a pathogen population due to the small number of initial colonists (Sakai *et al.* 2001, McDonald & Linde 2002). Thus introduced populations have lower levels of diversity compared to those from which they were derived (Sakai *et al.* 2001, Dlugosch & Parker 2008). For example, the pine pitch canker

pathogen *Fusarium circinatum,* which causes pitch canker of pine, has limited genetic variation in its areas of introduction compared to that in its native geographic range (Wikler & Gordon 2000). Likewise, Carlier *et al.* (1996) showed that *Mycosphaerella fijiensis,* the causal agent of Black leaf streak disease of banana, has high genetic diversity and large numbers of private alleles at low frequency in its area of origin within South-East Asia. Reduced levels of genetic diversity were, however, observed in recently established populations in Latin America, the Pacific Islands and Africa. The majority of alleles observed for the pathogen in these countries were also present in South-East Asia (Carlier *et al.* 1996).

Reduced genetic diversity is thought to have a negative effect on populations because they would be more susceptible to genetic drift (McDonald & Linde 2002). Genetic drift increases the risk of losing alleles that might confer adaptive abilities for organisms to survive in their new environment (Sakai *et al.* 2001, Allendorf & Lundquist 2003). This is, however, not a limiting factor for some plant pathogenic fungi, especially where they are able to reproduce asexually or where clonal lineages of one mating type commonly dominate an area. In such cases, substantial disease occurs together with an expansion in the geographic range of the pathogen (Goodwin 1997, Milgroom *et al.* 2008). *Cryphonectria parasitica,* for example, has recently been shown to have colonized south-eastern Europe and it is spreading through many adjacent countries. These populations are all considered clonal based on low levels of genetic diversity and vegetative compatibility types and the presence of one dominating mating type. Milgroom *et al.* (2008) proposed that these "clones have greater fitness than others" and that they are able to spread because they are well adapted to the new environmental conditions.

2.2.2 Multiple introductions

Not all introduced populations are characterized by reduced genetic diversity. Many population genetic studies have found substantial levels of variation in fungal populations outside their area of origin, suggesting multiple introduction events (Dlugosch & Parker 2008). Multiple introductions can increase the diversity of the population through the introduction of different alleles. This is especially apparent where the introduction events are from different geographic sources. This situation can result in invasive populations that are more genetically diverse than a single source population. In a study by Gobbin *et al.* (2006), high levels of genetic diversity due to large numbers of alleles were found within and amongst introduced *Plasmopara viticola* populations from Europe. They suggested that several genotypes had been introduced into Europe and that these introductions occurred multiple times. The source of these introductions was probably with grapevine propagation material

that was introduced into Europe multiple times since the early 1800's. Similarly, the European race of *Gremmeniella albietina* that causes scleroderris canker has been introduced into North America multiple times as indicated by RAPD marker analyses (Hamelin *et al.* 1998). Likewise, multiple introductions have also been suggested for *Phaeosphaeria nodorum* in North America (Stukenbrock *et al.* 2006) and *Diplodia pinea* in South Africa (Burgess *et al.* 2001b).

2.2.3 Population structure and reproductive mode

Introduced populations typically display changes in population structure and reproductive mode (Sakai *et al.* 2001, Desprez-Loustau *et al.* 2007). For example, where sexual reproduction is common in a fungus, random association amongst alleles is expected (Milgroom 1996). Newly colonized populations could show evidence of linkage disequilibrium (non-random associations between unlinked markers) due to influences such as small populations, genetic drift or the introduction of populations with different allele frequencies (Gladieux *et al.* 2008). Linkage disequilibrium created by drift can be reduced, over time, through continual migration of individuals amongst populations, gene flow and growth of the population (Slatkin 1987, Carlier *et al.* 1996, Dlugosch & Parker 2008, Gladieux *et al.* 2008). Shifts in the reproductive mode could, however, be hampered if only one mating type in a sexually reproducing pathogen is introduced, resulting in the establishment of asexual populations (Gladieux *et al.* 2008). The presence of only one mating type is, therefore, an obvious indication that a pathogen has been introduced (Goodwin *et al.* 1994, Engelbrecht *et al.* 2004).

2.3 Establishment

Many factors combined determine whether a pathogen population will become successfully established in a new environment. Thus, effective establishment is determined by various life-history characteristics of the pathogen, properties of the host such as susceptibility and genetic diversity, as well as by the environment (Sakai *et al.* 2001).

2.3.1 Competition

One of the main factors contributing to successful establishment of plants and animals lies in their ability to compete. Thus, invader species typically have enhanced capabilities to utilize the local resources as compared with native species (Sakai *et al.* 2001, Allendorf & Lundquist 2003). Although this characteristic has been observed in plant pathogens, its contribution to

invasion success is poorly understood and only a few examples have been documented. In Africa, Latin America and the Pacific Islands, the introduced fungal pathogen *Mycosphaerella fijiensis* dominates and has replaced populations of *Mycosphaerella musicola*, which causes Sigatoka disease of banana (Carlier *et al.* 1996). Likewise, it has been hypothesized that *Cryphonectria radicalis*, a local saprophytic species in Europe, is being replaced by the introduced pathogen *C. parasitica* (Hoegger *et al.* 2002). The classic example is that of the Dutch elm disease in Europe and North America where the more aggressive species, *Ophiostoma novo-ulmi* out-competes the non-aggressive strain *O. ulmi* (Houston 1985, Brasier 2001). This is due to the fitness advantage that *Ophiostoma novo-ulmi* has over *O. ulmi,* which includes traits such as competitive antagonism, enhanced ability to utilize host resources (pathogenicity) and better adaptation to the climate (Brasier 2001). Thus, a situation has been reached where *O. ulmi* is no longer present in Great Britain due to its complete replacement by *O. novo-ulmi* (Houston 1985).

2.3.2 Host specificity and range expansion

One of the main contributing factors to the successful establishment of plant pathogens as invasives lies in their capacity to survive and proliferate in a new environment. This typically depends on the host specificity of the pathogen as well as the availability of a susceptible host (Barrett *el al.* 2008). These factors could impact on the rate of range expansion and the boundary of the species (Antonovics 1976, Crawley 1986). Many obligate pathogens are usually host specific and occur in very specific environments (Lajeunesse & Forbes 2002). This is likely because they have often evolved in parallel with their hosts (Barrett *et al.* 2008).

Forest pathogens provide some good examples where host specificity is often high. These pathogens may have high levels of virulence, resulting in devastating mortality, but this effect is limited by the range and spread of the host species (Loo 2008). For example, tree pathogens in North America that are limited to either a single or low number of host species, include *Ophiostoma ulmi* and *O. novo-ulmi* (Dutch elm disease), *Ceratocystis fagacearum* (oak wilt), *Sirococcus clavigignenti-juglandacearum* (butternut canker), *Cronartium ribicola* (white pine blister rust) and *Cryphonectria parasitica* (chestnut blight) (Sinclair & Lyon 2005). Pathogens that have narrow host ranges, or infect single host species are more likely to undergo recurrent extinction and re-colonisation events, especially if host populations are small and fragmented (Thrall *et al.* 2001). These processes might then advance the loss of genetic diversity within the pathogen populations (Barrett *et al.* 2008).

In contrast to host specific pathogens, facultative pathogens, including many that infect agricultural crops, generally have multiple hosts and they possess the ability to live or survive saprophytically. They are thus less restricted by the presence of a specific host and could significantly increase their geographical distribution through infection of multiple hosts (Barrett *et al.* 2008). This is clearly evident in some *Phytophthora* species. *Phytophthora ramorum,* for example, infects over 100 native and non-native North American species of trees and shrubs, and over 30 in the UK and Europe, ranging in symptoms from leaf blights to shoot diebacks (Brasier 2008, Loo 2008). Pathogens with broad host ranges experience more stable environments and host population dynamics, and can, therefore, retain higher levels of within-population genetic variation (Barrett *et al.* 2008).

