Reducing the risk of pitch canker disease (caused by *Fusarium circinatum*) to *Pinus patula* in South Africa

by

Richard Glen Mitchell

Submitted in partial fulfilment of the requirements for the degree

Philosophiae Doctor

In the faculty of Natural and Agricultural Science
University of Pretoria
Pretoria

May 2012

Study leaders;
Prof. Teresa Coutinho
Prof. Emma Steenkamp
Prof. Mike Wingfield

Declaration

I, the undersigned, hereby declare that this thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria, contains my own independent work and has hitherto not been submitted by me for a degree at this or any other tertiary institution.

Richard Glen Mitchell

May 2012

Table of contents

Acknowledgements

I would like to thank;

My supervisors, Mike Wingfield, Teresa Coutinho and Emma Steenkamp, particularly for their assistance in editing the chapters herein.

Bill Dvorak and Gary Hodge of Camcore, for their valuable contribution to many of the chapters.

Komatiland Forests for incurring the costs to screen the families and clones used in this study, and for making the data available for analysis and publication.

York Timbers for covering the University registration fees, traveling expenses, time to complete the manuscript, and for some of the data presented.

The University of Pretoria, the National Research Foundation (NRF) and members of the Tree Protection Co-operative Program (TPCP) for financial assistance.

Most importantly, my wife Nicky, for her patience and encouragement during the completion of these studies.

Preface

Pinus patula is South Africa's most important pine species due to it good growth and wood properties. However, for almost two decades the pitch canker fungus, *Fusarium circinatum*, has been the cause of large scale mortality of *P. patula* seedlings in nurseries and after planting. Historically the pathogen has been confined to nurseries in South Africa but the pathogen has now begun to infect mature trees and this appears to be an increasing threat to pine forestry in the country. In order to limit the negative effect that *F. circinatum* has on *P. patula* seedling survival, and to reduce the risk of infection on mature trees, it is accepted that the most effective approach will be to improve the tolerance of the planting stock.

In Chapter 1 of this thesis, previous work on *F. circinatum*, with particular emphasis on its occurrence in South Africa, is reviewed. Current methods used to control this pathogen on *P. patula* seedlings and young trees are discussed and long-term approaches to improve the resistance of the planting stock are suggested. In Chapter 2, *P. elliottii*, *P. maximinoi* and *P. tecunumanii*, which are well suited to subtropical sites, and *P. pseudostrobus*, which is suited to temperate sites, are inoculated with *F. circinatum* to determine their potential as a replacement for *P. patula* on these sites.

In Chapter 3, families of *P. patula x P. tecunumanii*, and various other pine hybrids, are tested via inoculations with *F. circinatum* to determine their tolerance. *Pinus patula x P. tecunumanii*, in particular, has been shown in previous studies to be the most promising hybrid and of particular importance in South Africa. In Chapter 4, the frost tolerance of *P. maximinoi* and *P. tecunumanii*, which are two of the most promising alternative species to *P. patula* on the warmer sites in South Africa, is considered. An additional objective in this chapter is to determine whether the frost tolerance of these two species can be improved thereby extending their planting range to include cooler sites.

Studies in Chapter 5 consider the variation between full-sib *P. patula* families in their tolerance to *F. circinatum*. The purpose of the study is to determine whether improvements can be made

by identifying specific parental combinations that are more resistant to infection by *F. circinatum*. In Chapter 6, *P. patula* families, as seedlings in repeated greenhouse studies and as trees, are inoculated with *F. circinatum* and the variation between and within families assessed. The value of screening families of *P. patula* in the greenhouse, by comparing the tolerance of these as seedlings with the tolerance of the same families as mature trees, is thus evaluated. Lastly, the tolerance of seedlings established from seed collected from the mature trees which were inoculated with *F. circinatum* is compared. In Chapter 7, the results of the aforementioned studies are summarised and, with these results in mind, the future of *P. patula* in South Africa with reference to *F. circinatum* is considered.

Note: throughout this thesis the term "tolerance" is used to describe the ability for the plant to resist infection by F. circinatum. Plants that displayed little or no disease expression after inoculation are referred to as "tolerant", whilst those that displayed a high degree of infection are referred to as "susceptible".

Published in Southern Forests (2011) vol: 73: 1-13

Chapter 1:

The pitch canker fungus, *Fusarium circinatum*; Implications for South African forestry

Abstract

Fusarium circinatum, the causal agent of pitch canker of mature pines and root/collar rot of pine seedlings/cuttings, has resulted in large scale losses to pine forestry in various parts of the world. The disease, caused by this fungus, is now regarded as one of the most important threats to pine plantations by a pathogen. *F. circinatum* was first discovered in South Africa in 1990 where it infected *Pinus patula* seedlings in a nursery. Subsequently, the pathogen spread to pine nurseries in all other parts of the country, where it affects several *Pinus* species. *F. circinatum* then appeared in the field where it has resulted in large-scale mortality of mostly young *P. patula* seedlings after planting. Pitch canker first appeared on mature *P. radiata* in 2006 and sporadic outbreaks of the disease have subsequently occurred on this species and on *P. greggii* in the Western, southern and north-east Cape provinces. *P. patula* is the most important softwood species grown in South Africa comprising 50% of all softwoods planted and is highly susceptible to *F. circinatum*. The pathogen, therefore, poses a potentially devastating threat to the future sustainability of the South African softwood industry. Strategic measures to minimize further spread are urgently needed. This review presents an overview of the impact that *F. circinatum* has had on South African forestry, and it considers the long term prospects for pine forestry in the country as this relates to the presence of the pitch canker fungus.

Keywords: *Pinus patula*, South Africa, economic impact, host tolerance, disease management

Introduction

Pitch canker is a serious disease of pines caused by the fungus *Fusarium circinatum* (=*Gibberella circinata*) (Nierenberg and O'Donnell, 1998; Britz et al. 2005). *F. circinatum* was first described on infected *P. virginiana* trees in North Carolina, USA (Hepting and Roth 1946). Subsequently, the pathogen has been identified in Haiti (Berry and Hepting 1959), all of southeast USA down to Florida (Dwinell et al. 1985), California (McCain et al. 1987), Japan (Kobayashi and Muramoto 1989), South Africa (Viljoen et al. 1994), Spain (Dwinell et al. 1998), Mexico (Guerra-Santos 1999), Chile (Wingfield et al. 2002b), Italy (Carlucci et al. 2007) and most recently Portugal (Bragança et al. 2009).

In most parts of the world, the pitch canker pathogen is best known for the damage that it causes to established trees. Although it was known to occur on nursery seedlings elsewhere (Barnard and Blakeslee 1980; Dwinell 1999), its emergence as a major nursery pathogen in South Africa was a problem unique to this country (Viljoen et al. 1994, 1997; Wingfield et al. 2002a, 2008; Bayley and Blakeway 2002), and later also in Chile (Wingfield et al. 2002b) and Portugal (Bragança et al. 2009). In South Africa, the impact of the pathogen in nurseries has extended beyond the direct losses to nursery plants. Indeed, it is the losses that are experienced during establishment of new plantations (Mitchell et al. 2004; Crous 2005; Wingfield et al. 2008) that represents the most important manifestation of *F. circinatum* in South Africa. Within 10 years of its first detection in South Africa, *F. circinatum* has emerged as one of the greatest constraints to pine plantation forestry, particularly to *P. patula*, in the country.

A considerable body of international research has been conducted on the pathogen and the disease that it causes (Bethune and Hepting 1963; Cashion 1975; Dwinell et al. 1977; Dwinell and Phelps 1977; Wilkinson et al. 1977; Blakeslee and Oak 1980; Fisher et al. 1981; Dwinell and Barrows-Broaddus 1981; Storer et al. 1998; Dwinell and Fraedrich 1999; Gordon et al. 2001). In this paper we summarise some of this research, focussing on those most important aspects relating to the future sustainability of *P. patula* in South Africa. Data, estimating the economic cost of *F. circinatum* on poor establishment, are also presented. We further link this to the contamination rate measured in nursery studies. The importance of breeding for host tolerance in

P. patula, and the use of hybrids and alternative species in effectively managing the disease, is discussed as the most effective long term solution.

Symptoms

In the nursery, the primary symptoms of infection by *F. circinatum* include an initial tip wilting of pine seedlings. This is followed by the discoloration of the area beneath the growing tip (usually purpling) and, as the disease progresses, the seedlings turn brown and die (Fig. 1a-b). During the early stages of infection, root tips die and the roots are no longer able to take up water. Collar rot is also often observed. In the advanced stages of infection, fungal growth can occasionally be seen on the seedling stem (Fig. 1c). Also, hedge plants are particularly susceptible to infection as they are frequently wounded during shoot harvesting (Fig. 1d).

On mature or established plantation trees, pitch canker displays symptoms including branch die-back, infected cones and the development of large resinous stem cankers and resin-soaked wood (Fig. 2a-f) (Bethune and Hepting 1963; Dwinell and Phelps 1977; Storer et al. 1998, 1999). In South Africa, this form of the disease has been known for only a short while (Coutinho et al. 2007) and is localized to moist coastal areas in the southern Cape, similar to those in California (Gordon et al. 2001), and on marginal sites of the north-eastern Cape (personal observation). Nevertheless, the pitch canker pathogen elicits markedly different symptoms on pines in the plantation and nursery environments, with the latter usually associated with much more rapid plant mortality (Barnard and Blakeslee 1980). To recognize these distinct forms of the disease caused by the pitch canker fungus, the nursery seedling form of the disease is referred to as *F. circinatum* infection, while the term “pitch canker” refers exclusively to the disease on mature or established plantation trees (Wingfield et al. 1999).

The disease symptoms that develop during plant establishment are similar to those in the nursery. Mortality usually commences within the first 3 months after planting, with maximum losses between month 3 and 6, which can persist up to 1 year (Crous 2005). In most cases, the greatest mortality is seen during the first winter (dry) season after good initial growth, indicating that the

pathogen is favoured by stressful conditions (Dwinell et al. 1977; Crous 2005). However, disease symptoms can also affect recently planted seedlings during the same planting season. In such cases, survival is worse on warm wet sites while better on the moist cool sites along the Mpumalanga escarpment, where there is constant air movement (Mitchell et al. 2009).

Infection and Dispersal

Infection of mature or established plantation trees by *F. circinatum* is reported to occur only through wounds that act as infection sites (Dwinell and Barrows-Broaddus 1981; Storer et al. 1998). These are commonly made by twig, bark and cone beetles such as species of *Ips*, *Conophthorus*, *Ernobius* and *Pissodes nemorensis*, which are also known to vector the pathogen (DeBarr and Barrows-Broadduss 1986; Storer et al. 1998; Coutinho et al. 2007). It is believed that the general absence of bark and twig-boring insects in South Africa is a possible reason that outbreaks of pitch canker on mature pine trees has remained absent for so long (Wingfield et al. 1999, 2002b). A notable exception is *P. nemorensis*, which is a well-established pest in South African pine plantations (Gebeyehu and Wingfield 2003). This insect was also found associated with the symptomatic trees examined in the Western Cape region where the first outbreak of pitch canker was detected (Coutinho et al. 2007). However, the exact role of *P. nemorensis* in the epidemiology of the disease in South Africa remains to be determined, because pitch canker could be facilitated by a number of other wounding or vectoring agents. For example, baboons have become a serious pest in stripping bark from trees (McNamara 2006). The pathogen can also gain access to the host through pruning wounds (Sakamoto and Gordon 2006). In the reported pitch canker outbreak on mature *P. radiata* in the southern Cape (Coutinho et al. 2007), stem cankers were frequently found developing around pruned branch whorls (Fig. 2d) and baboon damage sites (Fig. 2c). Based on these facts, it would seem that there is sufficient wounding on mature trees to facilitate the rapid spread of the disease in South Africa.

Fusarium circinatum has the ability to infect seeds both internally and externally (Storer et al. 1998; Dwinell and Fraedrich 1999), which is thought to be a major source of nursery infection (Storer et al. 1998; Dwinell and Fraedrich 1999; Wingfield et al. 2008). In fact, the pathogen was

probably introduced into South Africa through the importation of infected seed (Wingfield et al. 2008). This could have occurred on any one of the several introductions of *P. patula*, or of any other seed collected from Mexico, since the first introduction of *P. patula* in 1907 (Dvorak 1997). Most seed sourced from South African seed orchards, however, are still free of *F. circinatum* as these are not collected in heavily infested areas (Wingfield, unpublished) as seen elsewhere in the world (Storer et al. 1998). The high incidence of *F. circinatum*-associated disease in South African pine seedling nurseries is, therefore, attributed to contaminated nursery containers, irrigation water, media and plants are also a source of infection (Coutinho et al. 2007; Wingfield et al. 2008).

Once *F. circinatum* is established in the nursery, it is not fully understood how infection takes place as there is little evidence linking infection to wounding alone. Adult fungus gnats (*Bradysia* spp.) are known to vector plant pathogens such as *Botrytis cinerea* and *Fusarium proliferatum* in pine nurseries and their larvae feed on root material providing infection courts (James et al. 1995). As such it has been suggested that the fungus gnat (*B. difformis*), commonly occurring in South African pine nurseries, may be associated with *F. circinatum* (Hurley et al. 2007). However, this hypothesis has been tested and no link between the presence of *B. difformis* and seedling infection could be established (Hurley et al. 2007). Also, it has been well documented that increasing the amount of nitrogen to pines increases infection by *F. circinatum* (Cashion 1975; Wilkinson et al. 1977; Fisher et al. 1981) possibly due to stimulation of succulent shoot growth (Dwinell et al. 1977; Gordon et al. 2001). If succulent tissue is predisposed to infection, this may explain why young pine seedlings in the nursery display disease symptoms without wounding.

It is commonly assumed that field infections of young plants result from planting out contaminated or infected nursery seedlings. There is strong evidence of this from previous studies. Mitchell et al. (2009) isolated *F. circinatum* from non-sterilized roots of an average of 38% of *P. patula* seedlings at the time of leaving the nursery (Fig. 3) as well as from the roots of other species and hybrids, albeit in lower concentrations (Fig. 4). In these studies it was found that the average contamination rate in winter is lower compared to spring or summer (Fig. 3), with one exception from a bed sampled in June 2008 (Fig. 3). This is consistent with the general

experience of South African nurserymen and has been recorded elsewhere (Hammerbacher et al. 2005a). It is further relevant that the level of contamination for the different species or hybrids in the studies carried out by Mitchell et al. (2009) was consistent with the general level of tolerance of these species or hybrids in inoculation studies (Roux et al. 2007) (Fig. 4).

Whether the pathogen actually gains access to the host via wounds made during planting, or whether the host is already infected upon planting is not clear. Due to the fact that non-sterilized root tips were examined in the studies carried out by Mitchell et al. (2009), it may be possible that the pathogen exists as an inhabitant of the rhizosphere as described by others (Muramoto et al. 1993; Hammerbacher et al. 2005b). However, it has also been documented that plant tissue may become internally infected where *F. circinatum* can remain in a latent state before disease symptoms are expressed (Storer et al. 1998). It has been debated as to whether the presence of *F. circinatum* within healthy plant tissue can be described as an endophyte, in which case, the pathogen would have no effect on tree growth until stress results in disease development . Given the fact that the pathogen is not isolated from healthy tissues of established trees, it is very unlikely that *F. circinatum* is a typical endophyte. Although it is assumed that a reduction of spore loads in the nursery will result in fewer contaminated/infected seedlings, the observed benefits of systemic fungicides, like Benomyl (Mitchell et al. 2004; Crous 2005), may support the theory that internal infection also plays a role. Understanding the principal means of nursery to field transfer of this pathogen will, in the future, promote a better understanding of the epidemiology of *F. circinatum*.

Economic impact

Most information regarding the economic impact of the pitch canker fungus relates to the disease as it occurs on mature trees. Very little information has been amassed relating to the impact that *F. circinatum* has in its manifestation as a seedling pathogen in nurseries or during establishment. Although there is no doubt that much of the poor survival is related to contamination/infection by *F. circinatum* in South Africa, it is notable that other factors also play a contributing role in survival, especially with regards to *P. patula*. The effects of high

temperatures, drought, insects and other pathogens have all been known to affect the survival of *P. patula* (Morris 1990, 1991; Allan and Higgs 2000; Rolando and Allan 2004). An increase in herbicide damage to young *P. patula* plantings has also been noted (Mitchell, unpublished). These factors may make it difficult to quantify the impact of *F. circinatum* on survival.

To better understand the cost to the South African industry, where it remains predominantly a nursery and reestablishment problem, losses due to *F. circinatum* have been measured by rouging dying plants in the nursery and carrying out field survival counts. Roguing dying plants usually amounts to less than 1% of the crop (Mitchell, unpublished). However, field losses are significant. In a comprehensive study, covering 16 different sites, Crous (2005) reported that *F. circinatum* was isolated from 42% of all dying plants removed from the field in the months following establishment. The study was conducted over two planting seasons between 2003 and 2005. His data showed that mean survival was 36 - 53% after the first year. Based on these data, and the fact that survival of less than 30% is often recorded, it can be assumed that at least half of all measured mortality (50%) is due to *F. circinatum*. Thus, for every 100 plants planted, 25 would probably die because they were either infected or contaminated by *F. circinatum* in the nursery. The remaining 25 would die from other causes mentioned.

Using the information generated by Crous (2005), and the current establishment costs (pitting, planting and blanking), Mitchell et al. (2009) calculated this loss to be R602/ha for the saw timber industry, and R896/ha for the pulpwood industry (Table 1). Extrapolated over South Africa, where approximately 15,000 ha are planted annually to *P. patula* (DAFF 2008), the impact of *F. circinatum* on tree survival costs the local forest industry in excess of R11 million per year (Mitchell et al. 2009). Morris (2010) recently estimated this to be R12 million, when *P. radiata* is included together with *P. patula*.

At this stage, insufficient data exist to quantify the economic loss due to pitch canker outbreaks in the Southern and South Eastern Cape on *P. radiata* and *P. greggii*. Based on field infections elsewhere in the world, the most significant impact of pitch canker occurs due to the loss in volume growth. Reduction in growth of *P. elliottii* infected stands has been reported to be 15% - 72% depending of the severity of infection (Bethume and Hepting 1963; Dwinell and Phelps

1977; Arvanitas et al. 1984). In more tolerant species such as *P. elliottii* and *P. taeda*, not all infected trees die (Dwinell and Phelps 1977). However, in severe cases, mortality in *P. elliottii* stands can reach levels of 25% with up to 98% of all trees showing some signs of infection (Blakeslee and Oak 1980). Gordon et al. (2001) have also reported a 98% infection rate in *P. radiata* where, due to the susceptibility of the species, more trees would be expected to die. If the disease manifests itself as a serious problem in plantations in South Africa, losses are likely to be of a similar magnitude in the infected areas. However, as seen from examples in California (Gordon et al. 2001) and South Africa (Coutinho et al. 2007), infection is greatest on coastal areas. It also appears from the outbreak on *P. greggii* in the north Eastern Cape that dry marginal sites are also at risk (personal observation). Based on these experiences, it could be speculated that the areas on the Mpumalanga escarpment may be less threatened.

Disease management

Field

One of the main strategies for preventing the occurrence or spread of pitch canker in established pine plantations is to limit wounding. Although this may in part be possible through effective control of insect and baboon damage, appropriate silvicultural practices are also important. For example, it has been shown that pruning wounds increase the risk of infection by *F. circinatum* (Sakamoto and Gordon 2006), which may be particularly important in saw timber stands. Care should be taken not to damage standing trees by harvesting equipment and tree removal when thinning compartments. Although pitch canker has not become an established pathogen of seed orchard trees in South Africa, cones should be clipped and not sheared from the branch whorls when harvesting (Dwinell and Barrows-Broaddus 1981; DeBarr and Barrows-Broadduss 1986).

There are indications that water stress predisposes trees to infection (Dwinell et al. 1977) and thus, the stress associated with the high stocking density of pulpwood stands, has been observed (Dwinell and Phelps 1977; Blakeslee et al. 1992; Barnard and Blakeslee 2006). This can, therefore, be limited by avoiding the plantings of susceptible species such as *P. patula*, on sites prone to drought, and carrying out timely thinnings (Berry and Hepting 1959; Blakeslee and

Rockwood 1999). It has also frequently been reported that pitch canker outbreaks are often associated with the application of nitrogen (Cashion 1975; Wilkinson et al. 1977; Barnard and Blakeslee 2006). The benefits of improving growth through mid-rotation fertilizer application are being considered and practiced in some areas of South Africa (Carlson et al. 2008). If the fertilizer applied contains higher amounts of nitrogen, relative to the other macro nutrients in the fertilizer, this practice may be counterproductive in the cases where pitch canker has become established or where the risk of infection is high.

Nursery

Controlling *F. circinatum* in pine nurseries remains the most important means of reducing field mortality at establishment. Some nurseries report that the control of *F. circinatum* is particularly difficult, while others report better control. This may be because nurserymen do not wish to disclose the extent of the problem, but there may also be some scientific evidence for this observation. Previous studies on *F. oxysporum* have indicated large variation in virulence for different isolates on nursery seedlings (James and Gilligan 1984), and also differences in virulence of isolates collected from different nurseries (James et al. 1999). Similarly, the same has been shown for different isolates of *F. circinatum* (Dwinell, 1978; Gordon et al. 2001). This suggests that isolates, unique to specific nurseries in South Africa, may have resulted in the observed differences in the severity of outbreaks between other nurseries in South Africa. Nevertheless, nurserymen have now come to accept that once *F. circinatum* is present in their nurseries, eradication is almost impossible. At best, the disease can be managed through an integrated management approach. Maintaining effective nursery hygiene is practiced by nurserymen and nurseries are audited annually by the Seedling Growers Association of South Africa to ensure high standards. Nurserymen are required to regularly remove dying seedlings and dispose of them by removal from the site or burning (PFWG 2004).

Reported control of *Fusarium* on seed includes soaking seeds in diluted solutions of ethanol, sodium hypochlorite, hydrogen peroxide, in hot water (90 seconds at 55.5 ^{o}C) and imbibing seeds with a biological control agent (*Pseudomonas chlororaphis*) before cold stratification (Dumroese et al. 1988; Hoefnagels and Linderman 1999). The fungicides Benomyl and Thiram have also been used to treat seed (Dwinell 1999). Most South African nurseries sterilize their

trays by treating with steam (70-80 ^{o}C) for 60-90 minutes in sealed containers (Kruger pers. comm. Top Crop nursery, 2008). Some authors report that effective control of *Fusarium* from polystyrene trays can be achieved by using a combination of a hot water (90 seconds at 80 ^{o}C) and copper treatment (James and Wollen 1989; Dumroese et al. 2002). However, the most effective method to control *F. circinatum* in polystyrene trays is to make use of only new trays when sowing *P. patula*.

Ensuring that irrigation water is free from contamination is crucial to control *F. circinatum* in nurseries. Most South African nurserymen have used calcium hypochlorite (chlorine) to sterilise irrigation water before use. This can be achieved either by injecting chlorine gas into the irrigation water or by adding chlorine tablets or granules to the water. Although, the recommended rate of 0.5-1 µg 1^{-1} provides good control (Newman 2004), up to 10 µg 1^{-1} has been used both to sterilize the water and to disinfect the plants and growing medium before being allowed to dissipate from the water as a gas (Kruger pers. comm. Top Crop nursery, 2008). Effective control of various nursery associated pathogens has also been achieved by treating contaminated water with hydrogen peroxide and ozone (Newman 2004). Sterilants, such as Sporekill®, Prasin® and Sodium hypochlorite (household bleach), have also shown to be effective in controlling *Fusarium oxysporum* spores in laboratory studies (Nel et al. 2007).

Once established in a nursery, fungicides are commonly applied to reduce infection by *F. circinatum*. In South Africa, however, their application is increasingly being restricted to the few fungicides that pose little threat to the environment and human health (FSC 2002). The most widely applied fungicides in South African nurseries to control *F. circinatum* contain the active ingredient Benomyl (from the benzimidazole group). The strong control that Benomyl has on *F. circinatum* has been shown in laboratory studies (Dwinell and Barrows-Broaddus 1981; Mitchell et al. 2005), in nursery trials (Mitchell et al. 2004) and on the post-planting survival of *P. patula* after applications at planting (Crous 2005). However, the use of Benomyl has now been banned by the Forest Stewardship Council (FSC 2002). Other fungicides containing Tebuconazole, Prochloraz manganese, and Propiconazole have also provided good control of *F. circinatum* in

laboratory *in vitro* studies (Mitchell et al. 2005), although their application has not been tested *in vivo*. Few fungicides may, therefore, be suitable for controlling *F. circinatum* and the majority still remain largely untested. In addition to the environmental dangers associated with fungicide use, there is strong evidence that pathogens build up resistance to fungicides over time, which is especially true of fungicides containing Benomyl (Staub and Sozzi 1984). Although fungicides are still being applied in an attempt to control *F. circinatum* in South Africa, elsewhere in the world the use of fungicides is reported as ineffective (EMPPO 2005; Barnard and Blakeslee 2006).

Apart from the traditional application of fungicides, effective control of various *Fusarium* pathogens by using biological organisms has been extensively documented (Sylvia and Sinclair 1983; Barrows-Broaddus and Dwinell 1985; Sneh et al. 1985; Hoefnagels and Linderman 1999). The most commonly reported biocontrol agents are species of *Trichoderma* (Bell et al. 1982; Ordentlich et al. 1991) of which *T. harzianum* is reported to be especially effective (De La Cruze et. al. 1995; Bacon et al. 2001). Some studies indicate strong inverse relationships between the amount of naturally occurring *Trichoderma* and pathogenic *Fusarium* species found on Douglas-fir seed (James et al. 1987). Although biocontrol agents have in many cases been shown to control other species of *Fusarium*, control appears to be less effective against *F. circinatum*. Tests carried out *in vitro* showed very effective control of *F. circinatum* using a single *T. harzianum* strain (Mitchell et al. 2005) and applied in the nursery, *T. harzianum* is associated with improved survival and growth of the nursery plants (Mitchell et al. 2004). However, after receiving applications of *T. harzianum* in the nursery, no improvement in field survival could be seen and, when applied in the field, benefits were short-lived (Mitchell et al. 2004). Therefore, although there may be some benefits to applying biocontrol agents, they need to be applied within an integrated pest management approach to obtain satisfactory results (Axelrood 1990).

Long term management strategies

In order to reduce the risk of large scale plantation losses to pitch canker in the long term, several breeding strategies can be employed. First, the tolerance of some species over others (Viljoen et

al. 1995, Hodge and Dvorak 2000) suggests that more tolerant species could be planted as substitutes, where possible. Secondly, the susceptible species can be hybridised with more tolerant species in order to impart tolerance to the progeny (Roux et al. 2007). Thirdly, the use of more tolerant families or clones within a generally accepted susceptible species has shown improved tolerance (Rockwood et al. 1988). These can be selected and deployed, or used to control pollinate other individuals of the same species in breeding programs.

Strategy 1: Alternative species

Due to the variation between pine species in their tolerance to various diseases, the simplest genetic strategy to reduce risk in plantations of non-native trees is to plant a number of species and not rely on large scale plantings of a few or a single species (Wingfield 1999; Wingfield et al. 2001, 2002a). For South Africa, this is particularly true if the main species planted is known to be susceptible to *F. circinatum* such as is the case with *P. patula*, in the summer rainfall regions, and *P. radiata*, in the winter rainfall regions. Greenhouse studies and field observations have shown significant variation in host susceptibility to *F. circinatum* at the species, provenance, family and clonal level (Dwinell et al. 1977; Dwinell and Barrows-Broaddus 1982; Barrows-Broaddus and Dwinell 1984; Hodge and Dvorak 2000, 2007 and Roux et al. 2007). The first comprehensive study conducted on a large number of species from all sub-sections within the *Pinus* taxa, were published by Hodge and Dvorak (2000). Using the pine taxonomic classification system of Price et al. (1998), *P. radiata* in the Sub-section Attenuatae was the most susceptible of all the species tested. There was great variability in susceptibility within Sub-section Oocarpae, oocarpa group. *Pinus patula* and *P. greggii* from this group were the most susceptible but not as much so as *P. radiata*. *P. tecunumanii* in the oocarpa group from high elevation regions of Mexico and Central America was moderately susceptible, and *P. oocarpa*, *P. pringeli*, *P. jaliscana* and *P. tecunumanii* from low elevation areas showed high levels of tolerance (Hodge and Dvorak 2000). In the Ponderosae Subsection, pseudostrobus group, *P. maximinoi* was more tolerant than *P. pseudostrobus*. Species from Sub-section Australes were moderately susceptible with *P. elliottii* showing the least tolerance, *P. taeda* slightly more, and all varieties of *P. caribaea* showing the highest level of tolerance (Hodge and Dvorak 2000).

Due to the high level of tolerance of South Africa's other main pines, *P. elliottii* and *P. taeda*, companies have increased plantings of these significantly in the last 10 years in an effort to improve field survival. This is despite the fact that both the wood quality and, often the growth, of *P. elliottii* and *P. taeda* is inferior to that of *P. patula* (Kietzka 1988; Malan 2003). It is also unclear whether these losses in growth and wood quality are not costing the industry more than the cost of increased blanking operations when planting *P. patula*. There are, however, a number of alternative species that show good growth compared to *P. patula*, as well as tolerance to *F. circinatum*. Two such species are *P. tecunumanii* and *P. maximinoi* if planted on the warmer sites of South Africa (Kietzka 1988; Malan 1994; Galpare et al. 2001). Furthermore, studies have shown that these species have outstanding wood properties (Malan 1994, 2006). Other pines showing acceptable tolerance to *F. circinatum* and promising growth potential are *P. teocote* and *P. pseudostrobus*, suited to the cooler regions of the country and *P. pringlei*, suited to the sub-temperate regions of South Africa (Darrow and Coetzee 1983; Coetzee 1985; Malan 2003). The development of *F. circinatum* in South Africa has no doubt called for renewed interest in alternative species, and with advancements in tree breeding, will mean a likely increase in their deployment.

Strategy 2: Hybridization

A contemporary approach to dealing with diseases is to develop hybrids between resistant and susceptible plants. A good example of this already established technique can be seen in the *Eucalyptus* genera (Bayley and Blakeway 2002). Early plantings of pure *E. grandis* in the subtropical regions of South Africa were severely hampered by stem cankers caused by *Chrysoporthe austroafricana* and *Kirramyces zuluense* (Bayley and Blakeway 2002). By hybridising *E. grandis* with *E. urophylla* and *E. camaldulensis*, and selecting tolerant clones from these hybrids, significant improvement in tolerance was achieved (Wingfield 1999; van Zyl and Wingfield 1999; Wingfield et al. 2001; Bayley and Blakeway 2002; van Heerden et al. 2005). Added benefits of these hybrids were improved wood quality and drought tolerance (Malan 1993). These examples indicate that a similar approach can be used to improve the tolerance of susceptible pines to *F. circinatum*. Indeed, some studies have already shown this.

Hybrids between *P. patula* and more tolerant partners such as *P. tecunumanii* and *P. oocarpa* are significantly more tolerant to *F. circinatum* on 3-year-old trees (Roux et al. 2007).

The commercial deployment of pine hybrids in South Africa has been predominantly to *P. elliottii x P. caribaea* (Bayley and Blakeway 2002). However, in recent years, interest in *P. patula* hybrids, such as *P. patula x P. tecunumanii* and *P. patula x P. oocarpa*, have seen a drastic increase in an effort to overcome the survival issues in *P. patula*. Furthermore, these hybrids are performing exceptionally well in early field trials. This is especially true of *P. patula x P. tecunumanii* (Nel et al. 2006). Assuming that companies have access to pollen sources of species such as *P. tecunumanii* and *P. oocarpa* hybrids can be made within a 3-year period and can be easily mass propagated as cuttings. For companies that may not have access to selections from these hybrid partners, or the capacity to produce large quantities of control-pollinated hybrid seed, alternative species may be required.

Strategy 3: Breeding and selection

Many authors report that tolerance of individual clones to *F. circinatum* is under strong genetic control and that improvement in tolerance is best done through selecting and breeding with more tolerant clones (Rockwood et al. 1988; Gordon et al. 1999; Storer et al. 1999; Storer et al. 2001). Even in susceptible species, like *P. radiata*, individuals in natural stands show appreciable tolerance to *F. circinatum* (Storer et al. 1999; Gordon et al. 2001). Carrying out artificial inoculation studies, to identify host tolerance, is not easily done on mature trees. The ability of pathogens to elicit disease symptoms is largely dependent on the environmental conditions during infection (Dwinell and Barrows-Broaddus, 1981 1982; Wingfield et al. 2008). In addition, infection studies in the field increase the risk of contaminating surrounding trees not intended for experimentation (Storer et al. 1999). A popular, and more practical, method commonly used is to artificially inoculate large numbers of seedlings in a controlled environment such as a greenhouse (Gerhold 1970; Hodge and Dvorak 2000, 2007). In this way, many treatments can be screened to determine their tolerance to the pathogen with which they are being challenged and without confounding environmental effects. Consequently, tree breeders attempting to identify resistant families and clones to *F. circinatum* began employing this technique in the last decade.

The Bent Creek Experimental Station in Ashville, North Carolina, as well as the Forestry and Agricultural Biotechnological Institute (FABI) at the University of Pretoria, are used to screen several hundred pine families a year to assist breeders in identifying disease-tolerant species, families and clones.

Although there are numerous examples showing the value of greenhouse inoculation studies (Barrows-Broaddus and Dwinell 1984; Blakeslee and Rockwood 1999; Gordon et al. 1999; Vogler and Kinloch 1999), there is some concern that greenhouse screening studies in South Africa do not provide sufficiently meaningful results when screening the progeny of *P. patula* seed orchard clones against *F. circinatum*. Understanding the factors that may confound the results of greenhouse inoculation studies, will help to improve techniques and the value of these studies. A problem associated with greenhouse screening lies in the fact that young plants are apparently more susceptible to infection by *F. circinatum* than mature trees in the field. Furthermore, infecting trees with spore concentrations higher than those that might occur naturally can confound the results. For example, inoculating healthy 2-month-old *P. patula* seedlings with a spore drench will kill all seedlings within 4 weeks (Viljoen et al. 1994). However, when inoculating 4-year-old *P. patula* trees, signs of recovery can be seen 10 months from the inoculation date (Viljoen *et al.* 1995). Similarly, *P. patula*, well-known to be more tolerant than *P. radiata* (Hodge and Dvorak 2000, 2007; Roux et al. 2007), showed no difference in susceptibility compared with *P. radiata* when mycelial plugs from agar plates were placed directly onto wounded 1-year-old seedling stems (Viljoen et al. 1995). In another experiment, *P. radiata* seedling survival was 2.1% when “low” levels of inoculum (50,000 spores per ml) and 0.3% when “high” levels (100,000 spores per ml) were applied to topped seedlings, resulting in no measured variation in the tolerance of *P. radiata* to *F. circinatum* with either concentration (Hodge and Dvorak 2000). On the other hand, when studying the effect of *F. circinatum* on mature *P. radiata*, levels of as little as 25 spores per infection site (a 1.6 mm diameter wound on the branch) elicited sufficient disease symptoms to differentiate family tolerance (Storer et al. 1999). These examples suggest that applying sufficient quantities of spores to discriminate between family tolerances as seedlings, without killing all treatments in greenhouse studies, is an important consideration.

The effects of increasing maturation on reduced susceptibility to *F. circinatum* can be seen even at a young age where cuttings are often more tolerant to infection than seedlings of the same families (Mitchell, unpublished data). This has also been recorded for other diseases such as Red Band Needle Blight caused by *Dothistroma septosporum* (Ades and Simpson 1990), Western Gall Rust *(Endocronartium harknessii)* (Zagory and Libby 1985; Power et al. 1994) and Fusiform rust (*Cronartium quercuum*) (Frampton et al. 2000). This suggests that the comparison of different treatments, where some may be represented as seedlings and others as cuttings in the same inoculation study, will confound results.

Although the validity of greenhouse screenings to determine family tolerance to *F. circinatum* has been questioned in South Africa, there are examples indicating that greenhouse screening tests are useful. Studies on *P. virginiana* produced reasonable correlations in two consecutive greenhouse inoculation studies with *F. circinatum* with the observed susceptibility of the parent clones in a seed orchard (0.68 and 0.86). Although the correlation was weak when compared to a limited number of the families in a progeny test planted from the same orchard (Barrows-Broaddus and Dwinell 1984), the most tolerant family in the field study was related to the most tolerant clone in the orchard. In other studies, a susceptible *P. elliottii* clone is used as a standard control check in greenhouse screening studies where it consistently ranks as highly susceptible (Hodge and Dvorak 2000; 2007). This indicates that, although the juvenile (greenhouse) – mature (field) correlation may be weaker than hoped for, the relationship is sufficiently strong to be useful when identifying families at the extreme range of infection (Vogler and Kinloch 1999).

To overcome the problems associated with greenhouse inoculation studies, a backwards selection approach using mature trees may be more effective than forward selection. Rockwood et al. (1988) reported very strong heritability's from 5 field tests which averaged 0.67 in artificially and naturally infected tests. From this observation they suggest that, for those incidences where pitch canker is well established in field plantings, seed stands of more tolerant individuals can be achieved by rouging infested stands down to the most tolerant trees. Based on the encouraging results achieved from this and other (Storer et al. 1999; Storer et al. 2001) field studies South African Tree Breeders could consider inoculating plus trees in progeny trials before establishing new seed orchards with these. This should provide for more reliable and meaningful results.

Trees should be inoculated once seed and grafting material have been harvested from the selections to ensure their posterity.

The future of *Pinus patula* in South Africa

The susceptibility of *P. patula* to *F. circinatum* will severely impact the future deployment of the species in this country. Although the problem remains confined at this stage to reestablishment failure, there is the likelihood that *F. circinatum* could result in pitch canker on mature *P. patula* and other pine trees. This highlights the urgent need to reconsider the deployment of *P. patula*, not only to limit further spread in South Africa, but also to prevent the spread of the fungus into the rest of Southern Africa.

The current lack of effective control in nurseries and in young field plantings increases the risk and cost to propagate this species. Chemicals, biocontrol agents and even increased nursery hygiene appear to have limited effect. Selection and breeding more tolerant families appears to be the only feasible solution to the problem if breeders wish to continue with the deployment of *P. patula*. In order to improve the tolerance of the current breeding populations, greenhouse screening studies are the most simple and rapid technique available to breeders. Such studies should effectively distinguish those families that are highly tolerant from those that are highly susceptible in field infections (Vogler and Kinloch 1999). Due to the general lack of tolerance of *P. patula* to *F. circinatum*, especially at the seedling stage, it may be too ambitious to expect that, by deploying the more tolerant families from greenhouse studies, the field survival problem will be resolved. Although there may be some improvement in field survival the importance of greenhouse screening trials is to identify families which, as mature trees, will be more tolerant to pitch canker. A drawback of this method is that seed quantities are drastically reduced as the more tolerant clones are selectively harvested in orchards. Immediate deployment of these families, therefore, may have to rely on vegetative propagation (Gordon et al. 2001). The best long-term solution would be to establish new clonal seed orchards of selected clones.

Deployment of either *P. patula* hybrids or the replacement with alternative species will mean a change in the future distribution of *P. patula*. Hybrids with subtropical species such as *P. tecunumanii* and *P. oocarpa* will extend the range of *P. patula* to include lower altitude warmer sites. The susceptibility of *P. patula* to *Diplodia pinea* on low altitude sites, where hail is a common occurrence (Smith et al. 2002), is still to be determined in the hybrid. Planting of the hybrids may be limited to sites with light or no frost, leaving *P. patula* as the primary choice for very high altitude sites. The frost susceptibility of hybrids between *P. patula* and subtropical species may be overcome by breeding for cold tolerance. Significant effort has gone into breeding *P. patula* with some breeding programs including selections from 3rd generation progeny tests in new seed orchards (Dvorak 1997). Unless the tolerance of these high yielding selections is not improved by control pollination with known tolerant families, or through hybridization with tolerant species, their deployment may never be realised. Breeders will in the future have to increase efforts into breeding alternatives and *P. patula* hybrids to overcome the susceptibility of this species. These changes, and the inevitable reduction of plantings of pure *P. patula*, will raise new challenges and opportunities for the South African forest industry.

