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Pitch canker caused by *Fusarium circinatum* – a growing threat to pine plantations and forests worldwide

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Abstract. Pitch canker, caused by the fungus *Fusarium circinatum*, is one of the most important pathogens of *Pinus* species. Sporadic outbreaks and epidemics caused by this fungus have been reported from numerous countries. Symptoms differ depending on the host species, geographical region, climatic conditions and associated insects. Pitch canker represents a significant threat to countries where non-native and susceptible *Pinus* spp. are grown intensively in plantations. A thorough understanding of the ecology and epidemiology of the causal agent is an important prerequisite to managing this threat. The aim of this review is to summarise contemporary knowledge relating to the pitch canker pathogen, with a particular focus on its threat to plantation forestry.

Additional keywords: environmental influences, genetic diversity, host interactions, insect associations, symptoms, taxonomy and identification.

Introduction

Pitch canker, caused by *Fusarium circinatum* (teleomorph = *Gibberella circinata*) (Nirenberg and O'Donnell 1998), is a destructive disease of pines in many parts of the world (Hepting and Roth 1953; McCain *et al*. 1987; Muramoto and Dwinell 1990; Santos and Tovar 1991; Viljoen *et al*. 1994; Dwinell *et al*. 2001; Wingfield *et al*. 2002*b*; Landeras *et al*. 2005; Carlucci *et al*. 2007; Coutinho *et al*. 2007). The disease is frequently associated with reduced yields and high levels of tree mortality in certain areas, resulting in significant economic losses (Dwinell *et al*. 1985). The symptom most typically associated with the pathogen is large resinous cankers on main trunks and lateral branches of susceptible pine species (Barnard and Blakeslee 1980; Barrows-Broaddus and Dwinell 1985*a*), although other plant parts such as roots, shoots, female flowers and mature cones, seed and seedlings can also be affected (Barrows-Broaddus and Dwinell 1985*a*; Barrows-Broaddus 1990; Correll *et al*. 1991; Carey and Kelly 1994; Viljoen *et al*. 1994; Storer *et al*. 1998*a*). Symptom expression and disease severity are strongly correlated with host and prevailing biotic and abiotic conditions. The disease is, therefore, quite dynamic and every outbreak in a particular area usually has a unique case history. Here, we review the fundamental aspects of the biology of the pathogen, its interaction with the host, insects and the environment. Current knowledge on how these factors contribute to pitch canker outbreaks and epidemics, and the management thereof, are considered, with particular reference to the threat the pathogen poses to intensively managed plantations of non-native species.

Global importance of *F. circinatum*

In the south-eastern (SE) United States, the disease was first recorded in 1946 (Hepting and Roth 1946) and is currently known to occur from Florida to as far north as Virginia and westwards to Texas (Dwinell *et al*. 1985; Ridley and Dick 2000). In these regions, pitch canker occasionally causes epidemics (Blakeslee *et al*. 1979; Dwinell *et al*. 1985), which may be associated with abiotic stress (Lopez-Zamora *et al*. 2007). Usually, the disease causes economic losses only in managed stands, such as seed orchards and it is only rarely a problem in native pine stands (Blakeslee *et al*. 1979).

The pitch canker fungus was first recorded in California in 1986 (McCain *et al*. 1987). The most severely affected area was in Santa Cruz County, where *F. circinatum* caused branch dieback in *Pinus radiata*, *P. muricata*, *P. pinea* and *P. halepensis*. Initially, the disease appeared to be limited to landscape plantings, but by 1992, it was found to occur in native populations of *P. radiata* on the Monterey peninsula (Storer *et al*. 1994). Pitch canker now occurs throughout the coastal regions of California, from San Diego in the south, to Mendocino County north of San Francisco (Gordon *et al*. 2001). Unlike the

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disease distribution in the SE United States, pitch canker in California appears to be confined to near-coastal regions, with the exception of one site in the Sierra Nevada (Vogler *et al*. 2004). The severity of the disease in California has been attributed to favourable biotic and abiotic conditions for infection (Storer *et al*. 1999*b*; Gordon *et al*. 2001).

F. circinatum first appeared in South Africa in a single forestry nursery in 1990, causing a root disease on *P. patula* seedlings and cuttings (Viljoen *et al*. 1994). Since this initial outbreak, *F. circinatum* has now spread to most pine-growing forestry nurseries in South Africa, where it currently represents the most important pathogen of *Pinus* spp. in the nurseries (Britz *et al*. 2005). More recently, pitch canker has been discovered in established plantations of *P. radiata* in the Cape Peninsula (Coutinho *et al*. 2007). The slow establishment of pitch canker from the nurseries to plantations in South Africa is probably due to a variety of different factors including climate, low initial levels of airborne inoculum, absence of effective insect vectors and wounding agents, and the lack of associations between native biota and the plantation trees.

In 2002, the pitch canker pathogen was reported from Chile where *P. radiata* nursery seedlings and clonal hedge plants were affected (Wingfield *et al*. 2002*b*). Although symptoms typical of pitch canker have been observed on older trees in plantations in that country, those trees were probably planted from infected nursery stock. There is no evidence that pitch canker has become established as a plantation disease in Chile, but it seems likely this will eventually occur, as it has in South Africa. The risk in Chile is heightened by the fact that the highly susceptible *P. radiata* is widely planted in that country.

Pitch canker also occurs in other parts of the world. Hepting and Roth (1953) noted that the disease was abundant in Haiti on *P. occidentalis*. In the late 1980s, pitch canker was reported to cause trunk cankers and dieback of *P. luchuensis* on the islands of Amamiooshima and Okinawa in Japan (Kobayashi and Muramoto 1989). In Mexico, the disease is prevalent on planted *P. radiata* and *P. halepensis* and in natural stands of *P. douglasiana*, *P. leicophylla*, *P. durangensis* and other pine species (Santos and Tovar 1991; Guerra-Santos 1999). In northern Spain, *F. circinatum* has been reported as the causal agent of a severe root disease of nursery seedlings of exotic *P. radiata* and *P. pinaster* (Landeras *et al*. 2005; Pérez-Sierra *et al*. 2007). Pitch canker symptoms were also reported on *P. radiata* plantation trees. In Italy, pitch canker has recently been reported on *P. halepensis* and *P. pinea* (Carlucci *et al.* 2007). However, the extent of damage due to *F. circinatum* throughout this region and the rest of Europe is unclear, as is its presence in native *Pinus* spp. in this region. Further spread of *F. circinatum* is of great concern to many countries such as Australia and New Zealand, where highly susceptible *P. radiata* is grown extensively in plantations (Dick 1998).