2.3.3 Host jumps

For some fungal pathogens, range expansion is not limited by host. Host jumps represent a common adaptive feature of fungi, and the switching from one host species to another has often lead to the emergence of diseases that have been particularly devastating (Rizzo *et al.* 2005, Slippers *et al.* 2005, Woolhouse *et al.* 2005). The distribution of the rust pathogen *Puccinia psidii* includes Central America, South America, the Caribbean, USA (Florida) (Coutinho *et al.* 1998, Glen *et al.* 2007) and recently, Hawaii (Uchida *et al.* 2006). This pathogen has been recorded to infect guava (*Psidium guajava*) and over 15 genera and 30 species in the family Myrtaceae, which are native to most of these areas. During the 1940's in Brazil, *P. psidii* underwent a host shift to infect exotic Eucalyptus where it was noticed causing severe infection, malformation and mortality in seedlings, young trees and coppice (Glen *et al.* 2007). *P. psidii* is now recognised as one of the most serious threats to *Eucalyptus* plantations grown world-wide (Coutinho *et al.* 1998, Booth *et al.* 2000, Glen *et al.* 2007). Australia is particularly at risk to this pathogen as 70 genera and 1646 species in Myrtaceae are native to Australia as well as over 850 species of Eucalyptus (Booth *et al.* 2000, Glen *et al.* 2007).

The canker pathogen *Chrysoporthe austroafricana*, currently known only from the African continent (Nakabonge *et al.* 2006), occurs on native *Syzygium cordatum* and *S. guineense* in the family Myrtaceae and provides another intriguing example of a distinct host shift in a relatively host specific tree pathogen (Heath *et al.* 2006). In South Africa, the pathogen has greatly increased its geographic range by jumping onto the exotic host *Eucalyptus grandis* (Myrtaceae) which is widely established as an important plantation species (Wingfield *et al.* 1989, Wingfield 2003). In addition, further host jumps onto *Tibouchina granulosa* that reside

in a different family (Melastomataceae), and planted as ornamental trees in South Africa, have been recorded (Myburg *et al.* 2002).

2.3.4 Hybridisation

Hybridisation and recombination are important driving forces in the evolution of invasiveness in fungal pathogens (Allendorf & Lundquist 2003, Brasier 2000). Interspecific hybridisation between two fungal species can produce a hybrid species that has the ability to infect novel hosts with enhanced pathogenicity leading to greater levels of invasiveness than either of its parent species (Brasier 2001). For example, the hybridisation of introduced poplar rust pathogens *Melampsora medusae* and *M. larici-populina* in New Zealand resulted in a hybrid with a broader host range than that of the parent species (Spiers & Hopcroft 1994, Burdon *et al.* 2006). Similarly, the hybridisation of *M. occidentalis* and *M. medusae medusae* that infect only *Populus trichocarpa* and *P. deltoides* respectively, have hybridised to form the species *Melampsora* x*columbiana.* This phenotypically different species is capable of infecting both hosts mentioned above and hybrid clones of these hosts (Newcombe *et al.* 2000). Another example of a fungal hybrid is *Puccinia graminis tritici* and *P. graminis secalis* that can infect lines of *Hordeum vulgare* which are resistant to the parent species (Watson & Luig 1958, Burdon & Thrall 2008). In the oomycetes, an allopolyploid recombination between hybrids *Phytophthora alni uniformis* and *Phytophthora alni multiformis* resulted in a new species, *Phytophthora alni* ssp. *alni* (Brasier *et al.* 2004). This persistent subspecies is part of a new taxon (*P. alni*), and is spreading throughout European riparian forests, killing alders (Ioos *et al.* 2006).

Not all hybrid species are sufficiently fit to establish and cause disease or to survive. Rather, there commonly needs to be some selection to produce a hybrid that is more fit than its parents. For example, although *Ophiostoma ulmi* and *O. novo-ulmi* cause devastating disease on elm trees, hybrids of these two species found in Europe are relatively unfit, rare and thus short-lived (Brasier *et al.* 1998). They are unable to compete with their parents and, therefore, do not contribute towards the disease epidemics (Brasier *et al.* 1998).

2.4 Spread

Successful invasion relies on the ability of a pathogen to spread to new locations. The number of spores (propagule pressure) (Lockwood *et al.* 2005), dispersal mode, reproductive mode and survival rates are critical factors regulating the spread of invasive species (Giraud *et al.*

2008). Spread is either through short-distance dispersal, which would include gradual range expansion of the pathogen in the present location, or long distance founder events (Gladieux *et al.* 2008). In fungi, large numbers of spores can be dispersed locally by means of rain-splash, via soil movement, or they can be water-borne as in the case of *Phytophthora* spp. (Lacey 1967). Some also have insect vectors as is true for *Ceratocystis* spp. and *Ophiostoma* spp. (Moller & DeVay 1968, Wingfield *et al.* 1993). These restricted or local methods of dispersal could result in reduced dispersal as distance from the source increases and could be a factor that would generally decrease effective population size (Barrett *et al.* 2008).

Long-distance dispersal of plant pathogens is typically associated with either human intervention in transporting infected plant material and seeds, or naturally via wind-dispersed spores (Brown & Hovmøller 2002, Stukenbrock *et al.* 2006). Pathogens that have high levels of dispersal (wind-blown spores) and thus high geneflow, will tend to display higher levels of population genetic diversity and isolation over distance, than those with more limited modes of dispersal (Barrès *et al.* 2008, Barrett *et al.* 2008). Some soil-borne pathogens such as *Armillaria* spp. spread by means of vegetative growth via rhizomorphs and can clonally expand their range by a few meters each year (Richardson *et al.* 2005, Prospero *et al.* 2008). Using microsatellite fingerprinting profiles, Coetzee *et al.* (2001) showed that various *Armillaria mellea* samples collected from the Company Gardens in Cape Town, South Africa, form part of one genet. This genet is estimated to be between 108 and 575 years old, which has spread to a diameter of about 345 m since its introduction into the country by early European settlers (Coetzee *et al.* 2001).

3.0 THE INVASION OF PINES AND ASSOCIATED PATHOGENS

Forest ecosystems provide interesting examples for studies that consider the dynamics within and among host-pathogen populations. They are also useful in efforts to determine the factors that shape the genetic structure of host and pathogen populations (Hamelin *et al.* 1998). This is particularly true because trees have long life cycles and diseases can be studied over sufficient periods of time to be able to understand patterns of host pathogen interaction. For the purpose of this review, a focus on *Pinus* spp. and their pathogens has been chosen to exemplify patterns of invasion.

3.1 Pines as invasives

Pines are considered to be the most successful invasive hardwood trees (Moran *et al.* 2000). Their invasive success has largely been facilitated by humans who have exploited the ability of these trees to grow well and to adapt to new environments, especially in nutrient-poor habitats (Poynton 1977, Le Maitre 1998). Thus, they have been taken from their native ranges in the Northern Hemisphere and widely planted in exotic locations, especially in the Southern Hemisphere, for ornamental and commercial plantation purposes (Richardson *et al.* 1994, Richardson *et al.* 2007). Pines produce large quantities of seed and pollen that disperse effectively and isolated individuals can also give rise to colonies by selfing (Poynton 1977). As a result, *Pinus* spp. species are able to expand rapidly in new environments, and populations have exploded to the point where they have become invasive in both their native and introduced environments (Richardson & Higgins 1998). Southern Hemisphere countries are particularly severely affected with exotic pine species dominating indigenous landscapes, displacing native plant species and causing huge ecological disturbances (Richardson *et al.* 1994, Richardson & Higgins 1998). Twenty three *Pinus* species have become naturalized in the Southern Hemisphere, eighteen of which are invasive in South Africa, South America, Australia and New Zealand (Richardson & Higgins 1998, Richardson & Petit 2006).