Acknowledgements

We thank members of the Tree Protection Co-operative Programme (TPCP), the THRIP initiative of the Department of Trade and Industry (DTI), South Africa, Komatiland Forests and York Timbers, for financial support. We are also grateful to many colleagues linked to the South African Forestry Industry for useful discussions and ideas that have been captured in this overview.

References

Ades PK, Simpson JA (1990) Clonal selection for resistance to *Dothistroma* needle blight in *Pinus radiata*. *New Forests* 4: 27-35.

Allan R, Higgs G (2000) Methods of improving the survival of *Pinus patula* planted into harvest residues. *Southern African Forestry Journal* 189: 47-51.

Arvanitas LG, Godbee JF (Jr), Porta I (1984) Pitch canker impact on growth: A case study in slash pine plantations. *Southern Journal of Applied Forestry* 8: 43-47.

Axelrood PE (1990) Biocontrol of plant pathogens: principals and strategies. Paper presented at the first meeting of the IUFRO Working party. Diseases and Insects in Forest Nurseries. Victoria, British Columbia, Canada, August 22-30.

Bacon CW, Yates IE, Hinton DM, Filmore M (2001) Biological control of *Fusarium moniliforme* in maize. *Environmental Health Perspectives* 109: 325-332.

Barnard EL, Blakeslee GM (1980) Pitch canker on Slash pine seedlings: A new disease in forest tree nurseries. *Plant Disease* 64: 695-696.

Barnard EL, Blakeslee GM (2006) Pitch canker of southern pines. *Plant Pathology Circular* no. 302. Fla. Dept. of Agric. & Consumer Serv. Division of Plant Industry.

Barrows-Broaddus J, Dwinell LD (1984) Variation in susceptibility to the pitch canker fungus among half-sib and full-sib families of Virginia pine. *Phytopathology* 74: 438 – 444.

Barrows-Broaddus J, Dwinell LD (1985) Evaluation of *Arthrobacter* sp. for biological control of the pitch canker fungus (*Fusarium Moniliforme* var. *subglutinans*) on slash pines. *Canadian Journal of Microbiology* 31: 888 - 892.

Bayley AD, Blakeway F (2002) Deployment strategies to maximise value recovery from tree improvement: The experience of two South African companies. *Southern African Forestry Journal* 195: 11 – 22.

Bell DK, Wells D, Markham CR (1982) In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytophatology* 72: 379-382.

Berry CR, Hepting GH (1959) Pitch canker of southern pines. USDA, Forest Service, Forest Pest Leaflet No. 35.

Bethune JE, Hepting GH (1963) Pitch canker damage to South Florida Slash pine. *Journal of Forestry* 61: 517 – 522.

Blakeslee GM, Allen JE, Lante WE (1992) Post-thinning disease and growth responses of pitch canker infected slash pine in the flatwoods of Florida. Proceedings of the 7th Biennial Southern Silvicultural Research Conference, Mobile, AL. Nov. 17-19, 1992.

Blakeslee GM, Oak SW (1980) Significant mortality associated with Pitch canker infection of Slash pine in Florida. *Plant disease* 63: 1023 - 1025.

Blakeslee GM, Rockwood D (1999) Variation in resistance to pitch canker in slash and loblolly pines. Current and Potential Impacts of the Pitch Canker in Radiata pine: in Devey ME, Matheson AC, Gordon TR. Proceedings of the IMPACT Montery Workshop, Montery, California. pp. 70 - 75.

Bragança H, Diogo, E, Moniz, F, Amaro, P (2009) First report of pitch canker on pines caused by *Fusarium circinatum* in Portugal. *Plant Disease* 93: 1079

Britz H, Coutinho TA, Wingfield BD, Marasas WFO, Wingfield MJ. 2005. Diversity and differentiation in two populations of *Gibberella circinata* in South Africa. *Plant Pathology* 54: 46-52.

Carlson CA, Fox TR, Allen HL, Albaugh TJ (2008) Modelling mid-rotation fertilizer responses using the age-shift approach. *Forest Ecology and Management* 256:256-262.

Carlucci A, Colatruglio L, Frisullo (2007) First report of pitch canker caused by *Fusarium circinatum* on *Pinus halepensis* and *P. pinea* in Apulia (Southern Italy). *Plant Disease* 91: 1683

Cashion NL (1975) Effects of nutrition on susceptibility of Slash and Loblolly pines to *Fusarium moniliforme* var. *subglutinans* and studies on the fungus in vitro. M.Sc. Thesis, University of Georgia.

Coetzee H (1985) Provenance research on Mexican pines. Paper presented on the tenth regular meeting of the SARCCUS Standing Committee for Forestry, Sabie River Bungalows, 21-24 May 1985. *Southern African Forestry Journal*, December issue: 68-73.

Coutinho TA, Steenkamp ET, Mongwaketsi K, Wilmot M, Wingfield MJ (2007) First outbreak of pitch canker in a South African pine plantation. *Australasian Plant Pathology* 36: 256 – 261.

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal* 205: 3 – 8

DAFF (2008) Forestry report on commercial timber resources and primary round wood processing in South Africa. Directorate: Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Darrow K, Coetzee H (1983) Potentially valuable Mexican pines for the summer rainfall region of Southern Africa. *Southern African Forestry Journal* 124:23-35.

De la Cruze J, Pintor-Toro JA, Benitez T, Llobell A (1995) Purification and characterization of an Endo-B-1,6-Glucanase from *Trichoderma harzianum* that is related to its mycoparatism. *Journal of Bacteriology* 177: 1864 – 1871.

DeBarr GL, Barrows-Broaddus J (1986) Cone and seed pest problems in the United States. North American Forestry Congress.

Dumroese RK, James RL, Wenny DL (2002) Hot water and copper coatings in reused containers decrease inoculum of *Fusarium* and *Cylindrocarpon* and increase Douglas-fir seedling growth. *Horticultural Science* 37: 943-947.

Dumroese RK, James RL, Wenny DL, Gilligan CJ (1988) Paper presented at the combined meeting of the Western Forest Nursery Council, Intermountain Nursery Association and Forest Nursery Association of British Columbia. August 8-11, 1988. p. 155-160.

Dvorak W (1997) Improvement and Breeding of *Pinus patula*. In: Proc. of the 24th Biennial Southern Forest Tree Improvement Conference. Orlando, Florida, USA. June 9-12 ,1997. (Eds. White, T., Huber D. and Powell, G.). pp:53-68.

Dwinell DL (1999) Contamination of *Pinus radiata* seeds in California by *Fusarium circinatum*. Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

Dwinell DL, Fraedrich SW (1999) Contamination of pine seeds by the pitch canker fungus. In: Landis, T. D. and Barnett, J. P (eds). *National Proceedings: Forest and Conservation Nursery Associations*, General Technical Report SRS-25. Ashville, North Carolina, US Department of Agriculture, Forest Service, Southern Research Station: pp. 41-42.

Dwinell LD (1978) Susceptibility of southern pines to infection by *Fusarium moniliforme* var. *subglutinans*. *Plant Disease Reporter*, vol. 62: 108-111.

Dwinell LD, Adams D, Guerra-Santos JJ, Aquirre JRM (1998) In: Proceedings of the 7th International Congress of Plant Pathology Paper 3.7.30 Edinburg, Scotland.

Dwinell LD, Barrows-Broaddus J, Kuhlman EG (1985) Pitch canker: A disease complex of southern pines. *Plant Disease* 69: 270-276.

Dwinell LD, Barrows-Broaddus JB (1981) Pitch canker in seed orchards. Proceedings from the Sixteenth Southern Forest Tree Improvement Conference, Virginia Polytechnic Institute and State University, Blacksburg. pp. 234-240.

Dwinell LD, Barrows-Broaddus JB (1982) Variability of the host and pathogen in the pitch canker complex. In: *Resistance to Diseases and Pests in Forest Trees*, Heybrook HM, Stephan BR, von Weissenberg K. (eds). *Proceedings of the third International Workshop on the Genetics of Host-Parasite Interactions in Forestry*, Wageningen, Netherlands, 14-21 September 1982. pp. 283-287.

Dwinell LD, Phelps WR (1977) Pitch canker of Slash pine in Florida. *Journal of Forestry* 75: 488-489.

Dwinell LD, Ryan PL, Khulman EG (1977) Pitch canker of Loblolly pine in seed orchards. Proceedings: Fourteenth Southern Forest Tree Improvement Conference, Gainsville, Florida, June 1977 pp. 130-137.

European and Mediterranean Plant Protection Organization (EMPPO) (2005) Data Sheets on Quarantine Pests: *Gibberella circinata*. Bulletin 35: 383-386.

Fisher RF, Garbett WS, Underhill EM (1981) Effects of fertilization on healthy and pitch canker-infected pines. *Southern Journal of Applied Forestry* 5: 77-79.

Forest Stewardship Council (FSC) (2002) Chemical pesticides in certified forests: interpretation of the FSC principals and criteria. Forest Stewardship council, Policy and Standards Unit, document no. FSC-IP-0001, Oaxaco, Mexico.

Frampton JF, Li B, Goldfarb B (2000) Early field growth of Loblolly pine rooted cuttings and seedlings. *Southern Journal of Applied Forestry* 24:98-105.

Galpare WJ, Hodge GR, Dvorak WS (2001) Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia and South Africa. *Forest Genetics* 8:159-170.

Gebeyehu S, Wingfield MJ (2003) Pine weevil *Pissodes nemorensis*: threat to South African pine plantations and options for control. *South African Journal of Science* 99: 531-536.

Gerhold HD (1970) A decade of progress in breeding disease-resistant forest trees. *Unasylva*, no. 97-98, vol. 24 (2-3): Chapter 4. In: An international review of forestry and forest industries. Published by the Food and Agriculture Organization of the United Nations.

Gordon T, Wikler K, Clark S, Storer A, Bonello P (1999) Resistance to pitch canker in Monterey pine. Current and Potential Impacts of the Pitch Canker in Radata pine: in Devey, M. E., Matheson, A.C. and Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. pp. 87 - 89.

Gordon TR, Storer AJ, Wood DL (2001) The pitch canker epidemic in California. *Plant Disease* 85 (11): 1128-1139.

Guerra-Santos J (1999) Pitch canker in Monterey pine in Mexico. Current and Potential Impacts of the Pitch Canker in Radata pine: in Devey ME, Matheson AC, Gordon TR. Proceedings of the IMPACT Montery Workshop, Montery, California. pp. 58 - 61.

Hammerbacher A, Coutinho TA, Wingfield MJ, Wingfield BD (2005a) Influence of ambient temperature and humidity on infection by *Fusarium circinatum* in South African pine nurseries. In: Biology and Epidemiology of *Fusarium circinatum*, M.Sc. thesis, FABI, University of Pretoria, South Africa.

Hammerbacher A, Coutinho TA, Wingfield MJ, Wingfield BD (2005b) Saprophytic survival of *Fusarium circinatum* in soil and organic matter. In: Biology and Epidemiology of *Fusarium circinatum*, M.Sc. thesis, FABI, University of Pretoria, South Africa.

Hepting GH, Roth ER (1946) Pitch canker, a new disease of some southern pines. *Journal of Forestry* 44: 742-744.

Hodge GH, Dvorak WS (2007) Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests* 33: 193-206.

Hodge GR, Dvorak WS (2000) Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests* 19: 241-258.

Hoefnagels MH, Linderman RG (1999) Biological suppression of seed-borne *Fusarium* spp. during cold stratification of Douglas-fir seeds. *Plant Disease* 83: 845-852.

Hurley B, Govender P, Coutinho TA, Wingfield BD, Wingfield MJ (2007) Fungus gnats and other *Diptera* in South African forestry nurseries and their possible association with the pitch canker fungus. *South African Journal of Science* 103:43-46.

James RL, Dumroese RK, Wenny DL (1995) *Botrytis cinerea* carried by adult fungus gnats (*Diptera: Sciaridae*) in container nurseries. *Tree Planters' Notes* 46: 48-53.

James RL, Dumroese RK, Wenny DL, Myers JF, Gilligan CJ (1987) Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 1. Seed and seedling infection, symptom production, and disease progression. United States Department of Agriculture, Forest Pest Management Report no. 87-13.

James RL, Gilligan CJ (1984) Studies of *Fusarium* associated with containerized conifer seedling diseases: Pathonegicity tests of isolates from the Alpine Nursery, Kalispell, Montana. Forest Pest Management Report, no. 84-14.

James RL, Perez R, Dumroese RK, Wenny DL (1999) Virulence of *Fusarium oxysporum* on Douglas fir germinants: comparison of isolates from nursery soils and roots of healthy and diseased seedlings. In: Lija A, Sutherland JR (eds). Proceedings of the 4th meeting of IUFRO Working Party 7.03.04-Diseases and Insects in Forest Nurseries. Finnish Forest Research Institute Research Papers. July 25-28, 1999.

James RL, Wollen RL (1989) An evaluation of the efficacy of hot water-chemical treatments to clean styroblock containers. Champion Timberlands Nursery Plains, Montana. Forest Pest Management Report no 89-5.

Kietzka JE (1988) *Pinus maximinoi*: A promising species in South Africa. *South African Forestry Journal* 145: 33-38.

Kobayashi T, Muramoto M (1989) Pitch canker of *Pinus luchuensis*, a new disease of Japanese forests. *Forest Pests* 38:169-173.

Malan FS (1993) The wood properties and qualities of three South African-grown Eucalypt hybrids. *South African Forestry Journal* 167: 35-44.

Malan FS (1994) The quality and wood properties of 4 provenances of South African grown *Pinus tecunumanii*. *Annals of Forest Science* 51:203-212

Malan FS (2003) The wood properties of South African timber resource for high-value solid wood products and its role in sustainable forestry. *Southern African Forestry Journal* 198:53-62.

Malan FS (2006) The wood properties and sawn-board quality of South African-grown *Pinus maximinoi* (HE Moore). *Southern African Forestry Journal* 208: 39-47.

McCain AH, Koehler CS, Tjosvold SA (1987) Pitch canker threatens California pines. *California Agriculture* 41: 22-23.

McNamara L (2006) Nutrient concentration of inner bark tissue in pine trees in Mpumalanga in relation to baboon damage. A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science.

Mitchell RG, Hongwane P, Olivier R (2009) Internal Research Report 4/2009. Komatiland Forests (Unpublished).

Mitchell RG, Zwolinski J, Jones NB, Coutinho TA (2004) The effect of applying prophylactic measures on the post-planting survival of *Pinus patula* in South Africa. *Southern African Forestry Journal* 200: 51-58.

Mitchell RG, Zwolinski JB, Jones NB, Coutinho TA (2005) Alternatives to Benomyl fungicide for controlling *Fusarium circinatum*: results from *in vitro* studies. In: Factors affecting the successful deployment of *Pinus patula* as rooted cuttings, pp. 21-36. M.Sc. degree, Forestry Programme, School of Agricultural Science and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Morris A (1990) Harvesting slash, *Hylastes angustatus* and climate as factors influencing transplant survival. *Forest Research Document 9/90*. Swaziland, Usutu Pulp Company Forest Research (unpublished).

Morris A (1991) Recommendations to improve transplant survival for re-establishment in the Usutu forest. *Forest Research Document 3/91*. Swaziland, Usutu Pulp Company Forest Research (unpublished).

Morris A (2010) South African Pitch Canker Control Programme. Progress Report to Forestry South Africa, April 2010 (unpublished).

Muramoto M, Tashiro T, Minamihashi H (1993) Distribution in Kagoshima prefecture and pathogenicity to pines of *Fusarium moniliforme* var. *subglutinans*. *Journal of Japanese Forestry Society* 75: 1-9.

Nel A, Kanzler A, Dvorak W (2006) Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. *In* Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. *Edited by* Fikret Isik. pp 158-161.

Nel B, Steinberg C, Labuschagne N, Viljoen A (20070 Evaluation of fungicides and sterilants for potential application in the management of *Fusarium* wilt of banana. FABI (unpublished).

Newman S (2004) Disinfecting water for disease management. 20th Annual Conference on Pest Management on Ornamentals, February 20-22, 2004, Society of American florists, San Jose, California.

Nierenberg HI, O'Donnell K (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia* 90: 434-458.

Ordentlich A, Migheli Q, Chet I (1991) Biological control activity of three *Tricoderma* isolates against *Fusarium* wilts of cotton and muskmelon and lack of correlation with their lytic enzymes. *Phytopathology* 133: 177-186.

Pine *Fusarium* working Group (PFWG) (2004) Forestry takes bold steps towards combating *Fusarium circinatum*. Article in *Wood SA/Timber Times*, November 2004. http://www.timbersa.co.za.

Power AB, Dodd RS, Libby WJ (1994) Effects of hedging on maturation in Radiata pine: Western Gall Rust susceptibility. *Silvae Genetica* 43:1-7.

Price RA, Liston A, Strauss S (1998) Phylogeny and Systematics of *Pinus*. In: Ecology and Biogeogrpahy of *Pinus* (ed. D. M. Richardson). Cambridge University Press. UK 527 p.

Rockwood DL, Blakeslee GM, Lowerts GA, Underhill EM, Oak SW (1988) Genetic strategies for reducing pitch canker incidence in Slash pine. *Southern Journal of Applied Forestry* 12: 28-32.

Rolando CA, Allan R (2004) Survival and growth of *P. patula* at two years in response to harvest residue and pest management. *Southern African Forestry Journal* 200: 19-26.

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests* 33: 109 – 123.

Sakamoto JM, and Gordon TR (2006) Factors influencing the infection of mechanical wounds by *Fusarium circinatum* on Monterey pines (*Pinus radiata*). *Plant Pathology* 55: 130-136.

Schweigkofler W, O'Donnell K, Garbelotto M (2004) Detection and quantification of airborne conidia of *Fusarium circinatum*, the causal agent of pine pitch canker, from two

California sites by using a real-time PCR approach combined with a simple spore trapping method. *Applied and Environmental Microbiology* 70: 3512–3520.

Smith H, Wingfield MJ, Coutinho TA (2002) The role of latent *Sphaeropsis sapinea* infections in post hail associated die-back of *Pinus patula*. *Forest Ecology and Management* 164:177-184.

Sneh B, Agami O, Baker R (1985) Biological control of *Fusarium*-wilt in Carnation with *Serratia liquefaciens* and *Hafnia alvei* isolated from rhizophere of Carnation. *Phytopathology* 113: 271-276.

Staub T, Sozzi D (1984) Fungicide resistance: a continuing challenge. *Plant Disease* 68: 1026-1031.

Storer AJ, Bonello P, Gordon TR, Wood DL (1999) Evidence of resistance to the pitch canker pathogen (*Fusarium circinatum*) in native stands of Monterey pine (*Pinus radiata*). *Forest Science* 45: 500-505.

Storer AJ, Gordon TR, Clark SL (1998) Association of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*, with Monterey pine seeds and seedlings in California. *Plant Pathology* 47: 649-656.

Storer AJ, Wood DL, Gordon TR, Libby WJ (2001) Restoring native Monterey pine forests. *Journal of Forestry* May 2001: 14-18.

Sylvia DM, Sinclair WA (1983) Suppressive influence of *Laccaria laccata* on *Fusarium oxysporum* and on Douglas-fir seedlings. *Phytopathology* 73: 384-389.

van Heerden SW, Amerson HV, Wingfield BD, Wingfield MJ (2005) Relative pathogenicity of *Cryphonectria cubensis* on *Eucalyptus* clones differing in their response to *C. cubensis*. *Plant Disease* 89: 659-662.

van Zyl LM, Wingfield MJ (1999) Wound response of *Eucalyptus* clones after inoculation with *Cryphonectria cubensis*. *European Journal of Forest Pathology* 29: 161-167.

Viljoen A, Marasas WFO, Wingfield MJ, Viljoen CD (1997) Characterization of *Fusarium subglutinans* f. sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Res*earch 101: 437-445.

Viljoen A, Wingfield MJ, Kemp GH, Marasas WFO (1995) Susceptibility of pines to the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Plant Pathology* 44: 877-882.

Viljoen A, Wingfield MJ, Marasas WFO (1994) First report of *Fusarium subglutinans* f. sp. *pini* on pines seedlings in South Africa. *Plant Disease* 78: 309 – 312.

Vogler D, Kinloch B (1999) Variation in the Radiata pine – Western Gall Rust pathosystem: natural vs controlled inoculations. In: Devey ME, Matheson AC, Gordon TR, eds. *Current and Potential Impacts of Pitch Canker in Radiata Pine. Proceedings of the IMPACT Monterey Workshop, California, USA, 30 November−3 December 1998.* Kingston, Australia: CSIRO, Forestry and Forest Products, pp. 98–99.

Wilkinson RC, Underhill EM, McGraw JR, Pritchett WL, Schmidt RA (1977) Pitch canker incidence and fertilizer-insecticide treatment. *IFAS progress report* 77-1.

Wingfield MJ (1999) Pathogens in exotic plantation forestry. *International Forestry Review* 1: 163-168.

Wingfield MJ, Coutinho TA, Roux J, Wingfield BD. 2002a. The future of exotic plantation forestry in the southern Hemisphere: Lessons learnt from pitch canker. *Southern African Forestry Journal* 195: 79 – 82.

Wingfield MJ, Hammerbacher A, Ganley RJ, Steenkamp ET, Gordon TR, Wingfield BD, Coutinho TA (2008) Pitch canker caused by *Fusarium circinatum* – a growing threat to pine plantations and forests worldwide. *Australasian Plant Pathology* 37: 319 – 334.

Wingfield MJ, Jacobs A, Coutinho TA, Ahumada R, Wingfield BD (2002b) First report of the pitch canker fungus, *Fusarium circinatum*, on pines in Chile. *Plant Pathology* 51: 397.

Wingfield MJ, Roux J, Coutinho TA, Govender P, Wingfield BD (2001) Plantation disease and pest management in the next century. *Southern African Forestry Journal* 190: 67-71.

Wingfield MJ, Wingfield BD, Coutinho TA, Viljoen A, Britz H, Steenkamp ET (1999) Pitch canker: a South African perspective. In: Devey ME, Matheson AC, Gordon TR, eds. *Current and Potential Impacts of Pitch Canker in Radiata Pine. Proceedings of the IMPACT Monterey Workshop, California, USA, 30 November−3 December 1998.* Kingston, Australia: CSIRO, Forestry and Forest Products, pp. 62–9.

Zagory D, Libby WJ (1985) Maturation-related resistance of *Pinus radiata* to Western Gall Rust. *The American Phytopathological Society* 75: 1443-1447.

Table 1. Approximate cost of *Fusarium circinatum*-related mortality per ha based on a planting density of 1111 and 1667 stems per hectare (Mitchell et al. 2009).

Activity	*Establishment cost*		*Cost due to F. circinatum (@25%)*		*Mean cost of PCF*
	1111 stems	*1667 stems*	*1111 stems*	*1667 stems*	
Pitting	R633	R950	R158.25	R237.50	R187.88
Planting (incl. plants)	R1,143	R1,715	R285.75	R428.75	R357.25
Blanking (incl. plants)	R611	R917	R157.75	R229.25	R193.50
Total cost per ha	R2,388	R3,582	R601.75	R895.50	R738.63

Figure 1 (a-d). Nursery symptoms showing (a-b) wilting and discoloration of the seedling stem, (c) development of hyphae on dead seedling stem, and d) infected hedge plants.

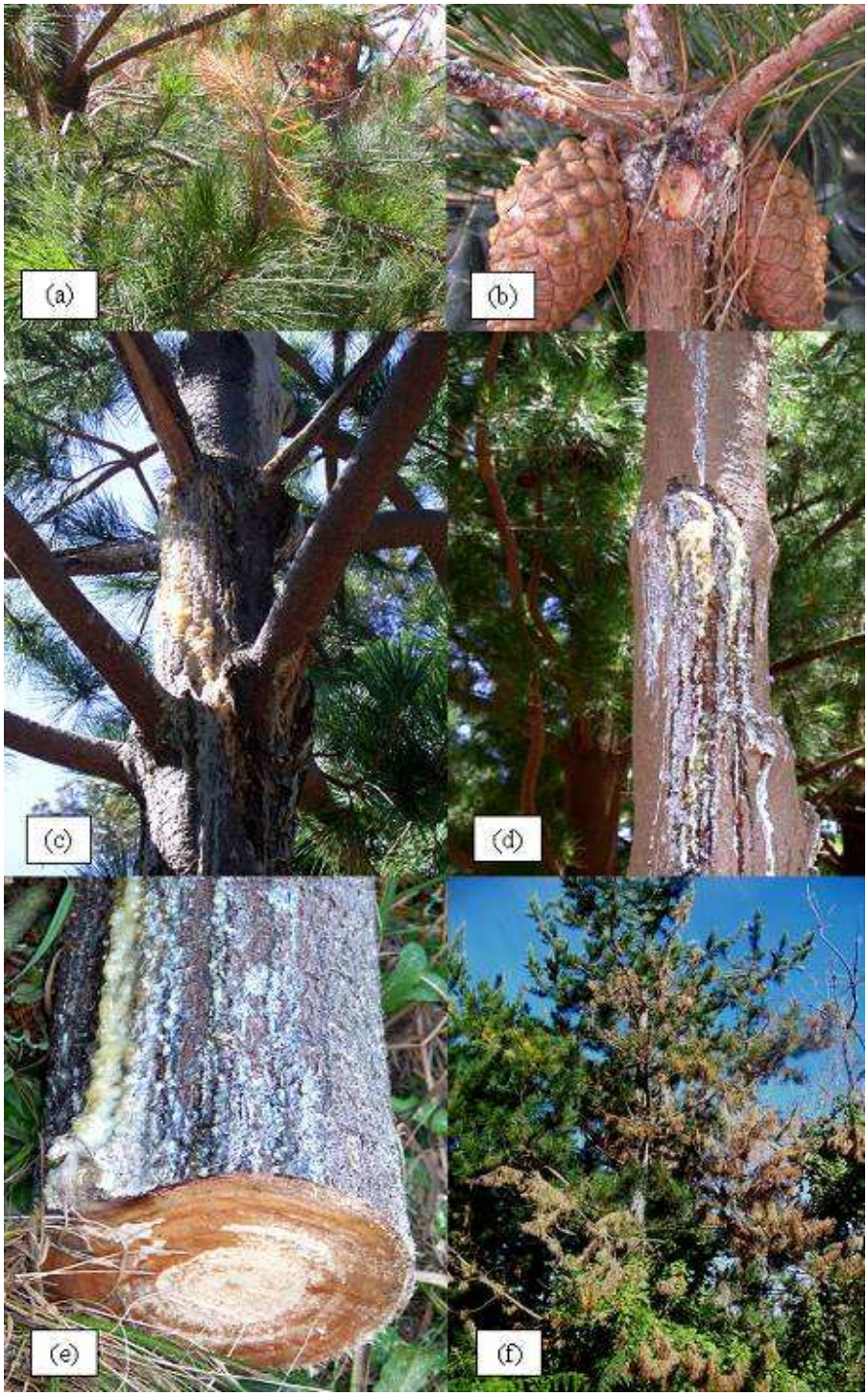

Figure 2 a-f. Pitch canker symptoms on *Pinus radiata* in South Africa; a) branch tip wilting and die-back, b) infected cones and seed, c) infected tree, producing copious amounts resin, also showing branch damage by baboons, d) infection at pruning wound sites, e) resin-soaked internal tissue, and f) tree death resulting from severe infection.

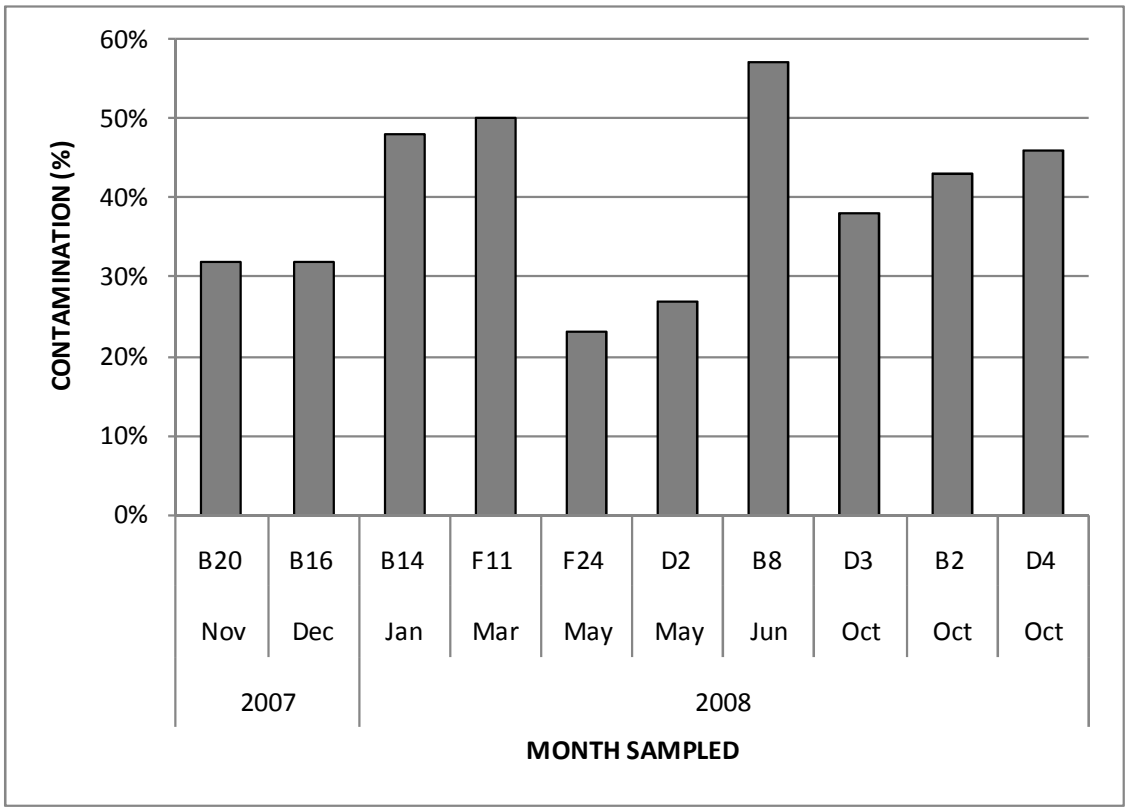

Figure 3. Percentage *Pinus patula* seedlings, from which *Fusarium circinatum* could be isolated, from seedling displaying a healthy appearance at the time of dispatch (Mitchell et al. 2009). (These results were obtained by assessing 30 - 50 asymptomatic seedlings on different occasions, over a 1-year period from various nursery beds (B20, B16, B14 etc.). The standard molecular identification method developed by Schweigkofler et al. (2004) was used to identify the presence of *F. circinatum*).

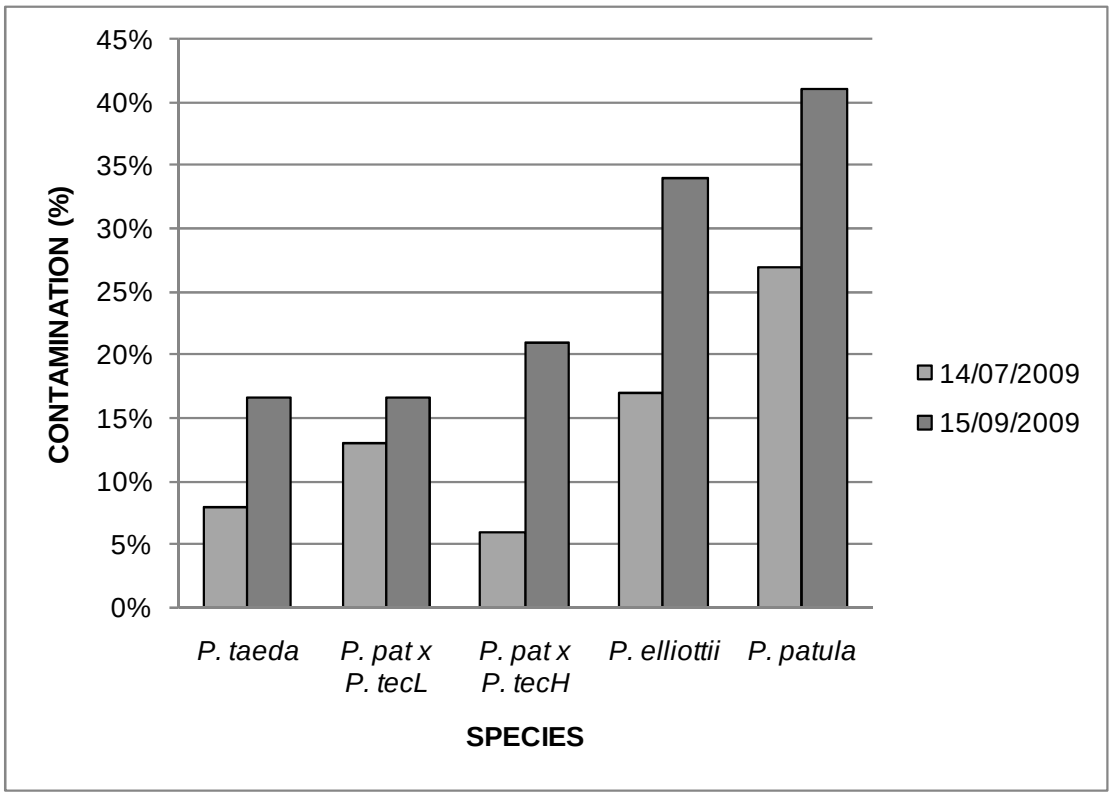

Figure 4. Percentage of asymptomatic plants from which *F. circinatum* was isolated from non-sterilised roots, of various pines, at the time of leaving the nursery (Mitchell et al. 2009). (These results were obtained by accessing 60 asymptomatic nursery plants per species/hybrid in 2 sampling periods. The standard molecular identification method developed by Schweigkofler et al. (2004) was used to identify the presence of *F. circinatum*).

(Published: New Forests, 2012, vol. 43:473-489).

Chapter 2:
Selection of *Pinus* spp. in South Africa for tolerance to infection by the pitch canker fungus

Abstract

The increasing threats from pests and diseases demand that the South African forest industry explores options to deploy alternative pine species in plantation development. This is especially true for species, such as *Pinus patula* Schiede & Deppe ex Schltdl. & Cham., which are highly susceptible to the pitch canker fungus *Fusarium circinatum*. Losses due to *F. circinatum* have been confined mostly to nurseries and at field establishment resulting in a significant cost to the industry. Although the fungus has not as yet resulted in stem and branch infections on established *P. patula* in South Africa, it has caused pitch canker on other, more susceptible species such as *P. radiata* D. Don., and *P. greggii* Engelm. ex Parl. As alternatives to *P. patula* on the warmer and cooler sites in South Africa, families of *P. elliottii* Engelm var. *elliottii*, *P. tecunumanii* (Schw.) Eguiluz & Perry, *P. maximinoi* H.E. Moore and *P. pseudostrobus* Lindl. were screened for tolerance to infection by *F. circinatum* in greenhouse studies. Seedlings were wounded and inoculated with spores of *F. circinatum*. Lesion development following inoculation was used to differentiate the levels of tolerance between families. The results showed that *P. maximinoi*, *P. pseudostrobus*, and the low elevation variety of *P. tecunumanii* are highly tolerant to infection with very little family variation. The narrow sense heritability estimates for these species were less than 0.06. In contrast, *P. elliottii* showed good tolerance with some family variation and a heritability of 0.22, while the high elevation source of *P. tecunumanii* showed a high degree of family variation and a heritability of 0.59. These results provide the industry with valuable information on pine species tolerant to *F. circinatum* that could be used as alternatives to *P. patula* in South Africa.

Keywords; CAMCORE, tree disease, screening for resistance, disease avoidance, plantation forestry

Introduction

Pitch canker is a serious disease of pines caused by the fungus *Fusarium circinatum* (anamorph) that has a sexual state (teleomorph) known as *Gibberella circinata* (Nierenberg and O'Donnell 1998; Britz et al. 2005). Although the exact origin of pitch canker is unknown, studies indicate that the pathogen may be endemic to Mexico (Guerra-Santos 1999), southeast USA (Wikler and Gordon 2000) or as far south as Central America (Dvorak et al., 2009). The first discovery of the disease in South Africa was 1991 (Viljoen et al. 1994) suggesting that *F. circinatum* is an introduced pathogen into this country (Wikler and Gordon, 2000). From vegetative compatibility group, and allelic frequency studies, the South African population has the third highest degree of genetic diversity after Mexico and the south-eastern USA (Viljoen et al. 1997; Wikler and Gordon 2000; Britz et al. 2005). Accepting that the South African population is in all probability young, several possibilities for its diversity have been suggested. Many genotypes could have been initially introduced (Viljoen et al. 1997), subsequent introductions have occurred (Britz et al. 2005) or sexual reproduction is occurring (Viljoen et al. 1997).

Pitch canker can result in branch die-back, stem cankers and in large-scale mortality (Dwinell and Phelps 1977; Bethune and Hepting 1963; Storer et al. 1999). Consequently, *F. circinatum* is considered one of the most serious pathogens threatening plantations of non-native *Pinus* spp. worldwide (Wingfield et al. 2008). The susceptibility of *Pinus* spp. to *F. circinatum*, however, varies considerably. Thus, some species, such as *Pinus radiata* and the northern provenances of *P. greggii* are highly susceptible whilst other species including *P. jaliscana* and *P. oocarpa* display high levels of tolerance to infection (Hodge and Dvorak 2000). This indicates that the threat of *F. circinatum* can be reduced by planting more tolerant species in high risk areas.

In South Africa, *P. patula* is the most important softwood planted (Department of Agriculture, Forestry and Fisheries 2008) and was the first pine species to be associated with the pitch canker fungus (Viljoen et al. 1994). The disease has now spread to most pine growing nurseries in the country where it is managed with varying degrees of success. The most significant effect of *F.*

circinatum on *P. patula*, however, can be seen on young seedlings that become infected and die after establishment (Crous 2005; Mitchell et al. 2011). More recently, pitch canker was discovered on mature *P. radiata* and *P. greggii* trees in the Southern and Eastern Cape provinces of South Africa (Coutinho et al. 2007; Roux 2007). Given the speed at which the nursery disease has progressed since it was first identified, and the current situation with growing areas of mature trees being infected, it seems reasonable to expect that pitch canker may spread to other areas and species, which could include mature *P. patula* trees.

In the long term, tree breeders in South Africa hope that the tolerance of *P. patula* to *F. circinatum* could be improved by including tolerance as a selection criterion in their breeding programmes (Mitchell et al. 2011). In the shorter term the tolerance of *P. patula* can be improved through hybridization with more tolerant species. The simplest immediate solution, however, is to deploy alternative species that are known to be tolerant to infection by *F. circinatum*, at least in high-risk sites (Mitchell et al. 2011).

South Africa is fortunate to have a wide range of site types that are suited to a number of *Pinus* species (Morris and Pallett 2000). Although *P. patula* is planted across many of these sites, *P. elliottii* and *P. taeda* have been planted in increasing numbers in the last decade as substitutes due to their tolerance to *F. circinatum* (Hodge and Dvorak 2000). In South Africa, *P. taeda* is considered highly tolerant to *F. circinatum* (Hodge and Dvorak 2000; Roux et al. 2007). However, due to its specific site requirements (well drained soils that are a minimum of 750 mm deep, in areas that receive a minimum of 950 mm of rain per year, and on sites below 1400 m (Schönau and Grey 1987; Morris and Pallett 2000; Zwolinski and Hinze 2000), few areas are suitable for this species. This, together with its tolerance to *F. circinatum*, makes it less important to screen the local population of *P. taeda*.

Pinus elliottii is less tolerant to *F. circinatum* than *P. taeda* (Hodge and Dvorak 2000) and is South Africa's second most important pine crop (Department of Agriculture, Forestry and Fisheries 2008). Elsewhere, large variation in tolerance to *F. circinatum* has been found between *P. elliottii* families, which have enabled breeders to improve the overall tolerance of the species

(Rockwood et al. 1988; Blakeslee and Rockwood 1999). This suggests that it would be important to screen the South African *P. elliottii* selections for tolerance to *F. circinatum*.