Taxonomy and identification

In 1946, when the pitch canker pathogen was first described, it was referred to as an undescribed species of *Fusarium* belonging to the section *Liseola* (Hepting and Roth 1946). Three years later, it was designated by Snyder *et al*. (1949) as *F. lateritium* f. sp. *pini*. In the 1970s, the pitch canker pathogen was identified as *F. moniliforme* var. *subglutinans* (section *Liseola*), based on the abundant microconidia on sympodially branching conidiophores and the absence of chlamydospores (Dwinell and Phelps 1977; Kuhlman *et al*. 1978). The fungus was then raised to species level in 1983 by Nelson *et al*. (1983) as *F. subglutinans.* Later, it was concluded that there was considerable justification for assigning strains of *F. subglutinans* pathogenic to pines to a *forma specialis*, based on differing restriction fragment length polymorphism (RFLP) patterns of mitochondrial DNA and host specificity (Correll *et al*. 1992). The unique nature of the pitch canker fungus was also supported by isozyme analysis (Huss *et al*. 1996) and random amplified polymorphic DNA profiles (Viljoen *et al*. 1997*a*). The pitch canker fungus was thus named *F. subglutinans* f. sp. *pini.*

Britz *et al*. (1999) introduced the biological species concept for diagnosing isolates of the pitch canker pathogen by demonstrating that it represents a discrete biological species or mating population (designated mating population H) of *Fusarium* section *Liseola*. Initially, the fungus was assigned to mating population B within this section (Kuhlman 1982), but crosses with B tester strains failed in later studies (Correll *et al*. 1992; Viljoen *et al*. 1994). The sexual structures and ascospores produced in the initial experiments were most probably due to the homothallic nature of some of the B mating population strains, as indicated by Britz *et al*. (1999). In their study, they showed conclusively that the pitch canker pathogen is heterothallic (Leslie 1995; Britz *et al*. 1999) and that mating is controlled by a single locus (*MAT*) with two idiomorphs (*MAT1–1* and *MAT1–2*). Later, DNA sequences located at the *MAT* locus were exploited to develop a PCR-based method to rapidly differentiate fungal isolates of opposite mating type (Steenkamp *et al*. 2000). This method, together with the discovery that reduced incubation temperatures enhance female fertility of *F. circinatum* isolates (Covert *et al*. 1999), greatly facilitated application of the biological species concept for differentiating the pitch canker pathogen from other fungi with similar morphology (e.g. *F. gutiforme*, *F. subglutinans* mating population E and *F. sacchari*).

Nirenberg and O'Donnell (1998) described the pine pitch canker fungus as a member of the so-called *Gibberella fujikuroi* complex, which roughly corresponds with the section *Liseola* of *Fusarium* (O'Donnell *et al*. 1998). Based on morphology, they named the asexual stage of the fungus *F. circinatum* and the sexual stage *Gibberella circinata* (Nirenberg and O'Donnell 1998). Based on a limited number of isolates, the authors differentiated *F. circinatum* from *F. subglutinans sensu stricto* pathogenic to maize, by its polyphialides with more than three openings (as opposed to fewer than three conidiogenous openings in *F. subglutinans*), proliferating conidiophores (those of *F. subglutinans* are strongly branched) and hyphal coils (absent in *F. subglutinans*, but present in *F. pseudocircinatum*). A later morphological study including a much larger collection of isolates (Britz *et al*. 2002), phylogenetic analyses based on histone *H3* (Steenkamp *et al*. 1999), and β-tubulin and translation elongation factor 1-α (O'Donnell *et al*. 1998; O'Donnell *et al*. 2000) sequences, supported designation of this pathogen as a distinct taxon in the *G. fujikuroi* complex.

Currently, two molecular methods are available for rapid and reliable identification of *F. circinatum*. The first of these is based

on PCR-RFLP of the histone *H3* gene (Steenkamp *et al*. 1999), which involves digestion of a specific portion of this gene with restriction enzymes *Cfo1* and *Dde1*. Following agarose gel electrophoresis, a unique fingerprint is generated for the pitch canker fungus. The other method is that of Schweigkofler *et al*. (2004) who sequenced the rRNA intergenic spacer region in a representative set of *Fusarium* isolates. From these data, *F. circinatum*-specific PCR primers were designed, which allow amplification of a 300 base pair fragment only in reactions containing DNA obtained from the pitch canker pathogen.

Genetic diversity of *F. circinatum*

F. circinatum reproduces both sexually and asexually. As with other heterothallic pathogens, each of these cycles affects the population structure differently. The asexual cycle results in clonal propagation, whereas the sexual cycle results in recombination leading to new genotypes (Leslie and Klein 1996; Britz *et al*. 1998). However, the outcome of sexual interactions between *F. circinatum* isolates is not only dependent on mating type compatibility as determined by the *MAT* locus, but also by female fertility. Like many other ascomycetes, isolates of the pitch canker fungus have clearly defined male–female roles, where only female fertile strains or so-called 'hermaphrodites' are capable of producing sexual fruiting structures upon fertilisation (Correll *et al*. 1992; Leslie 1995; Leslie and Summerell 2006). Because male-only strains are selected against during sexual recombination, populations undergoing frequent cycles of sexual reproduction usually have high numbers of female-fertile strains, while more male-only strains are expected under conditions where asexual reproduction predominates (Leslie and Klein 1996; Leslie and Summerell 2006). Unfortunately, previous experiments to score this trait in populations of *F. circinatum* were conducted at suboptimal conditions, suggesting that conclusions drawn on the reproductive mode and distribution of this phenomenon in natural populations of the pathogen should be viewed with caution (Britz *et al*. 1998, 2005; Covert *et al*. 1999). Nevertheless, the pathogen can be expected to be more diverse with limited male-only strains in its centre of origin and in regions where it is well established than in areas where it has been recently introduced. The population structure and the evolution of new genotypes by outcrossing also have implications for disease control using chemicals as well as for tree breeding for resistance against the pathogen.

Most studies of genetic diversity in *F. circinatum* examined distribution of mating type and vegetative compatibility groups (VCGs). The loci in the fungal genome that control VCGs are involved in determining the formation of somatic heterokaryons between isolates of the same species (Leslie 1993). VCG assays in sets of isolates representing the different populations of this fungus revealed high VCG diversities in the SE United States and Mexico (Correll *et al*. 1992; Gordon *et al*. 1996; Viljoen *et al*. 1997*b*; Wikler and Gordon 2000). Both mating types are also present in these populations, indicating that they are well established and may be reproducing sexually. However, many VCGs in Florida were shown to be clonally related (Wikler and Gordon 2000) and experimental evidence suggests that new VCGs of *F. circinatum* may arise spontaneously through somatic mutations (Petersen and Gordon 2005). Thus, it is possible that high levels of VCG diversity may be achieved in the absence of sexual reproduction. Both mating types and numerous VCGs of the pathogen have also been reported in South Africa (Viljoen *et al*. 1997*a*, 1997*b*; Britz *et al*. 1998, 2005; Wingfield *et al*. 2002*a*). Although these findings point towards an established outcrossing population, the authors noted that the observed diversity might also be due to a large and diverse founder population of the pathogen.