Although pines are considered noxious weeds in many countries, they have, along with *Eucalyptus* spp., become some of the world's best forest plantation species and are of great importance to local economies (Richardson & Petit 2006). Pines are not only highly valued for products such as solid wood, pulpwood and fuel wood, but the plantations provide employment and are a source of wealth creation (Toro & Gessel 1999, Moran *et al.* 2000, Richardson *et al.* 2007). In some situations, they have also been useful in stabilising eroded environments (Toro & Gessel 1999). One species in particular, *Pinus radiata*, native to a small belt along the coast of California and the offshore island of Guadeloupe (Poynton 1977, Rogers 2002), is an important commercial plantation species in the Southern Hemisphere. Over four million hectares of *P. radiata* have been established in South Africa, Chile, Argentina, Australia and New Zealand, with Chile and New Zealand having in excess of 1.5 million ha each (Toro & Gessel 1999, Rogers 2002).

3.2 Pine pathogens

Anthropogenic movement of pines has resulted in pine pathogens being moved over long distances through the movement of contaminated planting stock (Diekmann *et al.* 2002). For example, over 80 different *Pinus* species from various countries in Europe, Asia and North

America have been introduced into South Africa (RSA) since the European colonisation of the Cape more than 350 years ago (Poynton 1977), bringing with them over 115 exotic pests and diseases (Lundquist 1987). These introduced pathogens have in many cases become established and they have subsequently spread to cause serious disease problems (Harrington & Wingfield 1998, Wingfield 1999, Wingfield *et al.* 2001). The movement of pines and pathogens around the world, both in their native and non-native environments, presents a variety of examples illustrating the manner in which disease epidemics can occur. These patterns of invasion are discussed below.

3.3 Categories of invasion

3.3.1 Native host - native pathogen

In situations where both the host and pathogen are native, both would have co-evolved over long periods of time in their centres of origin (Gilbert 2002). Gene for gene resistance and other selective pressures would have maintained the environment in a state of equilibrium or general homeostasis (Parker & Gilbert 2004, Burdon *et al.* 2006). Epidemics are thus unlikely to occur due to these strong ecological pressures (Wingfield 1999, Burdon *et al.* 2006). In many cases, native pathogens are present without causing substantial disease, as is evident in natural forests, where the host trees are relatively resistant to cankers and foliage diseases resulting in these pathogens having only a minor impact (Harrington & Wingfield 1998).

If a native pathogen were to gain pathogenicity due to an evolutionary change that was maintained in the populations from events such as chromosome loss, somatic hybridisation, or recombination (Burdon *et al.* 2006), the state of equilibrium could be altered. In such cases, a native pathogen would gain the capacity to infect a native host, causing noticeable disease. Additionally, changes in the ecosystem could also alter the state of equilibrium. Stumps left open after the felling of pines in natural forests can, for example, create infection sites for root rot pathogens such as *Heterobasidion annosum* and *Armillaria* spp. (Hood *et al.* 1991, Harrington & Wingfield 1998, Sinclair & Lyon 2005). This could increase the pathogen inoculum to unnaturally high levels, which would escalate attacks on healthy trees (Wingfield 1999).

Climate change is another factor that can change the equilibrium state of an ecosystem where host-pathogen co-evolution is in effect (Ayres & Lombardero 2000, Burdon *et al.* 2006). This

is especially true for pathogens that are repressed from causing disease due to unfavourable historic climate conditions (Evans 1984). Increased temperatures might induce stresses on the host (e.g. drought) that would make it more susceptible to infection (Desprez-Loustau *et al.* 2006). In contrast, some pathogens are very sensitive to temperature in terms of infection potential and reproduction (Peterson 1973, Gadgil 1977). Increases in temperature could favour pathogen virulence, increasing opportunities for infection, and they could also lead to shortened life cycles leading to disease development (Anderson *et al.* 2004).

3.3.2 Native host - introduced pathogen

Where pathogens are introduced into new environments where there has been no host–pathogen co-adaptation (Parker & Gilbert 2004, Barrett *et al.* 2008), serious disease problems can emerge due to lack of appropriate resistance genes in the native hosts. These encounters are referred to as 'new encounter' or 'novel interaction' (Parker & Gilbert 2004) and they include some of the worlds most serious tree disease problems including Dutch elm disease, white pine blister rust and chestnut blight (Anagnostakis 1987, Brasier 2001, Kinloch 2003, Sinclair & Lyon 2005) .

White pine blister rust caused by *Cronartium ribicola* is probably the best example of this category of disease on pines caused by a fungal pathogen. The pathogen was first introduced into Europe from Asia and then, in the 1890's, into North America (Fernando & Owen 2004, Diekmann *et al.* 2002). In its area of origin in Asia, *C. ribicola* has co-evolved with the native pines which developed a high level of resistance to the pathogen (Butin 1995, Loo 2008), with only low levels of disease ever seen. In contrast, white pine blister rust is one of the most destructive tree diseases of all native North American white pines and has caused catastrophic disease epidemics (Butin 1995, Kinloch 2003, Fernando & Owen 2004). By killing adult trees, natural forests have been opened up, transforming the landscapes. Changes in local environments as a result of the forests being destroyed (Burdon *et al.* 2006), has also resulted in significant ecological damage (Fernando & Owen 2004, Loo 2008). Both the Grizzly bear (*Ursus arctors*) and the Clark's nutcracker (*Nucifraga columbiana*), for example, are highly dependent on the seeds produced by white bark pine, and the decline of white pines has had a concomitant negative effect on these animals (Loo 2008).

Although intercontinental spread of *C. ribicola* was through anthropogenic movement, population studies have shown that recombination and long-distance spore dispersal also appears to be an important mechanism for survival and spread for this pathogen (Et-Touil *et*

al. 1999, Hamelin *et al.* 2005). Furthermore, the ability of the pathogen to infect multiple host species in North America is most likely due to its outcrossing nature which would increase its evolutionary potential for survival (Richardson *et al.* 2005). A considerable amount of research has gone into breeding for resistance against this pathogen through selection and screening (Sniezko *et al.* 2001, Kinloch 2003). However, the possibility of new races of the pathogen being re-introduced from Asia, or new strains developing, is a constant threat (Sniezko *et al.* 2001).

In California, pitch canker disease caused by *Fusarium circinatum* (= *F. subglutinans*) represents another good example of an introduced pathogen affecting a native host (Aegerter *et al.* 2003, Gordon 2006), and it is considered one of the most important diseases of pine globally (Gordon 2006, Wingfield *et al.* 2008). The epidemic of this disease on native *Pinus radiata* in California has resulted in extensive mortality of the species. These losses are detrimental considering this area is the only existing source of genetic material of *P. radiata* used extensively for commercial forestry plantations (Rogers 2002).

Analyses of vegetative compatibility groups and RFLPs analyses of mitochondrial DNA have displayed low levels of diversity in the Californian populations of *F. circinatum*, consistent with recent introductions and clonal propagation (Correll *et al.* 1992, Gordon *et al.* 1996). These introductions are thought to have originated from South Eastern U.S.A where they shared common genotypes in high frequency (Wikler & Gordon 2000), and where the disease has been known since 1945 (Hepting & Roth 1946). Mexico is considered to be the origin of *F. circinatum* (Gordon 2006) due to high levels of genetic diversity that have been found for isolates of the pathogen collected in that country (Wikler & Gordon 2000). In Mexico, epidemics occur only in "off site" plantations, regardless of the pathogen being widespread there. This reinforces the notion that the fungus is in "host-pathogen equilibrium" with the native forests including many different pine species in that region (Wikler & Gordon 2000).

3.3.3 Introduced pine - native pathogen

When pines are moved out of their natural habitat, one of two distinct situations can arise. Either the exotic pine is infected by native pathogens or alternatively, they can be infected by non-native pathogens that have been co-introduced or introduced at a later stage (Parker & Gilbert 2004). The latter situation is most common in the Southern Hemisphere (Wingfield 1999, Wingfield *et al.* 2001). In both these situations, pathogens that are considered of minor

importance where the pines are native can have a devastating effect on pines planted outside their native range (Harrington & Wingfield 1998).