There are a number of other *Pinus* species that show potentially high levels of tolerance to *F. circinatum* in research trials and in commercial plantings. On warmer sites, *P. tecunumanii* and *P. maximinoi* are the most promising (Kietzka 1988; Dvorak et al. 2000, 2002; Galpare et al. 2001). Fewer species are available on the temperate and cooler sites that perform as well as *P. patula*. Similar performance can be achieved from the Mexican pine, *P. greggii*, on cold and dry regions in South Africa (Dvorak et al. 1996) but the susceptibility of the species to *F. circinatum* (Hodge and Dvorak 2000) does not make it a suitable alternative to reduce the risk of pitch canker. *Pinus pseudostrobus*, however, has also shown potential in these regions (Coetzee 1985) and is tolerant to *F. circinatum* (Hodge and Dvorak 2000). It is already being deployed commercially by one company. Although *P. tecunumanii*, *P. maximinoi* and *P. pseudostrobus* are known for their higher levels of tolerance to *F. circinatum* than *P. patula*, little is known about the degree of within family variation to infection by the pathogen.

In this study, families of *P. maximinoi*, *P. pseudostrobus*, *P. tecunumanii* and *P. elliottii*, were screened for their tolerance to *F. circinatum*. This was achieved using greenhouse inoculations. The objectives of the study were to determine whether meaningful family variation, in tolerance to *F. circinatum*, exists in these species which would indicate that their general tolerance could be further improved though breeding and selection.

Materials and Methods

Plant material

A number of Camcore trials were planted in the early 1980's testing the performance of unimproved *P. tecunumanii* and *P. maximinoi* families from various provenances (localities) in Mexico and Central America. Camcore is an international tree conservation and domestication programme at North Carolina State University, USA. The organization is actively involved in the

collection of wild populations of pine species for conservation and domestication in areas outside of their natural distribution. The trials were situated on Komatiland Forests' property, South Africa. The seed of these two species, collected for this study, was harvested from trees that had been selected for superior growth and stem form based on their breeding values in the Camcore trials. In the original field design, families were grouped by provenance and, therefore, the selections were likely to be pollinated by surrounding trees of the same provenance. However, there would also be some natural cross pollination between trees of different provenances. This is relevant because the seed collected would not necessarily represent a pure provenance.

Pinus tecunumanii

The *P. tecunumanii* seed was harvested, in 2004 and 2005, from 73 selected trees, representing 12 provenances in the Camcore trials (Table 1). Of the 73 trees, 49 were sampled from 4 provenances that occur below 1500 meters above sea level (low elevation or LE) in Honduras. The other 24 selections represented 8 provenances above this altitude (high elevation or HE). With the exception of one high elevation provenance (Las Trancas), which is found in Honduras, all others originate in Mexico and Guatemala.

The *P. tecunumanii* seed was sown in September 2006 in preparation for inoculating the seedlings in April 2007 (Table 2). When the seedlings were 6-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 88 seedlings or 22 seedlings per plot. Seedlings of *P. patula* and *P. elliottii*, from a commercial seed orchard, were included as controls in the trial.

Pinus maximinoi

The *P. maximinoi* seed was harvested, in 2005 and 2006, from 105 selected trees representing 13 provenances across Mexico, Guatemala and Honduras (Table 1). In this collection, most provenances were represented by 4 to 13 trees. The seed was sown in July 2007 in preparation for the March 2008 inoculation. When the seedlings were 7-months-old, they were packed out in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 56 seedlings. Seedlings of *P. tecunumanii* HE, *P.*

tecunumanii LE, *P. taeda*, *P. elliottii* and *P. patula* were included in the trial as controls (Table 2).

Pinus elliottii

Seed, from a total of 49 open pollinated 2nd generation *P. elliottii* families, was provided by Komatiland Forests from their breeding programme for this trial. Seed from the highly susceptible *P. elliottii* clone FA2 (Hodge and Dvorak 2000, 2007) was provided by Camcore and included in the study. All seed was sown in March 2009 in preparation for screening the seedlings in December 2010. When the seedlings were 8-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 44 seedlings. Commercial open pollinated seedlings of *P. patula*, *P. elliottii* and *P. taeda* were included as controls (Table 2).

Pinus pseudostrobus

Seed of 33 selected *P. pseudostrobus* trees, in a commercial stand, was supplied by Komatiland Forests for this trial. The seed was sown in May 2009 in preparation for screening the seedlings in December 2010, together with the *P. elliottii* trial. When the seedlings were 7-months-old, they were arranged in a randomised complete block design with 4 replications. Based on seedling availability, each treatment was represented by approximately 80 seedlings. As with the *P. elliottii* trial, open pollinated seed of *P. elliottii*, *P. taeda* and *P. patula* was included as controls (Table 2).

The seedlings in all the trials were raised in the Komatiland Forest's Research nursery at Sabie, South Africa (S25° 03.22', E30° 46.859'). The local climate can be described as warm temperate with a mean annual temperature of 15 °C and approximately 1300mm of rain predominantly during the summer months. Seedlings were raised in composted pine bark in the Unigro tray, consisting of loose inserts, under plastic covering. Each individual insert is square in shape with a top end width of 37 mm, length of 100 mm, and volume of 90 ml. The side walls have pronounced internal ridges to prevent root spiraling. Granular fertilizer (2:3:2 (N:P:K)) was applied as needed. No fungicides were applied to the seedlings during their establishment. Due to the different seed collection dates the trials were raised at different times in the nursery.

Inoculation procedures

The seedlings, for each trial, were transported to a greenhouse at the University of Pretoria, specifically erected for the purpose of screening pine families for tolerance to *F. circinatum*, on different occasions (Table 2). The greenhouse had a wet-wall cooling system and a separate heating system to maintain air temperatures of approximately 25^{o}C. The plants were left to acclimatise for 1 – 4 weeks before the seedlings were inoculated.

The inoculum was prepared on the same day that the seedlings were inoculated, using a equal mixture of conidia from three highly virulent South African isolates (CMW 3577, 3578, and 3579) of *F. circinatum* that are maintained by the Tree Protection Co-operative Programme (TPCP) in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. The isolates were cultured on half strength Potato Dextrose Agar (PDA; 2g potato extract, 10 g dextrose, and 7.5 g agar/l distilled water) under sterile conditions for 7 days at 25^{o}C prior to inoculation. Cultures were flooded with sterile water containing 15% glycerol and spores were dislodged using a glass “hockey stick”. The spore concentration was determined using a haemocytometer, viewed under a light microscope at 20x magnification, and adjusted to 50 000 spores/ml. The tubes of inoculum were maintained on crushed ice prior to inoculation.

In the greenhouse, the apical bud of each seedling was removed using sharp secateurs, and a 10 µl inoculum (500 spores per infection site) was immediately applied to the wounded tip using a micro-pipette. Once inoculated, plants in the trial were watered daily and assessed for lesion development 8-weeks after inoculation. The lengths of lesions were measured from the tips of the seedlings, at the point of inoculation, to the point where the tissue showed no visible necrosis. The seedling height, from the root collar to the wounded tip, was also measured. For each inoculated seedling the proportion of lesion length to the length of the seedling at the time of inoculation was expressed as a percentage of die-back. In many seedlings, new shoots (re-

sprouts) had formed below the lesions and the length of these was measured. All measurements were recorded in millimetres (mm).

Statistical Analyses

The statistical package SAS version 9.1.3 (SAS Institute, 2003) was used to carry out the data analysis. An analysis of variance (ANOVA) was conducted on the data (seedling height, percentage die-back, lesion length, and length of the re-sprout) for each of the four inoculation trials. In order to correct for the influence that the seedling size could have had on the variables, initial height was analysed as a covariate (Hodge and Dvorak 2000). A Pearson correlation coefficient analysis was carried out to determine the relationship that these variables had on each other. As the trials were analysed on separate occasions, different tests were used to determine treatment differences. A t-test was used to compare differences between provenance and species means in the *P. tecunumanii* data-set and a Student Newman Keuls (SNK) test was used to test for differences between family means. A Duncan Multiple Range test was used to determine treatment differences in the *P. maximinoi*, *P. elliottii* and *P. pseudostrobus* datasets. Narrow sense heritability was estimated following the method described by Dieters et al. (1995) for open-pollinated families ($h^2 = (3 \times \sigma^2_{female}) / \sigma^2_{phenotypic}$).

Results

After inoculation, lesions were observed in *P. patula* control seedlings within 7 days. Shortly thereafter, the *P. tecunumanii* (HE) treatments developed lesions followed by lesions in *P. elliottii*, families of *P. tecunumanii* (LE), *P. maximinoi* and *P. pseudostrobus*. Sprouts were seen developing below the lesion on those species that took longer to develop lesions. Previous studies have shown that lesion length and the percentage dieback can be influenced by the height of the seedling (Hodge and Dvorak 2000) or the diameter of an infected branch in field studies (Roux et al. 2007). In these cases taller seedlings, or thicker branches, reduced lesion development. It has also been found that lesion length and percentage dieback correlate well

(Hodge and Dvorak 2000). However, it has not been reported that the ability of the seedling to produce sprouts, below the lesion, may be related to the tolerance of the seedling.

Pinus tecunumanii

The effect of seedling height influenced percent die-back and the length of re-sprout significantly ($p<0.0001$), except for lesion length at the family level. The Pearson Correlation Coefficients between means at the provenance and family level indicate highly significant relationships for all comparisons. For provenance, the relationship between lesion length and percentage dieback was the strongest (r=0.99) and lesion length was used to rank treatments. The correlation between lesion length and the degree of re-sprout was strongly negative at the provenance (r= –0.78) and family (r= –0.76) levels, indicating that treatments with longer lesions produced shorter sprouts.

In this part of the study *P. tecunumanii* (LE) displayed the greatest level of tolerance to infection with a mean lesion length of 2.78 mm (Table 3). This did not differ significantly from the lesion lengths obtained for *P. elliottii* (3.11 mm). In contrast, *P. tecunumanii* (HE) had an average lesion length of 9.61 mm. This was significantly greater than lesions on *P. tecunumanii* (LE) and the *P. elliottii* control, but not as long as those on *P. patula* (22 mm) (Table 3). When assessing provenance variation, there were no differences in lesion lengths between the four low elevation provenances, while there were large differences among the high elevation provenances (Table 4). The high elevation provenance from Honduras, Las Trancas, was the most tolerant of this variety and similar in tolerance to the other *P. tecunumanii* (LE) provenances from Honduras (Table 4). There were also no significant differences in lesion lengths between the 49 low elevation families screened (SNK grouping not shown) and all low elevation families were similar to the *P. elliottii* control (Fig. 1). On the other hand, large family variation in lesion lengths occurred in the high elevation source (Fig. 1).

Pinus maximinoi

The effect of seedling height, at the time of inoculation, influenced percentage die-back moderately (r=-0.22) and had a weak, but significant, effect on the length of the re-sprout (r=-0.12). Seedling height had no effect on lesion length. The relationship between lesion length and percent die-back was extremely strong (r=0.92) and lesion length was used to rank treatments.

The overall mean lesion length of *P. maximinoi* (3.19 mm) was no different to that on *P. tecunumanii* (LE) (2.69 mm) (Table 5). Both of these species also showed the greatest ability to re-sprout. *P. maximinoi* (3.19 mm) was significantly more tolerant than *P. taeda* (4.95 mm), *P. elliottii* (5.91 mm), as well as *P. tecunumanii* (HE) (8.63 mm). The *P. patula* seed orchard control was more susceptible than all other treatments with an average lesion length of 21.04 mm (Table 5).

In this part of the study there was little variation between provenances (Table 6), or families (Fig. 2), of *P. maximinoi* in lesion length with only those at the extreme range of infection differing significantly (Duncan grouping not shown). In this comparison, provenances represented by fewer than 4 families, were excluded. The lack of family variation corresponded to a very low narrow-sense heritability estimate (h^2=0.014) for lesion length.

Pinus elliottii

The effect of seedling height influenced percent die-back caused by *F. circinatum* ($p<0.001$). Similar to the other studies, lesion length and percent dieback were strongly related (r=0.94) and lesion length was again used to rank treatments. Lesion length had a weak, but significant, relationship with the ability for the families to re-sprout (r= -0.28) indicating that families with shorter lesions produced longer shoots. Overall, *P. elliottii* produced the longest shoots followed by *P. patula* then *P. taeda*.

Pinus taeda ranked the most tolerant to *F. circinatum* with a mean lesion length of 4.2 mm (Table 7). Statistically, the lesion lengths were no different to the overall mean lesion length of *P. elliottii* (5.73mm). However, *P. taeda* was more tolerant than *P. elliottii* when comparing percentage die-back. *P. patula* was the most susceptible with a mean lesion length of 23.7 mm (Table 7). Family variation, among *P. elliottii*, was greater than in *P. maximinoi* and *P. tecunumanii* (LE) (Fig. 3). Most families ranked more susceptible than *P. taeda*. However, there were also a number of families that ranked more tolerant than *P. taeda*. The *P. elliottii* family FA2, previously used as a susceptible control by Hodge and Dvorak (2000, 2007), ranked as the 6th most susceptible in this study (Fig. 3). Narrow sense heritability was estimated at 0.22.

Pinus pseudostrobus

As with all the other trials, seedling height at the time of inoculation with *F. circinatum* influenced the percentage dieback. In this case, lesion development was also influenced by seedling height. Neither lesion length, nor the percentage die-back, influenced the ability of plants to re-sprout. Similar to all the other trials, lesion length and percentage dieback were strongly correlated (r=0.99) and lesion length was used to rank the families.

Compared to the controls, *P. pseudostrobus* was the most tolerant to *F. circinatum* with a mean lesion length of 3.7 mm followed by *P. taeda* (7.1 mm), *P. elliottii* (10.8 mm) and *P. patula* (24.6 mm) (Table 8). Family variation was low with 32 of the 33 *P. pseudostrobus* families ranked more tolerant than *P. taeda* (Fig. 4). Heritability was estimated at 0.06.

Discussion

The results of this study clearly show that the risk that *F. circinatum* poses to young and mature stands of *P. patula* in South Africa could be largely overcome by planting alternative species. *Pinus tecunumanii* (HE), suitable to warm-temperate and sub-temperate sites, showed the greatest variation in tolerance, where many families ranked more susceptible than *P. patula*. This indicates that screening families of this variety is strongly recommended before selecting those for deployment. This is particularly important if families of *P. tecunumanii* (HE) are used as hybrid partners with *P. patula*. *Pinus pseudostrobus*, a species that could be significantly improved in growth through breeding, showed considerable tolerance to *F. circinatum* in this study. This species could, therefore, become an important alternative on the temperate sites of South Africa in the future. *P. maximinoi*, and *P. tecunumanii* (LE), showed excellent tolerance to *F. circinatum* with little family variation indicating that these could be planted on the warmer sites, without concern that they may become infected with *F. circinatum*. *Pinus elliottii*, a highly adaptable species suitable to a wide range of sites, showed sufficient variation in tolerance to

suggest that screening families and selecting those with high levels of tolerance to infection by *F. circinatum* should be considered when deploying this species.

Pinus tecunumanii

The differences seen at the ecotype level where *P. tecunumanii* (LE) was significantly more tolerant to *F. circinatum* than *P. tecunumanii* (HE) has been previously reported (Hodge and Dvorak, 2000). In this study the mean lesion length of *P. tecunumanii* (HE) was longer than *P. elliottii* which is opposite to that reported by Hodge and Dvorak (2000). Although the tolerance of *P. tecunumanii* (LE) was similar to the mean of *P. elliottii*, where large family variation could be seen (Fig. 3), neither provenance nor family differences existed. This illustrates the high level of tolerance of this source. This was further demonstrated by the strong tendency to re-sprout after inoculation. The variation in tolerance of various *P. tecunumanii* (HE) provenances to *F. circinatum* has also been reported elsewhere (Hodge and Dvorak, 2007).

The very large variation in tolerance of the 24 *P. tecunumanii* (HE) families proved to be strongly heritable (h^2=0.59). A similar heritability estimate (h^2=0.58) was calculated for 14 *P. tecunumanii* (HE) families collected from a seed orchard in Colombia (Isaza 2008). Although a few *P. tecunumanii* (HE) families in this study were more susceptible than the *P. patula* control (Fig. 1), the overall mean for this variety indicated much higher levels of tolerance than *P. patula*. Furthermore, the high level of heritability indicates that the tolerance of *P. tecunumanii* (HE) could be improved upon relatively easily. It also suggests that families of *P. tecunumanii* (HE) should be screened for susceptibility before deployment, while those from the low elevation ecotype need not be screened. The high degree of provenance variation in the high elevation ecotype, compared to the lack of variation in the low elevation ecotype, has also been reported for traits other than susceptibility to infection by *F. circinatum*. For example, Malan (1994) found marked differences in mean inter-node length, branch diameter, and air-dried wood density between different high elevation provenances whilst no significant variation could be found for these traits amongst low elevation provenances.

In recent years tree breeders have learnt that *P. tecunumanii* hybridizes easily with *P. patula* and that the hybrid can outperform *P. patula* in field trials (Nel et al. 2006). Field inoculation studies on 3-year-old trees have shown that the tolerance of this hybrid to *F. circinatum* is superior to that of *P. patula* (Roux et al. 2007). The higher level of tolerance to *F. circinatum* is clearly seen by improved survival of the hybrid over *P. patula* in areas of little or no frost (Mitchell, unpublished). However, the results of this study suggest that not all of the hybrids made with families of the high elevation variety will be more tolerant to *F. circinatum*. Therefore, due to the susceptibility to frost, particularly in the low elevation ecotype (Dvorak et al. 2000), as well as susceptibility to *F. circinatum* in the high elevation ecotype (Hodge and Dvorak 2000), care should be taken when planting this hybrid on a large scale without prior selection.

Pinus maximinoi

Kietzka (1988) recommended planting *P. maximinoi* in South Africa in order to broaden the species base planted "should one of our major pine species (*P. patula*, *P. elliottii*, and *P. taeda*) become subject to major pest or disease problems". Although the tolerance of *P. maximinoi* to *F. circinatum* was probably unknown at the time, it was later established that it is more tolerant than *P. patula* (Hodge and Dvorak 2000).

The main constraints to planting *P. maximinoi* are its susceptibility to frost during establishment (Dvorak et al. 2000) and the production of heavy branch whorls that reduce sawn board timber quality (Malan 2006). However, saw millers are now able to remove the knot clusters to produce long lengths of clear timber (Malan 2006). Although *P. maximinoi* is sensitive to frost, which can result in establishment failure, it often survives better than *P. patula*. In a number of Camcore trials planted during 2008, the survival of *P. maximinoi* was consistently better than *P. patula* with an average survival across all trials of 96% compared with 80% for *P. patula* (data not shown). This indicates that many of the warmer and wetter sites currently planted to *P. patula*, could be replaced with *P. maximinoi*. These would include areas between 15 and 17^{o}C mean annual temperature with >1000 mm mean annual rainfall. This constitutes approximately 11% of the summer rainfall regions of South Africa where *P. patula* would be planted (Mitchell unpublished).

Pinus elliottii

Due to the greater tolerance to *F. circinatum*, *P. elliottii* survives well after planting, making it a popular species among foresters. However, the general tolerance of this species may not be sufficient to eliminate the risk of a pitch canker outbreak in South Africa. In the southern USA, mortality of infected *P. elliottii* stands has reached levels of 25% with as much as 98% of all trees showing infection (Blakeslee and Oak 1980). Fortunately, large genetic variation in tolerance to *F. circinatum* exists in the species, which has allowed breeders to identify and select tolerant individuals (Rockwood et al. 1988). Blakeslee and Rockwood (1999) reported narrow-sense heritability estimates around 0.25 in both greenhouse and field studies, similar to the h^2=0.22 estimated in this study, so genetic improvement of resistance within *P. elliottii* is certainly possible. This was supported by the fact that the susceptible family (FA2), used in the studies by Hodge and Dvorak (2000, 2007), ranked as the 6th most susceptible treatment in the present trial. Using family FA2 as a benchmark, several families were equally susceptible in this sample, highlighting the importance of screening *P. elliottii* in South Africa. Although the *P. elliottii* commercial seed orchard seedlot was more tolerant than family FA2, a number of families ranked more tolerant than the commercial *P. elliottii* seedlot and also *P. taeda* (Fig. 3).

Due to the adaptability of *P. elliottii* to a wide range of sites in South Africa, the species will continue to be planted on a large scale until alternative species and hybrids are deployed in greater numbers. The species has been planted in all areas where *P. patula* has been planted including sub-tropical sites not suited to *P. patula*. However, the growth of the species is inferior to that of *P. patula* on most sites, particularly those in temperate regions (Darrow and Coetzee 1983; Morris and Pallet 2000). Relative to alternative species, such as *P. tecunumanii* and *P. maximinoi*, the easy accessibility of seed makes *P. elliottii* an attractive alternative to *P. patula*. However, it should be cautioned that the slower growth of *P. elliottii* compared to *P. patula* (Morris and Pallett 2000) could cost more than the cost that *F. circinatum* might cause to *P. patula* (Mitchell et al. 2011).

Pinus pseudostrobus

Currently, few other species, suited to the cold regions of South Africa, grow as well as *P. patula* (Darrow and Coetzee 1983; Coetzee 1985; Morris and Pallett 2000). The limited choice is particularly challenging for breeders as the majority of South Africa's afforested regions experience mild to severe frost in the winter months. Of all the Mexican and Central American pines suited to colder regions, *P. pseudostrobus* shows the greatest tolerance to *F. circinatum* (Hodge and Dvorak 2000).

Hodge and Dvorak (2000) found that *P. pseudostrobus* was moderately resistant to infection by *F. circinatum,* where it was more tolerant than *P. elliottii*, somewhat less tolerant than the high elevation variety of *P. tecunumanii* and significantly less tolerant than *P. taeda*. The *P. pseudostrobus* seed used in the present study came from selections in a commercial stand with good growth such as described by Coetzee (1985). Little is known regarding the origin of these selections, however, in this study most families ranked more tolerant than *P. taeda*. One possible explanation is related to seedling size at the time of inoculation. In the Hodge and Dvorak (2000) study, the *P. pseudostrobus* seedlings were much smaller than most of the other species and the covariate adjustment for height may have not been sufficient to correct for this factor and to properly compare the tolerance of *P. pseudostrobus*. In contrast, in the present study, the *P. pseudostrobus* seedlings were of very similar height to the *P. taeda*, *P. elliottii* and *P. patula* controls.

Conclusion

In this study, the subtropical species, *P. tecunumanii* (LE) and *P. maximinoi*, were highly tolerant to infection by *F. circinatum* with little meaningful family variation. This suggests that they could be deployed with little concern of infection by the pathogen. Due to their susceptibility to frost, these species will be limited to the warmest areas where *P. patula* is currently planted. The sub-tropical to warm temperate species, *P. elliottii*, was less tolerant than *P. tecunumanii* (LE) and *P. maximinoi*, and displayed sufficient family variation with strong heritability. This result suggests that *P. elliottii* families should be screened and selected for tolerance to *F. circinatum* prior to decisions being made for large scale deployment. This species

is highly adaptable and probably the most suitable alternative to *P. patula* for planting on warm temperate sites. The warm to sub-temperate species *P. tecunumanii* (HE) was less tolerant to infection *F. circinatum* than *P. elliottii* and displayed very large family variation with extremely high heritability. This indicates that it should be screened for tolerance to *F. circinatum* prior to deployment. Disease screening will be especially important where *P. tecunumanii* is considered for hybridization with *P. patula*. In contrast, the cold temperate species, *P. pseudostrobus*, showed extreme tolerance to *F. circinatum* with little to no family variation. Through further breeding and selection for growth and other good characteristics, this species may become an alternative to *P. patula* on cold sites.

Overall results of this study have shown that there is good opportunity to develop alternative species to *P. patula* in areas where the pitch canker fungus limits the successful production of this species. The results are generally consistent with those from prior studies, field observations and prior studies where established trees have been used in inoculations (Roux et al. 2007). It is, however, important to recognise that this study was based on seedling evaluations, which clearly provide an indication of resistance to *F. circinatum*, but may not fully encompass the field situation. It is well-known that trees respond differently to infection by pathogens at different stages of development and field experiments extending the limits of this study need to be conducted.

Acknowledgements

We thank Komatiland Forests for providing the seed and incurring the costs involved in raising and screening the plant material. We also acknowledge the assistance of Bernice Porter who cared for the plants and coordinated the screening activities at the University of Pretoria. Members of the Tree Protection Co-operative Programme (TPCP) and members of the Technology and Human Resources for Industry Programme (THRIP), an initiative of the Department of Trade and Industry (DTI), are thanked for financial support.

References

Bethune JE, Hepting GH (1963) Pitch canker damage to South Florida Slash pine *Journal of Forestry* 61: 517 – 522.

Blakeslee GM, Oak SW (1980) Significant mortality associated with Pitch canker infection of Slash pine in Florida. *Plant Disease* 63: 1023 – 1025

Blakeslee GM, Rockwood D (1999) Variation in resistance to pitch canker in slash and loblolly pines. Current and Potential Impacts of the Pitch Canker in Radiata pine: in Devey ME, Matheson AC, Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. p. 70 - 75.

Britz H, Coutinho TA, Wingfield BD, Marasas WFO, Wingfield MJ (2005) Diversity and differentiation in two populations of *Gibberella circinata* in South Africa. *Plant Pathology* 54: 46-52.

Coetzee H (1985) Provenance research on Mexican pines. *Southern African Forestry Journal* 135: 68-73

Coutinho TA, Steenkamp ET, Mongwaketsi K, Wilmot M, Wingfield MJ (2007) First outbreak of pitch canker in a South African pine plantation. *Australasian Plant Pathology* 36: 256 – 261

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal* 205: 3 – 8

Darrow K, Coetzee H (1983) Potentially valuable Mexican pines in the summer rainfall region of Southern Africa. *South African Forestry Journal* 124 :23-35

Department of Agriculture, Forestry and Fisheries (DAFF) (2008) Report on commercial timber resources and primary roundwood processing in South Africa 2006/7. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Dieters M J, White TL, Hodge GR (1995) Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). *Canadian Journal of Forest Research* 25: 1397-1408

Dvorak WS, Gutiérrez EA, Galpare WJ, Hodge GR, Ororio LF, Bester C, Kikuti P (2000) *Pinus maximinoi*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by

the CAMCORE Cooperative, College of Natural Resources, NCSU, Raleigh, NC, USA. Pp 107 – 127

Dvorak WS, Hamrick JL, Furman BJ, Hodge GR, Jordan AP (2002) Conservation strategies for *Pinus maximinoi* based on provenance, RAPD and allozyme information. *Forest Genetics* 9: 263 – 274.

Dvorak WS, Kietzka JE, Donahue JK (1996) Three year survival and growth of provenances of *Pinus greggii* in the tropics and subtropics. *Forest Ecology and Management* 83: 123-131

Dvorak WS, Potter KM, Hipkins VD, Hodge GR (2009) Genetic diversity and gene exchange in *Pinus oocarpa*, a Mesoamerican pine with resistance to the pitch canker fungus (*Fusarium circinatum*) *International Journal of Plant Sciences* 170: 609-626.

Dwinell LD, Phelps WR (1977) Pitch canker of Slash pine in Florida. *Journal of Forestry* 75: 488-489.

Gapare WJ, Hodge GR, Dvorak WS (2001) Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia, and South Africa. *Forest Genetics* 8: 159-170

Guerra-Santos J (1999) Pitch canker in Monterey pine in Mexico. Current and Potential Impacts of the Pitch Canker in Radata pine: in Devey, M. E., Matheson, A.C. and Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. p. 58 - 61.

Hodge GR, Dvorak WS (2000) Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests* 19: 241-258

Hodge GR, Dvorak WS (2007) Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests* 33:193 – 206

Isaza N (2008) Flower promotion in *Pinus maximinoi* and *Pinus tecunumanii* in a tropical environment and artificial screening of high-elevation *P. tecunumanii* for resistance to *Fusarium circinatum*. M.Sc. thesis, North Carolina State University, Raleigh, North Carolina

Kietza JE (1988) *Pinus maximinoi*: A promising species in South Africa. *Southern African Forestry Journal* 145: 33 – 38

Malan FS (2006) The wood properties and sawn-board quality of South African-grown *Pinus maximinoi* (HE Moore). *Southern African Forestry Journal* 208: 39 – 48

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Morris AR, Pallett R (2000) Site requirements and species matching: Pines. In: Owen D (ed) *South African Forestry Handbook, South African Institute of Forestry*, vol 1: pp 80-84.

Nel A, Kanzler A, Dvorak W (2006) Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. In: Fikret Isik (ed) Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. pp 158-161

Nierenberg HI, O'Donnell (1998) New *Fusarium* species and combinations in the *Gibberella fujukuroi* species complex. *Mycologia* 90:434 – 458

Rockwood DL, Blakeslee GM, Lowerts GA, Underhill EM, Oak SW (1988) Genetic strategies for reducing pitch canker incidence in Slash pine. *Southern Journal of Applied Forestry* 12: 28 – 32.

Roux J (2007) Further outbreaks of pitch canker in South Africa. http://www.fabinet.up.ac.za/tpcp/pitch_canker_mature_trees

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, 33: 109 – 123

SAS Institute (2003) Cary, North Carolina, 27513, USA.

Schönau APG, Grey DC (1987) Site requirements for exotic tree species. In *Southern African Institute of Forestry*, *Forestry Handbook* pp. 82-94.

Storer AJ, Bonello P, Gordon TR, Wood DL (1999) Evidence of resistance to the pitch canker pathogen (*Fusarium circinatum*) in native stands of Monterey pine (*Pinus radiata*). *Forest Science* 45: 500-505.

Viljoen A, Wingfield MJ, Marasas WFO (1994) First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. *Plant Disease* 78: 309 – 312.

Viljoen A, Wingfield MJ, Gordon TR, Marasas WFO (1997) Genotypic diversity in a South African population of the pitch canker fungus *Fusarium subglutinans f.sp. pini*. *Plant Pathology* 46: 590-593.

Wikler K, Gordon TR (2000) An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. *Canadian Journal of Botany* 78: 709 – 717.

Wingfield MJ, Hammerbacher A, Ganley RJ, Steenkamp ET, Gordon TR., Wingfield BD, Coutinho TA (2008) Pitch canker caused by *Fusarium circinatum* – a growing threat to pine plantations and forests worldwide. *Australasian Plant Pathology* 37: 319 – 334.

Zwolinski JB and Hinze WHF (2000) Silviculture regimes: Pines. In: Owen D (ed) *South African Forestry Handbook, South African Institute of Forestry,* vol 1: pp 116-120.

Table 1. *Pinus tecunumanii* and *P. maximinoi* provenances screened for tolerance to *Fusarium circinatum*.

Pinus tecunumanii				*Pinus maximinoi*		
Ecotype	Country	Provenance	Families	Country	Provenance	Families
High	Guatemala	KM 47	1	Guatemala	Coban	13
High	Guatemala	San Jerónimo	5	Guatemala	San Jerónimo Guatemala	19
High	Guatemala	San Lorenzo	1	Guatemala	San Juan Sacateqequez	18
High	Honduras	Las Trancas	3	Guatemala	San Lorenzo	1
High	Mexico	Chempil	8	Honduras	Dulce Nombre de Copan	8
High	Mexico	Jitotol	3	Honduras	El Portillo	16
High	Mexico	Montebelo	6	Honduras	Marcala	4
High	Mexico	Napite	1	Honduras	Tatumbla	6
Low	Honduras	Jocón	3	Mexico	Altamirano	4
Low	Honduras	San Esteban	9	Mexico	Coapilla	1
Low	Honduras	San Francisco	15	Mexico	La Cañada	1
Low	Honduras	Villa Santa	22	Mexico	Monte Cristo	1
				Mexico	San Jerónimo Chiapas	13
			77			105

Table 2. Details of plant material, trial layout and dates for inoculation of seedlings inoculated with *F. circinatum*.

	P. tecunumanii	***P. maximinoi***	***P. elliottii***	***P. pseudostrobus***
Date sown	09/2006	07/2007	03/2009	05/2009
Date inoculated	03/04/07	19/03/08	01/12/09	01/12/09
Date assessed	30/05/07	21/05/08	04/02/10	04/02/10
Families tested	49 LE** and 24 HE*	104	49	33
Mean plot size	22	14	11	20
Replications	4	4	4	4
Mean plants per treatment	88	56	44	80
Range of plants per tmt	58-96	12-64	20-64	51-95
Controls	*P. elliottii*	*P. taeda*	*P. elliottii*	*P. elliottii*
	P. patula	*P. elliottii*	*P. patula*	*P. patula*
		P. patula	*P. taeda*	*P. taeda*
		*P. tecH**		
		*P. tecL***		

* *P. tecunumanii* (high elevation)

** *P. tecunumanii* (low elevation)

Table 3. Mean values of the variables measured for the species tested as controls, against *P. tecunumanii*.

Species	Height	Variables measured		
	(mm)	Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula	136.67 ^{C}	22.00 ^{C}	16.89 ^{C}	2.49 ^{C}
P. tec (high)	124.24 ^{B}	9.61 ^{B}	8.20 ^{B}	9.11 ^{B}
P. elliottii	148.34 ^{A}	3.11 ^{A}	2.41 ^{A}	10.66 ^{B}
P. tec (low)	147.68 ^{A}	2.78 ^{A}	2.37 ^{A}	21.78 ^{A}

Species that share the same letter (Duncan grouping) are not significantly different.

Table 4. Mean values of the variables measured for the species tested as controls, and *P. tecunumanii* provenances.

				Variables measured		
Provenance	**Ecotype**	**Country**	**Families**	**Lesion**	**Dieback**	**Re-sprout**
P. patula	Control		Mix	22.01 ^{A}	16.89	2.48
San Jerónimo	HE	Guatemala	5	11.48 ^{B}	10.65	7.56
Montebello	HE	Mexico	6	10.88 ^{B}	9.19	9.31
Chempil	HE	Mexico	8	10.19 ^{B}	8.48	8.95
Jitotol	HE	Mexico	3	8.1 ^{C}	6.28	10.83
Las Trancas	HE	Honduras	3	5.72 ^{D}	4.76	8.99
San Esteban	LE	Honduras	9	3.1 ^{D}	2.68	21.42
San Francisco	LE	Honduras	15	2.79 ^{D}	2.41	20.87
P. elliottii	Control		Mix	3.09 ^{D}	2.39	10.66
Jocón	LE	Honduras	3	2.73 ^{D}	2.25	22.79
Villa Santa	LE	Honduras	22	2.61 ^{D}	16.89	22.41

Provenances that share the same letter (Duncan grouping) are not significantly different.

Table 5. Mean values of the variables measured for the species tested as controls, against *P. maximinoi*.

Species	**Height**	**Variables measured**		
	(mm)	**Lesion length (mm)**	**Dieback (%)**	**Re-sprout (mm)**
P. patula	139.92 ^{A}	21.04 ^{A}	16.39 ^{A}	18.99 ^{BC}
P. tecHE	107.37 ^{C}	8.63 ^{B}	8.93 ^{B}	22.00 ^{BA}
P. elliottii	109.66 ^{C}	5.91 ^{C}	6.02 ^{C}	17.27 ^{C}
P. taeda	123.33 ^{B}	4.95 ^{C}	4.22 ^{D}	15.87 ^{C}
P. maximinoi	95.68 ^{D}	3.19 ^{D}	3.84 ^{DE}	25.44 ^{A}
P. tecLE	136.47 ^{A}	2.96 ^{D}	2.19 ^{E}	24.11 ^{A}

Species that share the same letter (Duncan grouping) are not significantly different.

Table 6. Mean values of the variables measured for the species tested as controls, and *P. maximinoi* provenances (represented by at least 4 families).

Provenance	Country of origin	Families	Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula (control)		Mix	21.04 ^{A}	16.39 ^{A}	18.99 ^{FE}
P. tecunumanii - high (control)		Mix	8.63 ^{B}	8.93 ^{B}	22.00 ^{DEC}
P. elliottii (control)		Mix	5.91 ^{C}	6.02 ^{C}	17.27 ^{F}
P. taeda (control)		Mix	4.95 ^{DC}	4.22 ^{DFE}	15.87 ^{F}
Altamirano	Mexico	4	3.64 ^{FE}	4.00 ^{DFE}	21.64 ^{DE}
San Juan Sacateqequez	Guatemala	18	3.32 ^{FE}	4.07 ^{DFE}	25.96 ^{BAC}
San Jerónimo Chiapas	Mexico	13	3.28 ^{FE}	3.28 ^{FE}	25.16 ^{BDAC}
El Portillo	Honduras	16	3.21 ^{F}	3.21 ^{F}	24.80 ^{BDAC}
Dulce Nombre de Copan	Honduras	8	3.20 ^{F}	3.20 ^{F}	24.78 ^{BDAC}
San Jerónimo Baja Verapaz	Guatemala	19	3.17 ^{F}	3.91 ^{DFE}	25.02 ^{BDAC}
Coban	Mexico	13	3.06 ^{F}	3.87 ^{DFE}	26.73 ^{BA}
Tatumbla	Honduras	6	2.98 ^{F}	3.92 ^{DFE}	24.98 ^{BDAC}
Marcala	Honduras	4	2.91 ^{FG}	3.35 ^{GFE}	27.39 ^{BA}
P. tecunumanii – low (control)		Mix	2.70 ^{FG}	2.19 ^{G}	24.11 ^{BDAC}

Provenances that share the same letter (Duncan grouping) are not significantly different.

Table 7. Comparison of the responses to inoculation with *F. circinatum* on *P. elliottii* with those on *P. patula* and *P. taeda* based on lesion length, die-back and the ability of plants to re-sprout.

Species inoculated	Height (mm)	Disease characteristics		
		Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula (control)	148.34	23.7 ^{A}	9.46 ^{A}	10.40 ^{B}
P. elliottii	124.24	5.3 ^{B}	2.44 ^{B}	24.19 ^{A}
P. taeda (control)	136.67	4.2 ^{B}	1.53 ^{C}	9.97 ^{B}

Table 8. Comparison of the responses of inoculation with *F. circinatum* on *P. pseudostrobus* with those on *P. patula*, *P. elliottii* and *P. taeda* based on lesion length, die-back and the ability of plants to re-sprout.

Variety	Height	Variables measured		
	(mm)	Lesion length (mm)	Dieback (%)	Re-sprout (mm)
P. patula	136.67	24.6 ^{A}	21.7 ^{A}	10.6 ^{A}
P. elliottii	124.24	10.8 ^{B}	10.2 ^{B}	42.6 ^{B}
P. taeda	148.34	7.1 ^{C}	5.8 ^{C}	17.3 ^{C}
P. pseudostrobus	147.68	3.7 ^{D}	2.6 ^{D}	21.3 ^{C}

Species that share the same letter (Duncan grouping) are not significantly different.