Populations of the pitch canker pathogen in California and Japan are extremely low in VCG diversity. Since the fungus was first reported in California, the population had been dominated by one VCG and the number of VCGs has only increased slightly (Gordon *et al*. 1996). Although both mating types are present, comparison of VCG diversity in progeny produced from laboratory crosses of Californian isolates to that in field populations indicated that sexual reproduction is rare or absent in the Californian population of *F. circinatum* (Gordon *et al*. 2006*a*). Similarly, the population in Japan has very low VCG diversity, suggesting the fungus was recently introduced into these regions with limited, if any, outcrossing (Wikler and Gordon 2000).

More recently, the genotypic diversity of the pitch canker pathogen has been determined using genomic polymorphic markers. The two sets of markers that are currently available have been developed using different strategies. Wikler and Gordon (2000) used a genomic subtraction procedure that involved enrichment of a genomic library of two isolates for fragments unique to one of the isolates using a series of DNA–DNA hybridisations. Following this approach, they developed eight polymorphic markers, four of which are detectible by PCR and four that can be assayed with Southern hybridisation (Wikler and Gordon 2000). Britz *et al*. (2002) developed the second marker set, which is based on polymorphic genomic sequences that are situated between simple sequence repeats (SSRs). The set of nine polymorphic markers are detectable by PCR.

The polymorphic marker set generated by genomic subtraction was applied to a selection of *F. circinatum* isolates from California, SE United States, South Africa, Mexico and Japan, associated with known but different VCGs (Wikler and Gordon 2000). The results indicated that the genetic diversity of *F. circinatum* was highest in Mexico, suggesting that the pathogen probably originated in this region. Furthermore, despite being assigned to different VCGs, some isolates representing different populations shared multilocus genotypes. Specifically, populations in California, SE United States and Japan were found to share common genotypes (Wikler and Gordon 2000). Representatives of the South African and Mexican populations of the fungus also shared a genotype. However, no genotypes associated with United States–Japanese isolates were shared with the South African–Mexican isolates. Wikler and Gordon (2000) concluded that pitch canker most likely spread from the SE United States to California and the disease may have reached Japan either directly from the SE United States or indirectly through California. They further concluded that the South African population of *F. circinatum* probably originated in Mexico, supporting previous suggestions that the disease was brought

into South Africa on contaminated seed collected in Mexico (Britz *et al*. 2001).

The Britz *et al*. (2002) marker set was used to compare the *F. circinatum* population isolated during the initial outbreak of the disease in South Africa (Viljoen *et al*. 1994) to those of a population collected more recently in 1996–98 (Britz *et al*. 2005). Analyses of the results revealed the presence of unique alleles in the more recent population and an increase in allelic diversity of the pathogen subsequent to the initial outbreak (Britz *et al*. 2005), supporting the notion that the fungus is well established and reproducing sexually in South Africa. However, limited genetic differentiation between the initial and recent populations was also reported, which is consistent with the fact that the two populations of the fungus shared a remarkable number of VCGs. Britz *et al*. (2005) concluded that the observed genetic diversity is unlikely to be the result of later additional introductions of the pathogen from external sources, but rather that the occurrence of disease at multiple South African pine nurseries reflected an expansion of the original infestation.

Symptoms

F. circinatum can infect the vegetative and reproductive parts of susceptible pine hosts of all ages. Shoots, branches, cones, seeds, stems and exposed roots may all become infected. Infections by the pathogen can also occur at any time of the year. However, the specific symptoms expressed may vary from one pine host to another and are dependent on the prevailing biotic and abiotic conditions (Dwinell *et al*. 1985; Gordon 2006).

The first symptoms of pitch canker are usually the wilting and discolouration of needles, which eventually turn red and fall off, resulting in branch dieback (Storer *et al*. 1995; Gordon *et al*. 2001). Dieback usually occurs from the tips of branches to the infection sites (Fig. 1*a*, *b*), due to the obstruction of water flow by girdling cankers that develop at the site of infection (Gordon *et al*. 2001). The tissues associated with these cankers are soaked with resin and copious resin typically also bleeds from infection sites (Fig. 1*c*, *d*). Because the fungus usually does not grow far proximally from the infection site on branches, the pathogen is unlikely to reach the tree bole and damage proximal to a canker is minimal (Barrows-Broaddus and Dwinell 1983; Gordon *et al*. 2001). Infections can occur at any point along a branch (Fig. 1*e*, *f*), although succulent, current-year growth tends to be more susceptible than woody tissue (Barrows-Broaddus and Dwinell 1983; Correll *et al*. 1991; Gordon *et al*. 2001).

Infections of reproductive structures with the pitch canker fungus are associated with mortality of female flowers and mature cones, and seed deterioration (Barrows-Broaddus 1990). Infected cones may be misshapen and smaller than normal and can abort before reaching maturity (Correll *et al*. 1991). *F. circinatum* may even be present in cones from healthy branches where seeds are only contaminated superficially with the pathogen (Storer *et al*. 1998*a*). *F. circinatum* may cause visible deterioration in infected seed and can significantly reduce seedling emergence. However, under certain conditions, infected seed that shows no apparent symptoms can germinate and produce asymptomatic seedlings from which the fungus can be isolated (Storer *et al*. 1998*a*). The ability of the pathogen to infect and survive in or on the reproductive organs of *Pinus* spp. greatly facilitates its spread into new areas.

F. circinatum causes pre- and post-emergence damping-off of seedlings (Fig. 2*a*), as well as mortality of established seedlings (Viljoen *et al*. 1994). If pre-emergence damping-off occurs, seed coats and coleoptiles of germinating seedlings are heavily colonised by the pathogen (Viljoen *et al*. 1994). Storer *et al*. (1998*a*) found that internal seed contamination by *F. circinatum* causes higher rates of pre-emergence damping-off than superficially contaminated seed. In the case of *F. circinatum* associated post-emergence damping-off, root collars of seedlings are girdled (Barnard and Blakeslee 1980). Mortality of established seedlings tends to be lower than that of newly germinated seedlings. The disease typically causes wilting of the seedlings (Fig. 2*b*–*e*) and roots are frequently underdeveloped with discoloured lesions and necrosis of the cortex evident (Viljoen *et al*. 1994). Seeds that germinate or seedlings growing in infested soil can develop pitch canker associated root rot or damping-off (Barnard and Blakeslee 1980; Viljoen *et al*. 1994; Gordon *et al*. 2001). Seedling infections can also remain undetected, since asymptomatic seedlings can harbour the pathogen as an endophyte (Storer *et al*. 1998*a*).