There are various examples of introduced pines being affected by native pathogens. A well recognised example is found with the introduced *Pinus contorta* in Northern Sweden, native to North America and the native pathogen *Gremmeniella abietina* (Richardson *et al.* 2005). The pathogen causes the disease known as Scleroderris canker and it results in large-scale infection and damage to these introduced trees (Karlman *et al.* 1994). In contrast, it has relatively little impact on the two native forests species *P. sylvestris* and *Picea abies* (Barklund & Rowe 1981, Hellgren & Barklund 1992).

There are various examples of native pathogens infecting introduced pine species in Southern Hemisphere pine plantations (Gibson 1979). Certainly the best example is found in the case of Armillaria root rot, which is found in most Southern Hemisphere countries where pines are grown as non-natives in plantations (Coetzee *et al.* 2000, Wingfield *et al.* 2001). Because they are native, the species of *Armillaria* spp. are different in these countries, but the disease problems and general symptoms are very similar (Hood *et al.* 1991, Shaw & Kile 1991). Armillaria spp. have very wide host ranges and non-native pines have been seriously damaged by them in most countries where they have been established in plantations.

3.3.4 Introduced pine - introduced pathogen

A good example of the co-introduction of pines and fungal pathogens is found in the case of *Diplodia pinea* (syn. *Sphaeropsis sapinea*) in Southern Hemisphere plantations. This pathogen is known to exist as an endophyte in healthy pine tissue (Smith *et al.* 1996, Stanosz *et al.* 1997) and has clearly been introduced into the Southern Hemisphere along with its hosts (Gilmour 1966, Gibson 1979, Burgess & Wingfield 2002). It is also, however, an opportunistic pathogen that infects pines when they are stressed (Wingfield 1999). The stress factors can either be as a result of new and unsuitable planting environments, severe wounding due to hail damage or when the trees suffer from drought (Swart & Wingfield 1991). *D. pinea* has been particularly destructive in plantations of the non-native and susceptible host, *P. radiata*, in RSA, New Zealand and Australia (Chou 1976, Currie & Toes 1978, Swart *et al.* 1987) causing shoot blight, stunting and deformation of trees and branch and trunk cankers (Gibson 1979, Swart & Wingfield 1991).

Diplodia pinea also provides a good example where molecular tools have been useful in determining the correct taxonomic position of the pathogen (Burgess *et al.* 2001a, de Wet *et al.* 2003). This has allowed for an improved understanding of how the pathogen has been introduced around the world (Burgess *et al.* 2001b, Burgess *et al.* 2004a). This has been particularly important as a foundation for population genetic studies.

Four morphotypes of *D. pinea* have been described and these have been provided with the designations A, B, C and I (Smith & Stanosz 1995, Hausner *et al.* 1999, de Wet *et al.* 2000). Multiple gene genealogies and microsatellite markers subsequently revealed that morphotype "B" represents a discrete taxon known as *Diplodia scrobiculata* (de Wet *et al.* 2003). This species is limited to North America and Europe where it is genetically highly diverse (Burgess *et al.* 2004b). Using simple sequence repeat (SSR) markers, Burgess *et al.* (2001a) further showed that morphotype "I" represents *Botryosphaeria obtusa*. Only two morphotypes (A and C) are thus responsible for the disease caused by *D. pinea.* These morphotypes differ in morphology, distribution, host specificity and virulence (de Wet *et al.* 2003).

In the case of *D. pinea,* SSR markers have revealed that the A morphotype has a global distribution but is relatively clonal, suggesting that only some genotypes are successful as endophytes (Burgess *et al.* 2004a). The low diversity of the pathogen could be advantageous in disease management strategies where trees are selected for tolerance or bred for resistance. In contrast, the more virulent C morphotype is known only to Indonesia (de Wet *et al.* 2002). The introduction of this morphotype into other countries should clearly be avoided by strict quarantine regulations.

4.0 DOTHISTROMA NEEDLE BLIGHT (DNB) AS A PINE PATHOGEN CASE STUDY

4.1 Introduction

Dothistroma needle blight, caused by a haploid ascomycete, *Dothistroma septosporum*, is considered to be one of the most important pathogens affecting exotic *Pinus* species, especially in the tropics and Southern Hemisphere (Gibson 1972, Ivory 1987, Harrington & Wingfield 1998, Bradshaw 2004). This fungal pathogen causes the well known disease commonly known as red band needle blight of pines or Dothistroma needle blight, and is

characterised by needle infections, defoliation, retarded growth and in severe cases, tree death (Gibson *et al.* 1964). The disease is identified by erumpent black conidiomata or fruiting bodies underneath the epidermis of the needles, which are normally surrounded by red or brown bands (Gibson 1972).

Dothistroma septosporum provides an excellent example to illustrate an introduced pathogen that has seriously damaged an introduced pine species. It further provides a good example of how the alteration of an environment by man has mediated both the introduction of a pathogen and the development of the disease. One of the key reasons why *Dothistroma* has become such an important pine disease problem globally, lies in the fact that extensive monocultures of a susceptible host have been established in areas where the environmental conditions are optimal for disease development.

The following section is not intended as a review of the literature pertaining to DNB as this topic has been treated extensively in previous reviews (Gibson 1972, Bradshaw 2004). Rather, this section serves as an introduction to the studies that make up this thesis, which includes investigations on the taxonomy and population genetics of *D. septosporum*. Here, I include the relevant characteristics of *Dothistroma*, especially in the Southern Hemisphere, in relation to the processes of invasion of plant pathogens as discussed above and presented in Figure 2. Furthermore, areas where research on the pathogen is lacking are highlighted.

4.2 Origin of the pathogen

Dothistroma septosporum has been known since the turn of the last century, where it was first described from *P. mugo* in Russia in 1911 (Doroguine 1911). Since then, the pathogen has been reported in over 45 countries in Eurasia, Africa, Oceania and the Americas (EPPO: http://www.eppo.org/QUARANTINE/fungi/Mycosphaerella_dearnessii/SCIRSP_ds.pdf, Ivory 1994) and infecting over 70 different species of pine (Bednářová *et al.* 2006). There have been no studies to ascertain the original, natural range of the species, although two hypotheses regarding its origin have been proposed. Evans (1984) suggested that the pathogen might be native to the high cloud forests of Central America. This was due to the presence of the pathogen on native pines, without causing epidemics, in secluded areas, far removed from anthropogenic activities that might have introduced the pathogen. Similarly, its occurrence in remote areas of the indigenous blue pine forests in the Himalayas prompted Ivory (1994) to suggest that it might also be native to these areas.

4.3 Introductions into new environments

There are no clear records detailing the introduction of Dothistroma into the Southern Hemisphere. However, as pines are not native to the Southern Hemisphere, it is reasonable to assume that *Dothistroma septosporum*, which is a pine specific pathogen, is also exotic in these regions. While pines were deliberately introduced into the Southern Hemisphere, the introduction of *Dothistroma* would have been accidental through infected pine seeds or plant material. It has been speculated that Dothistroma was introduced into New Zealand, for example, by forestry officials who visited East Africa in 1957 to observe the epidemics of the disease (Hirst 1997). The fungus was discovered five years after this visit, causing disease in central North Island forests (Gilmour 1967).

The increase in air traffic and development of pine plantations, especially after World War II, marks the period that Dothistroma was probably introduced into many different parts of the Southern Hemisphere (Table 3) (Gibson 1972). The first reports of *D. septosporum* in Central Africa were during the 1930's and 1940's (Barnes 1970, Gibson 1972), followed by Chile in 1957 (Gibson 1972), New Zealand in 1962 (Gilmour 1967) and Australia in 1975 (Edwards & Walker 1978). In each case, an epidemic of the disease was observed only years after the initial discovery. In all cases, the presence of Dothistroma was observed only after extensive plantations of the susceptible host, *P. radiata,* had been established. This was despite the fact that many other pine species were present in these countries for more than a century (Table 3). This could be attributed to a lag period which is sometimes required to allow for adaptive evolution to occur or, alternatively, the lag period could be attributed to ecological factors that are required for successful colonisation e.g. optimal temperature, correct inoculum pressure and host availability (Sakai *et al.* 2001).