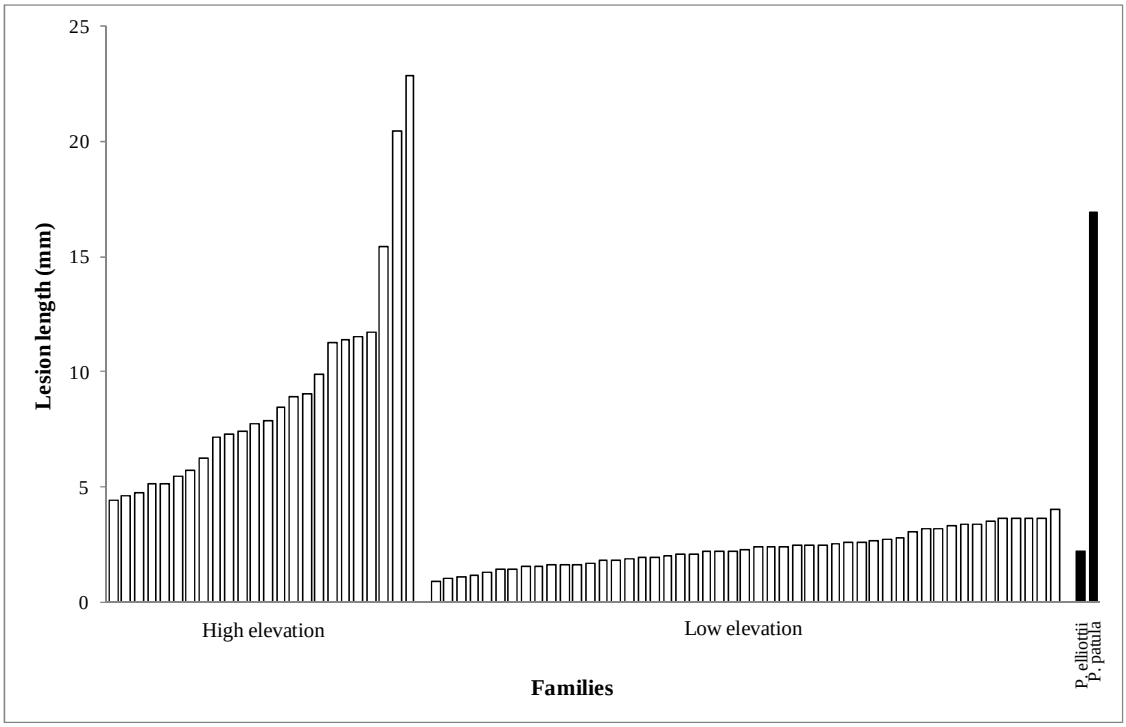

Fig. 1. Mean lesion length (corrected for height) for all *P. tecunumanii* families screened for tolerance to *F. circinatum* compared to the controls (*P. elliottii* and *P. patula*). Within ecotype, treatments are ranked from most to least tolerant. Narrow-sense heritability (Dieters et al., 1995) was calculated at 0.59 for the high elevation ecotype and 0.01 for the low elevation ecotype.

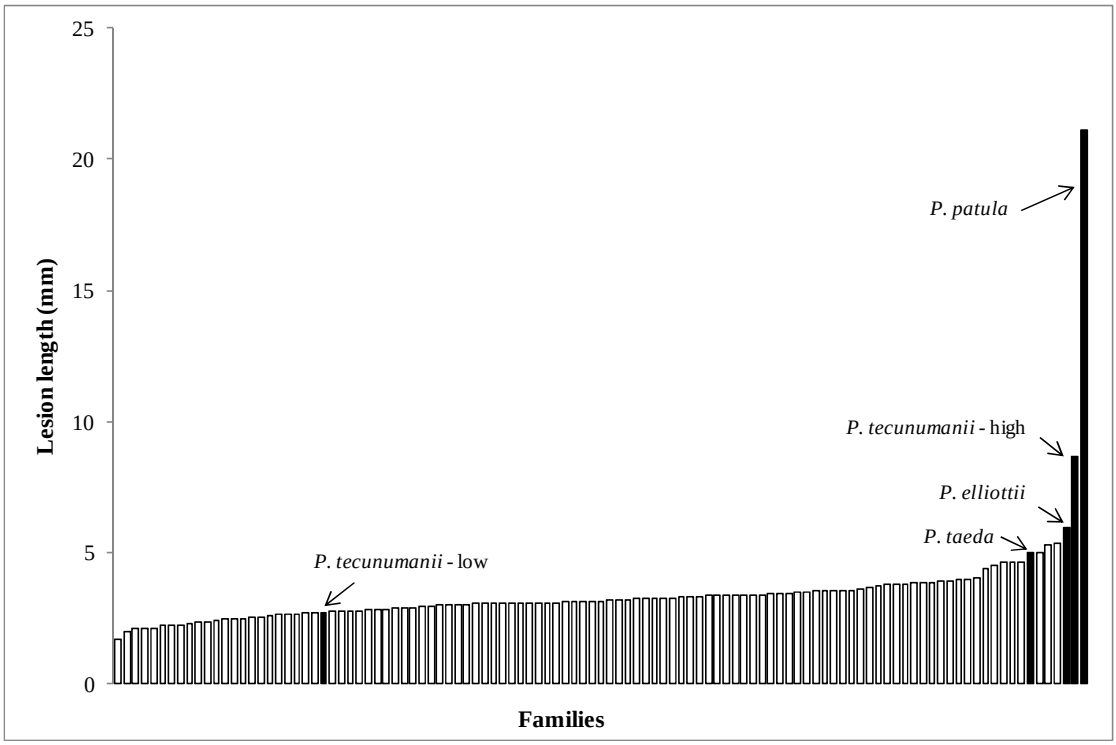

Fig. 2 Mean lesion length (corrected for height) for all *P. maximinoi* families screened compared to the controls. Treatments are ranked from most to least tolerant. Narrow-sense heritability (Dieters et al., 1995) was calculated at 0.014.

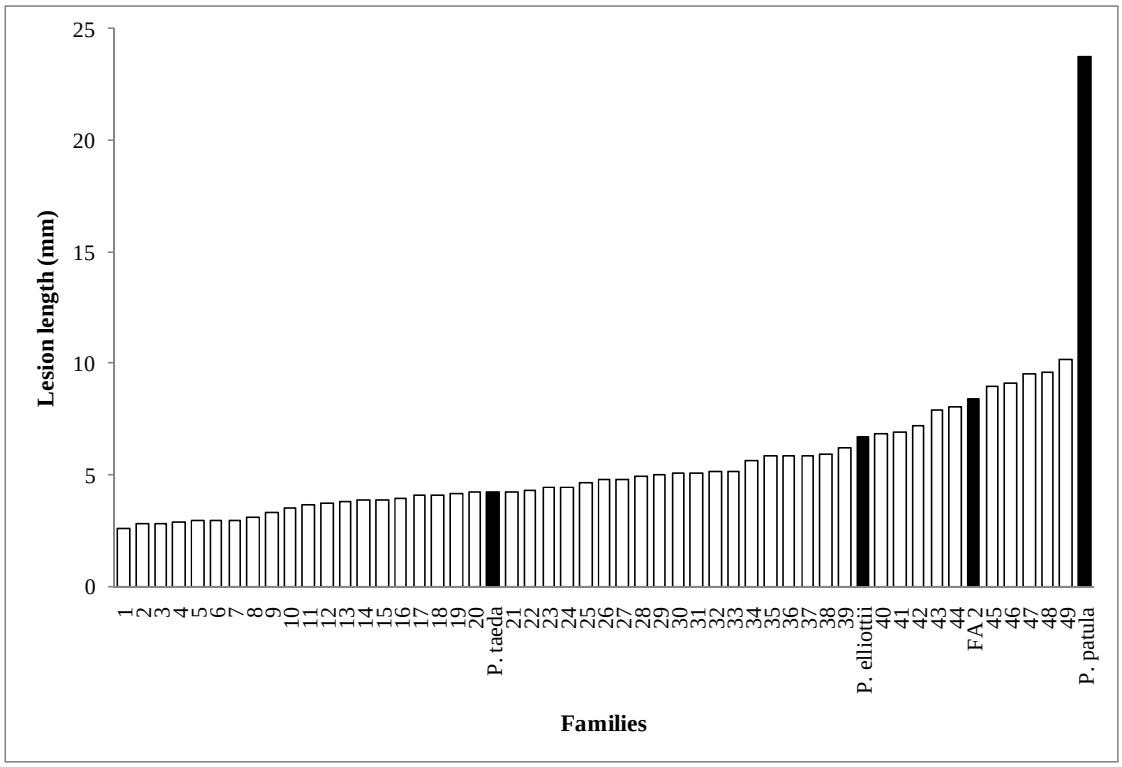

Fig. 3 Mean lesion length (corrected for height) for all *P. elliottii* families inoculated with *F. circinatum* compared to the *P. patula* and *P. taeda* controls and the susceptible *P. elliottii* control (FA2). Treatments are ranked from most to least tolerant. Narrow-sense heritability (Dieters et al., 1995) was calculated as 0.22.

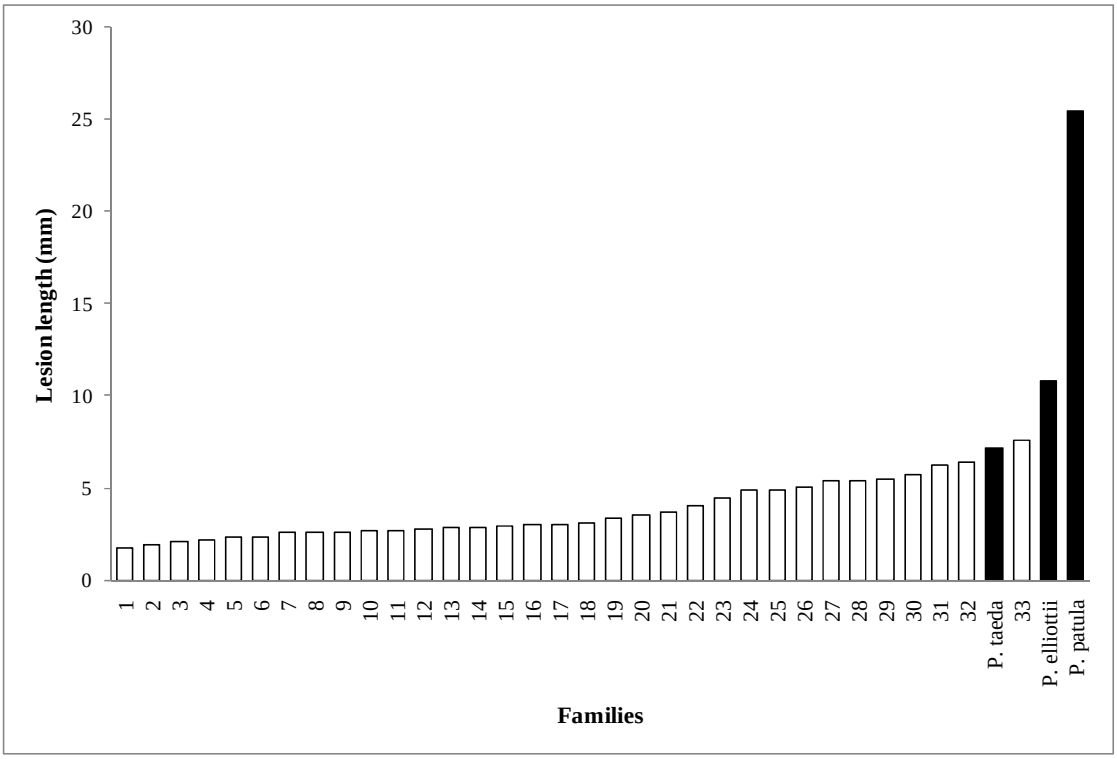

Fig. 4 Mean lesion length (corrected for height) for all *P. pseudostrobus* families screened compared to the controls. Treatments are ranked from most to least tolerant. Narrow-sense heritability (Dieters et al., 1995) was calculated at 0.06.

(Published: New Forests, 2012, DOI: 10.1007/s11056-012-9355-3).

Chapter 3:

The tolerance of *Pinus patula x Pinus tecunumanii*, and other pine hybrids, to *Fusarium circinatum* in greenhouse trials

Abstract

The field survival of *Pinus patula* seedlings in South Africa is frequently below acceptable standards. From numerous studies it has been determined that this is largely due to the pitch canker fungus, *Fusarium circinatum*. Other commercial pines, such as *P. elliottii* and *P. taeda*, show good tolerance to this pathogen and better survival, but have inferior wood properties and do not grow as well as *P. patula* on many sites in the summer rainfall regions of South Africa. There is, thus, an urgent need to improve the tolerance of *P. patula* to *F. circinatum*. Operational experience indicates that when *P. patula* is hybridized with tolerant species, such as *P. tecunumanii* and *P. oocarpa*, survival is greatly improved on the warmer sites of South Africa. Field studies on young trees suggest that this is due to the improved tolerance of these hybrids to *F. circinatum*. In order to test the tolerance of a number of pine hybrids, the pure species representing the hybrid parents, as well as individual families of *P. patula* x *P. tecunumanii*, a series of greenhouse screening trials were conducted during 2008 and 2009. The results indicated that species range in tolerance and hybrids, between *P. patula* and these species, are intermediate in tolerance to *F. circinatum*. Within *P. patula x P. tecunumanii*, large family variation exists when pollen from the high elevation source of *P. tecunumanii* is used. The results of these studies illustrate the importance of developing pine hybrid breeding programs to overcome the susceptibility of our pure species to pathogens such as *F. circinatum*.

Keywords; Forestry, disease tolerance, hybrids, greenhouse screening

Introduction

The successful establishment of South Africa's most important pine species, *Pinus patula* (DAFF 2010), is severely hampered by the pitch canker fungus, *Fusarium circinatum* (Mitchell et al. 2011). The greatest loss is seen in the field during the first six months post planting (Crous 2005). Currently, breeders are trying to improve the tolerance of *P. patula* to *F. circinatum* by screening for superior families using greenhouse inoculation studies. However, the level of tolerance within *P. patula* open-pollinated families does not seem to consistently translate into increased post-planting survival (A. Nel, Sappi forests, pers. com). In the immediate term, the problem can be overcome by planting *P. elliottii* and *P. taeda* on sites where *P. patula* is best suited. These, however, do not grow as well as *P. patula* on temperate sites, and have inferior wood properties for some applications (Kietzka 1988; Morris and Pallett 2000; Malan 2003).

As an alternative to planting pure species, there is a growing interest in South Africa to identify hybrids between *Pinus patula* and other pines to reduce the susceptibility of young *P. patula* seedlings to *Fusarium circinatum*. *Pinus patula x P. tecunumanii* is exhibiting good tolerance to *F. circinatum* (Roux et al. 2007) and growth (Nel et al. 2006; Kanzler et al. 2012), with numerous forestry companies deploying this hybrid on a commercial scale. Although *P. patula* x *P. tecunumanii* is exhibiting improved tolerance operationally, no selection for tolerance to *F. circinatum* has been carried out within the hybrid. Previous studies have shown that there is large provenance variation for tolerance to *F. circinatum* in both *P. patula* and *P. tecunumanii* (Hodge and Dvorak, 2006). This variation is closely related to the altitude where provenances naturally occur (Hodge and Dvorak, 2006). Provenances of *P. tecunumanii*, occurring below 1500 m (low elevation, *P. tecunumanii* (L)) in their home range are more tolerant to *F. circinatum* than those occurring above this altitude (high elevation, *P. tecunumanii* (H)) (Hodge and Dvorak 2000; 2006). Therefore, the variation in tolerance to *F. circinatum* among *P. patula* and *P. tecunumanii* suggests that *P. patula* x *P. tecunumanii* families are also likely to vary in tolerance. In addition to *P. tecunumanii*, *P. patula* has been successfully hybridized with a number of other species in South Africa including *P. oocarpa, P. elliottii, P. pringlei, P. greggii, P. taeda, P. herreare, P. maximinoi* and *P. caribaea*. A number of these, and other hybrids not necessarily with *P. patula*,

may in the future be planted in order to capture good characteristics which would include tolerance to *F. circinatum*.

The primary objective of this study was to examine the genetic control of *F. circinatum* tolerance among *P. patula x P. tecunumanii* hybrid families. A secondary objective was to assess the tolerance of a wide array of different pine hybrids in order to identify other potential hybrids that could be used to replace *P. patula* to reduce losses associated with *F. circinatum*. These objectives were investigated using artificial inoculation of seedlings and cuttings in a series of three greenhouse screening trials carried out in 2008 and 2009.

Materials and Methods

Pinus patula x Pinus tecunumanii

The first greenhouse trial (Trial 1: *P. pat x P. tec*) was conducted in November, 2008, and focused on the *P. patula x P. tecunumanii* hybrids. Included in the study were 75 *P. patula x P. tecunumanii* (L) families (*P. pat x P. tec*L) produced from crossing 13 *P. patula* parents and 12 *P. tecunumanii* (L) parents. There were also 24 *P. patula x P. tecunumanii* (H) families (*P. pat x P. tec*H) produced from crossing 10 *P. patula* parents and 7 *P. tecunumanii* (H) parents. All hybrid crosses were made using *P. patula* as the female parent, and *P. tecunumanii* as the male parent (see Appendix 1 for the specific crossing design). All hybrid families were screened for tolerance to *F. circinatum* using cuttings from juvenile seedling hedges. In addition, the trial also contained control-pollinated seedlings of 21 of the 75 *P. pat x P. tec*L and 6 of the 24 *P. pat x P. tec*H families. Finally, the trial also included open pollinated pure-species *P. patula* seedlings from the 13 *P. patula* mothers that were used as female parents of the hybrids, and bulk seedlings of a *P. elliottii* control (Table 1).

Other hybrids and species

In the second trial (Trial 2: Hybrids), cuttings from 14 hybrids (produced from 9 *Pinus* species), were screened in March 2009 (Table 1). Of the 14 hybrids, 6 were produced using *P. patula* as the female parent. Open-pollinated *P. patula* and *P. elliottii* seedlings, from commercial seed orchards, were included as controls. In order to compare the tolerance of the hybrids with the

pure species used to produce the hybrids, open-pollinated seedlings, representing 8 of the 9 parent species, were inoculated in a third trial (Trial 3: Species) in December 2009 (Table 1).

Plant growing conditions

The plants were raised in the Komatiland Forests Research nursery near the town of Sabie. Both plant types were grown in composted pine bark in plastic seedling containers with removable individual inserts of 0.09 dm3 capacity (Unigro98©), under covered greenhouse plastic and irrigated and fertilized where necessary. Cuttings were raised for approximately 9 months whilst seedlings were raised for approximately 7 months. At the end of the nursery phase, treatments were arranged in a randomized complete block design across, in most cases, 4 replications. Subject to plant availability each plot consisted of 12 - 22 plants. After packing the trials out, the plants were transported to the greenhouse screening facility at the University of Pretoria.

Inoculation procedure

Inoculation was carried out by removing the plant's apical bud with sharp secateurs and applying 500 spores of *F. circinatum* in a water solution to the wounded surface. The solution contained an equal mixture of 3 highly virulent South African isolates (CMW 3577, 3578, and 3579). Once inoculated, the trial was watered daily and assessed for lesion development 8 weeks after inoculation. Lesion development was read in mm from the tip of the seedling at the point of inoculation, to the point where the tissue showed no further visible necrosis. The seedling height, from the root collar to the wounded tip, was also measured. The proportion of lesion length, to the length of the seedling, was expressed as a percentage dieback. New shoot development, below the infected area, was measured in the *P. patula x P. tecunumanii* and species trial and is referred to as "resprout".

Statistical analysis – Trial 1: P. pat x P. tec

The statistical software package, SAS (SAS Institute, 2003) was used to analyse the data generated from the *P. patula x P. tecunumanii* trial (Trial 1). Pearson product moment correlations of family means for lesion length, percentage dieback, resprout and initial plant height were calculated, primarily to determine if there was a need to include height as a covariate

in subsequent models. Following this, a series of analyses of variance (ANOVAs) were done using SAS Proc GLM, and a series of variance component analyses were done using SAS Proc Mixed.

First, an analysis was done to examine if there were differences among the different species or hybrids and plant types (i.e., seedlings or cuttings). The data set included the open-pollinated seedlings of *P. patula* and *P. elliottii*, and both the seedlings and cuttings of *P. pat x P. tec*L and *P. pat x P. tec*H. The linear model included fixed effects for replicate (rep), species, and plant type, and random effects for family(species), type*family(species), rep*type*family(species), and initial height as a covariate. Duncan's multiple range tests were used to examine differences among species/hybrids - plant type combinations.

Next, a more focused analysis was done on the hybrid plants to examine if there was a difference in tolerance among the type of plant, i.e., seedlings and cuttings. The linear model for the combined data set (with both hybrids) included fixed effects for replicate, hybrid, plant type, and hybrid*type, and random effects for family(hybrid), type*family(hybrid), rep*type *family(hybrid), and initial height as a covariate. Separate ANOVAs were also done for the *P. pat x P. tec*L and *P. pat x P. tec*H data sets, with a reduced model eliminating effects for hybrid and hybrid*type. An ANOVA was also done on the open-pollinated *P. patula* data set to test for family differences in tolerance. The linear model included fixed effects for replicate, and random effects for family and rep*family, and initial height as a covariate.

Finally, variance component analyses for lesion length were done using SAS Proc Mixed to estimate genetic parameters for tolerance to *F. circinatum*. The analysis for the open-pollinated *P. patula* families used a model including fixed effects for replicate, and random effects for family and rep*family, and initial height as a covariate. Narrow-sense heritability (h^2) was estimated for lesion length for *P. patula* according to Dieters et al. 1995 as $h^2 = (3 \times \sigma^2_{female}) / \sigma^2_{phenotypic}$, where σ^2_{female} = variance due to *P. patula* female parent, and $\sigma^2_{phenotypic}$ = total phenotypic variance for lesion length. Separate variance component analyses were done for the *P. pat x P. tec*L and *P. pat x P. tec*H data sets using all plants of both types (cuttings and seedlings). The linear model included a fixed effect for replicate, and random effects for the *P.*

patula female parent, the *P. tecunumanii* male parent, the interaction of *P. patula x P. tecunumanii* parents, type*family, and type*rep*family, along with initial height as a covariate. For the hybrid data sets, the variance associated with *P. patula* female parent was taken to be the variance of general hybridizing ability (GHA) for *P. patula* ($\sigma^2_{GHA\text{-}pat}$), the variance associated with *P. tecunumanii* male parent was taken to be the GHA variance for *P. tecunumanii* ($\sigma^2_{GHA\text{-}tec}$), and the variance associated with the *P. patula x P. tecunumanii* interaction was taken to be the variance of specific hybridizing ability (SHA) ($\sigma^2_{SHA\text{-}pat\ x\ tec}$). Total phenotypic variance was estimated as the sum of all the variance components, and the percentage of variance accounted for each component was calculated. Total genetic variance among full-sib hybrid families was estimated as $\sigma^2_{G\text{-}FS} = \sigma^2_{GHA\text{-}pat} + \sigma^2_{GHA\text{-}tec} + \sigma^2_{SHA\text{-}pat\ x\ tec}$. Following Dieters et al. (1997), two separate estimates of heritability were calculated for the hybrid data sets as follows:

$$h^2_{pat} = (4 \times \sigma^2_{GHA\text{-}pat}) / \sigma^2_{phenotypic}$$

$$h^2_{tec} = (4 \times \sigma^2_{GHA\text{-}tec}) / \sigma^2_{phenotypic}$$

In addition, proportion of dominance (d^2) was calculated for the hybrid data sets as:

$$d^2_{pat\ x\ tec} = (4 \times \sigma^2_{SHA\text{-}pat\ x\ tec}) / \sigma^2_{phenotypic}$$

Statistical analysis – Trial 2: Hybrids, Trial 3: Species

The statistical software package GenStat 7.22 (2008) was used to analyse the data generated from the hybrid and species trials (Trials 2 and 3). Pearson product moment correlations of family means for lesion length, percentage dieback, resprout and initial plant height were calculated, primarily to determine if there was a need to include height as a covariate in subsequent models. Following this, an ANOVA was done on percentage dieback, lesion length, and length of the resprout with a model containing initial height as a covariate, and fixed effects for replicate, species/hybrid, and rep*species/hybrid. A Duncan's Multiple Range test was used to distinguish differences between species and hybrids.

Results

Trial 1: P. pat x P. tec

Plant height correlated significantly ($p<0.001$) with lesion length ($r = -0.41$), percent dieback ($r = -0.63$) and resprout ($r = -0.30$), and was used as a covariate in all analyses. Percent dieback and lesion length were highly correlated ($p<0.001$, $r = 0.92$) and lesion length was used to rank and compare treatments. There were clear differences for lesion length among species and hybrids. *P. elliottii* was the most tolerant (5.7 mm) followed by *P. pat x P. tec*L (7.5 mm), *P. pat x P. tec*H (17.1 mm) and *P. patula* (29.4 mm) (Table 2). There was no evidence for differences in tolerance among plant types (i.e., cuttings and seedlings) for lesion length (Tables 2, 3). When comparing percentage dieback, cuttings were significantly more tolerant than seedlings. Since lesion lengths for the cuttings and seedlings were similar, this seems attributable primarily to the fact that the cuttings were more than twice as tall as the seedlings. There was good evidence for significant differences in tolerance among hybrid families (Table 3). When each hybrid was analysed separately, families differed at the 10% level ($p\approx0.07$) and when the data was combined, differences among families were highly significant ($p=0.006$) (Table 3).

The range of family tolerance in *P. pat x P. tec*H was large where 23 out of the 24 families displayed lesions ranging from 5.5 to 25.4 mm in length, with the most susceptible family measuring 37 mm. Fifty percent of the *P. pat x P. tec*H families (centered around the median) displayed lesion lengths that ranged from 9 to 18 mm (Fig 1). The range in family tolerance of *P. pat x P. tec*L was smaller where 74 out of 75 families had lesion lengths ranging from 3.4 to 14.9 mm with the most susceptible family measuring on average 25.6 mm. Fifty percent of the *P. pat x P. tec*L families (centered around the median) displayed lesion lengths that ranged from 6 to 8 mm (Fig. 1). The range in tolerance of the 13 *P. patula* families was reflected by lesions from 17.3 to 40.3 mm in length where fifty percent (centered around the median) ranged from 25 to 32mm. Despite the large range, family differences in *P. patula* were not statistically significant ($p = 0.43$) (Table 3).

Family differences in *P. patula x P. tecunumanii* were primarily due to a result of the specific interaction between the *P. patula* and *P. tecunumanii* parents (i.e., $\sigma^2_{SHA\text{-}pat\ x\ tec}$), and to a lesser

extent the general ability of *P. patula* parents or *P. tecunumanii* parents to confer tolerance to their hybrid offspring (i.e., $\sigma^2_{GHA\text{-}pat}$ or $\sigma^2_{GHA\text{-}tec}$). This can be seen in Table 4 by comparing the *P. patula* and *P. tecunumanii* parental variance components to the *P. pat x P. tec* interaction variance component. In the case of *P. pat x P tec*H, SHA variance accounted for 9.6% of the phenotypic variance, while $\sigma^2_{GHA\text{-}pat}$ accounted for 6.4% of the phenotypic variance, and $\sigma^2_{GHA\text{-}tec}$ accounted for only 2.1% of the phenotypic variance (Table 4). The total genetic variance for hybrid families of *P. pat x P tecH* was 18.1% of the phenotypic variance. In the case of *P. pat x P tec*L, even though there was relatively little genetic variance among hybrid families (6.8% of the total phenotypic variance), SHA variance accounted for the bulk of that variance (4.2% of the phenotypic variance). The variation due to the *P. patula* and *P. tecunumanii* parents accounted for only 0.8% and 1.8% of the phenotypic variance, respectively (Table 4). Among the *P. patula* families, there was little genetic variation observed, corresponding to a narrow sense heritability of $h^2 = 0.06$ (i.e., 6% of the total phenotypic variance, Table 4). In contrast, there was more genetic control of tolerance in the *P. pat x P tec*H data set, with $h^2_{pat} = 0.25$ and $h^2_{tec} = 0.08$ (Table 4).

Trial 2: Hybrids, Trial 3: Species

Similar to the *P. patula x P. tecunumanii* data, lesion length correlated strongly with percent dieback both in the hybrid ($p<0.0001$, $r = 0.959$) and in the pure species trial ($p<0.0001$, $r = 0.946$), and was accordingly used to rank treatments. Seedling height had a significant negative effect on percent dieback in the hybrid ($p <0.001$, $r = -0.217$) and pure species trial ($p<0.0001$, $r = -0.263$) but had no effect on lesion length. Lesion length and resprout correlated negatively ($p<0.001$, $r = -0.450$) in the species trial indicating that the more tolerant species produced longer shoots after wounding and infection.

Pinus greggii var. *greggii* (*P. greg*N) ranked most susceptible (mean lesion length = 38 mm) in the species trial followed by *P. patula* (mean lesion length = 29.8 mm), *P. greggii* var. *australis* (*P. greg*S) (mean lesion length = 18.5 mm), *P. tecunumanii* (H) (mean lesion length = 9.2 mm), *P. elliottii* (mean lesion length = 8.8 mm), *P. caribaea* (mean lesion length = 8 mm), *P. tecunumanii* (L) (mean lesion length = 5.1 mm), *P. oocarpa* (mean lesion length = 4.2 mm) and *P. pringlei* (mean lesion length = 3.8 mm) (Table 5). All hybrids made with *P. patula* were

significantly more tolerant than *P. patula* (Fig. 2). In general, there was some correspondence between the tolerance of pure species, and the tolerance of *P. patula* hybrids. Hybrids made with *P. tecunumanii* (H), *P. greggii* (S), and *P. pringlei* were the most susceptible (Fig. 2) and more susceptible than *P. elliottii*. Hybrids made with *P. patula* and some of the most tolerant species (*P. oocarpa, P. tecunumanii* (L) and *P. elliottii*) were similar in tolerance to *P. elliottii*, and no different than hybrid combinations made between two tolerant species (Fig. 2). One surprising exception to this general pattern was that *P. patula* x *P. pringlei* was similar in tolerance to *P. pat* x *P. tec*H despite the fact that *P. pringlei* is highly tolerant as a pure species (Hodge and Dvorak 2000) which was also seen in Trial 3 (Table 5). This may be explained by the fact that there were an insufficient number of plants (28) of the hybrid, which were only represented across 2 replications, to obtain an accurate indication of the tolerance of this hybrid. This was also supported by a large standard error of the mean (3.44 mm).

Discussion

Standard quantitative genetics theory is based on assumptions of random mating populations of a pure species in genetic equilibrium. Hybrid populations are not in genetic equilibrium, and it is not clear that the concepts of additive genetic variance or heritability are appropriate for F_1 hybrids, or that hybrid heritabilities would predict genetic gain from forward selection of the best offspring of F_1 hybrids (Gordon 1999). However, there are no such difficulties when using hybrid progeny test data and genetic parameters to identify superior parents of hybrid families and to predict genetic gain from backward selection of the best hybrid parents and families. The parents of those families could then be re-crossed, and the resulting progeny mass propagated.

The data in this study suggest that substantial improvement in tolerance in *P. pat x P. tec*H can be made through identification of superior families. In this case family variation in tolerance is due mostly to the combination of specific parents (i.e., high $\sigma^2_{\text{SHA-pat x tec}}$, see Table 4), and this indicates that it will be necessary to screen all *P. pat x P. tec*H families. Relative to pure *P. patula*, there was improved tolerance of the *P. pat x P. tec*H hybrid to *F. circinatum.* Overall, however, *P. pat x P. tec*H was not as tolerant as *P. pat x P. tec*L. There was also much larger

variation seen among the 24 *P. pat x P. tec*H families tested, and some families were as susceptible as *P. patula* (Fig. 2).

With the very small amount of genetic variation observed among a large number of *P. pat x P. tec*L families tested, it is clear that it is not necessary to screen families of *P. pat x P. tec*L for *F. circinatum* tolerance. Although this hybrid is doing very well in field trials, it is restricted to warm temperate sites and is therefore likely to replace only a small portion of area planted to *P. patula*. It will, however, provide a good alternative to a number of other species such as *P. elliottii* and the popular *P. elliottii x P. caribaea* hybrid. Also, studies have shown that the tolerance of *P. tecunumanii* to frost is under genetic control (Mitchell et al. 2012) indicating that through selection the planting range of *P. pat x P. tec*L could be increased.

In the *P. patula x P. tecunumanii* trial, although the cuttings had slightly shorter lesion lengths, these differences were not statistically significant. There is, however, other evidence to suggest that cuttings are more tolerant, which concurs with general experience. In the nursery, cuttings appear less affected during outbreaks of *F. circinatum* (unpublished) and often survive better after planting (Mitchell et al. 2004). If this is so, it may be related to an increase in the maturation state of the plant (Zagory and Libby 1985; Mitchell et al. 2004). Elsewhere, studies on *P. radiata* have shown cuttings to be more tolerant than seedlings to *Endocronartium harknessii* (Power et al. 1994) and *P. taeda* cuttings show more tolerance to *Cronartium quercuum* than seedlings (Frampton et al. 2000). Further work in this field is needed.

Analysis of variance revealed no statistically significant variation in tolerance to *F. circinatum* amongst *P. patula* families (Table 3), and variance component analysis indicated low (although non-zero) heritability ($h^2 = 0.06$, Table 4). This lack of variation in tolerance could be explained by the fact that many of the *P. patula* seedlings died after inoculation due to their small size (average 68 mm), or from the limited number (13) families tested leading to an unbalanced representation across replications. Although the planting of *P. pat x P. tec*H may be extended to include some temperate sites, *P. patula* will remain an important species on high altitude sites due to its good growth and wood properties once successfully established. Therefore, if it is

possible, it would be very useful to identify *P. patula* families that are tolerant to *F. circinatum* although the genetic parameter estimates from this study suggest that this will be a challenge.

The tolerance of hybrids generated with tolerant species, and the improvement in tolerance when *P. patula* is hybridized with tolerant species, has been seen in field studies (Roux et al. 2007). The ranking of species in susceptibility to *F. circinatum* in our study is similar to that of Hodge and Dvorak (2000). Although, *P. greggii* var. *greggii* (*P. greg*N) was not used to make any of the hybrids tested, it was included in the species trial because it is known to be more susceptible than *P. patula* (Hodge and Dvorak 2000). It appears that, in general, tolerance to *F. circinatum* in pure species is closely related to the amount of tolerance that species is able to bring to a *P. patula* hybrid. Thus, one could expect a *P. patula x P. jaliscana* hybrid to exhibit good tolerance, based on the extreme tolerance of *P. jaliscana* as a pure species (Hodge and Dvorak 2000).

The success of other hybrids such as *P. elliottii x P. caribaea* (van der Sijde and Roelofsen 1986) and *P. patula x P. greggii* (Kietzka 2002) illustrates the potential that hybrid forestry offers as an alternative to pure species in South Africa. The *P. pat x P. tec*H hybrid is likely to become very important in the future due to its superior tolerance to frost compared to *P. pat x P. tec*L. *P. patula x P. tecunumanii* (H) has already been planted over a wide range of altitude classes including 1700 m above sea level with good success. Currently, it is the best hybrid alternative to *P. patula* on sites that experience light frost events and, by selecting provenances and families that are more frost tolerant (Dvorak et al. 2000; Mitchell et al. 2012), its planting range may be extended to include temperate sites. Although the immediate need is to identify hybrids between *P. patula* and species more tolerant to *F. circinatum*, there is a possibility that other hybrids may be deployed in the future in preference to *P. patula* on many sites. Currently, members of Camcore are testing a large number of hybrids over many sites in which *P. patula* is included as a control. More than 48 Camcore hybrid trials have been planted across Southern Africa and South America in the last 3 years testing over 15 different hybrids (J. Lopez pers. com 2011) with one local company testing approximately 40 pine hybrids. From these trials several hybrids are likely to show potential as an alternative to planting *P. patula*.

The findings presented here provide good evidence that the tolerance of *P. patula* to *F. circinatum* can be significantly improved by hybridizing it with *P. tecunumanii* and other tolerant species. Since *F. circinatum* is a major cause of seedling mortality after planting, the improved tolerance of *P. patula* hybrids may explain their better survival operationally. In the case of *P. patula x P. tecunumanii* the significant improvement in tolerance when the pollen is sourced from low elevation *P. tecunumanii* indicates that this hybrid need not be screened. However, there is large variation in the tolerance of hybrid families to *F. circinatum* when pollen is sourced from high elevation *P. tecunumanii* and care should be taken to screen these before large-scale commercial deployment. All other hybrids between *P. patula*, and species more tolerant to *P. patula*, display a significant improvement in tolerance to *F. circinatum*. Some of these hybrids are being tested in field trials and may prove to be valuable alternatives to *P. patula* in the future.

Acknowledgements

We wish to thank, in particular, Komatiland Forests for providing the seed and incurring the costs involved in raising and screening the plant material. We also thank Bernice Porter who coordinated much of the screening activities at the University of Pretoria and ensured that plants were watered in the greenhouse. Lastly, we thank the members of the Tree Protection Co-operative Programme (TPCP) and the THRIP initiative of the Department of Trade and Industry (DTI), for financial support.

References

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal*, vol. 205: 3 – 8.

Department of Agriculture, Forestry and Fisheries (DAFF) (2010) Report on commercial timber resources and primary roundwood processing in South Africa 2008/9. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Dieters MJ, Nikles DG, Toon PG, Pomroy P (1997) Genetic parameters for F1 hybrids of Pinus caribaea var. hondurensis with both *Pinus oocarpa* and *Pinus tecunumanii*. Canadian Journal of Forest Research, 27(7): 1024-1031.

Dieters MJ, White TL, Hodge GR (1995) Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). *Canadian Journal of Forest Research* 25: 1397-1408.

Dvorak WS, Hodge GR, Gutiérrez EA, Osorio LF, Malan FS, Stanger TK (2000) *Pinus tecunumanii*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative. College of Natural Resources, North Carolina State University. 188-209.

Frampton JF, Li B, Goldfarb B (2000) Early field growth of Loblolly pine rooted cuttings and seedlings. *Southern Journal of Applied Forestry*, Vol. 24: 98–105.

Gordon, IL (1999). Quantitative genetics of intraspecific hybrids. Heredity 83:757-764.

Hodge GR, Dvorak WS (2000). Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests*, 19: 241-258.

Hodge GR, Dvorak WS (2006). Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests*, 33:193 – 206.

Kanzler A, Payn K, Nel A (2012) Performance of two *Pinus patula* hybrids in Southern Africa. *Southern Forests* 74:19-25.

Kietzka JE (1988) *Pinus maximinoi*: A promising species in South Africa. *Southern African Forestry Journal* 145: 33 – 38

Kietzka JE (2002) “New” pine species and hybrids: Is there still potential? *Southern African Forestry Journal* 195: 89-92.

Malan FS (2003) The wood properties of South African timber resource for high-value solid wood products and its role in sustainable forestry. *Southern African Forestry Journal*, vol. 198:53-62.

Mitchell RG, Jones NB, Zwolinski J (2004) A review of on the effects of donor maturation on rooted conifer cuttings. *South African Forestry Journal*, vol. 204: 53-64.

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Mitchell RG, Wingfield MJ, Hodge GR, Dvorak W, Coutinho TA (2012) Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa. New Forests DOI: 10.1007/s11056-012-93.

Morris AR, Pallett R (2000) Site requirements and species matching: Pines. In: Owen D (ed) *South African Forestry Handbook, South African Institute of Forestry*, vol 1: pp 80-84.

Nel A, Kanzler A, Dvorak W (2006). Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. *In* Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. *Edited by* Fikret Isik. p 158-161.

Power AB, Dodd RS, Libby WJ (1994) Effects of hedging on maturation in Radiata pine: Western Gall Rust Susceptibility. *Silvae Genetica* 43: 1-7.

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, Vol. 33: 109 – 123.

SAS Institute (2003) Cary, North Carolina, 27513, USA.

van der Sijde HA, Roelofsen JW (1986) The potential of pine hybrids in South Africa. *Southern African Forestry Journal*, March 1986: 5-14

Zagory D, Libby WJ (1985) Maturation-related resistance of *Pinus radiata* to Western Gall Rust. *The American Phytopathological Society*, 75: 1443-1447.

Table 1. The list of treatments in the three trials examining tolerance of *P. patula* and other species and hybrids to artificial inoculation with *Fusarium circinatum*.

Details	*Trial 1: P. patula x P. tecunumanii*	Trial 2: Various hybrids	Trial 3: Species trial
Date inoculated	11/2008	03/2009	12/2009
Date assessed	01/2009	06/2009	02/2010
Treatments	75 *P. patula x P. tecunumanii* (L)[1]	*P. patula x P. tecunumanii (L)*[1]	*P. patula*
	24 *P. patula x P. tecunumanii* (H)[2]	*P. patula x P. tecunumanii (H)*[2]	*P. elliottii*
	13 *P. patula*	*P. patula x P. oocarpa*	*P. greggii (N)*[4]
	P. elliottii (control)	*P. patula x P. elliottii*	*P. greggii (S)*[3]
		P. patula x P. pringlei	*P. tecunumanii (H)*[2]
		P. patula x P. greggii (S)[3]	*P. tecunumanii (L)*[1]
		P. elliottii x P. caribaea	*P. oocarpa*
		P. elliottii x P. taeda	*P. caribaea*
		P. elliottii x P. tecunumanii (H)[2]	*P. pringlei*
		P. tecunumanii (H)[2] *x P. caribaea*	
		P. tecunumanii (L)[1] *x P. caribaea*	
		P. tecunumanii (H)[2] *x P. oocarpa*	
		P. caribaea x P. oocarpa	
		P. caribaea x P. tecunumanii (L)[1]	
		P. elliottii (control)	
		P. patula (control)	

[1]*P. tecunumanii* (low elevation source), [2]*P. tecunumanii* (high elevation source)

[3]*P. greggii* var. *australis* (from southern Mexico), [4]*P. greggii* var. *greggii* (from northern Mexico)

Table 2. The mean values for the parameters assessed among *P. patula*, and *P. patula x P. tecunumanii* represented as cuttings and seedlings, after inoculation with *F. circinatum* in Trial 1.