Expression of pitch canker symptoms is almost always associated with significant economic losses. In the nursery setting, the pitch canker pathogen is associated with extensive seedling mortality (Fig. 2*f*), especially for susceptible species such as *P. radiata* and *P. patula*. In planted and natural *Pinus* stands, pitch canker is frequently associated with a reduction in growth volume (Bethune and Hepting 1963; Arvanitis *et al*. 1984). This is largely attributable to loss of foliage due to shoot dieback and water stress. Although individual branch infections are unlikely to kill a tree, multiple infections can cause extensive dieback in the canopy. Stem cankers are usually indicative of an advanced diseased state where tree girdling may result in large-scale tree mortality (Blakeslee and Oak 1979). In addition to girdling stem cankers and crown dieback, mortality can also, in some cases, be attributed to secondary factors such as bark beetles (Storer *et al*. 2002*b*). To date, infection of *Pseudotsuga menziesii* has been characterised by twig dieback only, with swollen resinous tissue apparent at the infection point on the twigs (Storer *et al*. 1994).

Host–pathogen interactions

Trees that are considered susceptible to pitch canker include 57 species of *Pinus* (Hepting and Roth 1953; Muramoto *et al*. 1993; Storer *et al*. 1994, 1997; Clark and Gordon 1999; Guerra-Santos 1999; Hodge and Dvorak 2000; Enebak and Carey 2003; Enebak and Stanosz 2003) and *Pseudotsuga menziesii* (Douglas-fir), the only recorded non-pine host (Gordon *et al*. 2006*b*). Indications are that *P. radiata* could be the most susceptible of all pine species (Hodge and Dvorak 2000; Gordon *et al*. 2001). A variety of other plant species, including trees and herbaceous plants, have been tested for susceptibility to *F. circinatum*, but all have been found to be highly resistant (McCain *et al*. 1987; M. A. Dick and J. A. Simpson, unpubl. data). Barrows-Broaddus and Dwinell (1980) suggested that the pitch canker fungus

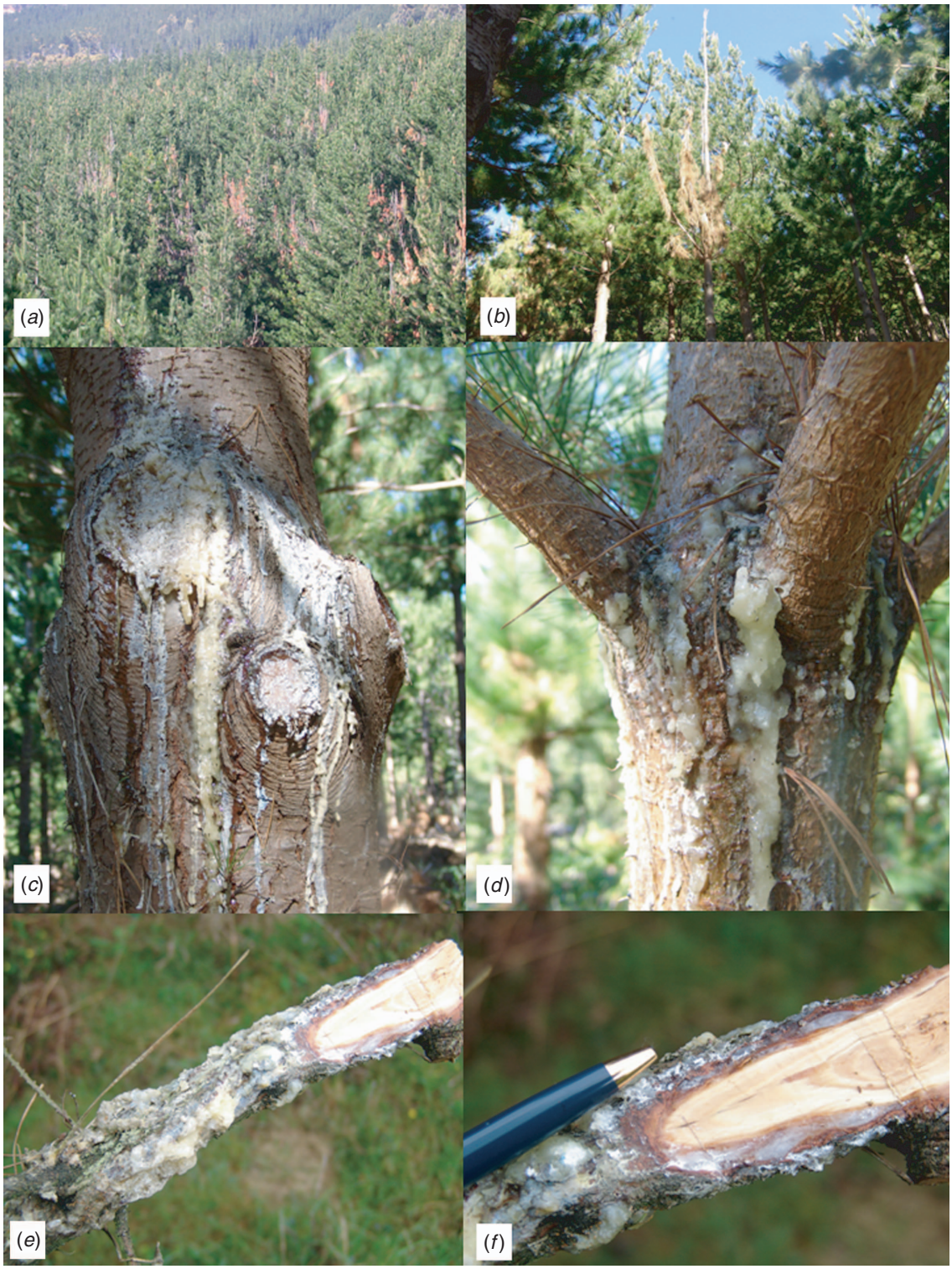

Fig. 1. Pitch canker symptoms on mature pine trees: (*a*) plantation of infected trees showing dieback symptoms; (*b*) shoot tip dieback; (*c*) resinous cankers on the main stem; (*d*) resinous cankers at the first branch whorl; (*e*) infected branch showing both external and internal symptoms; and (*f*) infected branch showing the pitch soaking of the wood of an affected branch.

may also be pathogenic to gladiolus corms, although this was later refuted by Viljoen *et al*. (1995*a*), who showed that the fungus on gladiolus was *F. proliferatum*. Thus, the available evidence suggests that *F. circinatum* is exclusively a pathogen of coniferous trees, and most specifically *Pinus* spp.