4.4 Pathogen Colonisation

For the invasion process to be secured, conditions allowing successful colonisation, establishment and spread must be optimal for Dothistroma. In terms of colonisation, the infection process is important (Gadgil 1967); for establishment and spread, the availability and the susceptibility of the pine species is essential (Gibson 1972). In all cases, the environment is a critical factor (Gilmour 1981).

4.4.1 Infection process

Inoculum pressure is important for successful infection, and for *D. septosporum* on mature *P. radiata,* this has been estimated to be about 100 conidia/mm^2 (Hirst 1997). Conidia on the needle surfaces germinate and the germ tubes enter the host via stomata (Gadgil 1967, Peterson & Walla 1978), which is a process that can take three or more days (Gadgil 1967). Once infection has occurred, inter- and intracellular hyphal growth takes place within the necrotic mesophyll tissue. Tissue necrosis is a result of the production by the pathogen of the toxin dothistromin, which either kills the tissue directly or induces a host response resulting in cell death (Bradshaw 2004). This toxin is also responsible for the development of the typical red colour (Shain & Franich 1981) that is associated with the symptoms of this disease. Black conidiomata full of conidia develop within these red bands and further symptoms include necrosis of the entire needles causing premature defoliation (Gibson *et al.* 1964).

4.4.2 Environmental conditions

Temperature and moisture are the main environmental factors influencing the success of an infection in *D. septosporum*. Cool, wet conditions, such as several days of rain or overcast, humid weather, favour germination and spread of the pathogen (Gilmour 1981, Peterson 1973). Germination of conidia occurs only between 8-25 °C with an optimum at 18 °C (Ivory 1967b) and where there is high humidity (> 96 %) or extensive periods of needle wetness (Peterson 1973, Gadgil 1977, Gilmour 1981). Light does not affect conidial germination or growth on the needle surface, but it may affect the degree of infection and symptom expression (Ivory 1967b, Gibson *et al.* 1967) which may be related to dothistromin production (Bradshaw 2004). Gadgil & Holden (1976) noticed that shaded areas are less affected by disease than those exposed directly to the light and suggested it might be related to a host response. Soil nutrition is another factor that has an effect on host susceptibility. Soil deficient in available sulphur or high in nitrogen increases the host's vulnerability to infection by Dothistroma (Edwards & Walker 1978, Lambert 1986).

4.4.3 Life-cycle

Temperature has a significant effect on the life-cycle of *D. septosporum*. The period between germination and the appearance of symptoms has been reported in Kenya and Tanzania to range from between five weeks (in warmer climates) to four months. After the first symptoms have appeared, it can take a further 1- to- 2 weeks before conidia are produced, completing the life cycle (Gibson *et al.* 1964, Hocking & Etheridge 1967, Gibson 1972). If symptoms only appear in autumn, sporulation may be delayed until the following spring. In New

Zealand, the life-cycle of *D. septosporum* has been noted to be as short as two weeks in optimal environmental conditions (Gadgil 1974). *D. septosporum* in the Southern Hemisphere can thus have multiple generations in one growing season, increasing its infection and epidemic potential. In the colder climates of Europe, it can take a year or two before the *D. septosporum* life-cycle is complete. Infection usually occurs during the late spring or summer months and the pathogen over-winters in infected needles. Sporulation occurs only the following year during the spring and summer months (Butin 1985). Thus, in these colder climates, it can take years of continual infection before the effects can be seen because increment loss is proportional to the level of infection (Van der Pas 1981).

4.5 Establishment

4.5.1 Competition and niche opportunity

The ability of a species to out-compete other organisms that might potentially occupy the same niche could be advantageous to an invasive pathogen. *D. septosporum* is known as an ecologically obligate parasite, with the host providing a habitat that is non-competitive, rather than for a specific nutrient required by the pathogen. This is due to the slow growth, lack of specific nutrient requirements and inability of *D. septosporum* to survive for extended periods when competing with other saprophytes (Gibson 1972). Recently, Schwelm *et al.* (2008) showed that the toxin dothistromin, which is produced during early infection and colonisation, may have a role in competition. This would allow Dothistroma "to protect its niche from other micro-organisms" (Schwelm *et al.* 2008) and it could explain why Bradshaw *et al.* (2000) found higher levels of the toxin (5 to 500 fold more) in strains from the Bavarian Alps compared to isolates from New Zealand. In the Northern Hemisphere, where pines and this pathogen are native, *D. septosporum* would be under higher selection pressure to produce the toxin and protect its niche against many other pathogens and saprophytes. Non native pines would have a reduced number of pathogens and possibly endophytes, which would provide a greater number of niches for an invading pathogen to occupy. The loss of toxin production over time in New Zealand could, therefore, be due to a lack of selection pressure on the pathogen to retain increasing levels of virulence.

4.5.2 Species richness

Probably the single most important factor that has contributed to the success of *D. septosporum* as an invasive species in the Southern Hemisphere is the "niche opportunity" available for extensive establishment and spread. There has been a long history of different

pine species (over 80 in South Africa) being introduced into Southern Hemisphere countries (Table 3). Seeds and plant material have been obtained from various sources world-wide including seed merchants in Europe (Austria, France and Italy), America and Japan, along with other introductions from Holland, Portugal, Guatemala and Mexico (Poynton 1977). *D. septosporum* could have been introduced into the Southern Hemisphere any number of times from these Northern Hemisphere counties. It was, however, only after large areas of the susceptible host, *P. radiata* were planted, mainly as monocultures, that the pathogen established itself and spread, causing severe epidemics. The countries most affected are Chile, New Zealand and Australia, which combined, produce over 92.03 % of the total world production of *P. radiata* (Rogers 2002). It is interesting however, that *P. radiata* is not susceptible to the disease in its natural range in California, but that it does cause disease epidemics on non-native *P. radiata* plantations just a few kilometres up the North coast of California (Cobb *et al.* 1969).

4.5.3 Range expansion

Under optimal environmental conditions, *D. septosporum* is capable of extending its range within a relatively short period of time. Within seven years of the first report of it affecting the foliage of young *P. radiata* in the Usumbara Mountains of Tanzania, the disease was documented to have spread into all major plantations of *P. radiata* in Kenya, Malawi and Uganda (Gibson *et al.* 1964). Similarly, in New Zealand, range expansion of the epidemic was rapid with over 127 000 acres being infected within three to four years (Gilmour 1967).

Certain host factors could curb expansion of a DNB epidemic. The age of the host seems to be a critical factor for infection. *P. radiata* in particular shows a certain amount of resistance to pathogen infection once they reach maturity at an age of approximately 15 years or older (Gibson 1972, Ivory 1972). Seedlings and young trees are, therefore, most susceptible. Also, the establishment of large monocultures of pine does not necessarily mean that the plantation will be affected. For instance, no signs of *D. septosporum* have been observed on large plantations of the more resistant *P. patula* and *P. elliottii* in South Africa, despite the plantations being established for many years (Anonymous 2001).

4.5.4 Host jumps

Host jumps do not represent a mechanism whereby *D. septosporum* would increase its range as its host range is limited to pine species. Although the pathogen is known to infect over 70 different pine species (Bednářová *et al.* 2006), various levels of susceptibility are known.

Pinus radiata, P. nigra and *P. ponderosa* are the most susceptible while *P. patula* has been recorded as being one of the most resistant or immune species (Cobb & Libby 1968, Gibson 1979). *D. septosporum* has rarely been reported infecting other conifers including *Pseudotsuga menziesii* (Dubin & Walper 1967), *Larix decidua* (Bassett 1969), *Picea abies* (Lang 1987), *Picea sitchensis* (Gadgil 1984) and *Picea omorika* (Karadžić 1994). In all of these cases however, the trees were adjacent to highly infected sites with high inoculum pressures.