Species/hybrid[1]	*Type*	*Freq.*	*Height (mm)*	*Lesion (mm)*	*Dieback (%)*	*Resprout (mm)*
P. elliottii	Seedlings	59	163.1	5.7 ^{A}	3.7 ^{A}	46.2 ^{A}
P. patula	Seedlings	461	68.7	29.4 ^{B}	42.6 ^{B}	30.6 ^{B}
*P. pat x P. tec*H	Cuttings	1275	155.9	16.1 ^{C}	12.1 ^{C}	28.3 ^{B}
*P. pat x P. tec*H	Seedlings	307	73.2	21.4 ^{C}	29.5 ^{D}	25.5 ^{C}
*P. pat x P. tec*L	Cuttings	4625	177.5	7.4 ^{A}	5.0 ^{A}	20.4 ^{E}
*P. pat x P. tec*L	Seedlings	1173	81.8	8.2 ^{A}	10.8 ^{E}	43.2 ^{E}

[1]High and low elevation *P. patula x P. tecunumanii* are indicated with H and L, respectively. For a given variable, means with different letters are statistically different ($p<0.05$).

Table 3. The results of an ANOVA on lesion length to determine family and type of plant (cutting *vs* seedling) differences for the two hybrids (*P. pat x P. tec*H, *P. pat x P. tec*L) and for *P. patula* to *F. circinatum* tolerance in Trial 1.

Source	DF	SS	MS	FValue	ProbF
a) *P. pat x P. tec*H[1]					
Rep	3	7578.2	2526.1	6.60	0.0004
Type (cutting/seedling)	1	22.2	22.2	0.06	0.8194
Family	23	57120.0	2483.5	4.04	0.0693
Type*Family	5	2903.3	580.7	1.43	0.2198
Rep*Type*Family	87	37839.0	434.9	2.26	0.0000
Height	1	24.6	24.6	0.13	0.7206
b) *P. pat x P. tec*L[2]					
Rep	3	4194.7	1398.2	5.92	0.0006
Type (cutting/seedling)	1	20.9	20.9	0.08	0.7835
Family	74	62258	841.3	1.78	0.0745
Type*Family	20	9178.2	458.9	1.89	0.0131
Rep*Type*Family	283	70760	250	4.46	0.0000
Height	1	881.9	881.9	15.75	0.0001
c) P. pat x P. tec combined[3]					
Rep	3	7687.9	2562.6	9.19	0.0000
Hybrid	1	41351	41351	65.83	0.0000
Type (cutting/seedling)	1	3.2	3.2	0.01	0.9161
Hybrid*Type	1	90.1	90.1	0.26	0.6131
Family (Hybrid)	97	119325	1230.1	2.51	0.0055
Type*Family(Hybrid)	25	11889	475.6	1.60	0.0345
Height	1	567	567	6.66	0.0099
Rep*Family(Hybrid*Type)	373	114237	306.3	3.60	0.0000
d) P. patula[4]					
Rep	3	4008.9	1336.3	1.85	0.1534
Family	12	10752	895.9	1.04	0.4356
Rep*Family	35	35255	1007.3	6.90	0.0000
Height	1	9213.7	9213.7	63.08	0.0000

1. ANOVA on all *P. patula x P. tecunumanii* treatments (from high elevation provenances only)
2. ANOVA on all *P. patula x P. tecunumanii* treatments (from low elevation provenances only)
3. ANOVA on all *P. patula x P. tecunumanii* treatments
4. ANOVA on all *P. patula* treatments

Table 4. Variance components[1] and genetic parameters for lesion length (assessing tolerance to *F. circinatum*) for hybrids of *P. patula* x *P. tecunumanii* High-elevation sources, *P. patula* x *P. tecunumanii* Low-elevation sources, and pure species *P. patula*.

	Data Set					
	*P. pat x P. tec*H		*P. pat x P. tec*L		*P. patula*	
Source	mm^2	%	mm^2	%	mm^2	%
P. patula parent	16.5	6.4	0.6	0.8	26.0	2.1
P. tecunumanii parent	5.3	2.1	1.4	1.8	-	-
P. patula x P. tecunumanii	25.0	9.7	3.3	4.2	-	-
P. pat * *P. tec* * type	0.2	0.1	3.2	4.1	-	-
Plot	18.4	7.2	13.1	16.9	99.1	39.6
Residual	192.3	74.6	56.0	72.1	146.1	58.3
Phenotypic	257.7	100.0	77.7	100	250.5	100
h^2	-	-	-	-	0.06	-
h^2_{pat}	0.25	-	0.03	-	-	-
h^2_{tec}	0.08	-	0.07	-	-	-
$d^2_{pat\ x\ tec}$	0.39	-	0.17	-	-	-

[1]Variance components for lesion length expressed in the units of measurement (mm^2) and percent of total phenotypic variation (%). Hybrids were tested both as two plant types, cuttings and seedlings. Estimated heritability (h^2) for *P. patula* was calculated as $(3 \times \sigma^2_{female}) / \sigma^2_{phenotypic}$ following Dieters et al. 1995.

Table 5. The mean lesion length, percent dieback and the ability to resprout of various pine species after inoculation with *F. circinatum*.

Species	Freq.	Height (mm)	Lesion (mm)	Dieback (%)	Resprout (mm)
P. greggii (N)	67	126.9	37.97 ^{A}	27.91 ^{A}	16.5 ^{A}
P. patula	57	118.3	29.65 ^{B}	23.38 $^{B/}$	18.4 ^{A}
P. greggii (S)	95	165.6	18.51 ^{C}	12.25 ^{C}	43.2 ^{B}
P. tecunumanii (H)	95	177.9	9.16 ^{D}	7.01 ^{DE}	59.2 ^{C}
P. elliottii	95	183.5	8.82 ^{D}	6.77 ^{D}	86.3 ^{D}
P. caribaea	87	149.7	7.97 ^{DE}	6.31 ^{DF}	81 ^{D}
P. tecunumanii (L)	89	150.8	5.07 ^{EF}	3.91 ^{FG}	96.9 ^{E}
P. oocarpa	95	158.1	4.21 ^{F}	3.28 ^{G}	117.1 ^{F}
P. pringlei	93	111.5	3.81 ^{F}	1.87 ^{H}	39.6 ^{B}

Note. Treatments are ranked from most to least tolerant based on lesion length. Treatments with different letters are significantly ($p<0.05$) different (Duncan grouping).

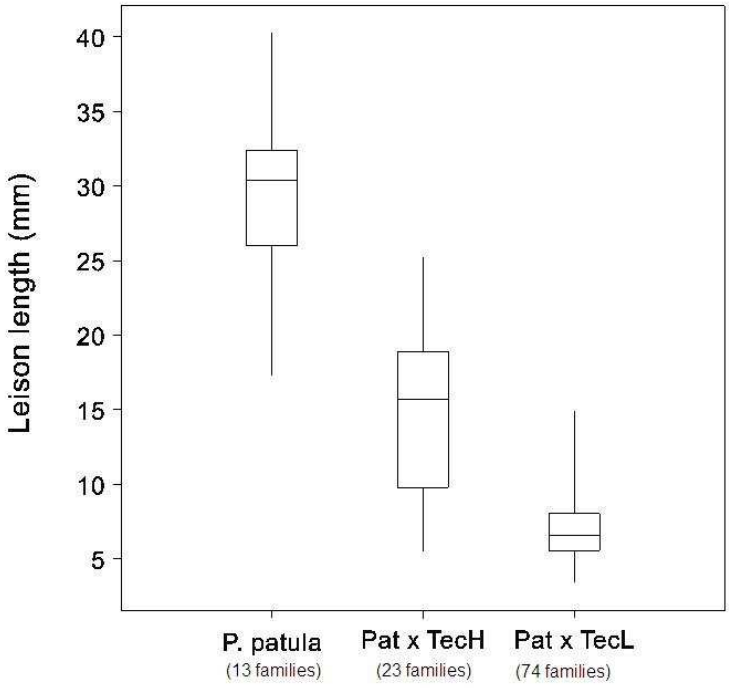

Figure 1. Box and Whisker plot showing family variation in *P. patula* and *P. patula x P. tecunumanii*. The blocks represent the center 50% (interquartile) values recorded. Horizontal lines within the blocks represent the median, vertical lines either ends of the box represent the range in susceptibility.

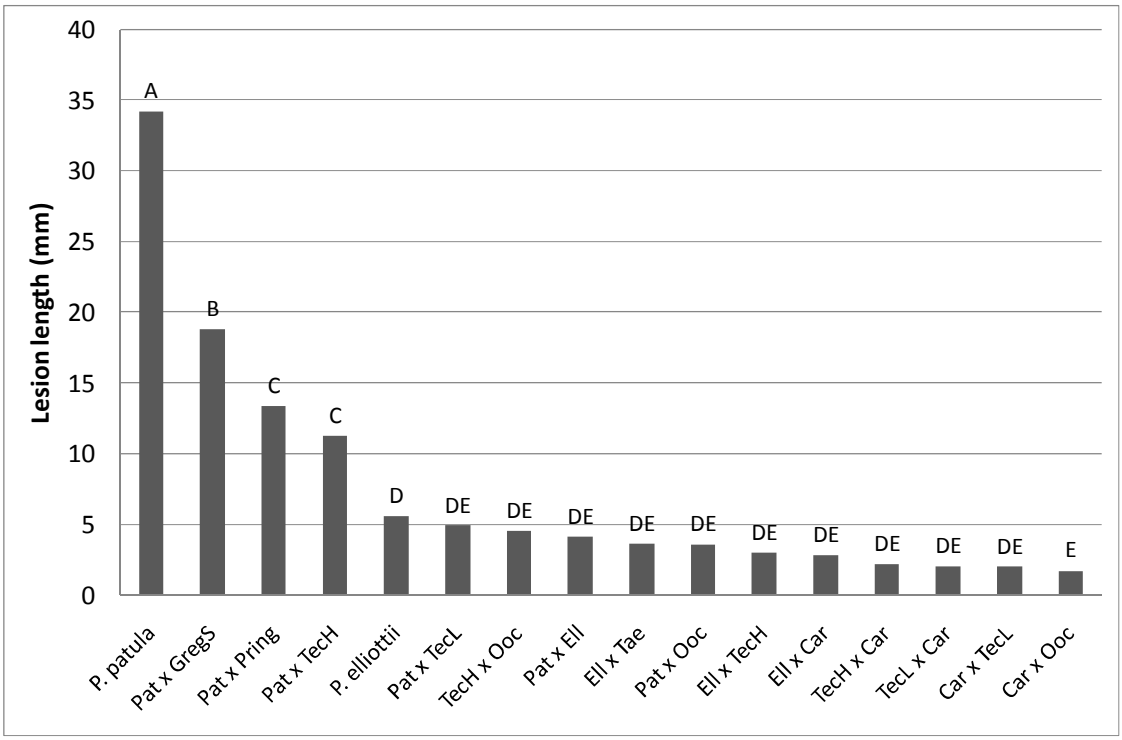

Figure 2. The variation in tolerance of a number of hybrids and *P. patula* and *P. elliottii* to *Fusarium circinatum* in Trial 2.

Appendix 1. The specific mating design used to produce a total of 99 *P. patula x P. tecunumanii* full-sib families with 13 *P. patula* female parents and 7 high elevation and 12 low elevation *P. tecunumanii* pollen parents.

	Female													
Male	Pat 1	Pat 2	Pat 3	Pat 4	Pat 5	Pat 6	Pat 7	Pat 8	Pat 9	Pat 10	Pat 11	Pat 12	Pat 13	Total
TecH 1		*			*	**				*	**	*		6
TecH 2											*			1
TecH 3						**	*			*	**			4
TecH 4		*	*				**					*	*	5
TecH 5		**		*			*					*	*	5
TecH 6			*											1
TecH 7		*			*									2
TecL 1			**	*			**			*		*		5
TecL 2					*						*			2
TecL 3				*				*		*		*	*	5
TecL 4	*	*	*	**		*	*		*	*		*		9
TecL 5	*	*	**					*		**		*		6
TecL 6			**	*			**	*						4
TecL 7	*	**	*	*			**			*		**		7
TecL 8	*			*			**			*		*	*	6
TecL 9	*		**	*				*		*		*		6
TecL 10	**	*	**	**		*	*	*	**	*			*	10
TecL 11		*	*				**	*		*		**	*	7
TecL 12	**		**	*		*	**	*				*	*	8
Total	7	9	11	10	3	5	11	7	2	11	4	12	7	99

A single asterisk indicates that the full-sib family was represented by cuttings only, while double asterisk indicates that the family was represented by both cuttings and seedlings. TecL = P. tec (low elevation), TecH = P. tec (high elevation).

(Published: New Forests, 2012 DOI: 10.1007/s11056-012-93)

Chapter 4:

Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa

Abstract

The future of South Africa's most important pine species, *Pinus patula*, is threatened by the pitch canker fungus, *Fusarium circinatum*. *Pinus maximinoi* and *P. tecunumanii* represent two subtropical species that provide an alternative to planting *P. patula* on the warmer sites of South Africa. Extending the planting range of *P. tecunumanii* and *P. maximinoi* to include higher and colder altitude sites will reduce the area planted to *P. patula* and the risk of *F. circinatum*. During 2007 progeny trials of *P. tecunumanii* and *P. maximinoi* were planted on a sub-tropical and sub-temperate site. Shortly after the establishment of these trials, unusually cold weather conditions were experienced across South Africa (-3^{o}C at the sub-temperate site) resulting in severe mortality. This provided the opportunity to assess the variation in survival as a measure of frost tolerance within these two species to determine whether it could be improved upon through selection. Results indicated that the variation in survival was under genetic control in *P. tecunumanii* ($h^2_{(0,1)}$ = 0.16, h^2_L = 0.27) and *P. maximinoi* ($h^2_{(0,1)}$ = 0.11, h^2_L = 0.23) at the sub-temperate site. Correlations in provenance ranking for survival across sites were high for both species. Moderate correlations in family survival for *P. tecunumanii* (r=0.52) were found at the two sites. Improvements in cold tolerance can thus be made in both species extending their planting range to include greater areas planted to *P. patula* thereby limiting the risk of *F. circinatum*.

Keywords; Camcore, genetic diversity, frost susceptibility, South Africa, *Pinus maximinoi*, *Pinus tecunumanii*.

Introduction

There is considerable interest in commercializing alternative pine species not commonly planted in South Africa. This emerges from a desire to improve several weaker characteristics of currently deployed species, including the susceptibility of *Pinus patula* to the pitch canker fungus, *Fusarium circinatum*. *Pinus tecunumanii* and *P. maximinoi* are two alternative species that have shown good growth (Kietzka 1988; Dvorak et al. 2000a, b; Galpare et al. 2001), wood properties (Malan 1994, 2006), and tolerance to *F. circinatum* (Hodge and Dvorak 2000, 2007; Mitchell et al. 2011). *P. tecunumanii* may be a particularly valuable species because it hybridises easily with South Africa's most important pine, *P. patula*, resulting in improved tolerance of the hybrid to *F. circinatum* (Roux et al. 2007).

Pinus tecunumanii and *P. maximinoi* are susceptible to frost that limits their planting to warmer sites. Populations of *P. tecunumanii* can be divided into those that occur below 1500 m altitude (low elevation subgroup) and those that occur above 1500 m (high elevation subgroup) in their natural origin in Central America and Mexico (Dvorak 1986; Dvorak et al. 2000a). High and low elevation subgroups of the species also are distinguishable genetically through molecular marker assessment (Dvorak et al. 2009). From a climatic standpoint, low elevation provenances are more tropical in nature and particularly sensitive to frost while high elevation provenances from southern Mexico and Guatemala can tolerate light frost (Dvorak et al. 2000a). Similarly, *P. maximinoi* grows best on tropical or sub-tropical sites free from frost (Dvorak et al. 2000b). Improving the frost tolerance of these two species would increase their planting range to include warm and sub-temperate sites currently planted to *P. patula*.

During 2007, progeny trials of *P. tecunumanii* and *P. maximinoi* were established just before a winter period. The survival of these trials was severely affected by a frost event resulting in their termination. In this study the survival data were examined and tolerance of these two species to frost was determined.

Methods

A group of 113 *P. tecunumanii* and 43 *P. maximinoi* trees (hereafter called "selections") were identified in 1st generation provenance / progeny trials planted by Komatiland Forests. Selection criteria were provenance and family growth, and individual tree growth and form. The trials were then heavily thinned, effectively converting the progeny tests into seedling orchards. Seed was collected from the selections, and was sown at the Komatiland Forest's nursery near Sabie in September 2006. Sufficient seed was sown to plant two second-generation trials of each species on two separate predetermined sites (Spitskop and Wilgeboom). The Spitskop site could be described as sub-temperate while the Wilgeboom site is sub-tropical. The 1st generation selections represented a number of the original provenances from different countries in Central America and southern Mexico (Table 1), which had been planted in blocks. The selections were, therefore, likely to have been pollinated by surrounding trees of the same provenance, but may also have been pollinated by trees further away and not necessarily of the same provenance.
Seedlings were raised in 128 Unigro plastic molded trays with an individual cavity size of 60 cc. Well-composted pine bark was used as the growing medium and 2:3:2 (22) N:P:K granular fertilizer was applied as required.

The trials were planted in mid-March 2007 when seedlings were 6-months-old. At each site, one *P. maximinoi* and one *P. tecunumanii* trial was planted. Based on the availability of seedlings, the Wilgeboom trials were comprised of the full set of families whilst the Spitskop trials contained fewer families. In the case of both species, the families in the Spitskop trials were common in the Wilgeboom trials. In each case, the trial design was a randomized complete block with 6 replications and 6 tree family row plots. Each family, therefore, was represented by 36 trees in each trial. In all the trials *Pinus elliottii* and *P. patula* seedlings from a commercial seed orchard were used as controls.

The trials were assessed for survival after 60 days and blanked to 100% stocking. Approximately 5 days after the blanking operation, extremely cold conditions were experienced across the country. The nearest weather station was at Graskop, a town approximately halfway between the two sites, and of similar altitude (1450m) and climate to the sub-temperate Spitskop site.

Weather data for the month of May showed that temperatures dropped to below freezing (0 to -3^{o}C) for four consecutive days, and below 5^{o}C for 9 consecutive days, between 21 – 29/05/07 at Graskop (Fig. 1). Soon after the frost event (75 days from the original planting), the trials were reassessed for survival. Severe mortality was recorded at the sub-temperate Spitskop site while less damage was recorded at the sub-tropical Wilgeboom site.

Statistical analyses

The statistical package SAS version 9.1.3 (SAS Institute 2004) was used to analyse the data in which case individual tree observations, recorded as dead (0) or alive (1), were used as the unit of analysis. Several analyses of variance were conducted using SAS Proc GLM on each of the four individual tests (*P. tecunumanii* and *P. maximinoi* on the Spitskop and Wilgeboom sites). In all analyses, the binomial survival data was used as the units of observation.

Although inter-mating among trees from different provenances would have occurred in the thinned 1st generation progeny tests, the original provenance of a family could very likely have an impact on frost tolerance of the second-generation families. To investigate this, an analysis of variance was done to compare the control species, *P. patula* and *P. elliottii*, to the country and provenance of origin for *P. tecunumanii* and *P. maximinoi*. The linear model contained *rep* and *country* treated as fixed effects, and *provenance, family(provenance),* and *rep*family(provenance)* treated as random effects. SAS Proc GLM was used to conduct the analysis of variance, and least squares means (LS means) were calculated along with the p-values testing for statistically significant difference for each pair of means. A second GLM analysis was done using only the species data sets on each test site (that is, removing the control species), in order to test for differences among provenance and family (provenance). As above, in this model, *rep* was treated as fixed effects, while *provenance, family(provenance),* and *rep*family(provenance)* were treated as random effects, and LS means calculated as described above. A Pearson correlation was calculated between provenance and family means on the two sites for each species separately

To examine the potential to breed for frost tolerance in the two species, genetic parameters for the populations were calculated with SAS Proc MIXED, using a linear model with *rep* treated as a fixed effect, and *family* and *rep*family* were treated as random effects. As the families were represented by open-pollinated seed collected in seedling seed orchards, converted from progeny tests, a coefficient of 3 provides a better estimate of additive genetic variance (Dieters et al. 1995). Heritability was calculated on the observed (binomial) scale, as $h^2_{(0,1)} = 3\,\sigma^2_f / (\sigma^2_f + \sigma^2_{plot} + \sigma^2_{error})$ where σ^2_f =estimated family variance, σ^2_{plot} = estimated rep*family(provenance) variance, and σ^2_{error} = estimated residual variance. The heritability estimate on the binomial scale was then converted to an estimate on the underlying liability scale (h^2_L) following the methodology of Chambers et al. (1996). Standard errors of the heritability were estimated using the approximation formula (Dickerson 1969), with the standard error of the family variance estimate calculated from the ASYCOV option in Proc MIXED.

Results

Due to the more extreme temperatures, fewer plants survived at Spitskop than at Wilgeboom. The mean survival for *P. tecunumanii* (LE) was 27% and 46% for *P. tecunumanii* (HE) at Spitskop compared to 85.1% and 93.6%, respectively, at the Wilgeboom site. In the *P. tecunumanii* trial at Spitskop, *P. patula* survived better (83%) than *P. elliottii* (69%) compared to 100% survival for both species at the Wilgeboom site. The survival of *P. maximinoi* at the Spitskop site was 19% compared to 87% at Wilgeboom. In the *P. maximinoi* trials *P. patula* survival (89%) was poorer than *P. elliottii* (97%) at Spitskop and similar to *P. elliottii* at Wilgeboom (97% vs. 94%).

In the *P. tecunumanii* trials, the means of the HE families, which originated from southern Mexico and Guatemala, were significantly better than the mean of the LE families from Honduras at both sites (Table 3). The single high elevation variety of *P. tecunumanii* representing Honduras (Las Trancas) was significantly poorer than the mean of the high elevation families from Guatemala and Mexico at the Spitskop site. In the *P. maximinoi* trials, families that originated from Honduras ranked better than those from Mexico that ranked better

than those from Guatemala at both sites (Table 3). However, there were no significant differences between the means for each country at either site.

An analysis of provenance differences, which were represented by at least 2 families at a single site, indicated that the *P. tecunumanii* high elevation provenances (San Lorenso, Chempil, Montebello and San Jerónimo) survived significantly better than the *P. tecunumanii* low elevation provenances (San Esteban, San Francisco, Jocon and Villa Santa) at both the Spitskop and Wilgeboom sites (Table 4). Within the *P. tecunumanii* high elevation subgroup, the San Lorenso and Montebello provenances survived significantly better than the San José and Las Trancas provenances planted at the Spitskop site and the San José and Jitotal provenances at the Wilgeboom site (Table 4). Within the low elevation subgroup, the Villa Santa provenance survived significantly more poorly than the other LE provenances at the Spitskop site (Table 4). When comparing the ranking of provenances represented by at least 2 families at each site, there was a high correlation between the survival means on the two sites (Table 4).

There was no significant difference for all provenances of *P. maximinoi* at either site (Table 4), despite the fact that the LS means for survival ranged from 13.3% to 27.8% at Spitskop, and from 83.8% to 93.5% at Wilgeboom. Despite there being no significant differences between *P. maximinoi* provenances at either site, there was a high correlation between the survival means on the two sites (Table 4).

There was large family variation in survival at the Spitskop site for *P. tecunumanii* of both the low (3-61%) and high (11-78%) elevation subgroups. At the Spitskop site, a number of high elevation families showed little frost tolerance and some low elevation families showed frost tolerance similar to the mean of the high elevation variety (Fig. 2). The range of family survival in *P. maximinoi* at the Spitskop site was smaller, with the most tolerant family measuring 38% survival (Fig. 3). There was little variation at the Wilgeboom site for both species and it is likely that only the most susceptible families showed some mortality. Narrow-sense heritability estimates for the trials at Spitskop were good for *P. tecunumanii* ($h^2_{(0,1)} = 0.16$, $h^2_L = 0.27$) and weaker for *P. maximinoi* ($h^2_{(0,1)} = 0.11$, $h^2_L = 0.23$) (Table 5). Narrow-sense heritability at the Wilgeboom site was poor for *P. tecunumanii* ($h^2_{(0,1)} = 0.05$, $h^2_L = 0.12$) and was nil for *P.*

maximinoi (Table 5). The correlation between *P. tecunumanii* families was stronger (r=0.52) than *P. maximinoi* (r=0.37) (Figs. 4-5).

Discussion

This study provides quantitative evidence that frost tolerance is under genetic control in *P. tecunumanii* and *P. maximinoi,* which is similar to that observed in other pine species (Rehfeldt 1989; Duncan et al. 1996; Howe 2006). A substantial amount of the observed family variance in the selected population for *P. tecunumanii* appears related to the original country and provenance origin. These provenance effects were consistent across two distinct environments that were widely different in their frost survival means.

In this study, frost tolerance was measured using a binomial trait. Survival for both species on both sites was near the low or high end of the scale (19% for *P. maximinoi* and 33% for *P. tecunumanii* at Spitskop, and 86% for both species at Wilgeboom). Despite there being no significant differences between *P. maximinoi* provenances (Table 4), the ranking in survival on the two sites were very similar. Heritability estimates on the binomial scale were rather low, but on the underlying liability scale, h^2_L estimates of 0.23 to 0.27 for the two species at the Spitskop site indicates that frost tolerance in both species could be improved through breeding and selection. *Pinus maximinoi* appeared to be more susceptible to cold damage than *P. tecunumanii*. Since breeding would begin with a lower mean tolerance in the population, it may require multiple generations to make any significant improvement in the frost tolerance of this species. Compared to *P. maximinoi,* the broader range of family tolerance in *P. tecunumanii* (particularly of the high-elevation subgroup) and slightly higher heritability suggests that advances in breeding for frost tolerance in *P. tecunumanii* would be more easily achieved.

The *P. tecunumanii* high elevation provenances, Montebello, San Jerónimo and Chempil, which ranked as some of the more frost tolerant provenances in this study, have been found to be significantly more susceptible to *F. circinatum* than the high elevation provenances of Jitotol and Las Trancas (Mitchell et al. 2011), which were less tolerant of frost. Similarly, Villa Santa, which was significantly more frost susceptible compared to other low elevation provenances in

this study, ranked as the most tolerant provenance to *F. circinatum* (Mitchell et al. 2011). This suggests that there is an inverse relationship between frost tolerance and tolerance to *F. circinatum* within *P. tecunumanii*. This means that selecting and breeding for frost tolerance in *P. tecunumanii*, and increasing the distribution of more frost tolerant selections to cool sites as a replacement for *P. patula*, may be limited by a decline in tolerance to *F. circinatum*.

Subsequent to the frost event in 2007, both species were replanted in February 2008 at the Wilgeboom site and *P. tecunumanii* was replanted at the Spitskop site in the same month. The survival was excellent at both sites. This illustrates that planting these species in a warmer month, followed by a normal winter period, can be successful. However, from our experience planting these species on sites, where frequent frost events are a normal occurrence and later than February in South Africa, should be avoided until their frost tolerance can be improved.

It is known that hybridizing frost-susceptible with tolerant species can provide an effective means to improve frost tolerance (Duncan et al. 1996). Therefore, to compliment breeding for frost tolerance, *P. tecunumanii* and *P. maximinoi* could be hybridized with tolerant species (such as *P. patula*). In such cases, whilst an improvement in frost tolerance can be seen, the frost tolerance of the hybrid may more closely resemble the susceptible parent (Duncan et al. 1996). This may be the experience in South Africa, where the *P. patula x P. tecunumanii* hybrid has become very popular due to its improved tolerance to *F. circinatum* (Roux et al. 2007), it remains susceptible to frost especially when the low elevation subgroup is used as the pollen parent. It is likely, however, that the susceptibility of *P. patula x P. tecunumanii* to frost can be improved upon by backcrossing it with *P. patula* as reported for other species (Lopez-Upton et al. 1999). Importantly, the tolerance of hybrid families to frost seems more reliant on the specific combining ability of the parents and not necessarily the tolerance of the parents themselves (Duncan et al. 1996). Therefore, it seems likely that families of *P. patula x P. tecunumanii*, and not only the parents, would need to be tested for frost tolerance as well as for tolerance to *F. circinatum* in the future. This would also be the case where *P. maximinoi* is hybridized with other frost tolerant species.

As seen in this study, and elsewhere (Lopez-Upton et al. 1999, Howe 2006, Dong et al. 2009), exposing young trees to cold temperatures in field trials may be an effective method to identify frost tolerant individuals. Based on our experience, temperatures slightly below freezing, for several hours per day for several days in the field should be sufficient to screen families for tolerance in *P. tecunumanii* and *P. maximinoi* in the field. However, a number of artificial screening methods have also been described (Rehfeldt 1989; South et al. 1993; Tinus et al. 2002; Mahalovich et al. 2006; Aldrete et al. 2008). Artificial tests using either seedlings/cuttings or needles, can be subjected to freezing temperatures in a controlled environment. After thawing, needles can simply be assessed for discoloration and bending ability and then the amount of damage scored (Rehfeldt 1989; South et al. 1993). Alternatively the amount of electrolyte leakage from the damaged tissue can be scored (South et al. 1993; Tinus et al. 2002; Mahalovich et al. 2006; Aldrete et al. 2008). The results of these artificial tests often compare well with observations in the field (Howe 2006; Dvorak pers. comm. 2010). Whichever method is chosen to identify tolerant provenances and individuals, it will become increasingly important to improve the tolerance of subtropical species, such as *P. tecunumanii* and *P. maximinoi* to cold temperatures in South Africa.

Conclusions and Outlook

There was good evidence from this study that the frost susceptibility of *P. tecunumanii* and *P. maximinoi* is under genetic control, and can be improved by selecting provenances and families that are more tolerant to frost. Our hope is that through selection, we can eventually build up a sizeable population of individual trees with good frost tolerance and good growth. In order to maximize the potential that *P. tecunumanii* and *P. maximinoi* offer, particularly to reduce the risk posed by *F. circinatum*, frost tolerance will have to be included as a future selection criterion. Results of this study show that his can be achieved by planting provenances and families in cold climates and then recording mortality. Alternatively, various laboratory techniques should be explored to rapidly screen provenance and families that are most cold hardy. These can then be more thoroughly tested in the field under natural climatic conditions.

Acknowledgements

We thank Komatiland Forests for making available the data from these trials for publication.

References

Aldrete A, Mexal JG, Burr KE (2008) Seedling cold hardiness, bud set, and bud break in nine provenances of *Pinus greggii* Englm. *Forest Ecology and Management* 255: 3672-3676.

Chambers PGS, Borralho NMG, Potts BM (1996) Genetic analysis of survival in *Eucalyptus globulus* ssp. *globulus*. *Silvae Genetica* 46(2-3): 107-112.

Dickerson GE (1969) Techniques for research in quantitative animal genetics. In: Techniques and procedures in animal science research. Am Soc Anim Sci, Albany, N.Y., pp 36–79.

Dieters MJ, White TL, Hodge GR (1995) Genetic parameter estimates for volume from full-sib tests of slash pine (*Pinus elliottii*). *Canadian Journal of Forest Research* 25: 1397-1408.

Dong J, LinY, Yu S, Wang Q, Lu A, Feng J, Liu Y, Man S, Qu Y (2009) Selection of superior provenance of eastern white pine by cold resistance. *Journal of Northeast Forestry University* 37: 4-6.

Duncan PD, White TL, Hodge GR (1996) First-year freeze hardiness of pure species and hybrid taxa of *Pinus elliottii* (Engelman) and *Pinus caribaea* (Morelet). *New Forests* 212: 223-241.

Dvorak WS (1986) Provenance/progeny testing of *Pinus tecunumanii*. In: Proc IUFRO Breeding theory, progeny testing and seed orchard management. Williamsburg, VA. October 12-17. pp: 299-309.

Dvorak WS, Gutiérrez EA, Galpare WJ, Hodge GR, Ororio LF, Bester C, Kikuti P (2000b) *Pinus maximinoi*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU, Raleigh, NC, USA. pp 107 – 127.

Dvorak WS, Hodge GR, Gutiérrez EA, Osorio LF, Malan FS, Stanger TK (2000a) *Pinus tecunumanii*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species

by the CAMCORE Cooperative. College of Natural Resources, North Carolina State University. 188-209.

Dvorak WS, Potter KM, Hipkins VD, Hodge GR (2009) Genetic diversity and gene exchange in *Pinus oocarpa*, a Mesoamerican pine with resistance to the pitch canker fungus (*Fusarium circinatum*). *International Journal of Plant Sciences* 170:609-626.

Gapare W, Hodge GR, Dvorak WS (2001) Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia, and South Africa. *Forest Genetics* 8: 159-170

Hodge GR, Dvorak WS (2000) Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests* 19: 241-258.

Hodge GR, Dvorak WS (2007) Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests* 33:193 – 206.

Howe K (2006) Identifying candidate genes associated with cold adaptation in Douglas-fir using DNA microarrays. Oregon State University in partial fulfillment of Master of Science.

Kietza JE (1988) *Pinus maximinoi*: A promising species in South Africa. *Southern African Forestry Journal* 145: 33 – 38.

López-Upton J, White TL, Huber DA (1999) Taxon and family differences in survival, cold hardiness, early growth, and rust incidence of Loblolly pine, Slash pine and some Pine hybrids. *Silvae Genetica* 48:303-313.

Mahalovich MF, Burr KE , Foushee DL (2006) Whitebark pine germination, rust resistance and cold hardiness among seed sources in the inland northwest: planting strategies for restoration. USDA Forest Service Proceedings RMRS-P-43; 91-101.

Malan FS (1994) The quality and wood properties of 4 provenances of South-African-grown *Pinus tecunumanii*. *Ann Sci For*. 51: 203-212.

Malan FS (2006) The wood properties and sawn-board quality of South African-grown *Pinus maximinoi* (HE Moore). *Southern African Forestry Journal* 208: 39 – 48.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET, Coutinho TA (2011) Selection of *Pinus* spp. in South African for tolerance to infection by the pitch canker fungus. *New Forests* (in print http://www.springerlink.com/content/j02181n158286311).

Rehfeldt GE (1989) Genetic variances and covariances in freezing tolerance of lodgepole pine during early winter acclimation. *Silvae Genetica* 38: 133-137

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests* 33: 109 – 123.

SAS Institute (2004) *SAS® 9.1.3 ETL Studio: User's Guide*. Cary, NC, USA.

South D, Donald DGM, Rakestraw JL (1993) Effect of nursery culture and bud status on freeze injury to *Pinus taeda* and *P. elliottii* seedlings. *South African Forestry Journal* 166:37-46.

Tinus RW, Sword M, Barnett JP (2002) Prevention of cold damage to container-grown longleaf pine roots. General Technical Report SRS-56, Ashville, NC, USDA Forest Service, Southern Research station, p 55-57.

Table 1: Number of families representing provenances

Species	*Ecotype*	*Provenance*	*State*	*Country*	*Wilgeboom*	*Spitskop*
Pinus maximinoi	NA	Coban	Alta Verapaz	Guatemala	6	5
		San Jeronimo	Baja Verapaz	Guatemala	10	9
		San Juan Sacatep.	Guatemala	Guatemala	6	5
		San Lorenzo	Zacapa	Guatemala	1	1
		Copan	Copan	Honduras	3	2
		Tatumbla	Fco. Morazán	Honduras	3	3
		Marcala	La Paz	Honduras	3	2
		El Portillo	Ocotepeque	Honduras	3	3
		Altamirano	Chiapas	Mexico	1	1
		La Canada	Chiapas	Mexico	2	2
		Monte Cristo	Chiapas	Mexico	1	1
		San Jerónimo	Chiapas	Mexico	4	4
Total					43	38
Pinus tecunumanii	High	San Jerónimo	Baja Verapaz	Guatemala	7	4
	High	KM 47	Guatemala	Guatemala	1	1
	High	La Soledad	Jalapa	Guatemala	1	1
	High	San Lorenzo	Zacapa	Guatemala	3	2
	High	Las Trancas	La Paz	Honduras	3	2
	High	Chempil	Chiapas	Mexico	13	9
	High	Jitotol	Chiapas	Mexico	3	3
	High	Las Piedrecitas	Chiapas	Mexico	2	1
	High	Montebello	Chiapas	Mexico	8	6
	High	Napite	Chiapas	Mexico	2	1
	High	San José	Chiapas	Mexico	2	2
	Low	Jocon	Yoro	Honduras	4	2
	Low	San Esteban	Olancho	Honduras	13	9
	Low	San Francisco	Olancho	Honduras	18	15
	Low	Villa Santa	El Paraiso	Honduras	33	26
Total					113	84

NA = Not applicable

Table 2: Site details where the *Pinus maximinoi* and *Pinus tecunumanii* trials were planted in South Africa

Trial site	*Wilgeboom*	*Spitskop*
Location	30° 56’ 19’’E; 24 57’ 5’’S	30° 50’ 23’’E; 25 9’ 37’’S
Description	Sub-tropical	Sub-temperate
Altitude	983 m	1480 m
Mean min temperature in coldest month	5°C	4°C
Mean annual temperature	18.5°C	15°C
Mean max temperature in warmest month	27°C	24°C
Mean annual precipitation	1100 mm	1266 mm
Plant date (day 0)	4-15/03/2007	12-13/03/2007
Blank date (day 60)	14/05/2007	15/05/2007
Assessment date (day 75)	30/05/2007	31/05/2007

Table 3. The least square mean survival by country ranked from best to worst for *P. tecunumanii* and *P. maximinoi*.

Species	*Country*	*Spitskop (%)*	*Wilgeboom (%)*
Pinus tecunumanii	*P. patula*	83.3 ^{A}	100 ^{A}
	P. elliottii	69.4 ^{A}	100 ^{A}
	Mexico (HE)	52.2 ^{B}	92.0 ^{A}
	Guatemala (HE)	48.3 ^{B}	95.4 ^{A}
	Honduras (HE)	34.7 ^{C}	88.9 ^{AB}
	Honduras (LE)	26.6 ^{C}	85.3 ^{B}
Pinus maximinoi	*P. elliottii*	97.2 ^{B}	94.4 ^{A}
	P. patula	88.9 ^{B}	97.2 ^{A}
	Honduras	21.4 ^{A}	89.8 ^{A}
	Mexico	18.8 ^{A}	86.1 ^{A}
	Guatemala	16.3 ^{A}	85.5 ^{A}

Treatments with different letters, for each species separately, are significantly different ($p<0.05$).

Table 4. The least square mean survival by provenance (represented by at least 2 families) of *P. tecunumanii* and *P. maximinoi* ranked from best to worst at the Spitskop site.