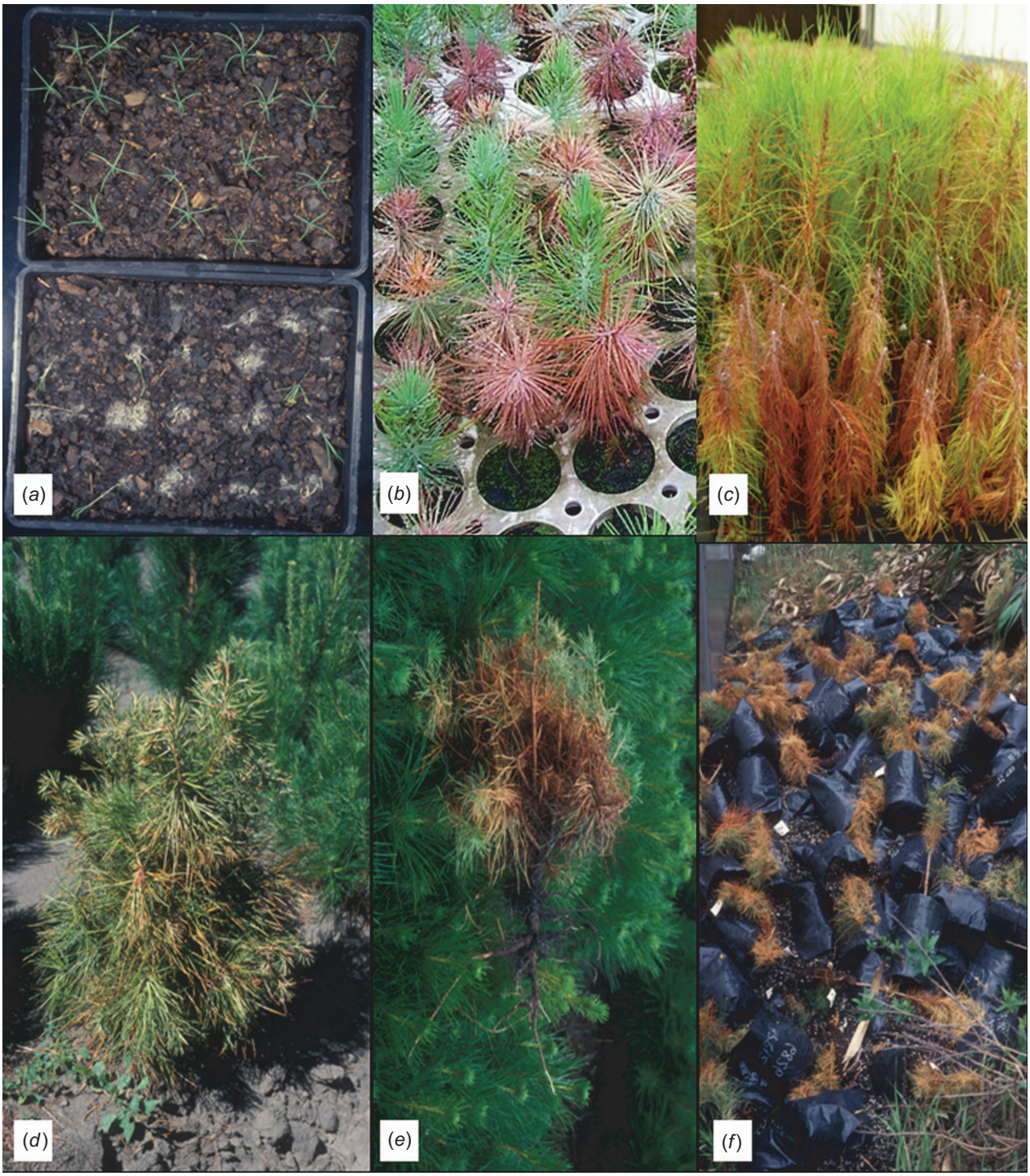

Fig. 2. Symptoms caused by *Fusarium circinatum* in young pine seedlings: (*a*) damping-off of germinated *Pinus patula* seedlings; (*b*) wilted and dead *P. patula* seedlings after natural infection; (*c*) inoculated pine seedlings showing dieback symptoms; (*d*) young *P. radiata* tree showing dieback of branch tips; (*e*) branch showing dieback symptoms; and (*f*) discarded *P. radiata* seedlings infected with *F. circinatum.*

Although the host range of *F. circinatum* extends to most *Pinus* spp., some appear to be resistant and quantitative differences have been documented within species that are susceptible (Rockwood *et al*. 1988; Viljoen *et al*. 1995*b*; Hodge and Dvorak 2007). *P. brutia* and several other pine species, especially those in the *Pinus* subspecies *Pinaster*, appear to have relatively high levels of resistance to the disease (Gordon *et al*. 1998*b*; Hodge and Dvorak 2000; Mead 2000). For some pines, such as *P. lambertiana* and *P. jeffreyi*, pathogenicity has been demonstrated in greenhouse experiments

but has not been observed in the field (Storer *et al*. 1994). However, studies of other pine species have shown that greenhouse susceptibility levels are correlated with the incidence of disease observed in the field. Thus, with sufficient exposure to the pathogen, susceptible species might be expected to display equivalent levels of disease in the field (Gordon *et al*. 1998*a*). Furthermore, species thought to be relatively resistant frequently develop a high incidence of disease under the influence of environmental stress and inoculum pressure (Blakeslee and Rockwood 1999; Lopez-Zamora *et al*. 2007). Thus, the relative resistance of different *Pinus* spp. is controlled by genetic as well as environmental factors.

Genetic resistance to pitch canker has been demonstrated in all pine species tested (Kelley and Williams 1982; Viljoen *et al*. 1995*b*; Hodge and Dvorak 2000) and within-species variation has been reported for pines grown in the SE United States (Dwinell and Barrows-Broaddus 1979; Bronson *et al*. 1992; Blakeslee and Rockwood 1999), California (Storer *et al*. 1999*a*), and Mexico and Central America (Hodge and Dvorak 2007). In the case of *P. radiata*, Matheson *et al*. (2006) confirmed the heritability of resistance to pitch canker in a study that included material from breeding programs in Australia, Chile and New Zealand. Similarly, Blakeslee and Rockwood (1999) found variation in resistance to *F. circinatum* in breeding families of *P. elliottii* and *P. taeda*. Families of *P. elliottii* and *P. taeda* in which resistance persists in adverse environmental conditions have been selected and are now used in operational plantations, advanced breeding programs and in research on mechanisms of resistance in the SE United States (Blakeslee and Rockwood 1999). The specific loci, alleles and gene regulation associated with resistance traits against pitch canker in these pine species are currently being investigated (Morse *et al*. 2004; Kayihan *et al*. 2005).

In 1988, it became apparent that some *P. radiata* in California were unaffected by pitch canker, despite their proximity to heavily infected trees. Direct inoculations of these asymptomatic trees showed that they supported a significantly slower rate of lesion expansion than susceptible trees (Correll *et al*. 1991). Subsequent studies showed that although most individuals of *P. radiata* are susceptible to pitch canker (Storer *et al*. 2002*b*), a small percentage of trees manifest resistance to the disease (Gordon *et al*. 1998*b*; Storer *et al*. 1999*a*). Although resistance can be detected in greenhouse tests of relatively young trees (<2 years old), resistance does not appear to be functional in emerging seedlings (Aegerter and Gordon 2006). In addition to inherent genetic resistance, systemic induced resistance (SIR) has been reported to occur in *P. radiata* in California (Storer *et al*. 1999*a*; Bonello *et al*. 2001*a*). This was the first report of SIR in coniferous trees and the molecular and biochemical pathways stimulated by this form of resistance are currently under investigation. Induced resistance in *P. radiata* appears to be a critical component of disease remission in areas where pitch canker was first observed in California (Gordon 2006). Thus, SIR may help to minimise damage to indigenous *P. radiata* pine forests and ensure the continued vitality of this valuable natural resource.