4.5.5 Hybridisation

As stated previously, hybridisation and recombination are important driving forces for the evolution of invasiveness amongst fungi (Allendorf & Lundquist 2003). Hybridisations have not been reported for *D. septosporum*, although three different varieties of the pathogen have been described based on differences in the average conidial length (Thyr & Shaw 1964, Ivory 1967a). There has, however, been considerable debate as to whether conidial size represents an appropriate character by which to distinguish among forms or varieties of *D. septosporum* (Funk & Parker 1966, Gadgil 1967, Sutton 1980). Evans (1984), studied a considerable collection of world-wide fungi, finding appreciable contrasts in both anamorph and teleomorph morphology. He contested the validity of varieties in *Dothistroma*, but acknowledged that morphotypes or ecotypes probably do exist. Bradshaw *et al.* (2000) also failed to support the distinction between the varieties based on morphology. They did find, however, in one of the first molecular analyses on this species, some ITS sequence differences between isolates from New Zealand, Europe, South America and parts of North America (Oregon and Canada) with those from North America (Minnesota and Nebraska). Because the numbers of isolates included in this study were low, no clear hypothesis could be formulated for the observation of sequence differences between isolates.

4.6 Spread

Once established, *D. septosporum* spreads via asexual conidia that are produced in the conidiomata on the needles. Short distance dispersal in the pathogen is very effective. When mature, the presence of water ruptures the epidermis and releases the spores (Peterson 1973), which are dispersed via run-off water from the needles or a splash take-off mechanism (Gibson *et al.* 1964, Ivory 1987). The spores can also become airborne in tiny mist droplets. Maximum conidial dispersal is thus found to occur under light rain or heavy mist conditions, where the spores can be carried over several distances. *D. septosporum* can be spread

effectively between trees in a plantation or nursery or even between plantations (Gibson *et al.* 1964, Ivory 1987), thus effectively expanding its range. Spores of *D. septosporum* can remain viable within dry plant material for many months depending on the environment. At 18 °C, spores can survive for up to a year, while at higher temperatures spores can survive between a few weeks up to five months (Gibson *et al.* 1964, Ivory 1967b). Spores will germinate again once exposed to water (Ivory 1987).

The introduction of *D. septosporum* into Australia is thought to have occurred via conidia present in mist clouds that blow over the Tasman Sea from New Zealand (Edwards & Walker 1978). This view is supported by that fact that stringent quarantine regulations in Australia would make it improbable that an introduction via plant material occurred (Edwards & Walker 1978, Bradshaw 2004). This hypothesis was substantiated when charred pieces of vegetation were found in New South Wales after a firestorm occurred during the burning of an infected plantation in New Zealand (Matheson 1985). The fungus in Australia is, therefore, hypothesised to be genetically the same as that in New Zealand.

Sexual ascospores are also produced by *D. septosporum* in ascostromata, but these generally occur later in the disease cycle and are produced primarily on dead needles or those that have been cast (Butin 1985). The sexual state of *D. septosporum*, which produces these sexual spores, known as *Mycosphaerella pini* (previously *Scirrhia pini*) (Funk & Parker 1966), has been observed only in certain countries in the Northern Hemisphere (Peterson & Graham 1974, Evans 1984, Kowalski & Jankowiak 1998). Thus, only the clonally produced asexual spores of *D. septosporum* have been found in the Southern Hemisphere.

4.7 Control

Three measures are currently utilized to inhibit spread and to control DNB. These include silvicultural practices to reduce inoculum, chemical spray applications and the development of DNB resistant *P. radiata* planting stock. Silvicultural practices include pruning lower branches to reduce the humidity within the trees, removing underbrush (weed control) and burning infected trees (Ahumada pers. communication, Bulman *et al.* 2004). Copper fungicides e.g. Bordeaux mixture (Cuprous oxide and copper oxychloride) applied aerially once or twice per year have been very effective (Peterson 1981, Gibson 1972) as they inhibit germination of spores (Franich 1988). Dothistroma Resistant (DR) *Pinus radiata,* bred in New Zealand, is available for commercial planting and is reported to have successfully

reduced mean infection levels by 12-15 % (Carson & Carson 1991, Dick 1989, Chou 1991, Bradshaw 2004). This DR breed might only be effective in New Zealand, however, as an extensive study on the *Dothistroma* populations in New Zealand showed them all to be clonal (Hirst 1997). Thus, resistance was bred against only one genotype. Based on the clonal reproduction of this pathogen, resistance in the host will be maintained unless a mutation which might confer greater pathogenicity is fixed during the frequent cycles of reproduction or a more virulent strain is introduced into the country.

4.8 Global epidemics

Most research on *D. septosporum* in the past focused on the epidemics that the pathogen caused in plantations of exotic pine species in the Southern Hemisphere (Ivory 1967a, Gibson 1972). Although the pathogen was known to be present in the Northern Hemisphere, there was no real cause for concern as the disease was not serious. This was partly due to natural selection pressures or temperatures and levels of humidity that were not optimal for disease development (Evans 1984). However, in the last two decades, an increase in the incidence and severity of DNB has been recorded in the Northern Hemisphere (Bradshaw 2004). Here, epidemic levels of the disease have been evident on susceptible, exotic plantations within affected countries: *P. nigra* subsp. *laricio* (Corsican pine) in the U.K. (Brown *et al.* 2003) and France (Aumonier 2002), *P. contorta* var. *latifolia* (lodgepole pine) in British Columbia (Woods 2003, Woods *et al.* 2005), and *P. nigra* (Austrian pine) in the Czech Republic (Jankovský *et al.* 2004) and Hungary (Koltay 2001) for example. Apart from these epidemics, new geographic areas and hosts have also been reported for this disease (Bradshaw 2004 and references therein, Bednářová *et al.* 2006). The concern in the Northern Hemisphere is that DNB may not be limited to pines grown in intensively managed plantations but that it may pose a serious threat to *Pinus* species in natural forests (Maschning & Pehl 1994, Aumonier 2002, Brown *et al.* 2003, Woods 2003, Woods *et al.* 2005). One of the explanations for this increase in epidemics, and the presence of *D. septosporum* on new hosts, is that global climate change has lead to previously unfavourable environments now being conducive to infection (Woods 2003). This has resulted in escalated international concern regarding this pathogen (Bradshaw 2004).

5.0 SUMMARY AND CONCLUSIONS

Biological invasions are caused by species that have been introduced into new areas and that threaten, or have the potential to cause harm in their new environment. In all cases, invasive species undergo a process that includes introduction into an area, colonisation, establishment and spread. These stages of invasions can either be studied in terms of the ecology of the species by studying life history traits and characteristics of the species or by studying the population genetics of the species.

Plant pathogens have not been as extensively studied as invasives to the degree that plants, animals and to some extent, insects have been treated. The more recent use of population genetics to study aspects related to invasions and the evolutionary potential of species has provided excellent opportunities to study the invasiveness of plant pathogens that otherwise might have been difficult.

In many cases for plant pathogens, every stage of the invasion process leaves a genetic 'imprint' in the genetic variation within and among populations (Gladieux *et al.* 2008). By studying the diversity and population structure of individuals within a population, information relating to origins of species, source and sink populations, recent population introductions etc, can be elucidated. All of these provide an improved understanding of the evolutionary, and thus invasion potential of these pathogens. Many examples have been provided in the text of this review.

The invasion process for plant pathogens can be categorised into four different situations. These include 1) Native host, native pathogen; 2) Native host, introduced pathogen; 3) Introduced host, native pathogen and 4) Introduced host, introduced pathogen. Using these different categories requires a clear understanding of the origin of the host and the origin of the pathogen. Pathogens in their native environments on native hosts generally do not cause disease, however, if the pathogen is then moved to a new environment it can either infect native or introduced plants to cause disease.

