

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

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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*) (Nirenberg 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-Broadus 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 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-Broadus 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-Broadus 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 Fraedich 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. Rouging 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 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-Broadbent 1981; DeBarr and Barrows-Broadbent 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 1969; 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 °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 °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 °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 l⁻¹ provides good control (Newman

2004), up to $10 \mu\text{g l}^{-1}$ 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-Broadus 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; 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. elliotii* 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. elliotii* 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. elliotii* 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 *Chrysosporthe 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-Broadus, 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-Broadus 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. elliotii* 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.

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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.

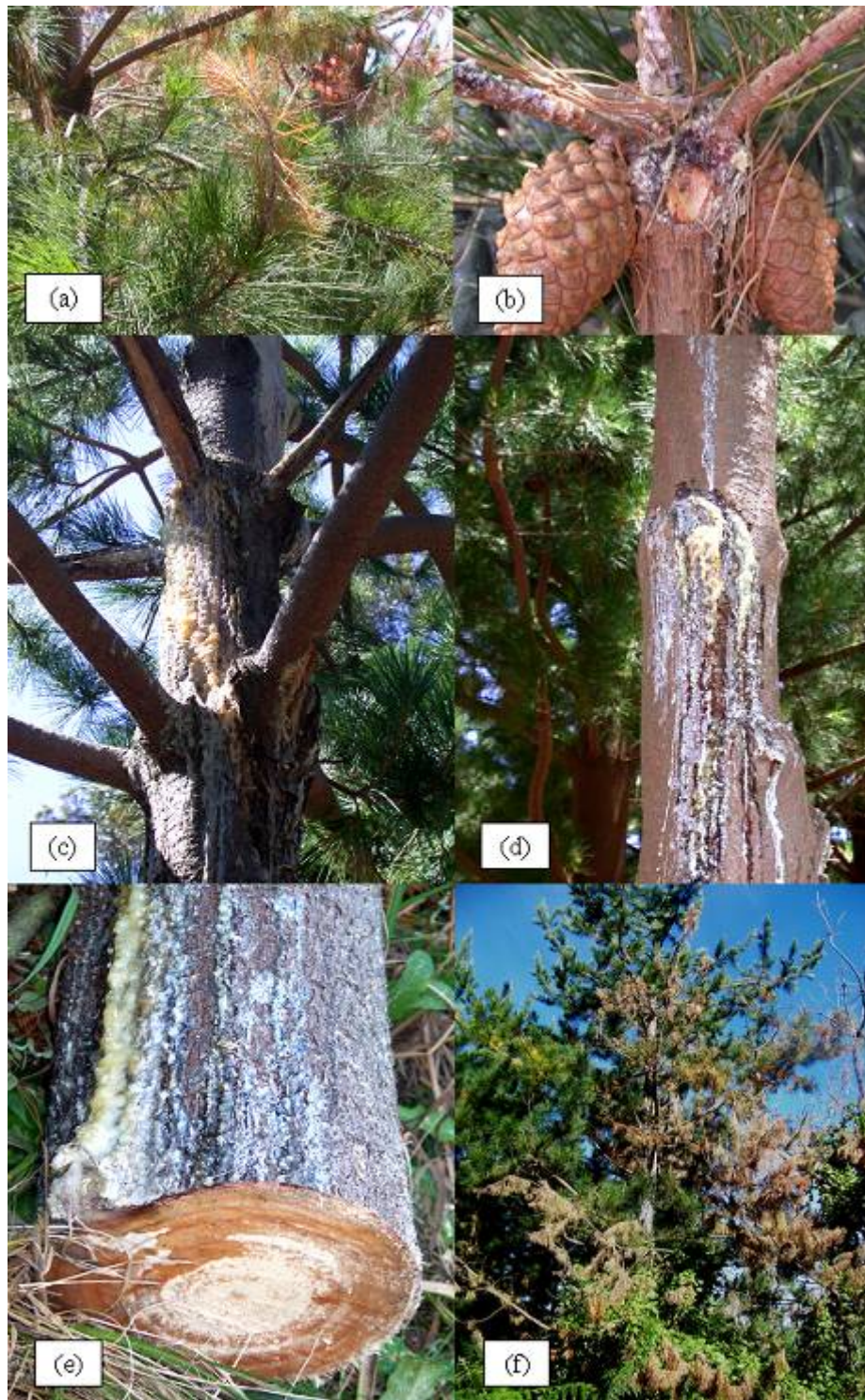


Figure 2 a-f. Pitch canker symptoms on *Pinus radiata* in South Africa; a) branch tip wilting and die-back, b) infected cones and seed, c) infected tree, producing copious amounts resin, also showing branch damage by baboons, d) infection at pruning wound sites, e) resin-soaked internal tissue, and f)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

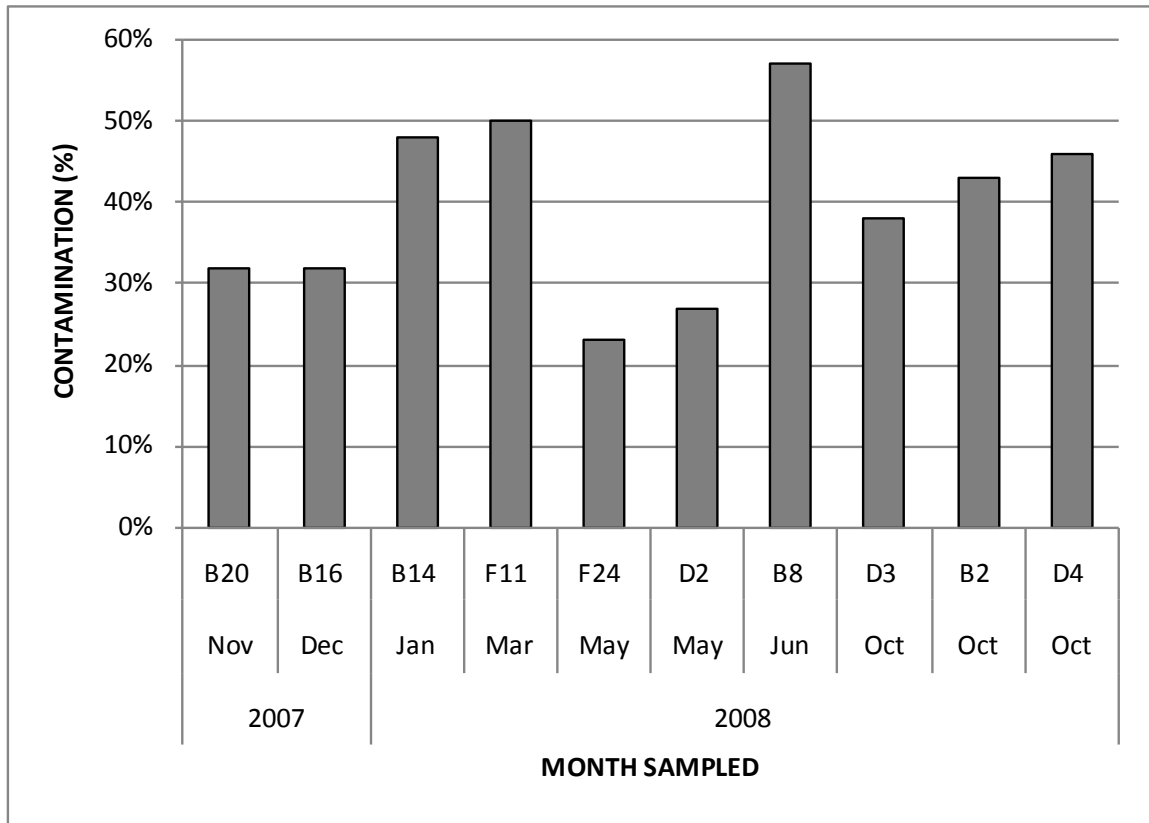


Figure 3. Percentage *Pinus patula* seedlings, from which *Fusarium circinatum* could be isolated, from seedling displaying a healthy appearance at the time of dispatch (Mitchell et al. 2009). (These results were obtained by assessing 30 - 50 asymptomatic seedlings on different occasions, over a 1-year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