Genetic resistance of pines to pitch canker is intrinsically linked with pathogen virulence and the durability of resistance may be affected by the ability of *F. circinatum* populations to generate novel pathotypes. Variation in virulence of *F. circinatum* populations has been detected (Barrows-Broaddus and Dwinell 1979; Viljoen *et al*. 1995*b*; Gordon *et al*. 2001) and the capacity to undergo sexual reproduction may enhance prospects for the appearance of pathogen genotypes capable of overcoming disease resistance. In South Africa, Mexico and the SE United States, *F. circinatum* populations are genetically diverse (Wikler and Gordon 2000; Britz *et al*. 2005), whereas in California and Japan there is no evidence that sexual reproduction is occurring. Similarly, *P. radiata* genotypes resistant to Californian strains of *F. circinatum* were susceptible to strains from Florida, Mexico and South Africa (Gordon *et al*. 2001). Breeding for resistance to the pitch canker fungus will prove effective only if a multi-faceted approach to resistance is employed, which can meet the challenge of novel pathotypes that might appear as a result of sexual recombination.

The genetic basis for *F. circinatum* virulence on *Pinus* spp. is not well understood. A genetic linkage map for a hybrid cross between *F. circinatum* and *F. subglutinans* was recently constructed by De Vos *et al*. (2007). Although F_1 progeny for this cross were avirulent on pine, backcross populations with specific F_1 individuals and the parental *F. circinatum* strain showed a wide range of virulence to pine (De Vos *et al*. 2007; Friel *et al*. 2007). This map, therefore, represents an important step towards clarifying the genetic basis for virulence in the pitch canker fungus. Research currently focuses on utilising the backcrossed populations to identify quantitative trait loci associated with pathogenicity of *F. circinatum* to pine.

Biology and ecology

Various aspects of the biology and ecology of *F. circinatum* have been investigated. However, in many cases these have been limited to single tree species or geographical regions and results have generally been extrapolated, despite relatively little knowledge regarding variation between hosts and countries. Biological parameters of *F. circinatum* that have been studied include: inoculum dynamics, dispersal, infection, colonisation, survival and sexual reproduction of the pathogen.

Inoculum of *F. circinatum* is available during all seasons of the year, although in the SE United States and California, the highest frequency of spores has been found to occur during the autumn–winter months (Kratka *et al*. 1979; Schweigkofler *et al*. 2004). Spores have also been found to survive longer during this period than in spring–summer (Blakeslee *et al*. 1978). In Florida, sporodochia containing macroconidia occurred commonly on infected branches in the upper crown of infected trees (Blakeslee *et al*. 1978) and were commonly observed on dead needles attached to infected shoots, where they possibly continue to serve as an inoculum source in needle litter (Barrows-Broaddus and Dwinell 1984). In California, airborne inoculum of *F. circinatum* was detected in abundance throughout the year in an area with a high incidence of pitch canker on *P. radiata*, but not in areas where there was no evidence of the disease (Correll *et al*. 1991). On the central coast of California, where pitch canker is known to be present, a bark wash survey revealed the presence of spores on both symptomatic and asymptomatic trees (Adams 1989). The superficial infestations found on seeds in cones borne on healthy trees are presumably due to deposition of airborne spores (Storer *et al*. 1998*a*). Spores of the pitch canker fungus

have been found in rainwater, in the air (Kuhlman *et al*. 1982), and in spore traps beneath asymptomatic trees (Fraedrich and Dwinell 1997).

Dispersal of *F. circinatum* spores occurs through wind, insect vectors, water splash, soil and movement of infected plant materials (Blakeslee *et al*. 1978, 1979; Dwinell and Barrows-Broaddus 1978; Viljoen *et al*. 1994; Hoover *et al*. 1996; Fraedrich and Dwinell 1997; Storer *et al*. 1998*a*; Wikler and Gordon 2000; Gordon *et al*. 2001). In the SE United States, natural infection of wounds by airborne spores has been found to be predominantly associated with mechanical damage, caused by branch removal and cone harvesters, and weather related injuries such as wounds created from hail or wind damage to the trees (Dwinell and Phelps 1977; Dwinell and Barrows-Broaddus 1981; Kelley and Williams 1982; Dwinell *et al*. 1985). By contrast, the frequency of infection of artificial or natural wounds in California is so low that this form of infection is not considered important. In the field, natural injuries have not been observed to be associated with pitch canker infections and likewise, mechanical wounds, such as pruning, or artificially wounded, non-inoculated branches either did not become infected (Correll *et al*. 1991; Gordon *et al*. 1998*a*), or sustained infections only at a very low rate (Sakamoto and Gordon 2006). Instead, insects are considered to be the most important agents of transmission of the fungus. It has been speculated that if windblown or rain-splashed spores do access natural or mechanical injuries, that the wound may dry out before a successful infection can occur, as the type of wound created and the age of the wound has been found to be significantly correlated with successful colonisation. Specifically, infection frequencies have been found to increase with fresh wounds compared with old wounds, with the presence of a water droplet and by deeper wounds (Barrows-Broaddus and Dwinell 1985*a*; Kuhlman 1987; Sakamoto and Gordon 2006; Inman *et al*. 2008).

F. circinatum spores are capable of surviving in soil and wood debris (Dwinell and Barrows-Broaddus 1978; Gordon *et al*. 2001). Studies have shown that the pathogen can survive for over 6 months in wet soil, up to 1 year in dry soil (T. R. Gordon and B. J. Aegerter, unpubl. data), and more than 3 years in soil under refrigeration (Barrows-Broaddus and Kerr 1981). *F. circinatum* can be readily isolated from the wood of infected trees and from slash piles near infested sites. Studies on the survival of the fungus in wood chips and branches in California found that it was still viable after 1 year and it was recoverable at a low frequency from 3-year-old branches (McNee *et al*. 2002). *F. circinatum* can be isolated from needle litter in pitch canker-infested stands of *P. radiata* (Aegerter and Gordon 2006) but the durability of inoculum in this substrate has not been assessed. Likewise, the length of time that *F. circinatum* spores can survive on insects is currently unknown.

Under certain conditions, the pitch canker fungus appears to be able to enter an apparently endophytic state during which it does not induce any symptoms in the host plant. In some cases, infected seed can germinate and produce asymptomatic seedlings from which the fungus can be isolated (Storer *et al*. 1998*a*). In Chile, it is relatively common for such infected, asymptomatic seedlings to be outplanted and, at some point, the fungus can switch from a latent to an active form of infection. At present, it is unknown whether all seedlings with latent infections would eventually display pitch canker symptoms or what mechanisms governs this change of behaviour.