A central observation to have emerged from this review is that in many cases, it has been humans, either directly or indirectly, that have perpetuated the emergence of disease. This has either been through travel and trade, thereby creating an introduction pathway for pathogens to spread, or through the alteration of the environment, such as via agriculture or plantation

forestry, which can lead to the rapid emergence of disease epidemics. *Dothistroma septosporum*, as evident in the case study, provides a useful example of where both situations apply to the pathogen and the subsequent disease epidemics that have been associated with it. Thus, *D. septosporum* has become one of the most successful invasive pathogens, especially in the Southern Hemisphere on the widely grown exotic *P. radiata.*

The ecology of *D. septosporum* has been very well characterised on the basis of infection processes, life-cycle and mode of dispersal, in an attempt to better understand the pathogen and to develop control strategies to reduce its impact. However, there is a distinct lack of knowledge relating to the genetics aspect of the pathogen, its pattern of spread or the source of introductions. Many hypotheses have been raised regarding patterns of spread of *D. septosporum*, but most remain unconfirmed. The presence of a teleomorph in the Northern Hemisphere indicates that sexual reproduction is occurring in that area of the world. In contrast, in the Southern Hemisphere, only the asexual state is known. This suggests that while in its native range, *D. septosporum* could be evolving due to the possibility of recombination but that the pathogen in the Southern Hemisphere is reproducing clonally. This has already been established for the populations present in New Zealand where DR forms of *P. radiata* have been planted. The accidental introduction of more virulent genotypes in this, or other Southern Hemisphere countries, could result in new disease outbreaks. There is thus a critical need to study the populations of *D. septosporum* and to determine the genetic diversity and structure globally.

Accurate taxonomy is clearly a key factor in understanding the global distribution and management of *D. septosporum*. At the time of embarking on this study, only one species of Dothistroma was known to cause DNB. However, different varieties of the species had been described based on conidia morphology and the possibility that different morphotypes or ecotypes existed was a distinct possibility. Furthermore, the report of differences in ITS sequences between isolates from different countries made it important to further investigate the relationships that might exist between these isolates at a genetic level.

The main focus of the studies presented in this thesis will be to validate or substantiate some of the issues raised above. Because the morphology of different *D. septosporum* isolates has already been extensively studied, the work presented in this thesis will focus on expanding the studies started by Bradshaw *et al.* (2000) and Ganley & Bradshaw (2001). Molecular phylogenetic tools will thus be used to determine whether morphotypes or ecotypes exist by

considering the phylogenetic relationships of isolates from different countries. In addition, we will determine whether DNA sequence data validates the separation of *D. septosporum* into different varieties. Detailed studies on populations of *D. septosporum* from a world-wide collection are likely to yield valuable information pertaining to population diversities and structure of this pathogen. These data, to be obtained using microsatellite markers, will hopefully be valuable in determining aspects related to the genetics of the pathogen which could aid in the development of future management and quarantine strategies for *D. septosporum.*

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Table 1: Lists of some websites, databases, books and journals that are dedicated to the topic of biological invasions

Acronym / Name		**URL / Editors / Authors / Publishers**
Websites:		
APHIS	Animal and Plant Health Inspection Service General information	www.aphis.usda.gov/invasivespecies
2010 BIP	The 2010 Biodiversity Indicators Partnership	www.twentyten.net/
CABI		www.cabi.org/datapage.asp?iDocID=173
DAISIE	Delivering Alien Invasive Species Inventories for Europe	www.europe-aliens.org
GISP	Global Invasive Species Programme	www.gisp.org/
ISSG	Invasive Species Specialist Group	www.issg.org/
NOBANIS	North European and Baltic Network on Invasive Alien Species	www.nobanis.org/
	Alien Invader Plants	www.geocities.com/wessaaliens/index.htm
	Alien Invasive Species in China	www.chinabiodiversity.com/shwdyx/ruq/ruq-index-en.htm
	Japanese Knotweed Alliance	www.cabi.org/japaneseknotweedalliance/
Databases:		
AgNIC	Agriculture Newwork Information Center	laurel.nal.usda.gov:8080/agnic/
AKEPMP	Alaska Exotic Plant Mapping Project	agdc.usgs.gov/akepic/
APIRS		plants.ifas.ufl.edu/search80/NetAns2/
	Regulated Pest List	www.invasivespecies.org/NewInitiatives.html
	Alien Plant Invaders of Natural Areas in the U. S.	www.nps.gov/plants/alien/list/a.htm
	Alien Species in Hawaii	www.hear.org/
	Alien Species in Poland	www.iop.krakow.pl/ias/
	Lithuanian invasive species database	www.ku.lt/lisd/species_lists/fungi_all.html
Go to: http://42explore.com/invasive.htm "The Topic: Invasive species" for a list of over 70 additional websites.		
Books:		
Handbook of Alien Species in Europe (2008)		DAISIE 2008
Invasion Ecology (2008)		Lockwood JL, Hoopes MF, Marchetti MP (2008)
Invasive Alien Species: A Toolkit of Best Prevention and Management Practices (2001)		Edited by Wittenberg R & Cock MJ (2001)
Invasive species in a changing world (2000)		Edited by Mooney HA, Hobbs RJ
Biological Invasions - A global perspective. Scope 37 (1989)		Edited by Drake JA, Mooney HA, diCastri F, Groves RH, Kruger FJ, Rejmanek M, Williamson M.
The ecology of invasions by *Pinus* (Pinaceae) and *Hakea* (Proteaceae) species, with special emphasis on patterns, processes and consequences of invasion in mountain fynbos of the southwestern Cape Province, South Africa (1989)		Richardson DM
The ecology and management of biological invasions in southern Africa (1986)		Macdonald IAW, Kruger FJ, Ferrar AA
Journal:		
Biological Invasions		Editor-in-Chief: Daniel Simberloff / Springer Netherlands

Table 2: The ecological/life history traits and possible population genetic structure of plant pathogens during the different stages of invasion.

INVASION STAGES	PLANT PATHOGEN TRAITS	
	ECOLOGY / LIFE TRAITS	POPULATION GENETIC EVIDENCE
Origin / Source	Centres of diversity	areas of origin have high genetic diversity, large no of private alleles
	Host-pathogen interactions	Co-evolution between host and pathogen
		can determine historical source and sink patterns of migration
		source populations can be identified if introduced population shares identical genotypes
Introductions	Man-mediated via endophytes in plants, plant parts e.g. germplasms, seeds	
	Single introductions	low leves of diversity/clonality/one mating type
	Multiple introductions	high levels of genetic diversity/both mating types
Colonisation	Small number of individuals are introduced or spread into a new area - host infection takes place	founder effects or bottleneck = reduced genetic diversity
	Reproduction	
	asexual (spores, vegetative spread)	Gametic disequilibrium / linkage disequilibrium / clonal
	sexual	gametic equilibrium / random association amongst alleles
	homothallic	inbreeding- clonal or reduced genetic diversity
	heterothallic	outbreeding- large amount of genotypic diversity
Establishment	Host specificity	
	nonobligate pathogens (wide host range)	maintain higher levels of within population genetic variation
	obligate parasites (narrow host range or host specific)	experience frequent local extinction and re-colonisation events = loss of genetic diversity
	Range expansion	migration and geneflow = increases genetic diversity reducing effects of drift
	Host jumping	recombination events
	Hybrids	hybridisation events or recombinations
Spread	Long distance dispersal (air-borne spores)	low population sub-structure
	Short distance dispersal (rain-splash)	high population sub-structure

Table 3: Dates are provided for when pines in general and more specifically, *P. radiata*, were introduced into some Southern Hemisphere countries. Dates are also recorded for when the susceptible species *P. radiata* was extensively grown in plantations and when the first reports and epidemics of Dothistroma needle blight (DNB) occurred.