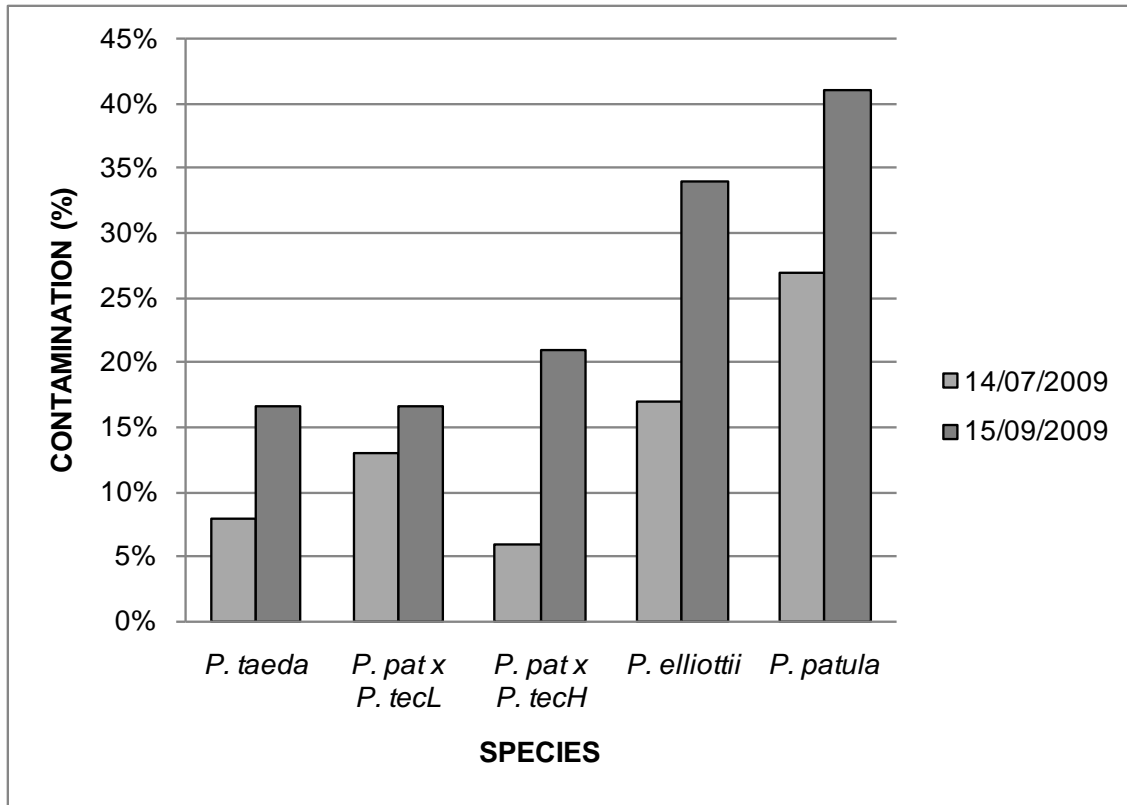


Figure 4. Percentage of asymptomatic plants from which *F. circinatum* was isolated from non-sterilised roots, of various pines, at the time of leaving the nursery (Mitchell et al. 2009). (These results were obtained by assessing 60 asymptomatic nursery plants per species/hybrid in 2 sampling periods.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

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).

<i>Activity</i>	<i>Establishment cost</i>		<i>Cost due to F. circinatum</i> (@25%)		<i>Mean cost of PCF</i>
	<i>1111 stems</i>	<i>1667 stems</i>	<i>1111 stems</i>	<i>1667 stems</i>	
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