Species	*Provenance*	*State*	*Country*	*Spitskop (%)*	*Wilgeboom (%)*
Pinus tecunumanii	*P. elliottii* control	Local source	S. Africa	83.3 ^{A}	100 ^{A}
	P. patula control	Local source	S. Africa	69.4 ^{AB}	100 ^{A}
	San Lorenso (HE)	Zacapa	Guatemala	52.8 (2) ^{BCD}	98.1(3) ^{AB}
	Montebello (HE)	Chiapas	Mexico	52.4 (6) ^{CD}	96.5 (8) ^{AB}
	San Jerónimo (HE)	Baja Verapaz	Guatemala	45.0 (4) ^{CDE}	93.7 (7) ^{ABC}
	Chempil (HE)	Chiapas	Mexico	44.0 (9) ^{CE}	94.0 (13) ^{ABC}
	Jitotol (HE)	Chiapas	Mexico	41.7 (3) ^{CEF}	88.0 (3) ^{CD}
	San José (HE)	Chiapas	Mexico	36.1 (2) ^{EFG}	87.5 (2) ^{CD}
	Las Trancas (HE)	La Paz	Honduras	34.7 (2) ^{EFG}	88.9 (3) ^{AC}
	Jocon (LE)	Yoro	Honduras	30.6 (2) ^{FGH}	86.8 (4) ^{D}
	San Esteban (LE)	Olancho	Honduras	26.7 (9) ^{GH}	85.3 (13) ^{D}
	San Francisco (LE)	Olancho	Honduras	26.1 (15) ^{GH}	83.6 (18) ^{D}
	Villa Santa (LE)	El Paraiso	Honduras	22.9 (26) ^{D}	85.7 (33) ^{D}
Pinus maximinoi	*P. elliottii* control	Local source	S. Africa	97.2 ^{B}	94.4 ^{A}
	P. patula control	Local source	S. Africa	88.9 ^{B}	97.2 ^{A}
	Marcala	La Paz	Honduras	27.8 (2) ^{A}	93.5 (3) ^{A}
	La Canada	Chiapas	Mexico	23.6 (2) ^{A}	86.1 (2) ^{A}
	Tatumbla	Fco. Morazán	Honduras	21.3 (3) ^{A}	88.9 (3) ^{A}
	San Juan Sacatepéquez	Guatemala	Guatemala	19.4 (5) ^{A}	86.1 (6) ^{A}
	San Jerónimo	Chiapas	Mexico	17.9 (4) ^{A}	85.3 (4) ^{A}
	San Jerónimo	Baja Verapaz	Guatemala	17.4 (9) ^{A}	86.8 (10) ^{A}
	El Portillo	Ocotepeque	Honduras	16.7 (3) ^{A}	87.0 (3) ^{A}
	Coban	Alta Verapaz	Guatemala	13.3 (5) ^{A}	83.8 (6) ^{A}

Figures in brackets are the number of families representing each provenance.

Treatments with different letters, for each species separately, are significantly different ($p<0.05$).

Table 5. Heritability estimates for *Pinus maximinoi* and *P. tecunumanii* at the two sites

Model	*Site*	*Species*	*h2(0,1)*	*se*	*h2(L)*	*se*
Family	Spitskop	*P. maximinoi*	0.11	0.04	0.23	0.09
Family	Spitskop	*P. tecunumanii*	0.16	0.06	0.27	0.10
Family	Wilgeboom	*P. maximinoi*	0.00	0.00	0.00	0.00
Family	Wilgeboom	*P. tecunumanii*	0.05	0.03	0.12	0.07

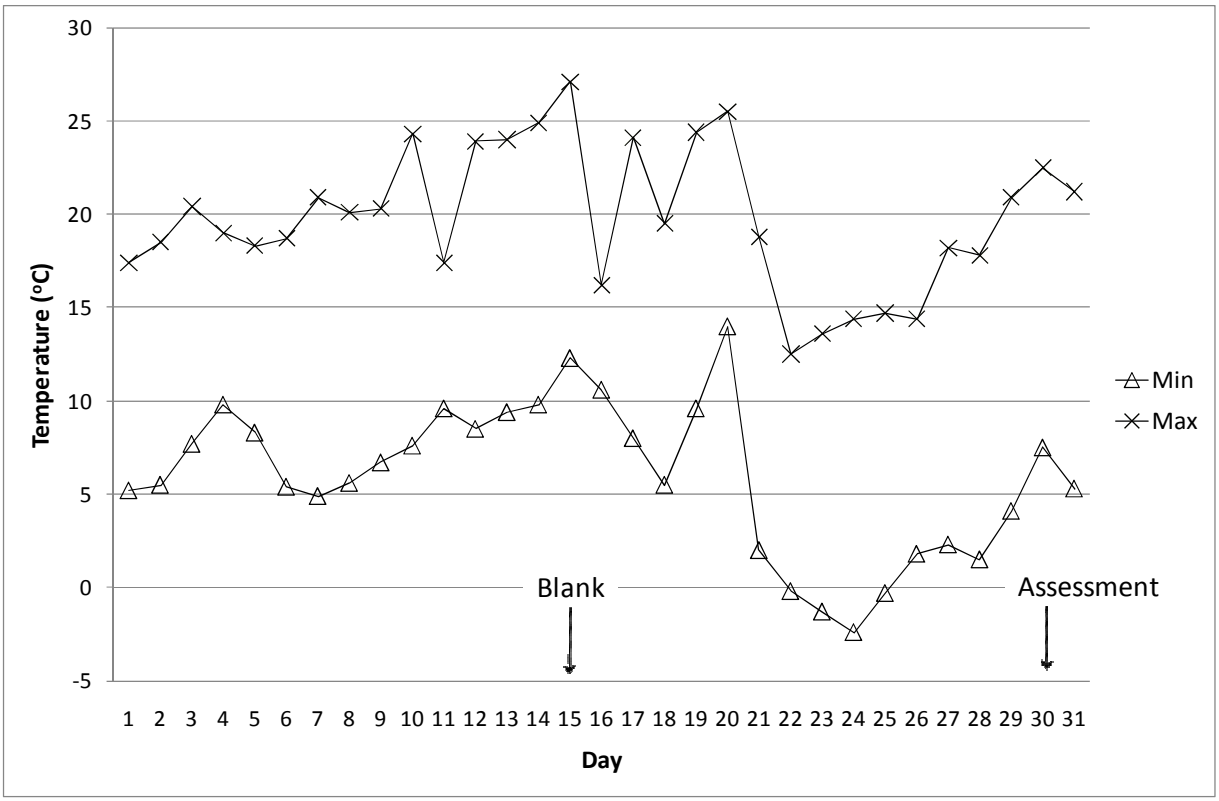

Figure 1. Minimum and maximum temperatures recorded, at a nearby weather station representative of the cool-temperate Spitskop site, for the month of May 2007.

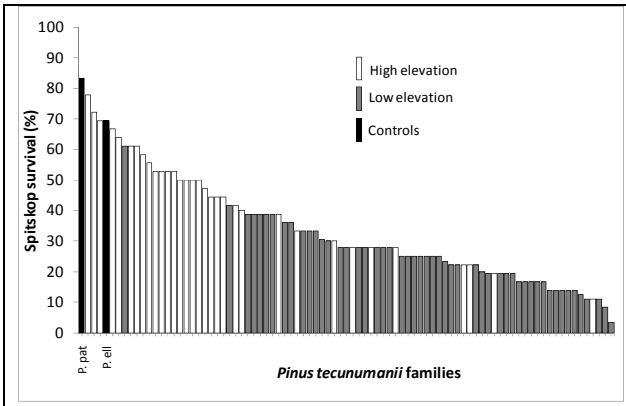

Figure 2. *P. tecunumanii* family survival on the cool-temperate site (Spitskop) ($h^2_{(0,1)}$ = 0.16, h^2_L = 0.27).

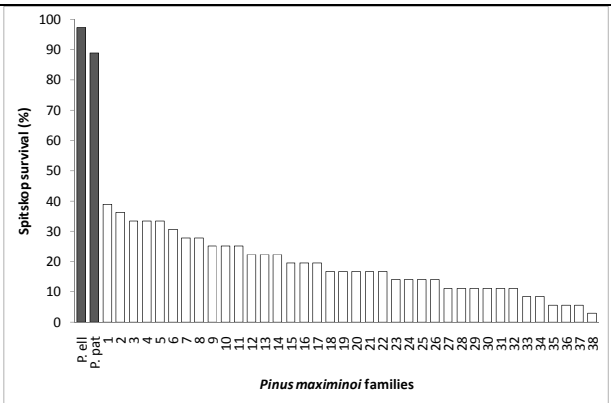

Figure 3. *P. maximinoi* family survival on the cool-temperate site (Spitskop) ($h^2_{(0,1)}$ = 0.11, h^2_L = 0.23).

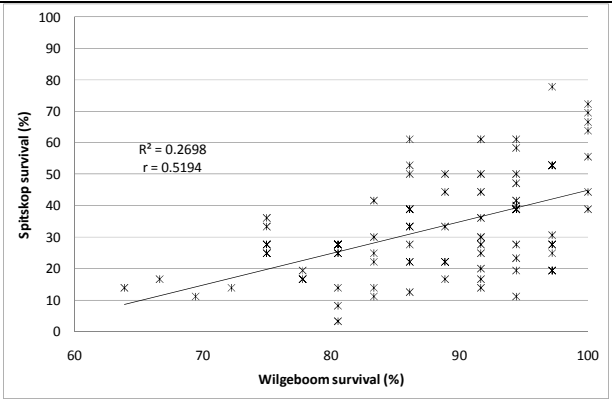

Figure 4. The correlation between all *P. tecunumanii* families at the two sites.

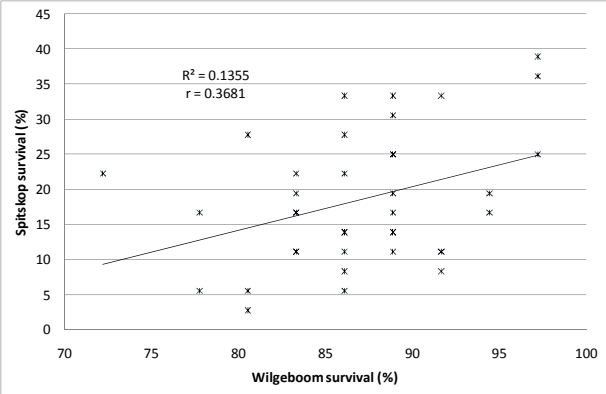

Figure 5. The correlation between all *P. maximinoi* families at the two sites.

Chapter 5:

Tolerance of *Pinus patula* full-sib families to *Fusarium circinatum* in a greenhouse study

Abstract

The pitch canker fungus, *Fusarium circinatum*, has resulted in large scale mortality of young *Pinus patula* seedlings in nurseries and after planting in South Africa since 1990. Tree Breeders have suggested that the only long-term solution to limit infection by this pathogen is to identify and deploy tolerant *P. patula* families. A commonly used technique to identify tolerant clones is to artificially inoculate open pollinated progeny from orchard clones with *F. circinatum* under greenhouse conditions. In these trials, large variation in tolerance to the pathogen between the open-pollinated seedlings, within each family, is observed and this could be an influence of the pollen parent. Therefore, identifying individual full-sib families where both parents are known, should improve the identification of tolerant families, which can then be repeated. In this study, cuttings from control-pollinated *P. patula* seedling hedges were inoculated with *F. circinatum* in a greenhouse. The results showed large family variation where some of the full-sib families were similar in tolerance to *P. elliottii* seedlings. It is, therefore, recommended that breeders focus on identifying specific family combinations that are more tolerant to *F. circinatum*.

Keywords; *Pinus patula*, *P. elliottii*, greenhouse inoculation, *Fusarium circinatum*, cuttings

Introduction

Pinus patula is the most important pine species used to establish plantations in South Africa (Department of Agriculture, Forestry and Fisheries 2008), due to its superior growth and wood properties (Morris and Pallett 2000, Vermaak 2007). However, during the last 20 years its deployment has been severely hampered by the pitch canker fungus, *Fusarium circinatum* which causes mortality of young seedlings in nurseries and after planting (Wingfield et al. 2002, Mitchell et al. 2011a). In order to reduce the negative impact of this pathogen on *P. patula*, forest owners can either replace this species with alternatives such as *P. elliottii*, *P. taeda* or *P. tecunumanii* (Mitchell et al. 2012c) or hybridize it with tolerant species (Mitchell et al. 2012a). In most cases, however, these alternative species or hybrids are best suited to sub-tropical and warm temperate sites. This complicates the replacement of *P. patula* as it currently remains the best species for planting in colder climates. In order to continue to deploy *P. patula* in these regions, without the risk of it becoming infected with *F. circinatum*, it is necessary to identify tolerant individuals that can be planted in new seed orchards, or used in a controlled pollination program.

In previous greenhouse studies, where open pollinated *P. patula* families have been screened as seedlings, large variation in tolerance has been observed between the seedlings from the same family. These differences could be due to the variation in genotypic diversity within an open pollinated family. Assuming that this variation is due to the influence of the pollen parent, then identifying those full-sib families that produce progeny with tolerance to infection will be more beneficial than identifying mother trees with good general combining ability. In these cases, tolerant full-sib families can repeatedly be made. Due to the limitation of control-pollinated seed, such families would likely be deployed as cuttings from seedling hedges.

In this study, cuttings from a number of control pollinated full-sib *P. patula* families were inoculated with *F. circinatum* in a greenhouse. A number of common parents were used that allowed us to determine the general tolerance of a parent. The overall aim was to assess the variation in tolerance to *F. circinatum* between full-sib families sharing a common parent and to identify full-sib families that could be used in a pine plantation breeding programme.

Methods and materials

Plant material

Sixty control-pollinated full-sib (identity of both parents known) *P. patula* families, from 29 parents, were established as seedling hedges in 2008 for the production of rooted cuttings. Of the 29 parents, 14 clones were used as the female parent and 23 were used as the male parent. Eight clones were used as both the female and male parent. The shoots from these hedges were routinely harvested and rooted. Those cuttings that had rooted during the first half of 2009 were used in this trial. The 60 families were arranged as treatments in a randomized complete block design with four replications. The average number of cuttings per treatment was 51. Open pollinated seedlings of *P. patula* and *P. elliottii* from commercial seed orchards were included as controls. In September 2009, the plants were transported to a greenhouse at the University of Pretoria.

Inoculations

The plants were inoculated with a combination of three *F. circinatum* isolates (CMW 3577, 3578 and 3579) maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) and which were shown to be highly virulent in a previously study. The isolates were grown on half strength Potato Dextrose Agar (PDA; 2g potato extract, 10 g dextrose, and 7.5 g agar/l distilled water) prior to inoculation. The cultures were flooded with sterile water and the spore concentrate diluted to 50 000 spores/ml. The diluted spore suspension of each isolate was then combined equally. The plants were wounded by removing the apical bud and inoculated by applying 10 µl (500 spores) of the mixed inoculum to the wounded surface. The inoculated plants were watered daily and assessed for lesion development 8 weeks after inoculation. At the time of assessment the seedling height, from the root collar to the wounded tip, was also recorded.

Statistical analysis

The statistical software package Genstat version 14 (2011) was used to analyse the data. The following factors were analysed; a) Group (APxAP families as cuttings, *P. patula* seedling control and *P. elliottii* seedling control), b) *P. patula* female parents, c) *P. patula* male parents and d) full-sib families. Summary statistics were carried out on the variables (Height, Lesion length and Dieback) to calculate means and the standard error of the mean (SEM) for each treatment. A correlation matrix was generated between Height, Lesion Length and Die-back to determine the relationship that each had on the other. The factors, Group, Female, Male and Full-sib family were analysed individually in separate models by analysis of variance (ANOVA) using lesion length as the variable. The control treatments were included in all analyses. Due to the small sample size of some of the individual full-sib families after mortality ($n<30$) standard errors of the mean (SEM) were generally large and the individual full-sib families with a standard error greater than 3 mm were excluded from the full-sib family comparison.

A separate ANOVA was carried out where families were nested within parents to assess family variation within a parent. A Duncan Multiple Range test was used to differentiate treatment differences at the 5% significance level in each case. Narrow-sense heritability, using lesion length as the variable, was calculated for the complete dataset of 60 families using the Model Least-Squares and Maximum Likelihood program (LSMLMW & MIXMDL PC-2 Version) developed by Harvey (1990), where a coefficient of relationship of 0.5 was used. The seedling controls (*P. patula* and *P. elliottii*) were excluded from heritability analyses.

Results

Lesions could be seen developing one week after inoculation. By the time that the trial was 8 weeks old approximately one third of the inoculated *P. patula* plants had died. This was most likely due to the very small size of the cuttings (82 mm) and seedlings (86 mm) (Table 1). None of the *P. elliottii* seedlings died. Although many of the *P. patula* cuttings had died, sufficient numbers, as seen by the analysis of variance and heritability test, remained to distinguish treatment differences and obtain meaningful results.

Lesion length and percentage die-back correlated positively ($r=0.911$, $p<0.001$). Height correlated negatively with die-back ($r=-0.217$, $p<0.001$) and had a small, but significant, influence on lesion length ($r=0.081$, $p<0.05$). A comparison of unadjusted values and those that were adjusted for height was made and, depending on the model, the adjusted lesion length for the *P. elliottii* seedling control (in particular) was not constant. Due to the small effect that height had on lesion length ($r=0.081$), unadjusted lesion length was used to compare treatment means.

The *P. elliottii* seedling control was significantly more tolerant (8.6 mm) than the combined mean of all *P. patula* cutting treatments (22.7 mm) which was more tolerant than the *P. patula* seedling control (30.5 mm) (Table 1). There were large differences between the parents tested. In the case of the female parents, which were represented by at least three full-sib progenies, lesion length ranged from 15.8 mm to 34 mm (Fig. 1). Although there were 23 male parents used in the study, only 10 were represented by at least three full-sib families. These produced lesions ranging from 18.8 mm to 26.8 mm (Fig. 1).

The greatest variation in tolerance was between the full-sib families assessed. The lesion lengths of 38 families (that had a SEM of less than 3mm) ranged from 5.8 mm to 30.8 mm (Fig. 2). Family AP12 x AP29 (13.4 mm), and those below it, was as tolerant as *P. elliottii* (8.6 mm) (Duncan Multiple Range Test) (Fig. 2). There was significant variation between full-sib families that shared a common parent where some full-sib families were more tolerant than either parent (Fig. 3). Narrow-sense heritability for the full-sib families was estimated at 0.199 ± 0.055.

Discussion

The results of this study have demonstrated that the variation in tolerance within open pollinated families to *F. circinatum* is partly due to the effect of the pollen parent. Although none of the parents (the mean of a minimum of 3 full-sib families with a common parent) were as tolerant as the *P. elliottii* seedlings, there were some full-sib *P. patula* families, tested as cuttings, which were as tolerant as *P. elliottii*. The combined mean for all the families as cuttings reflected a higher level of tolerance than the mean of the *P. patula* seedling control. This indicates that, if the full-sib families were tested as seedlings, fewer full-sib families would have been as tolerant as the *P. elliottii* control.

Because the progeny of several of the full-sib crosses were more tolerant than either parent, it is probable that the specific combination of two different clones (referred to specific combining ability or SCA) contributes more to the large phenotypic variation observed as has been reported for the *P. patula* x *P. tecunumanii* hybrid (Mitchell et al. 2012a). If this is true for *P. patula*, then it will be necessary to screen specific crosses and not only the parents for tolerance to *F. circinatum* in the future. None of the *P. patula* parents tested could be described as tolerant (ie. as tolerant as *P. elliottii*). Ideally, a greater number of parents of open pollinated families, which would be representative of a larger number of crosses, would enable better identification of tolerant parents with good general combining ability.

There has been some concern amongst breeders that the general susceptibility of *P. patula* to *F. circinatum* cannot be sufficiently improved to overcome the substantial damage due to nursery infection and subsequent post-planting mortality. However, the reasonable full-sib family heritability (0.2 ± 0.05) and wide range in tolerance with relatively few (38) full-sib families indicates that improvement can be made, particularly if individual full-sib families are screened.

Despite the fact that hybrids such as *P. patula* x *P. tecunumanii* are performing very well (Nel et al. 2006) and are more tolerant to *F. circinatum* than the pure species (Roux et al. 2007), *P. patula* still remains the most important softwood species on the cold sites of South Africa.

Therefore, it is imperative that breeders identify tolerant *P. patula* clones and family combinations that produce tolerant offspring. It is likely that due to the advanced breeding of *P. patula*, where 4th generation (F4) selections have been identified for orchard establishment in South Africa, further improvement in the species will be biased towards disease tolerance and its performance as a hybrid partner.

This study has focused on opportunities to produce *P. patula* that is not severely damaged by *F. circinatum* in nurseries or during establishment. Nursery and establishment infection currently accounts for the most significant losses due the pathogen in South Africa, but recent outbreaks of pitch canker on mature trees in plantations (Coutinho et al. 2007) represents a great threat. *Pinus radiata* has been most severely damaged by *F. circinatum* in plantations in the western and southern Cape Province and there have not been similar outbreaks in *P. patula* plantations. However, this manifestation of the disease remains a substantial threat to *P. patula* and providing resistance to infection, such as seems possible from this study, will be important (Wingfield et al. 1999).

Ultimately new seed orchards composed of clones tolerant to infection by *F. circinatum* will be planted in South Africa. Obtaining seed from such new orchards will not be possible for at least 10 years. In the interim, identifying specific parental combinations should provide a means of deploying *P. patula* that has some level of tolerance to infection by *F. circinatum*. Although it would be useful to repeat the trial on which this study was based, preferably by including additional families, other research has shown a relatively high genetic correlation (0.9) between repeated greenhouse experiments testing seedling tolerance to *F. circinatum* (Mitchell unpublished). This suggests that the specific full-sib families, that had similar levels of tolerance to *P. elliottii* in this study, should be propagated as cuttings and tested commercially.

Acknowledgements

We are grateful to Komatiland Forests for providing the plant material and incurring the costs involved in raising and screening the plants. We are also grateful to Bernice Porter who coordinated the inoculation procedure and who was responsible for the aftercare of the plants once inoculated.

References

Coutinho TA, Steenkamp ET, Mongwaketsi K, Wilmot M, Wingfield MJ (2007) First outbreak of pitch canker in a South African pine plantation. *Australasian Plant Pathology* 36: 256 – 261

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal*, vol. 205: 3 – 8.

Department of Agriculture, Forestry and Fisheries (DAFF) (2008) Report on commercial timber resources and primary roundwood processing in South Africa 2006/7. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

GenStat Release Edition 14.2 (2011). VSN International Ltd, Waterhouse Street, Hemel Hempstead, England.

Harvey WR (1990) Users guide for the PC-2 version of the LSM LMW Mixed Model least squares and maximum likelihood computer program. Columbus, USA: Ohio State University.

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011a) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET and Coutinho TA (2012a) Tolerance of *Pinus patula x Pinus tecunumanii*, and other pine hybrids, to *Fusarium circinatum* in greenhouse trials. *New Forests 2012 DOI: 10.1007/s11056-012-93*..

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET, Coutinho TA (2011b) Selection of *Pinus* spp. in South African for tolerance to infection by the pitch canker fungus. *New Forests* (http://www.springerlink.com/content/j02181n158286311).

Morris AR, Pallett R (2000) Site requirements and species matching: Pines. In: Owen D (ed) *South African Forestry Handbook, South African Institute of Forestry*, vol 1: pp 80-84.

Nel A, Kanzler A, Dvorak W (2006) Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. In: Fikret Isik (ed) Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. pp 158-161.

Roux J, Eisenberg B, Kanzler A, Nel, A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests* 33: 109 – 123.

Vermaak JA (2007) Genetic variation for growth and wood properties of *Pinus patula* families grown on six sites in South Africa. M.Sc. thesis, University of Stellenbosch, South Africa.

Wingfield MJ, Coutinho TA, Roux J, Wingfield BD (2002) The future of exotic plantation forestry in the tropics and southern Hemisphere: Lessons from pitch canker. *Southern African forestry Journal* 195: 79-82.

Wingfield MJ, Wingfield BD, Coutinho TA, Viljoen A, Britz H, Steenkamp ET (1999) Pitch canker: A south African perspective (1999) In: Devey ME, Matheson AC, Gordon TR, eds. *Current and Potential Impacts of Pitch Canker in Radiata Pine. Proceedings of the IMPACT Monterey Workshop, California, USA, 30 November−3 December 1998.* Kingston, Australia: CSIRO, Forestry and Forest Products, 62–9.

Table 1. Mean values and standard errors for height, lesion length and percentage dieback for all full-sib *P. patula* crosses (AP x AP), *P. elliottii* and *P. patula* controls.

Group	*Height (mm)*				*Lesion length (mm)*				*Dieback (%)*[1]			
	Mean	**SE**	**Duncan**	**n**	**Mean**	**SE**	**Duncan**	**n**	**Mean**	**SE**	**Duncan**	**n**
AP x AP	81.8	0.53	B	3020	22.7	0.41	A	1989	27.7	0.59	A	1989
P. elliottii	137.1	2.92	A	56	8.6	1.19	B	56	15.7	1.17	B	56
P. patula	86.0	2.79	B	56	30.5	2.78	C	42	35.9	3.50	C	42

Treatments with a different letter (Duncan) are significantly different, n = number of plants, [1]Dieback (%) was adjusted for height.

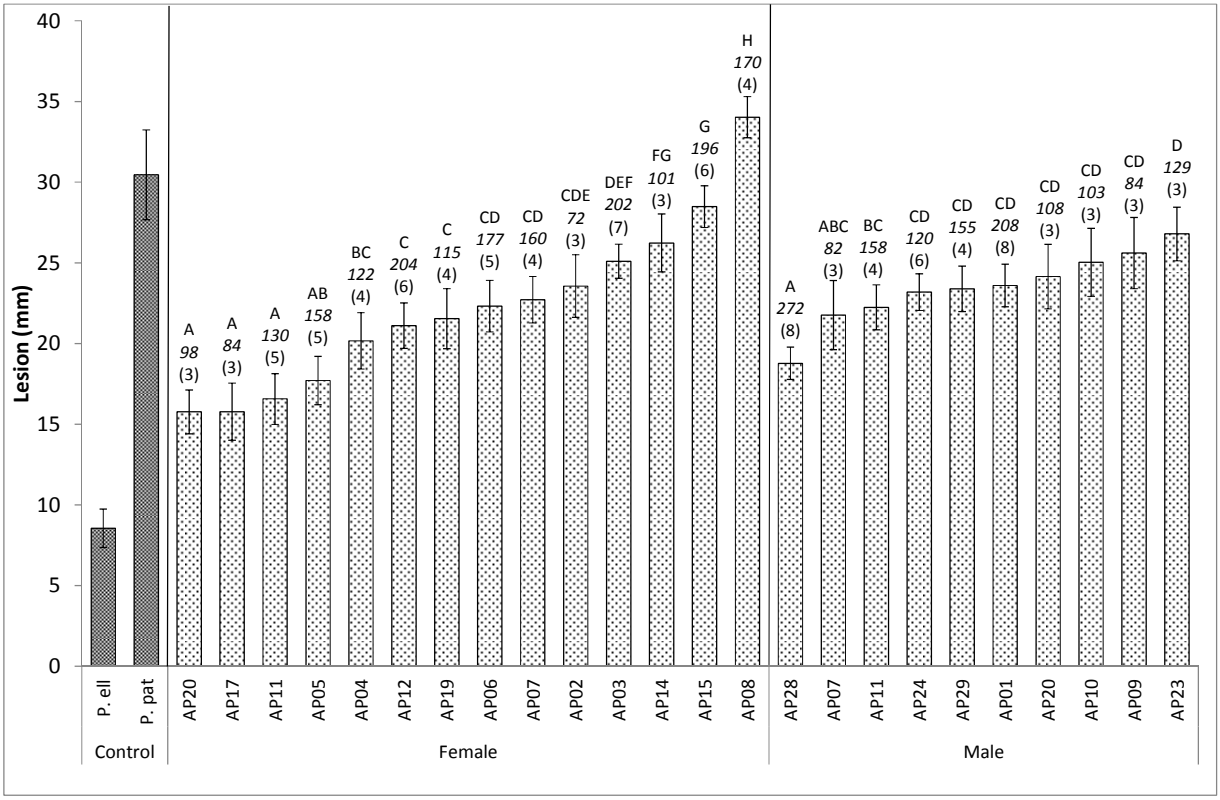

Figure 1. The ranking of the *P. patula* female and male parents as cuttings, relative to *P. patula* and *P. elliottii* seedling controls. Parent clones with a different letter (within Female or Male groups) are significantly different. The numbers in italics are the numbers of plants per parent clone and numbers in parenthesis are the number of full-sib families that share a common parent.

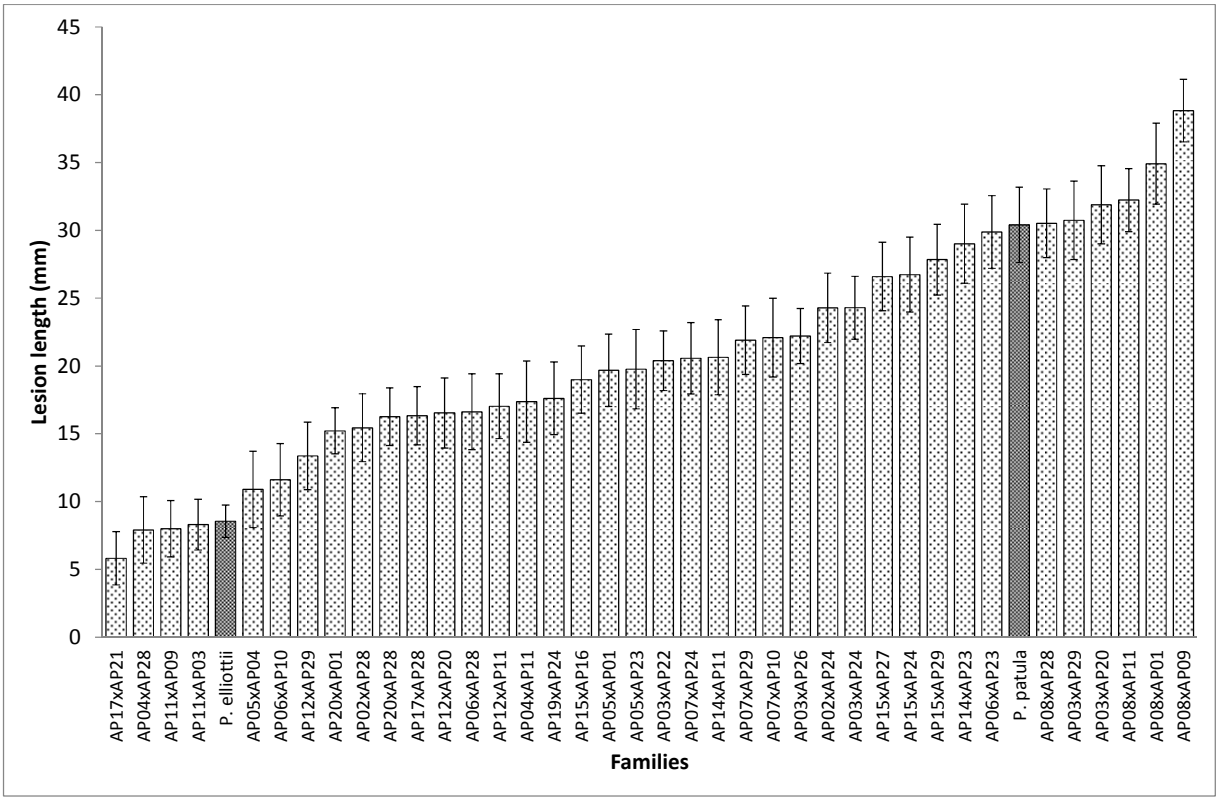

Figure 2. Full-sib *P. patula* family ranking from most to least tolerant. All families with a standard error of the mean greater than 3 mm are not shown. Narrow-sense heritability for the full-sib families was estimated at 0.199 ± 0.055.

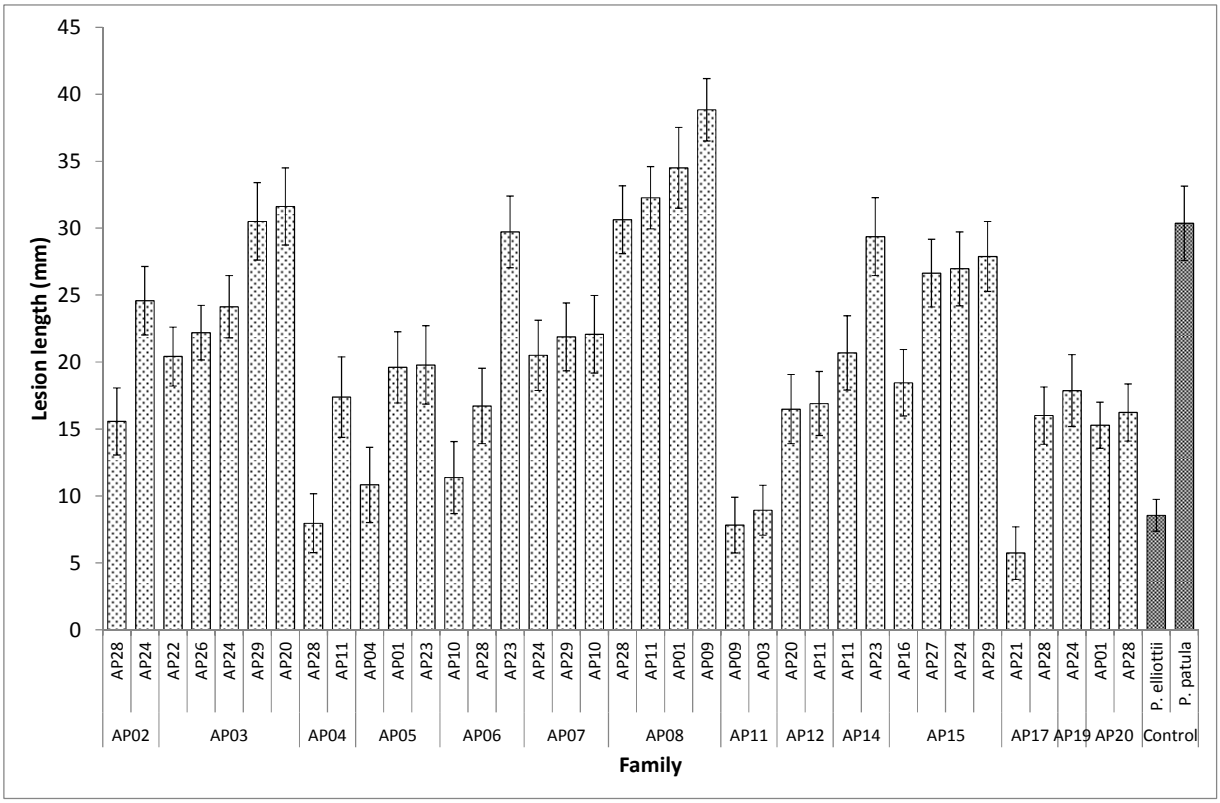

Figure 3. The variation in tolerance between *P. patula* full-sib families, which share a common female parent, relative to the *P. elliottii* and *P. patula* seedling control.

Chapter 6:

Tolerance of *Pinus patula* seedlings and established trees to infection by *Fusarium circinatum*

Abstract

Since the introduction of the pitch canker fungus, *Fusarium circinatum*, to South Africa, foresters have been challenged with poor field survival of seedlings at establishment. Possibly one of the best long term solutions is to improve the genetic tolerance of *P. patula* to infection by *F. circinatum*. Currently, large numbers of families are routinely screened for their tolerance to the pathogen by infecting open pollinated seedlings from orchard clones in a greenhouse and assessing lesion development. In this study, 9-year-old *P. patula* trees, from 96 families, were inoculated with *F. circinatum.* Their levels of tolerance were assessed and compared to that observed in seedlings originating from seed harvested from the same trees. The field results were also compared with those from previous greenhouse screening trials where seedlings from a number of the same families were inoculated with *F. circinatum*. The results show that there was a strong phenotypic ($r=0.712 \pm 0.002$) and genetic ($r_g =0.939 \pm 0.027$) correlation in the performance of the families common in both the greenhouse studies. A comparison of the tolerance of the families, screened as both seedlings and as trees, was also strong ($r=0.425 \pm 0.044$). Furthermore, the seedlings raised from seed collected from the infected *P. patula* trees, that ranked more tolerant than the mean of the *P. elliottii* trees, were similar in tolerance to *P. elliottii* seedlings in the greenhouse trial. It is thus believed that utilizing of seedlings from clones, known to be tolerant, will improve field survival and the tolerance of mature trees to pitch canker.

Keywords; *Pinus patula*, disease screening, pitch canker, seedlings, trees, *Fusarium circinatum*, heritability

Introduction

Pinus patula is South Africa's most important pine species with an annual planting of approximately 15,000 ha (Department of Agriculture, Forestry and Fisheries 2010) or 25 million seedlings. Although it is susceptible to fungal pathogens such as *Diplodia pinea* (Swart et al. 1985; Smith et al. 2002) and abiotic factors such as fire (de Ronde and du Plessis 2002) and high temperatures during the summer months (Allan and Higgs 2000), the most limiting factor is its susceptibility to the pitch canker fungus, *Fusarium circinatum* (Mitchell et al. 2011a).

Poor seedling survival (Crous 2005) and the cost of blanking young *P. patula* stands (Mitchell et al. 2011a) have caused foresters to question the continued planting of this species in the affected regions. It is accepted that the only long term solution will be to improve the genetic resistance of *P. patula* to *F. circinatum*. Success in creating tolerant germplasm has been achieved by hybridizing *P. patula* with subtropical species such as *P. tecunumanii* and *P. oocarpa* (Roux et al. 2007) and these hybrids have gained popularity in recent years. *Pinus patula* x *P. tecunumanii* and *P. patula* x *P. oocarpa* are, however, limited to the warmer sites of South Africa leaving *P. patula* as the preferred current species for the colder climatic regions (<15 ^{o}C mean annual temperature).

Breeders have attempted for a number of years to identify *P. patula* seed orchard clones that produce tolerant open pollinated progeny that can be commercially deployed. These studies are carried out in a greenhouse where seedlings are wounded and inoculated with spores of *F. circinatum*. In these studies, none of the *P. patula* families, when tested as seedlings, have been found to be as tolerant as *P. elliottii*. In other studies, inoculating seedlings of various species in the greenhouse (Hodge and Dvorak 2000, Mitchell et al. 2012c) compare well with the tolerance of the same species in the field (Roux et al. 2007). Therefore, it can be expected that if tolerant *P. patula* clones do exist, identifying them as seedlings in greenhouse inoculation studies will be a relatively easy and a cost efficient means of improving field survival as well as reducing the risk of pitch canker on mature trees.

The objectives of this study were to determine a) whether screening families as seedlings in a greenhouse will provide information about the tolerance of the same families as mature trees, and b) whether there is sufficient variation in the tolerance of *P. patula* to *F. circinatum* in order to identify highly tolerant families. To achieve these objectives two successive greenhouse trials were carried out testing a large number of families several of which were common to both greenhouse screening events. These results were then compared to the tolerance of the same families tested as 9-year old trees. Furthermore, we compare the tolerance of seedlings, raised from seed collected from the mature trees, to *F. circinatum* in the greenhouse.

Materials and Methods

Greenhouse studies

In this study we conducted 3 different greenhouse studies. The first two studies utilized open pollinated seed from 78 and 63, respectively, *P. patula* seed orchard clones. They were respectively sown early 2006 in preparation for inoculation in November 2006 and in the winter of 2006 in preparation inoculation in March 2007 (Table 1). Seventeen of the *P. patula* families were common to the two studies. Although the first study did not include a control species, the second trial included seedlings from open pollinated *P. elliottii* and *P. radiata* seed orchards, and cuttings of *P. patula* x *P. tecunumanii* (low elevation source or LE), *P. patula* x *P. oocarpa*, *P. patula* x *P. greggii* var. *greggii*, *P. patula x P. caribaea*, *P. elliottii* x *P. caribaea*, *P. tecunumanii* (LE) x *P. caribaea*, *P. tecunumanii LE x P. oocarpa*.

The material for the first two studies was raised in the Komatiland Forests Research nursery in composted pine bark in plastic Unigro 98 trays with square-shaped removable inserts. Each insert has a cavity size of 90 cc. The plants were fertilized with granular 2:3:2 (22) N:P:K fertilizer approximately 3 times during the nursery period. No fungicides were applied to the seedlings during their establishment. Once the seedlings/cuttings were ready for planting the treatments were arranged in a randomised complete block design with four replications (Table 1).