F. circinatum has been observed to reproduce sexually *in vitro* on agar medium (Britz *et al*. 1999) and on surface sterilised host tissue (Britz *et al*. 1999; Wikler *et al*. 2000). Nonetheless, sexual fruiting structures or perithecia of this pathogen have never been observed in the field. Yet, the results of genetic diversity studies suggest that certain populations of the pathogen are reproducing sexually, while others reproduce predominantly mitotically (Wikler and Gordon 2000; Wikler *et al*. 2000; Britz *et al*. 2005; Gordon *et al*. 2006*a*). Scarcity of hermaphrodite strains in the population would be expected to constrain the frequency of recombination through outcrossing. Another contributing factor may be a skewed distribution of mating type in the population, thus reducing the probability of contact between isolates of opposite mating type. Whereas both factors may be operative in the California population of *F. circinatum* (Gordon *et al*. 1996; Wikler *et al*. 2000), they seem less likely to limit outcrossing in South Africa (Britz *et al*. 2005). Yet notwithstanding intensive sampling, perithecia have never been observed in this area (Britz *et al*. 2005), or anywhere else under natural conditions. One possible explanation is that mating is favoured by low temperatures (Covert *et al*. 1999), whereas disease outbreaks occur during relatively warm periods. Thus, ambient conditions at the time samples were taken may not have been conducive to formation of perithecia (Britz *et al*. 2005). Also, the possibility that *F. circinatum* completes its sexual cycle on some other host or substrate cannot be discounted.

Environmental interactions

Several environmental factors are known to contribute to pitch canker disease establishment and severity. Weather-related injuries in the SE United States are known to provide infection courts for *F. circinatum* (Dwinell and Barrows-Broaddus 1982) and many of the outbreaks of pitch canker in this region have been associated with hurricanes (Kelley and Williams 1982; Dwinell *et al*. 1985). Nutrient levels can affect susceptibility to the pitch canker pathogen. Specifically, high levels of nutrients, both in soil and foliage, have been found to increase disease severity (Fisher *et al*. 1981; Blakeslee *et al*. 1999; Lopez-Zamora *et al*. 2007). The choice of planting site can have a pronounced effect on pitch canker epidemiology. Susceptibility to pitch canker increases during drought stress, waterlogging or shallow soils, especially when trees are planted at high-stand densities (Dwinell *et al*. 1985; Runion and Bruck 1986; Blakeslee and Rockwood 1999).

Air pollution can also play a role in development of disease. High ambient ozone concentrations were found to enhance canker development in susceptible trees, whereas in resistant trees, the interaction of the pathogen and the air pollutant caused stunted growth and decreased root mass (Carey and Kelly 1994). This might, in part, help explain the predominance of pitch canker incidence in California along highways and landscape plantings (Correll *et al*. 1991). In the SE United States, air pollution from chicken houses has been found to increase foliar nutrient levels, thus increasing disease susceptibility (Lopez-Zamora *et al*. 2007).

Climatic conditions are thought to play a major role in disease establishment and severity of pitch canker. Temperature is known to influence growth, spore germination and infection of susceptible hosts by *F. circinatum.* In culture, all tested isolates

of *F. circinatum* grew most rapidly at 25°C and progressively more slowly at 20, 15 and 10°C, whereas spore germination occurred most rapidly at 20°C and was slowest at 10°C (Inman *et al*. 2008). If lower temperatures extend the time required for germination and growth beyond the window of wound susceptibility a reduced infection frequency is expected. This limiting effect of temperature can explain the lower rate of infection resulting from inoculations in winter as compared with those performed during warmer spring months (Inman *et al*. 2008).

In addition to temperature, humidity may also influence the establishment of pitch canker. Infections may not occur even where temperatures are within the optimum range if sufficient moisture is not available. These factors could help account for differences in infections from injury-related wounds between the SE United States compared with California; the high humidity and temperature in the SE United States may be more conducive to infection. In coastal California fog can occur throughout the year, resulting in high humidity and condensation, both of which favour infection. The frequency and duration of fog is diminished at more inland locations and this correlates with a significantly lower incidence and severity of disease (Wikler *et al*. 2003). Furthermore, experiments have documented a significant effect of humidity on frequency of infection of insect-mediated wounds (Sakamoto *et al*. 2007). However, disease intensity also declines at more northerly latitudes, notwithstanding the regular occurrence of fog, and this may be due to progressively lower temperatures during periods when moisture is available. Elsewhere, low humidity in parts of Chile and South Africa where *F. circinatum* is present but pitch canker has not developed, may be part of the reason why the disease has not become established in these regions.

Insect associations

Insects can serve as wounding agents on trees or as vectors of *F. circinatum* both in the SE United States (Blakeslee *et al*. 1978) and in California (Gordon *et al*. 2001). When discussing different insect-pathogen associations, it is necessary to differentiate between insects indigenous to specific areas or specific pine hosts compared with insects that are generalists for many *Pinus* spp. or in an exotic setting. Likewise, it is necessary to distinguish whether insects are involved in wounding of the host or vectoring of *F. circinatum* to the wound site or both. The association of insects with pitch canker varies from region to region and is likely to reflect complex interactions between the host, insect wounding agents and/or vectors, and the environment. It is, therefore, difficult to predict the influence of insects, either native biota or introduced, host-coevolved insect communities, in the establishment and spread of pitch canker in forestry plantations located in exotic regions.

Insects indigenous to the SE United States

In the SE United States, pitch canker infections have predominantly been found associated with injury-related damage. It has been widely purported that insects are not a significant factor in the spread of the disease. However, several studies have shown an important association between insects and the occurrence of pitch canker in various *Pinus* spp. (Hepting and Roth 1953; Blakeslee *et al*. 1978, 1980; Blakeslee and Foltz 1981; Runion and Bruck 1985). Application of the systemic insecticide, carbofuran, resulted in a reduction of both insect shoot damage and pitch canker infections, suggesting a direct relationship between the two factors (Runion *et al*. 1993). Native insects of the SE United States that have been implicated in the spread of pitch canker include *Pissodes nemorensis* (deodar weevil), *Rhyacionia frustrana* (Nantucket pine tip moth) and other *Rhyacionia* spp., although, the frequency with which these insects are involved in wounding and/or vectoring of the pathogen is unclear. Specifically, *F. circinatum* has been successfully isolated from shoots exhibiting damage from *Rhyacionia* spp., as well as from larvae and pupae, suggesting that these insects can vector the pathogen and may also be capable of creating wounds sufficient for infection (Matthews 1962; Runion and Bruck 1985). A positive correlation between seedling terminals damaged by *Rhyacionia* spp. and the incidence of pitch canker infections has also been reported (Runion *et al*. 1993). Likewise, spores have also been isolated from *P. nemorensis* in Florida and feeding wounds were found to be correlated with pitch canker infections in plantations (Blakeslee *et al*. 1978; Blakeslee and Foltz 1981). Greenhouse inoculations using contaminated weevils have resulted in successful infections of pine seedlings whereas trees with feeding wounds from *F. circinatum*-free weevils remained disease free until artificially inoculated (Blakeslee and Foltz 1981).