	Pines introduced	*P. radiata* introduced	Extensive plantations of *P. radiata*	First reports of DNB	Epidemics of DNB	References
Kenya			1945-1960 (1)	1960 on *P. radiata* (from Tanzania) (2)	1964 (3)	1) Lavery & Mead 1998; 2) Gibson 1972; 3) Gibson et al 1964
Tanzania				1957 -young *P. radiata* Tanganyika (Tanzania) (1)	1960 (1)	1) Gibson 1972
Malawi			1928-1934 resumed again in 1955 (1)	1940's on *P. radiata* (1)	1962 (1)	1) Gibson 1972; 2) Lavery & Mead 1998
Zimbabwe		1902 (3)	1928-1934 (2) resumed again in 1955 (1)	Middle 1930's on *P. radiata* (4)	1962 (1)	1) Gibson 1972; 2) Lavery & Mead 1998; 3) Poynton 1977 4) Barnes 1970
South Africa	late 17th century (1)	1850 (2)	1884 (2)	1965 on *P. canariensis* (3)	1984 - increased occurance (4, 5)	1) Poynton 1977; 2) Lavery & Mead 1998; 3) Gibson 1972; 4) Lundquist 1987; 5) Ivory 1994
Chile		1885 (1)	1940's (1)	1957 on *P. radiata* (2)	1964 - 1965 (2)	1) Toro & Gessel 1999; 2) Gibson 1972
New Zealand	Shortly before 1830 (1)	1859 (2)	1870's (3)	1962 on *P. attenuata* x *P. radiata* but probably present since late 1950's (4)	1966 (5)	1) Richardson & Higgins 1998; 2) Hirst et al 1999; 3) Lavery & Mead 1998; 4) Gilmour 1967; 5) Gibson 1972
Australia	1770's with British colonisation (1)	1857 (1)	1875 (2)	1975 on *P. radiata* (3)	1977 (3)	1) Richardson & Higgins 1998; 2) Lavery & Mead 1998; 3) Edwards & Walker 1978

Figure 1: Number of publications returned from a search of the ISI Web of Knowledge using the "topic" criteria and the search words "invas*" and "ecology*", and "biological" and "invas*" for the years 1968 to 2008. An increasing trend is observed from the year 1992 onwards.

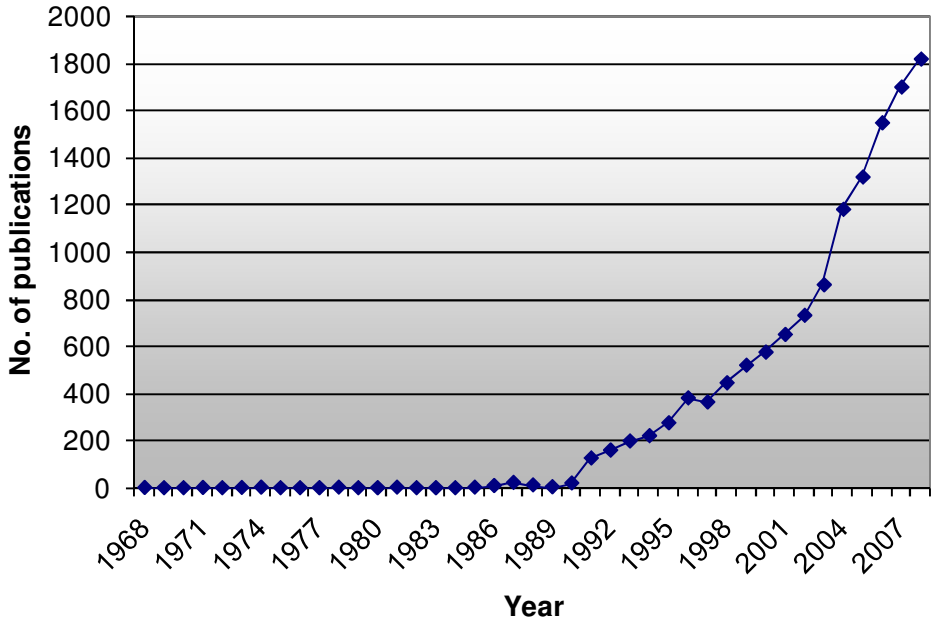
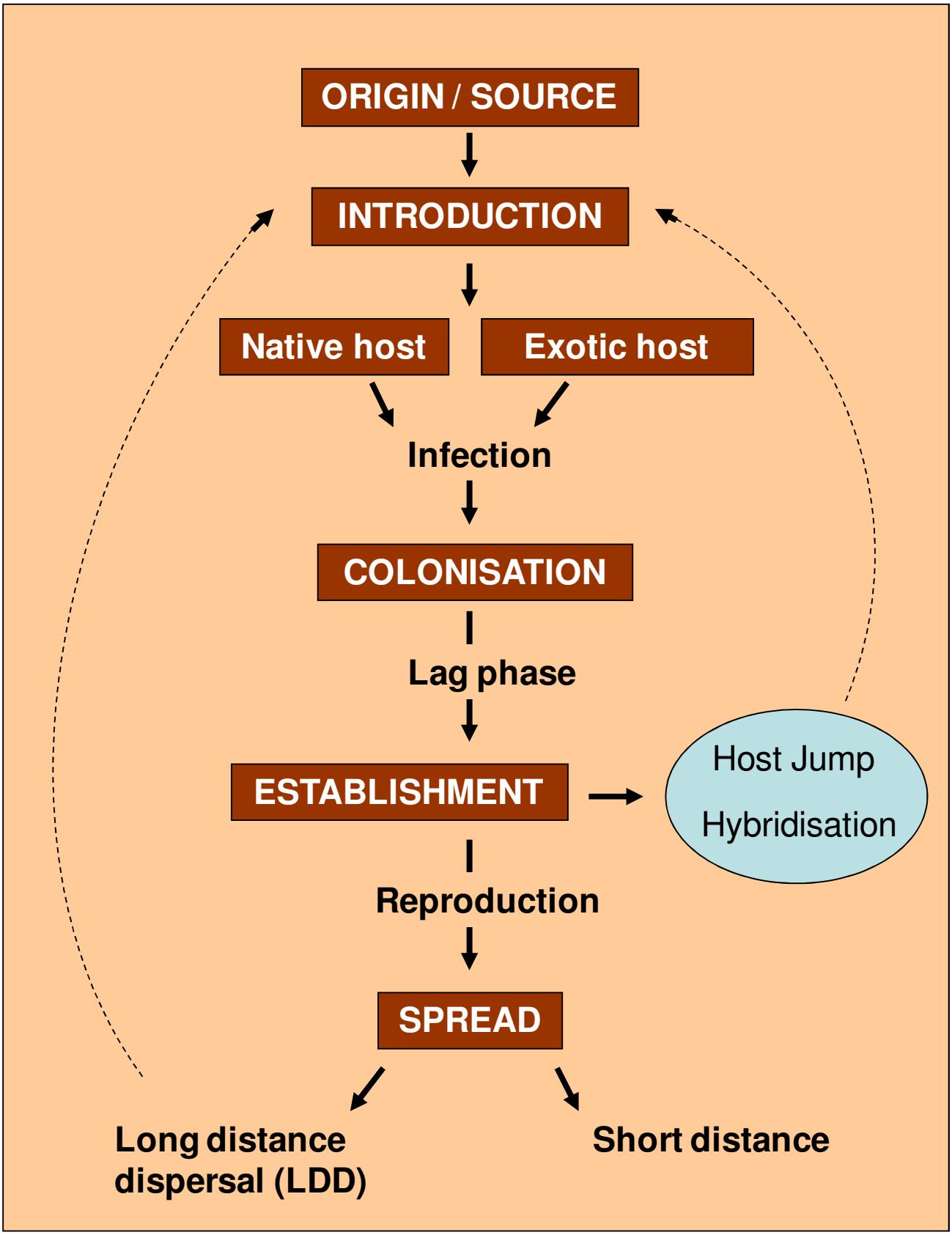

2000
1800
1600
1400
1200
1000
800
600
400
200
0
No. of publications
1968
1971
1974
1977
1980
1983
1986
1989
1992
1995
1998
2001
2004
2007
Year

Figure 2: The stages of invasion of a plant pathogen after it has been introduced onto a new area. Different scenarios of infecting native or exotic susceptible hosts, host-jumps or hybridisations are possible before the pathogen reproduces and spreads to new locations.


ORIGIN / SOURCE
INTRODUCTION
Native host
Exotic host
Infection
COLONISATION
Lag phase
ESTABLISHMENT
Host Jump
Hybridisation
Reproduction
SPREAD
Long distance dispersal (LDD)
Short distance