The third greenhouse study utilized seed collected in August 2009 from 17 open pollinated *P. patula* trees that were scored as either tolerant or susceptible to *F. circinatum* (see below). All the available seed from 6 tolerant trees and 12 susceptible trees was sown in May 2010 in the York Timbers Klipkraal nursery near Sabie. The seedlings were raised in composted pine bark in the Unigro tray described previously, and under similar conditions as the Komatiland nursery, for a period of 10 months. Open pollinated *P. elliottii* seedlings from a 2nd generation seed orchard were included as a control. At the end of the nursery phase, the seedlings from each tree were arranged in plots in a randomized complete block design and replicated 4 times. Depending on the number of seedlings available each plot consisted of 14 – 28 plants with an average plot size of 21 plants (84 seedlings per treatment).

After the seedlings for each of the three studies were packed out, the plants were transported to a greenhouse screening facility that is specifically used for inoculation studies at the Forest and Agricultural Biotechnology Institute (FABI) of the University of Pretoria. The plants in the first two studies were inoculated with a combination of three virulent isolates (CMW 3577, 3578 and 3579) of *F. circinatum*, while only one isolate (CMW 3579) was used in the third study. The reason for this is that, although CMW 3579 is highly virulent it is outcompeted by the CMW 3577 and CMW 3578 when applied to the same wound (Porter 2010). In all cases, inoculum was prepared by growing the fungi on half strength Potato Dextrose Agar (PDA; 2g potato extract, 10 g dextrose, and 7.5 g agar/l distilled water) for 7 days at 25^{o}C. The spores of each isolate were then harvested by flooding the cultures with sterile distilled water and quantified using a haemocytometer. For the first two studies, the spore suspensions for each the three isolates were mixed together equally to a final concentration of 50 000 spores/ml, while the third study utilized 50 000 spores/ml prepared from the single isolate. In the greenhouse, 10 µl (500 spores) of the freshly prepared inoculum as applied to the wounded surface of a seedling where its apical bud was removed using secateurs. Once inoculated, the plants were watered daily. After 8 weeks, lesion development and height of each seedling was recorded in mm.

Field study

During 2001 open-pollinated seedlings from 96 seed orchard clones of *P. patula* were planted in un-replicated square plots of 25 trees per family by Komatiland Forests. The family plots were

arranged in a rectangular shape (16 plots long and 6 plots wide) and the seedlings were planted 3 m from each other. When the trial was 9-years-old, 4-12 trees were chosen (based on the availability of cones and poor stem form) in each family plot and marked for use in this study. Most families were represented by 10 trees and a total of 923 trees were selected. Ten *P. elliottii* trees of the same age, from a compartment adjacent to the trial site, were selected as a control.

On the 15th December 2009 the trees were inoculated with the same three isolates (CMW 3577, 3578 and 3579) used for the first two greenhouse studies. Inoculum was prepared by growing the isolates on half strength PDA for 7 days at 25^{o}C. The trees were wounded at chest height by removing part of the thick outer bark and extracting a 5 mm plug of phloem to the depth of the cambium from three equally spaced sides of the tree. Each wound was inoculated with a separate isolate of the pathogen. A 5 mm agar plug with mycelium (removed with a cork borer from the actively growing culture) was placed so that the hyphae made contact with the cambium. The identity of each isolate was recorded by painting the stem of the tree above the wound unique to each isolate. The agar plugs were sealed with masking tape to protect the inoculum from desiccation. Twelve weeks later (15/03/2010) the wounds were exposed by removing the bark above and below the point of inoculation and recording the length of each lesion in mm. The circumference of each tree was also assessed at the height of inoculation.

In October 2011, 20 months after the trial had been assessed, the trees were felled to prevent the spread of *F. circinatum*. Bark and wood samples were collected from the inoculation sites on several of the fresh logs that had been stacked at the roadside. These were submitted to the Tree Pathology Cooperative Program (TPCP), of the University of Pretoria, where they were placed on *Fusarium* selective medium and assessed for the presence of *F. circinatum*.

Statistical analysis

The statistical software package SAS Enterprise Guide 4.3 (2010, copyright SAS Institute Inc., Cary, NC.) was used to analyse the data in all the trials. In keeping with previous studies (Mitchell et al. 2012c), lesion length was used as the variable when describing treatment differences.

Each of the greenhouse trials was analysed separately. After standardizing and correcting the data for the effect of replication, a Pearson correlation matrix was generated as a measure of the strength of the relationship of height, lesion length and percentage dieback. The data (lesion length) was subjected to an analysis of variance (ANOVA) to determine the level of significance between family means. A Duncan Multiple Range test was used to identify treatment differences at the 5% significance level. Narrow-sense heritability was calculated using the Model Least-Squares and Maximum Likelihood program (LSMLMW & MIXMDL PC-2 Version) (Harvey 1990) where a coefficient of relationship of 0.25 was used. The *P. elliottii* and *P. radiata* seedlings, as well as the cuttings of the various hybrids, were excluded from heritability analysis in the second trial.

After analysing the results from the first two greenhouse studies, the tolerance of the 17 families common to both trials, was compared. A Pearson correlation matrix was used as an indication of the strength of the relationship based on phenotypic observations. The proc varcomp procedure was carried out to estimate the family variance components in each dataset. Data sets containing the family means for each trial were created. The data sets were then merged and the correlation procedure (proc corr) was carried out to estimate the covariance of the family means. The genetic correlation and standard error of the genetic correlation, was calculated using the formulas (Falconer 1989) shown below where “Site A and Site B” refer to dataset A and B. These procedures were carried out on unadjusted greenhouse values compared with both adjusted and unadjusted field values in separate analyses.

$$r_{g_{siteA\_siteB}} = \frac{\mathrm{COV}_{f_{siteA\_siteB}}}{\sqrt{\sigma^2_{f_{siteA}} \times \sigma^2_{f_{siteB}}}} \qquad SE_{r_g} = \frac{1-r_g^2}{\sqrt{2}} \times \sqrt{\frac{SE_{h_x^2} \times SE_{h_y^2}}{h_x^2 \times h_y^2}}$$

For the field study, a Pearson correlation matrix was calculated between tree stem circumference and lesion length for each isolate, and the means of the combined isolates. As circumference correlated positively with the combined lesion length ($r=0.143$, $p<0.001$) the analyses of variance (ANOVA) tests were conducted on the corrected (for circumference) lesion length values testing family (n=97 (including the *P. elliottii* control), isolate (n=3) and their interaction in a single model (see Model 1 below). The nested effect of tree within family was tested in a separate model (Model 2). A Duncan Multiple Range test was used to distinguish family, tree and isolate differences.

Model 1

$w_{ijk} = \mu + f_i + s_j + fs_{ij} + e_{ijk}$

where;

w_{ijk} = wound or lesion length (either corrected or not) of isolate j of tree k of family i,

μ = the population mean,

f_i = family i's random effect,

s_j = the random effect of isolate k,

fs_{ij} = the interaction effect between family i and isolate j,

e_{ijk} = the random error effect.

Model 2

$w_{ijk} = \mu + f_i + t_j + e_{ijk}$

where;

w_{ijk} = wound or lesion length (either corrected or not) of tree k of family i,

μ = the population mean,

f_i = family i's random effect,

t_j = the random effect of tree j within family i,

e_{ijk} = the random error effect.

There were 12 families common to the first (2006) greenhouse trial and the field trial, and 16 families common to the second (2007) greenhouse trial and the field trial. The phenotypic

correlation between the families common to the greenhouse and field was determined using the same procedures described for the two greenhouse studies. This was done separately for each greenhouse study and the field. The data for both greenhouse studies were then merged to allow for a comparison of 23 common families between the greenhouse trials and the field trial.

Results

Greenhouse studies

In the first two greenhouse studies, seedling height correlated negatively ($p<0.021$, $r=-0.143$ and $p<0.001$, $r=-0.368$) with dieback but did not correlate well with lesion length in the first ($r=0.036$) or the second greenhouse study ($r=0.023$); unadjusted lesion length was used to compare treatments. The lesion length of the 78 families screened in the first (2006) greenhouse study ranged from 5 mm (AP044) to 14.7 mm. The narrow sense heritability estimate (h^2) was 0.248 ± 0.047 (Table 2). In the second (2007) greenhouse study, *P. elliottii* and those hybrids between *P. elliottii*, *P. patula*, *P. caribaea* var. *hondurensis*, *P. oocarpa* and *P. tecunumanii*, were significantly more tolerant than the mean of all *P. patula* families (Table 3) based on lesion length. *Pinus radiata*, and the hybrid between *P. patula* and *P. greggii* var. *greggii*, was significantly more susceptible than *P. patula* (Table 3). The lesion length of the 63 *P. patula* families in the second greenhouse trial ranged from 10.2 mm to 34.2 mm. The most tolerant family (AP004) was as tolerant as the *P. elliottii* control (9.1 mm) and 20 families were as susceptible as the *P. radiata* control (32.2 mm). The narrow sense family heritability (h^2) estimate was 0.521 ± 0.093 (Table 2).

The phenotypic correlation generated by comparing the 17 common families in the two greenhouse studies was strong ($r = 0.712 \pm 0.002$), as was the genetic correlation ($r_g = 0.939 \pm 0.027$) (Table 4, Fig. 2). Family AP004, which was as tolerant as the *P. elliottii* control in the second greenhouse study was the second most tolerant family in the first greenhouse study (Table 5). Families AP057 and AP064 were considered susceptible in both the first and second greenhouse studies (Table 5) and families AP067, AP036, AP039, AP065, AP055, which were intermediate, ranked similar in both studies (Table 5).

In the third greenhouse study, that employed progeny of the most tolerant and susceptible trees identified in the field study (see below), seedling height had a negative effect on percentage dieback ($r=-0.2756$, $p<0.001$) and correlated positively with lesion length ($r=0.11568$, $p<0.001$). Lesion length and percentage dieback correlated strongly ($r=0.88793$, $p<0.001$). The lesion length and dieback values were thus adjusted for height. The seedlings of the 3 *P. patula* trees which ranked more tolerant than *P. elliottii* trees in the field (AP11-6, AP29-2 and AP12-3) were equally tolerant to the *P. elliottii* seedlings in the greenhouse (Table 6). All of the *P. patula* trees that had a lesion length longer than the mean length of the 10 *P. elliottii* trees (55 mm), produced progeny that were more susceptible than *P. elliottii* seedlings (Table 6). The lesions on the seedlings from the most susceptible trees continued to develop, and by month 4, most of them were dead whilst those from the most tolerant trees were producing new shoots beneath the lesion (Fig. 3). None of the seedlings in the *P. elliottii* control died (Fig. 3).

Field inoculation study

The strength of the correlation between the families screened in the greenhouse and as established trees, was influenced by adjusting the field data for the significant effect that tree circumference had on lesion length ($r=0.143$, $p<0.001$). Results are discussed on both adjusted and unadjusted field values.

Overall, the mean lesion length of all the *P. patula* trees was 95.9 ± SE 0.67 mm and the mean of the *P. elliottii* trees was 54.5 ± SE 2.25mm. There was large variation between the families with the lesion length of the most tolerant family (AP168) measured 70.1 ± SE 5.89 mm and most susceptible family (AP163) measured 121.0 ± SE 4.74 mm (Fig. 2). The lesion length for the trees of the most tolerant family (AP168) ranged from 38.0 mm to 111.0 mm and within the most susceptible family (AP163) ranged from 99.7 mm to 150.5 mm. The most tolerant *P. elliottii* tree had a lesion length of 36.0 mm and the most susceptible *P. elliottii* tree had a lesion length of 67.2 mm. The most tolerant *P. patula* tree in the trial had a mean lesion length of 30.3 mm and the most susceptible *P. patula* tree had a mean lesion length of 162.7 mm. Of the 923 *P. patula* trees, 30 had lesion lengths that were smaller than those of the 10 *P. elliottii* trees (54.5 mm) and

67 trees had lesion lengths smaller than the most susceptible *P. elliottii* tree (67.2 mm). Approximately 5% of the trees had lesion lengths less than 60 mm and were thus considered tolerant based on the mean lesion lengths the 10 *P. elliottii* trees. The 30 most tolerant trees (with lesion lengths less than 55 mm) were from 25 of the 96 families.

Isolate CMW3579 was significantly more aggressive (98.3 mm) than CMW 3578 (96.2 mm) which was more aggressive than CMW 3577 (92.1 mm). There was no family x isolate ($p=0.454$) interaction. Although the trees produced resin around the inoculation points, the infection sites never developed into large cankers. In addition, trees in the field did not develop any typical symptoms of pitch canker such as shoot and branch dieback seen elsewhere (Coutinho et al. 2007). Furthermore, *F. circinatum* could not be re-isolated from the wood samples 22 months after the inoculation date.

A comparison of the 12 families in the first (2006) greenhouse trial, that were common in the field trial, produced a phenotypic correlation (r) of 0.555 ± 0.061 (not adjusted) or 0.444 ± 0.149 (adjusted) (Table 4). The phenotypic correlation (r) between the 16 common families in the second (2007) greenhouse trial and the field trial was 0.471 ± 0.066 (not adjusted) or 0.467 ± 0.068 (adjusted) (Table 4). The phenotypic correlation (r) between the 23 families in the combined greenhouse studies (2006 and 2007) and field was 0.425 ± 0.044 (not adjusted) or 0.397 ± 0.060 (adjusted) (Table 4).

Discussion

Results of this study show clearly that there is sufficient variation within *P. patula* to identify families that can be considered comparable to *P. elliottii* in tolerance to *F. circinatum*. The strong genetic correlation (0.94) between the two repeated greenhouse studies, and good (0.25) to very strong (0.52) narrow-sense family heritability seen in each, indicates that more tolerant clones can be identified as seedlings by screening a large number of open pollinated families in a greenhouse trial. Despite the fact that the correlation between the greenhouse studies was strong, the tolerance of some families was dissimilar. Therefore, it would be wise to repeat a greenhouse screening event at least once before identifying tolerant families.

The meaningful comparison of the ranking of the 23 common families represented in the field and the greenhouse trials (r=0.425 ± 0.044), indicates that greenhouse screening provides an indication of the tolerance of mature trees to *F. circinatum*, as has been reported for other pines (Barrows-Broaddus and Dwinell 1984, Blakeslee and Rockwood 1999). This was especially evident by the fact that the most tolerant trees in the field produced seedlings that were as tolerant as *P. elliottii* to infection. This is supported by other studies where heritability values of up to 0.86 have been recorded from field trials (Rockwood et al. 1988, Blakeslee and Rockwood 1999).

It is evident that a very large number of clones of *P. patula* need to be screened in order to identify those that are similar to the general tolerance of *P. elliottii* in the field. In this study we estimate that approximately 5% of *P. patula* trees, currently deployed, are likely to be as tolerant *P. elliottii*. This figure is also quoted for planted *P. radiata* trees (Storer et al. 1999). The 30 trees that ranked more tolerant to *F. circinatum* than the mean of *P. elliottii* in the field trial represent just over 3% of the total number of *P. patula* trees screened. It is likely that the family which was as tolerant as *P. elliottii* in the second greenhouse study represented, by random selection, the upper range of tolerance.

In previous field inoculation studies, susceptible *P. radiata* trees developed signs of pitch canker after inoculating branches with *F. circinatum* (Storer et al. 1999). Perhaps it is because we inoculated stems and the fact that *P. patula* is more tolerant than *P. radiata* to *F. circinatum*, (Hodge and Dvorak 2000), that typical signs of pitch canker were not observed. The recovery of young *P. patula* trees, after being inoculated with *F. circinatum*, has also been reported (Viljoen et al. 1995). This clearly demonstrates that trees are more tolerant to infection than seedlings.

Our results in the field confirm those of Porter (2010) that isolate CMW 3579 is more aggressive than CMW 3577 or CMW 3578. Together with the fact that there was no interaction between host and isolate, we concur with Porter (2010) that inoculating *P. patula* seedlings or trees with only CMW 3579 in future screening studies should achieve rapid and reliable results. This may not be the case for different species of pine. Whilst Gordon et al. (1999) also reported an absence

of interaction between the host and isolate for *P. radiata*, Blakeslee and Rockwood (1999) reported that this phenomenon exists for susceptible *P. elliottii* and *P. taeda* families. This indicates that further research on this topic is necessary.

The results of this study provide a basis for estimating the number of families, and individuals per family, which should be screened for their tolerance to *F. circinatum* in order to identify a sample of trees for a new orchard comprised of tolerant clones (see Rockwood et al. 1988). If we consider 25 families, of the 96 families tested in the field (i.e. ± 25%), produced the 30 most tolerant trees based on a sample size of 10 trees per family, then potentially 50-60 trees may have been identified from the 96 families if 20 trees per family were inoculated. In this case it is likely that these tolerant trees would not be restricted to only 25 families. Several studies have shown that there is a good correlation between the ranking of clones in repeated inoculation events (Gordon et al 1999, Storer et al. 1999) particularly if they were considered tolerant when they were inoculated for the first time (Sammon et al. 1999). Therefore, we can expect that "tolerant" clones will retain their classification once grafted in a new orchard.

The slight positive correlation between tree circumference and lesion length in the field trial suggests that faster growing trees may be more susceptible to *F. circinatum*. However, other studies show this not to be the case (Rockwood et al. 1988, Blakeslee and Rockwood 1999). Although tree survival was not recorded for each family, the poor survival in some plots would have resulted in larger trees which may explain the positive correlation.

Historically, disease tolerance has not been considered a selection criterion in *P. patula* but it is becoming increasingly important to do so. We expect that future breeding efforts will focus on identifying a sub-population of clones that are tolerant to *F. circinatum*, and new selections will be identified from their progeny which grow well. Similar to the improved tolerance of the *P. patula* x *P. tecunumanii* hybrid to *F. circinatum* (Table 3), which is due to the good tolerance of *P. tecunumanii* to *F. circinatum* (Mitchell et al. 2012c), tolerant *P. patula* clones could be used to control pollinate *P. patula* clones with good growth but poor tolerance to *F. circinatum*. Including genetic material from more recent seed introductions will become increasingly

important to continue the deployment of *P. patula* as a pure species, as will identifying hybrid partners and alternative species, which can replace some of the area currently suited to *P. patula*.

Acknowledgements

We thank Komatiland Forests for providing the seed and incurring the costs involved in raising and screening the plant material; Phillip Hongwane and his team, for collecting the seed from the trees in the field trial; Bernice Porter who coordinated the greenhouse screening activities and aftercare of the plants; the students, from the University of Pretoria, who helped inoculate the trees in the field. Financial support for laboratory work was provided by the members of the Tree Co-operative Programme (TPCP) supported by the THRIP initiative of the Department of Trade and Industry.

References

Allan R, Higgs G (2000) Methods of improving the survival of *Pinus patula* planted into harvesting residues. *Southern African Forestry Journal* 189: 47-51.

Barrows-Broaddus J, Dwinell LD (1984) Variation in susceptibility to the pitch canker fungus among half-sib and full-sib families of Virginia pine. *Phytopathology*, vol. 74: 438 – 444.

Blakeslee GM, Rockwood D (1999) Variation in resistance to pitch canker in slash and loblolly pines. Current and Potential Impacts of the Pitch Canker in Radiata pine: in Devey, M. E., Matheson, A.C. and Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. p. 70 - 75.

Coutinho TA, Steenkamp ET, Mongwaketsi K, Wilmot M, Wingfield MJ (2007) First outbreak of pitch canker in a South African pine plantation. *Australasian Plant Pathology* 36: 256 – 261.

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal*, vol. 205: 3 – 8.

de Ronde C, du Plessis M (2002) Determining the relative resistance of selected *Pinus* species to fire damage. Forest Fire Research & Wildland Fire Safety, Viegas (ed.) 2002 Millpress, Rotterdam, ISBN 90-77017-72-0

Department of Agriculture, Forestry and Fisheries (DAFF) (2010) Report on commercial timber resources and primary roundwood processing in South Africa 2008/9. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Falconer DS (1989) Introduction to Quantitative Genetics. Longman Scientific & Technical, U.K. 438pp

Gordon T, Wikler K, Clark S, Storer A, Bonello P (1999) Resistance to pitch canker in Monterey pine. Current and Potential Impacts of the Pitch Canker in Radata pine: in Devey, M. E., Matheson, A.C. and Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. p. 87 - 89.

Harvey WR (1990) Users guide for the PC-2 version of the LSM LMW Mixed Model least squares and maximum likelihood computer program. Columbus, USA: Ohio State University.

Hodge GR, Dvorak WS (2000) Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests* 19: 241-258.

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011a) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET and Coutinho TA (2012) Tolerance of *Pinus patula x Pinus tecunumanii*, and other pine hybrids, to *Fusarium circinatum* in greenhouse trials. *New Forests, DOI: 10.1007/s11056-012-9355-3*.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET, Coutinho TA (2011b) Selection of *Pinus* spp. in South African for tolerance to infection by the pitch canker fungus. *New Forests* 43:473-489.

Porter B (2010) *In planta* competition between three *Fusarium circinatum* isolates. In: Pathogenicity and competition studies on *Fusarium circinatum*, a pathogen of pine trees. M.Sc. thesis. University of Pretoria pp 53-80.

Rockwood DL, Blakeslee GM, Lowerts GA, Underhill EM, Oak SW (1988) Genetic strategies for reducing pitch canker incidence in Slash pine. *Southern Journal of Applied Forestry*, 12: 28-32.

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, Vol. 33: 109 – 123.

Sammon S, Adams D, Gordon T, Frankel S, Ringnes D (1999) Early results for screening for pitch canker resistance in Cambria population of Monterey pine (*Pinus radiata*). Current and Potential Impacts of the Pitch Canker in Radiata pine: in Devey, M. E., Matheson, A.C. and Gordon, T. R. Proceedings of the IMPACT Montery Workshop, Montery, California. p. 85 - 87.

Smith H, Wingfield MJ, Coutinho TA (2002) The role of latent *Sphaeropsis sapinea* infections in post hail associated die-back of *Pinus patula*. *Forest Ecology and Management* 164:177-184.

Storer AJ, Bonello P, Gordon TR, Wood DL (1999) Evidence of resistance to the pitch canker pathogen (*Fusarium circinatum*) in native stands of Monterey pine (*Pinus radiata*). *Forest Science*, vol. 45: 500-505.

Swart WJ, Knox-Davies PS, Wingfield MJ (1985) *Sphaeropsis sapinea*, with special reference to its occurrence on *Pinus* spp. in South Africa. Southern African Journal of forestry, December, 1985; 1-8.

Viljoen A, Wingfield MJ, Kemp GHJ, Marasas WFO (1995) Susceptibility of pines to the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Plant Pathology*, vol. 44: 877-882.

Table 1. Trial layout and dates for inoculation of *Pinus patula* seedlings with *F. circinatum*.

Details	***Greenhouse 1***	***Greenhouse 2***	***Field***	***Greenhouse 3***
Date inoculated	Nov 2006	Mar 2007	Dec 2009	Dec 2010
Date assessed	Jan 2007	May 2007	Mar 2010	Feb 2011
Families tested	78	63	96	17
Mean plot size	22 (seedlings)	14 (seedlings)	10 (trees)	21 (seedlings)
Replications	4	4	1	4
Mean no. obs. per family	88	56	30	84
Controls	*None*	*P. elliottii*	*P. elliottii*	*P. elliottii*
		P. patula		*P. patula*
		P. radiata		
		P. pat x P. tecL		
		P. pat x P. ooc		
		P. pat x P. carH		
		P. pat x P. greN		
		P. tecH x P. ooc		
		P. tecL x P. carH		
		P. ell x P. carH		

Table 2. The narrow-sense family heritability (h^2) estimates of the greenhouse studies.

Screening event	*Families*	$h_2 \pm$ *standard error*
2006 greenhouse	78	0.248 ± 0.047
2007 greenhouse	63	0.521 ± 0.093
2006 + 2007 greenhouse	35	0.395 ± 0.096

Table 3. The tolerance of *P. patula*, compared with *P. elliottii*, *P. radiata* and several hybrids, in the 2007 greenhouse trial.

Treatment group	*N*	*Height ± SE (mm)*	*Lesion ± SE (mm)*	*Dieback ± SE (%)*
P. tecunumanii LE x P. oocarpa	22	93.95 ± 5.14	3.22 ± 0.29 ^{A}	4.40 ± 0.42 ^{D}
P. tecunumanii LE x P. caribaea	52	128.4 ± 2.12	5.14 ± 0.69 ^{A}	4.16 ± 0.56 ^{A}
P. patula x P. caribaea	23	110.3 ± 3.08	5.217 ± 0.41 ^{A}	4.78 ± 0.39 ^{A}
P. elliottii	53	180.6 ± 2.08	5.60 ± 0.56 ^{A}	3.11 ± 0.32 ^{A}
P. elliottii x P. caribaea	44	193.4 ± 3.03	6.11 ± 0.67 ^{A}	3.22 ± 0.35 ^{A}
P. patula x P. oocarpa	45	124.0 ± 5.42	7.49 ± 1.14 ^{A}	6.21 ± 0.93 ^{A}
P. patula x P. tecunumanii LE	67	116.6 ± 3.34	8.15 ± 0.75 ^{A}	7.62 ± 0.81 ^{A}
P. patula	4371	142.5 ± 0.41	24.57 ± 0.20 ^{B}	18.53 ± 0.17 ^{B}
P. radiata	66	99.5 ± 2.63	34.65 ± 1.81 ^{C}	35.23 ± 1.90 ^{C}
P. patula x P. greggii var. *greggii*	35	63.8 ± 2.17	36.89 ± 2.22 ^{C}	50.18 ± 3.28 ^{D}

Table 4. A summary of the phenotypic (r) and genetic (r_g) correlations of the families common in the 2006 and 2007 greenhouse trials, and the phenotypic (r) correlation between those the families common to the greenhouse and field trial.

Site A	*Site B*	*Common Families*	$r_{(phenotypic)}$	$r_{g\ (genetic)}$
G/H 1	G/H 2	17	0.712 ± 0.002	0.939 ± 0.027
G/H 1	Field	12	0.555 ± 0.061 (0.444 ± 0.149)	
G/H 2	Field	16	0.471 ± 0.066 (0.467 ± 0.068)	
G/H 1 and 2	Field	23	0.425 ± 0.044 (0.397 ± 0.060)	

Values in parenthesis are based on lesion length after adjusting for circumference

Table 5. The ranking of families common in different screening events.

G/house 1	*n*	*Lesion*	*G/house 2*	*n*	*Lesion*	*Field*	*n*	*Lesion*
AP044$^{(1,2)}$	21	5.92	AP004$^{(1,2)}$	44	7.51	AP094$^{(2,F)}$	30	75.50
AP004$^{(1,2)}$	57	7.83	**AP063$^{(1,2,F)}$**	62	16.37	AP073$^{(1,F)}$	30	76.93
AP076$^{(1,2)}$	74	8.52	AP082$^{(2,F)}$	77	16.54	**AP011$^{(1,2,F)}$**	30	80.26
AP011$^{(1,2,F)}$	55	8.94	**AP011$^{(1,2,F)}$**	61	16.72	AP035$^{(1,F)}$	30	82.30
AP029$^{(1,F)}$	74	9.02	AP044$^{(1,2)}$	80	16.97	**AP067$^{(1,2,F)}$**	30	83.43
AP053$^{(1,2)}$	42	9.1	AP088$^{(2,F)}$	73	18.13	AP014$^{(1,F)}$	30	86.20
AP073$^{(1,F)}$	90	9.17	AP111$^{(2,F)}$	81	19.52	AP113$^{(2,F)}$	30	86.33
AP036$^{(1,2,F)}$	72	9.2	AP053$^{(1,2)}$	30	21.03	**AP063$^{(1,2,F)}$**	30	90.10
AP067$^{(1,2,F)}$	49	9.3	**AP067$^{(1,2,F)}$**	61	21.06	AP088$^{(2,F)}$	30	90.44
AP062$^{(1,F)}$	23	9.33	AP076$^{(1,2)}$	76	21.98	AP013$^{(1,F)}$	30	91.50
AP014$^{(1,F)}$	92	9.81	**AP036$^{(1,2,F)}$**	68	23.1	AP082$^{(2,F)}$	30	91.93
AP063$^{(1,2,F)}$	87	10.01	AP038$^{(1,2)}$	39	23.69	AP099$^{(2,F)}$	30	92.60
AP035$^{(1,F)}$	86	10.12	AP094$^{(2,F)}$	82	23.99	AP089$^{(2,F)}$	30	95.20
AP068$^{(1,F)}$	40	10.17	AP115$^{(2,F)}$	68	24.18	**AP036$^{(1,2,F)}$**	30	95.90
AP039$^{(1,2)}$	52	10.26	AP061$^{(1,2)}$	54	25.67	AP062$^{(1,F)}$	30	98.53
AP013$^{(1,F)}$	87	10.99	AP039$^{(1,2)}$	83	25.76	AP068$^{(1,F)}$	30	100.87
AP065$^{(1,2)}$	46	11.08	AP043$^{(1,2)}$	53	25.93	AP093$^{(2,F)}$	30	103.56
AP055$^{(1,2)}$	37	11.87	AP065$^{(1,2)}$	80	25.95	AP111$^{(2,F)}$	30	103.59
AP078$^{(1,2,F)}$	71	12.16	AP110$^{(2,F)}$	66	27.33	AP105$^{(2,F)}$	30	104.90
AP043$^{(1,2)}$	91	12.19	AP093$^{(2,F)}$	83	27.59	AP115$^{(2,F)}$	30	106.47
AP064$^{(1,2)}$	82	12.91	**AP078$^{(1,2,F)}$**	45	28.06	AP029$^{(1,F)}$	30	108.80
AP061$^{(1,2)}$	85	12.97	AP055$^{(1,2)}$	80	28.17	AP110$^{(2,F)}$	30	110.97
AP038$^{(1,2)}$	90	13.1	AP089$^{(2,F)}$	83	28.32	**AP078$^{(1,2,F)}$**	30	114.13
AP057$^{(1,2)}$	62	13.35	AP057$^{(1,2)}$	74	29.17			
			AP113$^{(2,F)}$	64	29.21			
			AP099$^{(2,F)}$	77	30.66			
			AP105$^{(2,F)}$	85	30.68			
			AP064$^{(1,2)}$	83	31.89			

[1]Numbers in parenthesis indicate which trial the family is common in *viz*. 1 = 1st greenhouse screening, 2 = 2nd greenhouse screening and F = field screening. [2]Families in bold are common across all three screening events.

Table 6. A comparison of the 6 tolerant (lesion <65 mm) and 12 susceptible (lesion >126 mm) *P. patula* trees with the tolerance of their open-pollinated progeny as seedlings in the greenhouse.

	Field		***Greenhouse***		
Tree	**Rank**	**Lesion ± SE (mm)**	**N**	**Lesion ± SE (mm)**	**Dieback ± SE (%)**
AP11-6	7	41.0 ± 5.97	79	20.0 ± 1.8^{FG}	21.8 ± 2.0^{F}
AP29-2	23	51.0 ± 1.77	88	15.7 ± 2.1^{G}	18.1 ± 2.3^{F}
AP12-3	26	52.0 ± 1.44	78	16.6 ± 1.9^{G}	17.3 ± 2.3^{F}
P. elliottii[1]		54.5 ± 2.25	75	17.5 ± 1.9^{G}	20.8 ± 1.9^{F}
AP84-2	48	63.7 ± 3.18	93	27.4 ± 2.1^{DE}	31.0 ± 2.5^{DE}
AP69-5	51	64.0 ± 9.71	28	33.9 ± 2.7^{BCD}	37.7 ± 3.3^{BCD}
AP21-3	54	64.7 ± 7.36	68	31.2 ± 2.5^{CDE}	36.1 ± 2.6^{CDE}
P. patula[1]		95.9 ± 0.67	75	32.4 ± 2.2^{CD}	35.5 ± 1.6^{CDE}
AP22-1	812	126.3 ± 13.98	87	31.1 ± 2.2^{CDE}	34.8 ± 2.3^{CDE}
AP51-2	865	127.3 ± 1.58	51	36.0 ± 2.3^{ABC}	40.3 ± 2.6^{ABC}
AP73-2	870	127.7 ± 15.34	73	24.4 ± 2.1^{EF}	29.5 ± 2.0^{E}
AP15-10	873	128.3 ± 3.49	74	43.2 ± 2.3^{A}	46.8 ± 2.3^{A}
AP22-2	885	130.7 ± 12.16	44	35.1 ± 2.5^{BC}	40.1 ± 3.1^{ABC}
AP81-1	895	132.7 ± 5.19	31	36.6 ± 2.2^{ABC}	41.1 ± 2.7^{ABC}
AP15-2	896	133.3 ± 8.22	73	40.3 ± 1.8^{AB}	44.7 ± 1.9^{AB}
AP03-2	898	134.7 ± 7.64	79	32.8 ± 2.3^{BCD}	36.3 ± 2.2^{CDE}
AP51-3	901	135.0 ± 5.35	52	34.5 ± 3.0^{BCD}	38.7 ± 3.1^{BCD}
AP56-2	907	138.0 ± 10.95	80	36.5 ± 1.8^{ABC}	40.6 ± 1.9^{ABC}
AP87-2	911	141.0 ± 16.78	81	38.6 ± 1.8^{ABC}	42.4 ± 1.8^{ABC}
AP61-8	920	162.7 ± 7.22	87	30.9 ± 2.2^{CDE}	36.2 ± 2.5^{CDE}

[1]The mean values for the *P. elliottii* and *P. patula* treatments in the field are based on the mean of the 10 *P. elliottii* trees and the mean lesion of all 920 *P. patula* trees.

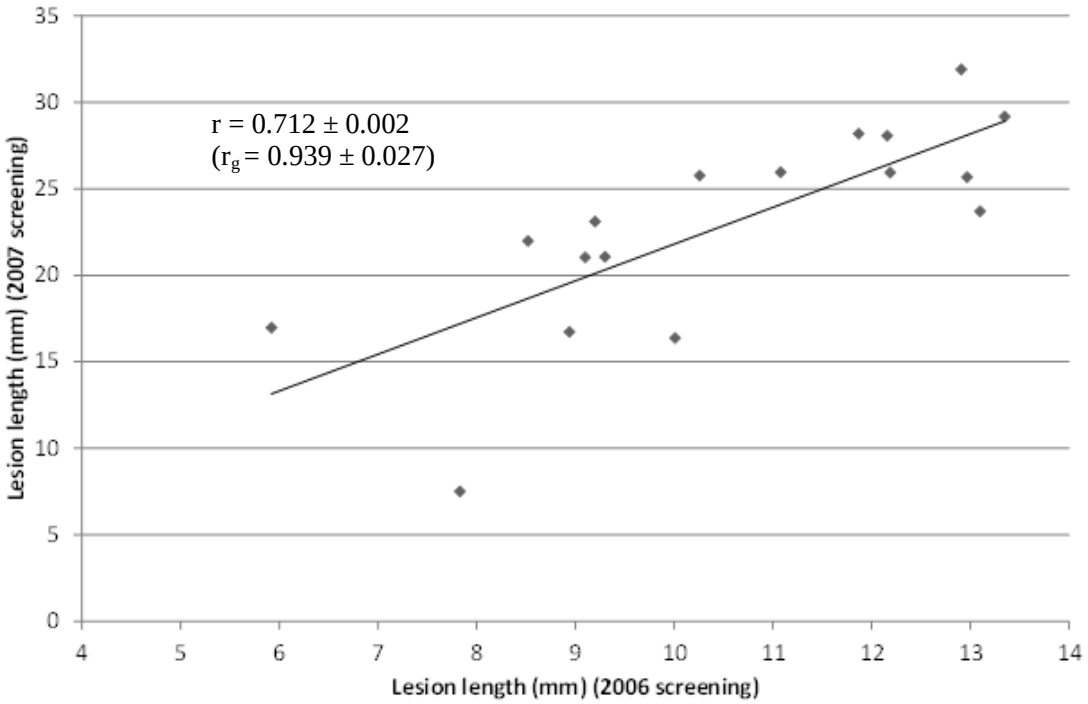

Figure 1. The phenotypic correlation (based on lesion length) of the 17 family's common in the two greenhouse studies. The figure in parenthesis represents the genetic correlation.

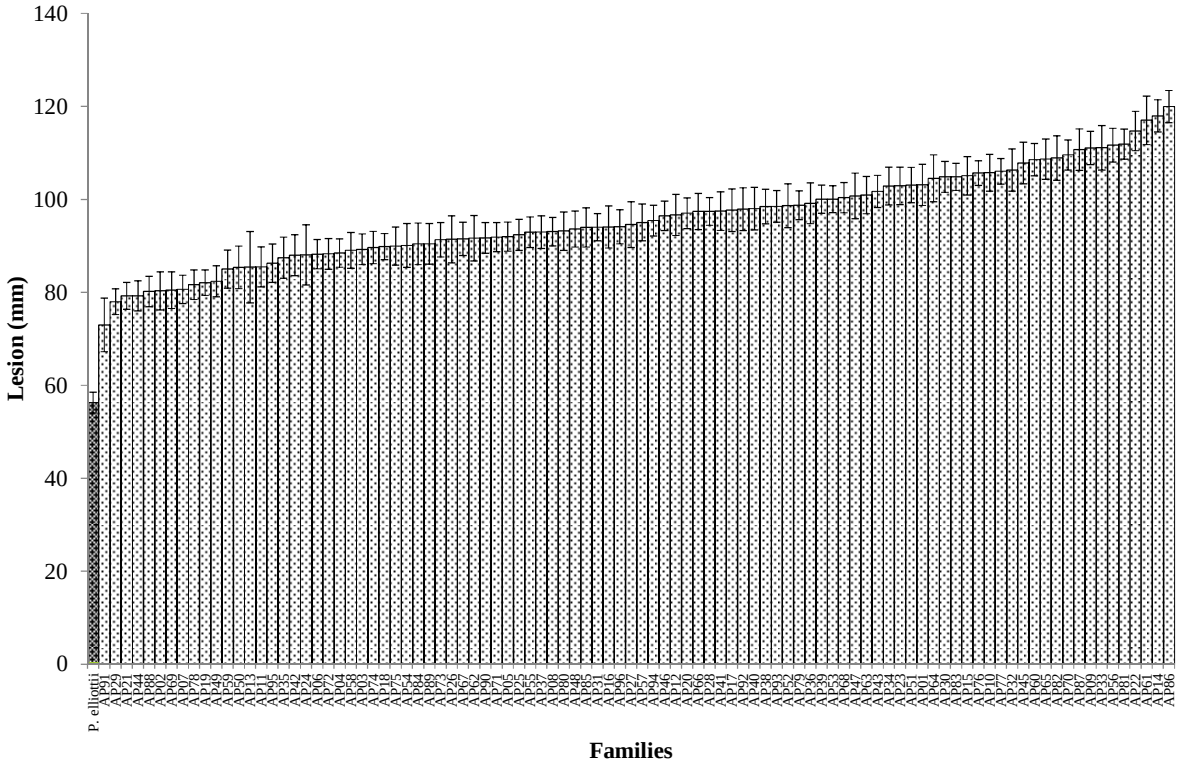

Figure 2. The mean lesion length of the 96 *P. patula* families tested in the field, from most to least tolerant. The *P. elliottii* control is far left.

Figure 3: The individual trees in the field trial ranged from tolerant (top left) to highly susceptible (top centre and top right). Bottom; seedlings from the seed collected from the most susceptible tree (bottom left), and from the 7th most tolerant tree (bottom centre), against *P. elliottii* seedlings (bottom right).

Chapter 7:

Future outlook for *Pinus patula* in South Africa in the presence of the pitch canker fungus (*Fusarium circinatum*)

Abstract

Approximately 50% of the area planted to softwood trees in South Africa has been established with *Pinus patula* making this the most important pine species in the country. More effort has gone into developing this species for improved growth, tree form and wood properties than with any other species. This substantial investment has been threatened in the last 10 years by the pitch canker fungus, *Fusarium circinatum*. The fungus infects and contaminates nursery plants and, once transferred to the field, causes severe mortality of young trees in the first year after establishment. Although nurserymen have some control of the disease, it is recognized that the best long-term solution to mitigate damage due to *F. circinatum* infection is to identify tolerant species, clones and hybrids for deployment in plantations in the future. Research has shown that alternatives such as *P. tecunumanii, P. maximinoi* and *P. elliottii* are suitable for warm sites. Pine hybrids, particularly between *P. patula* and the high elevation sources of *P. tecunumanii,* appear to be a suitable replacement on sub-temperate and temperate sites. Although these alternative species and hybrids are sensitive to sub-freezing temperatures, extending their planting range can be achieved by including frost tolerance as a selection criterion. Future breeding efforts will most certainly focus on improving the tolerance of pure *P. patula* to *F. circinatum,* which can be achieved by identifying specific family crosses and tolerant clones. The commercial deployment of tolerant control-pollinated *P. patula* and hybrid families will most likely be established as rooted cuttings, which requires more advanced propagation technology. In the long term, new seed orchards comprised of *P. patula* clones tolerant to *F. circinatum* will be used to produce seed for seedling production.

Keywords; Camcore, *Pinus patula*, *Pinus patula x Pinus tecunumanii*, *Fusarium circinatum*, Site-species matching

The history of *P. patula* in South Africa

Pinus patula was originally introduced into South Africa in 1907 (Kotze 1926, Burgers 1975, Wormald 1975, Dvorak 1997). Further introductions were made in 1911 and 1928 (Burgers 1975) but it is not known exactly where this seed was collected in Mexico. One report is that the third introduction came from Guajmalpa in the State of Mexico (Burgers 1975, Butterfield 1990). Other possible locations include the states of Hidalgo and Veracruz because the original roads in these areas often followed old Aztec trails that were in close proximity to natural stands of *P. patula* making for easily accessible seed collections (Dvorak, pers. comm.). These early introductions formed the basis of the commercial deployment of the species in South Africa, and the initial *P. patula* breeding programs (Adlard 1981) that started in the late 1950s (Coetzee 1985). The species performed exceptionally well in the summer rainfall region and had superior growth, stem form and wood properties (Poynton 1979). The selections made in the early plantings responded well to tree improvement efforts (Darrow and Coetzee 1983) and by 1970 223,600 ha was planted to *P. patula* (Nyoka 2003).

Several introductions of *P. patula* seed were made at a later stage. In 1969/70 Coetzee and Fisk, of the South African Department of Forestry, made collections again in Hidalgo and northern Oaxaca and also in Puebla from five provenances and 40 trees (Darrow and Coetzee 1983). Many families from these collections outperformed the yield from commercial plantations at the time (Darrow and Coetzee 1983). A comprehensive seed collection was also carried out by Barrett (1972) from Argentina, who sent some seed to South Africa where a single trial was established. Several trials were also established in 1971 in Zimbabwe (then Rhodesia) from seed introduced in 1969 (Barnes and Mullin 1984). South Africa also received provenance material of *P. patula* from the Food and Agriculture Organization (FAO) in the 1980s. Although the majority of the selections in the South African orchards originate from the commercial plantings made in the 1920s (Coetzee 1985), selections from the provenance trials planted in South Africa and Zimbabwe have also been included in some breeding programs.

The largest collection of *P. patula* seed was made by Camcore at North Carolina State University (formally known as Central American and Mexican Coniferous Resources Cooperative, now

known as the "International Tree Breeding & Conservation Program") between the years 1986 and 1994 where 22 populations/provenances and over 500 selected trees across Mexico were sampled (Dvorak et al. 1995, Dvorak 1997). The seeds from these trees were distributed to companies in Brazil, Colombia, South Africa and Zimbabwe where trials were established using the same field design (Dvorak 1997). Similar to the collection by Coetzee and Fisk (Darrow and Coetzee 1983) many of the selections outperformed commercial *P. patula* orchard material for volume which, by this stage, had undergone further improvement (Dvorak et al. 1995). To date, 289 F1 (1st generation) selections, from 18 provenances, have been identified in the South African Camcore trials (Camcore, unpublished) and are available to members. These selections have not been commercially deployed and local breeding programs have only just begun testing their progeny. Considering that many of the selections outperformed advanced generation orchard material, it can be expected that these selections would add much value to local breeding programs.

Current status

Currently 340,000 ha are planted to *P. patula* in South Africa which is approximately 52% of the total area planted to pine (650,000 ha) (Department of Agriculture, Forestry and Fisheries 2010). The tree performs exceptionally well in the afforested regions between Stutterheim in the Eastern Cape and Tzaneen in the Limpopo province where mean annual temperatures are less than or equal to 16.5^{o}C and rainfall is greater than 880 or 780 mm/annum at its warmest and coolest planted limits, respectively (Fig 1 – derived from gridded data supplied by Schulze *et al*, 2007). Although *P. patula* has proven to be an excellent species on these sites, it is particularly susceptible to a number of biotic and abiotic stress factors. Due to its thin bark (Dvorak et al. 2000c) *P. patula* dies easily after fire damage (de Ronde and du Plessis 2002) and it is very susceptible to drought and high temperatures during the first year of establishment (Allan and Higgs 2000). Commercial stands of *P. patula* are also frequently affected by pathogens. In the early years of the commercialization of the species, foresters learnt that it was particularly susceptible to infection by the blue stain fungus, *Diplodia pinea* (Swart et al. 1985), which could result in the loss of both young and mature stands after hail damage.

Today, the susceptibility of *P. patula* to *F. circinatum* is the most significant reason for poor survival after planting and the cause of death of young trees (Crous 2005). One company has measured a constant annual decline in survival of *P. patula* seedlings from approximately 88% in the year 2000 to approximately 64% in 2007 (Morris 2011) and it is estimated that 25% of all seedlings die in the first year in those nurseries where the disease has reached epidemic proportions (Crous 2005). It is clear that seedling mortality in the field results from contaminated or infected nursery plants (Mitchell et al. 2011) and, therefore, it is crucial that the pathogen is controlled in the nursery. It has also been noted that the correct planting of seedlings, which may be carrying *F. circinatum* spores, reduces the risk of infection and seedling mortality (Crous 2005) highlighting the importance of good silvicultural practice.

Opportunities to improve tolerance

Operational experience indicates that the most effective method to manage *F. circinatum* infections is to plant tolerant stock. This is best done by planting alternative pines such as *P. elliottii* and *P. taeda* that are more tolerant to infection (Hodge and Dvorak 2000, Mitchell et al. 2012c). Although the most popular alternative, *P. elliottii*, is known to be susceptible to *F. circinatum* as seedlings (Barnard and Blakeslee 1980), poor ranking families are still significantly more tolerant than the general tolerance of *P. patula* in South Africa (Mitchell et al. 2012c). Due to the good availability of *P. elliottii* and *P. taeda* seed, many forest companies have increased the planting of these two species in areas which were previously planted predominantly to *P. patula*. An analysis of the area planted by York Timbers for the past 6 years clearly shows this trend (Fig.2).

As an alternative to *P. patula* on the subtropical sites, *P. maximinoi* and *P. tecunumanii* have shown outstanding growth (Dvorak et al. 2000a and b, Gapare et al. 2001), excellent wood properties (Malan 2006, 2010) and good tolerance to *F. circinatum* (Hodge and Dvorak 2000). The tolerance of families of *P. maximinoi* and *P. tecunumanii* from low elevation (LE) provenances to *F. circinatum* is so high (Mitchell et al. 2012c) that they need not be screened to

identify tolerant families for deployment. On the other hand, there is large variation between provenances (Hodge and Dvorak 2007) and families (Mitchell et al. 2012c) of the high elevation (HE) source of *P. tecunumanii*. A number of *P. tecunumanii* (HE) provenances (Hodge and Dvorak 2007) and families (Mitchell et al. 2012c), as seedlings, are as susceptible as the general susceptibility of *P. patula* indicating the need to screen families of this source of *P. tecunumanii* to *F. circinatum*. Other sub-tropical species in the Oocarpa group (Price et al. 1998), such as *P. pringlei, P. jaliscana* and *P. oocarpa* are also tolerant to infection by *F. circinatum* in greenhouse trials (Hodge and Dvorak 2000). These have not been field-tested as extensively as *P. tecunumanii* and *P. maximinoi*, but have shown potential for commercial deployment (Darrow and Coetzee 1983). The only species that can tolerate frost and has shown good tolerance to *F. circinatum* in greenhouse trials is *P. pseudostrobus* (Hodge and Dvorak 2000, Mitchell et al. 2012c). Generally, the species does not perform as well as *P. patula*, although some families show similar growth to *P. patula* in first generation studies testing unimproved material (Camcore unpublished). This indicates potential for further improvement and commercial deployment of the species.

Hybrids between *P. patula* and tolerant species such as *P. tecunumanii, P. oocarpa, P. elliottii* and *P. pringlei* (Hodge and Dvorak 2000) are significantly more tolerant to infection by *F. circinatum* than *P. patula* (Mitchell et al. 2012b, Roux et al. 2007). Greenhouse screening studies of these hybrids have shown that there is substantial tolerance in *P. patula x P. tecunumanii* (LE) families. Also, despite significant variation among hybrid families of *P. patula x P. tecunumanii* (HE), this hybrid is more tolerant than *P. patula* (Mitchell et al. 2012b). The most susceptible *P. patula x P. tecunumanii* (HE) families are similar to the mean tolerance of *P. patula*. Trial results also indicate that the variation in susceptibility of *P. patula* x *P. tecunumanii* (HE) families is mostly due to the specific combination of the two parents. An added benefit of the *P. patula* x *P. tecunumanii* hybrid is the improvement in frost tolerance over *P. tecunumanii* (Hodge 2011) due to the frost tolerance of *P. patula* (Dvorak et al. 2000c). This has been recorded for other hybrids (Duncan et al. 1996) and consequently it is predicted that hybrids will be more tolerant of climate change (Warburton and Schulze 2006).

Significant variation in the tolerance to *F. circinatum* exists within *P. patula*. Provenances such as El Cielo, Yextla and Conrado Castillo are three of the most tolerant provenances in greenhouse trials (Hodge and Dvorak 2007). Inclusion of material from these provenances in seed orchards should improve the tolerance of commercial plantings. It is also possible to identify tolerant *P. patula* clones within those currently deployed as both trees and seedlings (Mitchell unpublished). Tolerance, however, is limited to 5% (Mitchell unpublished) which indicates that large numbers of clones need to be tested to identify a sufficient number for the initiation of a new seed orchard comprised of tolerant clones. The tolerance of *P. patula* can also be improved by identifying specific full-sib families, as opposed to identifying open-pollinated families, which produce more tolerant progeny (Mitchell, unpublished). Such crosses can be repeated annually. The combined results of these studies indicate that screening large numbers of *P. patula* families and clones for tolerance to *F. circinatum*, in greenhouse and field trials, can identify those with improved tolerance which can be used to establish new seed orchards. This is the easiest long-term solution to controlling the disease in *P. patula*.

Screening for tolerance to *F. circinatum* will become an increasingly important consideration when making future selections in *P. patula*. Advanced generations of *P. patula* have been developed for improved growth but the deployment of this material is severely restricted due to the presence of *F. circinatum*. It is, therefore, likely that breeders will begin focusing on identifying sub-populations of clones tolerant to *F. circinatum*. Due to the good growth of *P. patula* x *P. tecunumanii* and *P. patula* x *P. oocarpa*, breeders are already extensively testing specific family full-sib crosses between these hybrids. This will likely extend to selecting those that are also more tolerant of frost.

Large scale production of improved material

Until tolerant clones and hybrids are developed, good nursery hygiene is critical to ensure the successful deployment of *P. patula* (Pine *Fusarium* Working Group 2004). This is best addressed by ensuring that *F. circinatum* is controlled at each step in the plant production process. This includes ensuring that the growing medium, trays, sowing shed, wooden nursery beds, soil beneath the nursery beds, and any equipment used in the plant production process are free of the pathogen. It is highly recommended that the grow-out area is sterilized between each cycle before the next crop is placed on the beds. This can effectively be done by applying a strong solution of chlorine to the area and follow up applications of chlorine can be applied to the soil beneath the seedlings during the growing period. It is also important to ensure that all plants adjacent to the newly established seedlings are free of the disease. Only when such rigorous steps are taken, can one expect to see an improvement in the control of *F. circinatum*.

Due to the limited availability of seed, tolerant *P. patula* clones, families, and hybrids, will most likely be deployed as rooted cuttings. Historically, nurseries have focused on producing large numbers of seedlings that are relatively easy to produce. The production of cuttings is more complicated. For example, newly placed shoots need to receive regular misting and have elevated root zone temperatures to improve rooting success (Mitchell 2002). Also, the size of the pot and nutritional status of the parental hedged plant is important in determining the quantity and quality of shoots harvested. Hedges have limited lifespans that differ between species and hybrids. *Pinus patula*, for example, can be kept as seedlings in a hedged state for a maximum of 2.5 years before hedges must be replaced (Mitchell et al. 2004, Mitchell and Jones 2006). The result is that controlled pollinated families, which are tolerant to *F. circinatum*, need to be annually reproduced in order to continually supply the nursery with juvenile hedge material. Less is known about the maturation period for the *P. patula* hybrids and the large scale commercial deployment of these must be accompanied by research on this topic. The technology to improve the rooting success and high throughput of cuttings is changing rapidly and nurserymen will be required to keep abreast of these changes.

Operational deployment

With the addition of alternatives, particularly hybrids between *P. patula* and species tolerant of *F. circinatum*, significant changes to future site-species recommendations will need to be made. These alternatives and hybrids will outperform *P. patula* on many sites and will each occupy a specific niche where *P. patula* has historically been planted. In most cases, species and provenances that are more tolerant to *F. circinatum* (Hodge and Dvorak 2007) are more susceptible to frost (Mitchell et al. 2012). Therefore, if not exposed to frost, especially in the first year after planting, species like *P. tecunumanii* and *P. maximinoi* will survive better than *P. patula* due to their good tolerance to *F. circinatum*. This tendency has been observed in a number of Camcore trials (Table 1).

The *P. patula x P. tecunumanii* (LE) and *P. patula x P. oocarpa* hybrids have become a popular alternative to planting *P. patula* on the warmer sites of South Africa where they also survive better than *P. patula* (Table 2). Undoubtedly, the *P. patula* x *P. tecunumanii* (HE) hybrid is proving to be the most suitable alternative to *P. patula* on a wide range of sites which include those that are temperate (Mitchell unpublished). Considering that the hybrid is not as frost tolerant as *P. patula* (Hodge 2011) it is likely to be best suited to sites with a mean annual temperature of between 15.0 and 17.0 C° (Fig. 3). Not only does the *P. patula* x *P. tecunumanii* hybrid grow well (Nel et al. 2006), and is more tolerant to *F. circinatum* (Roux 2007), but it also has solid wood properties similar to *P. patula* (Malan 2010).

Although the susceptibility of *P. patula* to *F. circinatum* has caused the loss of many millions of Rands in the poor survival of seedlings (Mitchell et al. 2011) this has expedited the testing and development of pine hybrids and alternative species (Dvorak 2012). As has been seen with *Eucalyptus* hybrids, not only are these in many cases more tolerant to diseases (Bayley and Blakeway, 2002), they are also showing improved growth and wood properties (Malan, 1993). It is quite possible, therefore, that the added benefits of pine hybrids and alternative species far outweigh the losses that *F. circinatum* has caused the South African forest industry.

Acknowledgements

We are grateful to Bill Dvorak (Camcore), for his useful comments and editing of this paper. We are also grateful to York Timbers for providing the information on the proportion of *P. patula, P. elliottii* and *P. taeda* planted over the last 6 years.

References

Adlard PG (1981) Mexico: Forest or Desert? *Commonwealth Forestry Review* 60: 127-131.

Allan R, Higgs G (2000) Methods of improving the survival of *Pinus patula* planted into harvest residues. *Southern African Forestry Journal*, vol. 189: 47-51.

Barnard EL, Blakeslee GM (1980) Pitch canker on Slash pine seedlings: A new disease in forest tree nurseries. *Plant Disease*, vol. 64: 695-696.

Barnes RD, Mullin LJ (1984) *Pinus patula* provenance trials in Zimbabwe: seventh year results. In: RD Barnes & GL Gibson (Eds) Provenance and genetic improvement strategies in tropical forest trees. Proc. IUFRO. Mutare, April 9-14, pp. 151-152.

Barrett WHG (1972). Variación de caracteres morfológicos en poblaciones naturales de Pinus patula Schlect. et. Cham. en Mexico. IDIA. *Suplemento Forestal* No. 7: 9-35

Bayley, A. D. and Blakeway, F. 2002. Deployment strategies to maximise value recovery from tree improvement: The experience of two South African companies. *Southern African Forestry Journal*. vol. 195: 11 – 22.

Burgers TF (1975) Mexican origins of *Pinus patula* (Schiede et Deppe). Seeds introduced in South Africa. *Forestry in South Africa* no. 16.

Butterfield, M. K. 1990. Isoenzyme variation in a South African and Mexican population of *Pinus patula* Scheide & Deppe. MS Thesis. Department of Genetics, University of Natal, South Africa. 97 pp.

Coetzee H (1985) Provenance research on Mexican pines. *Southern African Forestry Journal* 135: 68-73.

Crous JW (2005) Post establishment survival of *Pinus patula* in Mpumalanga, one year after planting. *Southern African Forestry Journal* 205: 3 – 8

Darrow K, Coetzee H (1983) Potentially valuable Mexican pines in the summer rainfall region of Southern Africa. *South African Forestry Journal* 124 :23-35

de Ronde C, du Plessis M (2002) Determining the relative resistance of selected *Pinus* species to fire damage. Forest Fire Research & Wildland Fire Safety, Viegas (ed.) 2002 Millpress, Rotterdam, ISBN 90-77017-72-0

Department of Agriculture, Forestry and Fisheries (DAFF) (2010) Report on commercial timber resources and primary roundwood processing in South Africa 2008/9. The Directorate, Forestry Technical and Information Services, Private Bag X93, Pretoria, 0001, South Africa.

Duncan PD, White TL, Hodge GR (1996) First-year freeze hardiness of pure species and hybrid taxa of *Pinus elliottii* (Engelmann) and *Pinus caribaea* (Morelet). *New Forests* 212: 223-241.

Dvorak WS, Donahue JK, Vasquez JA (1995) Early performance of Camcore introductions of *Pinus patula* in Brazil, Colombia and South Africa. *Southern African Forestry Journal* 174:23-33.

Dvorak WS, Gutiérrez EA, Galpare WJ, Hodge GR, Ororio LF, Bester C, Kikuti P (2000b) *Pinus maximinoi*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU, Raleigh, NC, USA. Pp 107 – 127

Dvorak WS, Hodge GR, Gutiérrez EA, Ororio LF, Malan F, Stanger TK (2000a) *Pinus tecunumanii*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU, Raleigh, NC, USA. Pp 107 – 127.

Dvorak WS, Hodge GR, Kietzka JE, Malan F, Osorio LF, stanger TK (2000c) *Pinus patula*. In: Conservation & Testing of Tropical & Subtropical Forest Tree Species by the CAMCORE Cooperative, College of Natural Resources, NCSU, Raleigh, NC, USA. Pp 148 – 173.

Dvorak, W. S. 1997. The improvement and breeding of *Pinus patula*. In: Proc. 24th Southern Forest Tree Improvement Conference. June 9-12, Orlando, FL. pp: 53-68.

Dvorak, W.S. 2012. The Strategic Importance of Applied Tree Conservation Programs to the Forestry Industry in South Africa. In Press: *Southern Forests*, 2012 vol 741.

Galpare WJ, Hodge GR, Dvorak WS (2001) Genetic parameters and provenance variation of *Pinus maximinoi* in Brazil, Colombia, and South Africa. *Forest Genetics* 8: 159-170

Hodge GR (2011) Cold hardiness of *Pinus patula x Pinus tecunumanii* (LE) hybrids and their parents. Camcore Annual Business and Technical Meeting, December 4-10, 2011, Mantra Hotel, Mooloolaba, Queensland, Australia.

Hodge GR, Dvorak WS (2000) Differential responses of Central American and Mexican pine species and *Pinus radiata* to infection by the pitch canker fungus. *New Forests* 19: 241-258

Hodge GR, Dvorak WS (2007) Variation in pitch canker resistance among provenances of *Pinus patula* and *Pinus tecunumanii* from Mexico and Central America. *New Forests* 33:193 – 206

Kotze JJ (1926) *Pinus patula*, Schl & Cham., Its introduction into and growth in South Africa. South African Journal of Science. Vol. XXXIII. Pp:455-466.

Malan FS (1993) The wood properties and qualities of three South African-grown Eucalypt hybrids. *South African Forestry Journal* 167: 35-44.

Malan FS (2006) The wood properties and sawn-board quality of South African-grown *Pinus maximinoi* (HE Moore). *Southern African Forestry Journal* 208: 39 – 48

Malan FS (2010) Corewood in South African pine: necessity and opportunities for improvement. *Southern Forests* 72(2): 99–105.

Mitchell RG (2002) The effect of bottom heat on rooting *Pinus patula* and *Pinus elliottii x Pinus caribaea* stem cuttings in South Africa *Southern African Forestry Journal* 196:21-26.

Mitchell RG, Jones NB (2006) The effects of ontogenetic maturation in *Pinus patula* – Part II: Hedge cycling and field performance *Southern African Forestry Journal* 207: 3-6

Mitchell RG, Zwolinski J, Jones NB (2004) A review on the effects of donor maturation on rooting and field performance of conifer cuttings. *Southern African Forestry Journal* 201: 53-64.

Mitchell RG, Steenkamp ET, Coutinho TA, Wingfield MJ (2011) The pitch canker fungus: implications for South African forestry. *Southern Forests* 73: 1-13.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET and Coutinho TA (2012a) Susceptibility of provenances and families of *Pinus maximinoi* and *Pinus tecunumanii* to frost in South Africa. *New Forests*, DOI: 10.1007/s11056-012-9306-z.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET and Coutinho TA (2012b) Tolerance of *Pinus patula x Pinus tecunumanii*, and other pine hybrids, to *Fusarium circinatum* in greenhouse trials. *New Forests*, DOI 10.1007/s11056-012-9355-3.

Mitchell RG, Wingfield MJ, Hodge GR, Steenkamp ET, Coutinho TA (2012c) Selection of *Pinus* spp. in South Africa for tolerance to infection by the pitch canker fungus. *New Forests* 43:473-489

Morris A (2011) The Pitch Canker Fungus in South Africa and what we are doing about it. Camcore Annual Business and Technical Meeting, December 4-10, 2011, Mantra Hotel, Mooloolaba, Queensland, Australia.

Nel A, Kanzler A, Dvorak W (2006) Development of a commercial breeding program for *Pinus tecunumanii* in South Africa. In: Fikret Isik (ed) Proceedings of the IUFRO Division 2 Joint Conference: Low Input Breeding and Conservation of Forest Genetic Resources: Antalya, Turkey, 9-13 October 2006. pp 158-161

Nyoka, BI (2003) *Biosecurity in forestry:* a *case study on the status of invasive forest trees species in Southern Africa.* Forest Biosecurity Working Paper FBS/1E. Forestry Department. FAO, Rome (*unpublished*).

Pine *Fusarium* working Group (2004) Forestry takes bold steps towards combating *Fusarium circinatum*. Article in *Wood SA/Timber Times*, November 2004. http://www.timbersa.co.za.

Poynton RJ (1979) Tree planting in Southern Africa. Vol 1: The Pines. Department of Forestry, Pretoria, South Africa.

Price RA, Liston A, Strauss SH (1998) Phylogeny and systematics of Pinus. In: Ecology and Biogeography of *Pinus*. (Ed. DM Richardson). Cambridge University Press. UK. pp:49-68.

Roux J, Eisenberg B, Kanzler A, Nel A, Coetzee V, Kietzka E, Wingfield MJ (2007) Testing of selected South African *Pinus* hybrids and families for tolerance to the pitch canker pathogen, *Fusarium circinatum*. *New Forests*, 33: 109 – 123

Schulze, R.E.(Ed.), Maharaj, M., Warburton, M.L., Gers, C.J., Horan, M.J.C., Kunz, R.P., and Clark, D.J. (2007), *South African Atlas of Climatology and Agrohydrology*, Water Research Commission, WRC Report 1489/1/08.

Swart WJ, Knox-Davies PS, Wingfield MJ (1985) *Sphaeropsis sapinea*, with special reference to its occurrence on *Pinus* spp. in South Africa. Southern African Journal of forestry, December, 1985; 1-8.

Warburton M, Schulze R (2006) Climate change and the South African commercial forestry sector: and initial study. Report to Forestry SA, *ACRUcons Report 54*, December 2006. School of Bioresources Engineering and Environmental Hydrology University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Wormald TJ (1975) *Pinus patula*. Tropical Forestry Paper No. 7. Oxford Foresrty Institute, University of Oxford, UK. P 172.

Table 1. Three month survival of *P. maximinoi and P. tecunumanii*, compared with *P. patula* and other pines, in CAMCORE progeny trials established during early 2008.

Details	Location							
Plantation	Spitskop, B13	Tweefontein, A84		Brooklands, G2		Wilgeboom, C2		
Altitude	1470	1260		1160		980		
Mean annual temperature (°C)	16	17		18		19		
Mean annual precipitation (mm)	>1300	>1300		850-1050		1050-1300		
Climatic zone	Temperate	Sub-temperate		Warm-temperate		Sub-tropical		
Trial number	16X08A	15X08A	16X08B	15X08C	16X08C	15X08B	16X08D	Mean survival
Month planted	01/08	01/08	01/08	03/08	03/08	02/08	02/08	
P. maximinoi	100.0	93.3	91.7	95.5	97.2	94.2	100.0	96.0
P. patula	83.3	72.2	69.4	86.1	86.1	91.7	75.0	80.5
P. taeda	100.0	-	94.4	-	100.0	-	94.4	97.2
P. elliottii	91.7	91.7	86.1	100.0	94.4	100.0	97.2	94.4
P. tecunumanii – var. low	94.6	97.2	92.2	94.4	98.8	94.4	94.7	95.2
P. tecunumanii – var. high	96.7	97.2	92.5	97.2	98.6	94.4	93.6	95.7

Table 2. Three month survival results of hybrids between *P. patula* and *P. oocarpa* and *P. tecunumanii* compared with *P. patula* and *P. elliottii* on two sites free of frost.

Trial	*98-10-H01A3*	*98-10-H01A1*
Plantation	Spitskop, B31b	Wilgeboom C2b
Altitude	1300	970
Climate zone	Warm temperate	Sub-tropical
Plant date	Nov-08	Feb-08
P. elliottii	62%	98%
P. patula	55%	64%
P. patula x P. oocarpa	73%	97%
P. patula x P. tecunumanii (HE)	81%	98%
P. patula x P. tecunumanii (LE)	76%	98%

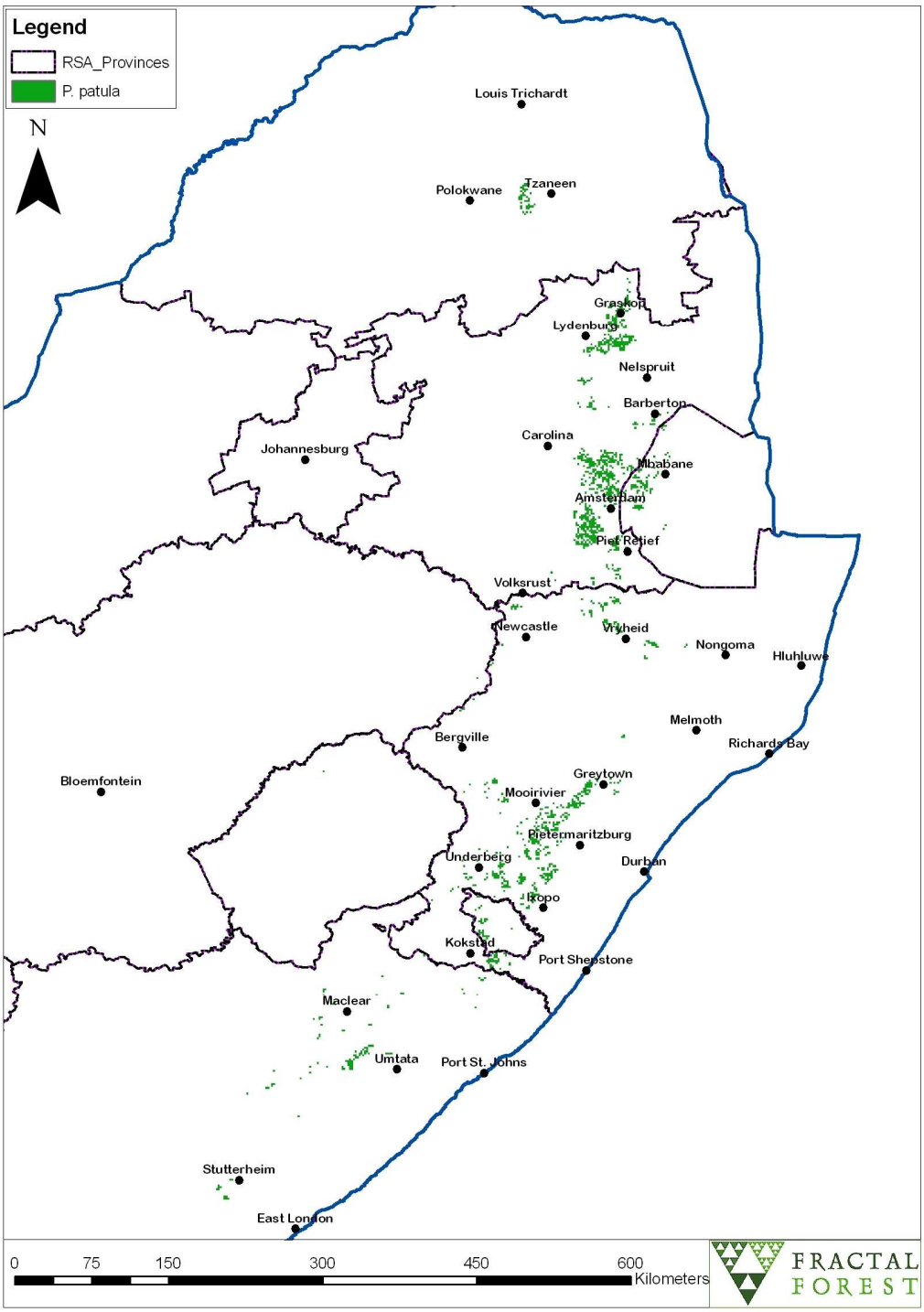

Figure 1. The optimal climatic distribution of *Pinus patula* within the current afforested regions along the eastern escarpment of South Africa.

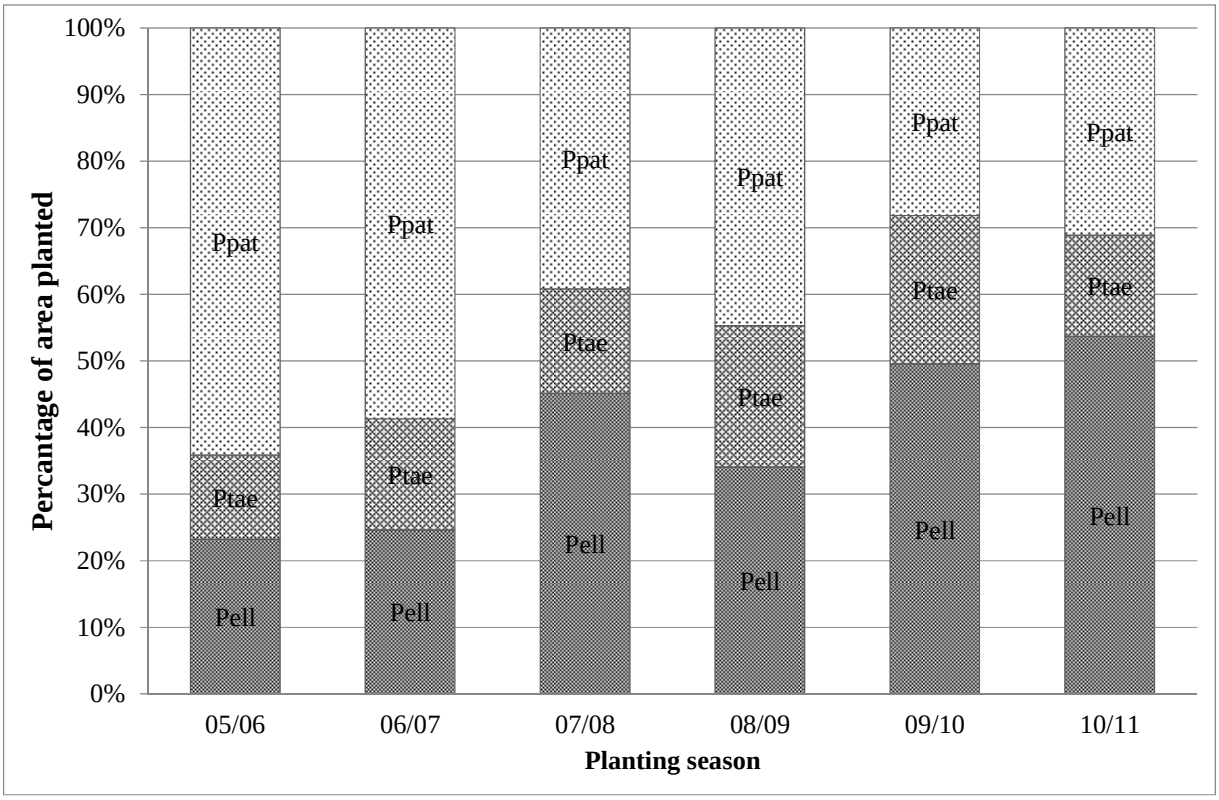

Figure 2. The proportion of *P. elliottii*, *P. taeda* and *P. patula* that was planted between July 2005 and June 2011 by York Timbers.

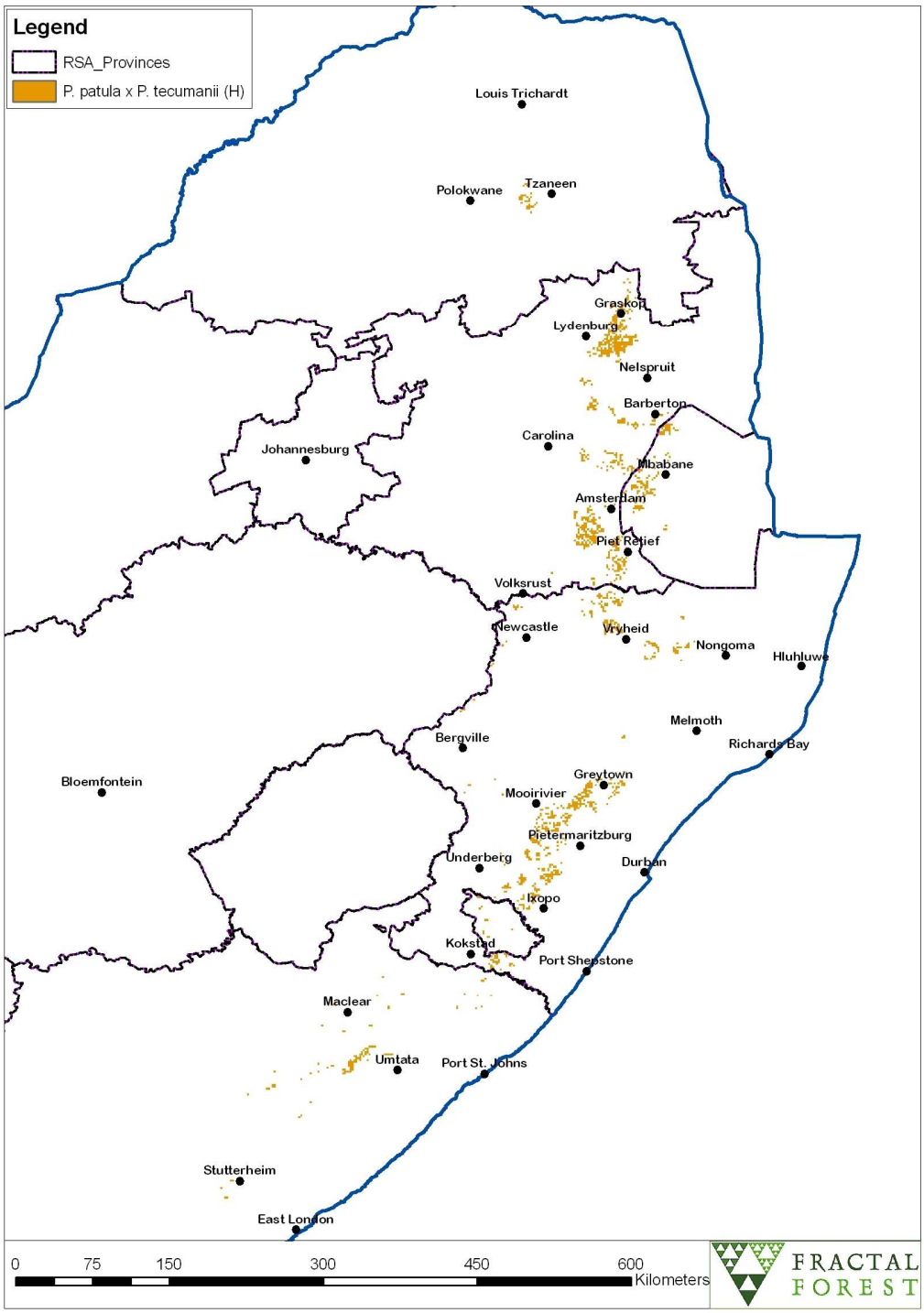

Figure 3. The predicted distribution for those afforested areas which will be climatically well suited to the *P. patula x P. tecunumanii* (HE) hybrid (15 – 17 ^{o}C mean annual temperature based on early trial results). These cover a large portion of land also suitable to *P. patula*.

Summary

The principal objective of this research has been to reduce the potential impact of *Fusarium circinatum* on young and mature *Pinus patula* trees in South Africa. The results provide new knowledge concerning the genetic variation within *P. patula*, and suggest alternative species and hybrids, with resistance to infection by the pitch-canker pathogen *F. circinatum*. The infection of nursery plants is the principal cause of dying seedlings after planting and controlling the disease in the nursery is paramount to achieving good post-planting survival of *P. patula*. A large number of alternative pines, which are more tolerant to the pathogen, exist. *Pinus elliottii* is the most versatile alternative due to the ready availability of seed and the fact that it can be planted on a wide range of sites. Although the species is more tolerant than *P. patula*, individual families vary in their tolerance to *F. circinatum* and care should be taken to eliminate the more susceptible families. *Pinus maximinoi* and the low elevation (LE) source of *P. tecunumanii* are highly tolerant and provide an excellent alternative to *P. patula* on sites free of frost. The high elevation (HE) source of *P. tecunumanii*, which is more tolerant of cold than the low elevation source, is significantly more tolerant to *F. circinatum* than *P. patula*. However, large variation in the tolerance of individual *P. tecunumanii* (HE) families to *F. circinatum* exists and tolerant families of this source need to be identified before commercial deployment. Although *P. maximinoi* and *P. tecunumanii* are sub-tropical pines and sensitive to frost, meaningful variation in the tolerance of individual families to frost has been observed which indicates that these species can be bred for improved frost tolerance. On the colder sites, *P. pseudostrobus* may become an important alternative to *P. patula* due to its excellent tolerance to *F. circinatum*. In all cases hybrids between *P. patula* and pines more tolerant to *F. circinatum*, are significantly more tolerant than *P. patula*. Of these the *P. patula x P. tecunumanii* hybrid is the most promising. Due to the excellent tolerance of *P. tecunumanii* (LE) all families of the *P. patula* x *P. tecunumanii* (LE) hybrid are tolerant of *F. circinatum*. On the other hand, families of the *P. patula* x *P. tecunumanii* (HE) vary greatly in their tolerance to *F. circinatum* and the specific combination of the parents appears to play an important role in determining tolerance. Although only 5% of the current *P. patula* growing stock is of similar tolerance to *F. circinatum* as *P. elliottii* heritability for tolerance to *F. circinatum* is high in *P. patula* indicating that

improvements can be achieved through breeding. Good control can also be achieved by identifying specific full-sib *P. patula* families that are tolerant to *F. circinatum*, rather than the use of open pollinated seed. It is anticipated that large areas, currently well suited to *P. patula*, will in the future be replanted with pine hybrids and species more tolerant of *F. circinatum*. *Pinus patula*, which has been bred for improved tolerance to *F. circinatum*, will be limited to the most temperate regions of South Africa.