Several insects of secondary importance have been identified as possible vectors of *F. circinatum* in the SE United States. Wounds caused by *Contarinia* sp. (needle midge) are common on pine in seed orchards and plantations and are often colonised by the pitch canker pathogen (Dwinell *et al*. 1985). Cone and seed feeding insects have also been identified, which may contribute to pitch canker dissemination and infection. These include *Leptoglossus corculus*, *Tetrya bipunktulata* (seedbugs), *Laspreyresia* spp. (seed worms) and *Megastionus atedius* (seed chalcids) (Dwinell *et al*. 1985). No association of the pathogen with *Ips* spp. (bark beetles) has been demonstrated in the SE United States (Blakeslee and Oak 1979).

Insects indigenous to California

The spread of pitch canker in California has been found to be strongly correlated with native insects that feed on or are associated with *P. radiata*. Numerous insects are capable of creating wounds or carrying *F. circinatum* spores (Fox *et al*. 1991; Hoover *et al*. 1996; Storer *et al*. 1997; Gordon *et al*. 2001; McNee *et al*. 2002) However, establishing the exact interaction between the insects and pitch canker infections has been more difficult. *Conophthorus radiatae* (Monterey pine cone beetles), *Pityophthorus* spp. (twig beetles), and *Ernobius punctulatus* (death-watch beetles), are known to be capable of vectoring *F. circinatum* as well as creating wounds, through their feeding activities, that can result in pitch canker infections (Correll *et al*. 1991; Hoover *et al*. 1995, 1996; McNee *et al*. 2002). These insects predominantly feed in the crown of *P. radiata* (Furniss and Carolin 1977; Ohmart 1979) and are believed to initiate the majority of branch canker infections observed. Repeated infestations by these insects can lead to intensification of the disease and can severely weaken the tree. *E. punctulatus* probe trees by excavating entrance tunnels that are

abandoned if resin flow is prolonged and abundant. It has thus been suggested that this 'tasting' phenomenon may enhance transmission of the disease by the beetles to apparently healthy trees (Fox *et al*. 1990; Bonello *et al*. 2001*b*). Several *Ips* spp. are also known to carry *F. circinatum* and are responsible for causing infections on large branches or the main bole, as this is where they establish galleries (Fox *et al*. 1991). *Ips* spp. are attracted to stressed trees thus, they are more likely to be involved in killing already weakened trees and spreading the disease to adjacent trees, rather than initiating infections in uninfected, healthy stands (Furniss and Carolin 1977; Fox *et al*. 1990, 1991; Storer *et al*. 2002*a*). Bark beetles can cohabitate with *F. circinatum* and in this way it appears that a new association has developed between these insects and the pathogen (Fox *et al*. 1990; Storer *et al*. 2002*a*).

A substantial body of work supports a key role for twig beetles (*Pityophthorus* spp.) in the establishment and intensification of pitch canker in *P. radiata*. These insects are well known as colonisers of dead and declining branches. Their association with pitch canker was supported by a high rate of recovery from symptomatic branches, with most of the emerging beetles carrying propagules of the pitch canker pathogen (McNee *et al*. 2002). However, the life history of *Pityophthorus* spp. seemed inconsistent with a role in disease transmission because emerging adults immediately seek weakened host material in which to breed. Consequently, there was not an obvious opportunity for them to visit a healthy branch and thereby establish an infection. This contrasts with a well known vector of Dutch elm disease, *Scolytus multistriatus*, which feeds on healthy trees before establishing a gallery in a declining (and possibly diseased) tree (Webber 1984).

In the case of pitch canker, twig beetles appear to meet the requirements for vectoring in a more subtle manner, reflecting limitations on their ability to locate a substrate in which to breed. Field experiments conducted by Bonello *et al*. (2001*b*) showed that *Pityophthorus* spp. could not identify branches that were suitable for colonisation, before landing. This suggests that *Pityophthorus* spp. will, by chance, occasionally land on healthy branches and thereafter may 'taste' them to assess their acceptability. To determine if this activity might result in wounds that could serve as infection courts, trees to which spores had been applied were exposed to twig beetles (*P. setosus*) that were allowed to move freely over the surface of the trees. Subsequent examination revealed a high frequency of pitch canker infections on trees explored by *P. setosus*, whereas control trees not visited by insects had no infections (Sakamoto *et al*. 2007). Similar results were obtained using *P. carmeli* (Sakamoto *et al*. 2007). In aggregate, these results indicate that two common twig beetle species are capable of transporting the fungus and creating infection courts on healthy branches. That this actually occurs under natural conditions is supported by field studies showing that trees baited with pheromones attractive to *Pityophthorus* spp. sustained significantly higher infection rates than control trees, which were not baited (Storer *et al*. 2004).

Several other insects have been identified that potentially have a role in vectoring or creating infection courts. Spores of *F. circinatum* have been isolated from a variety of insects that are not known to feed on pines such as flies, wasps and beetles (Correll *et al*. 1991). The importance of these insects in the epidemiology of the disease is unknown although they may visit wounds on trees and incidentally transmit the pathogen. Conversely, *Aphrophora canadensis* (spittlebug) has been implicated as a wounding agent for pitch canker infections but infections may be caused by spores deposited from the air that get trapped in the spittle masses, rather than by spores transported to the infection court by *A. canadensis* (Storer *et al*. 1998*b*).

Insects with a wide host range may provide a means for expansion of an infestation to include new hosts. Thus, *Pityophthorus* spp. may have contributed to the high incidence of pitch canker on bishop pine (*P. muricata*), where this species co-occurs with *P. radiata* (Erbilgin *et al*. 2005). However, some *Pityophthorus* spp. are associated with both *P. radiata* and *Pseudotsuga menziesii* (Douglas-fir), but limited susceptibility to pitch canker in the latter species makes the consequences of this connection less obvious (Erbilgin *et al*. 2005; Gordon *et al*. 2006*b*).

Insects associated with Pinus *spp. in South Africa, Chile and Spain*

In South Africa, fungus gnats (*Bradysia difformis*) were suspected to vector *F. circinatum* in pine nurseries. However, Hurley *et al*. (2007*a*) showed that although these insects are present in nurseries, they do not play a significant role in transmitting diseases to pine seedlings. The exact role of the larvae of these insects in facilitating wounding and thus transmission of the pitch canker fungus still has to be ascertained.

In the Tokai plantation, in the Western Cape Province, where the first outbreak of pitch canker was reported on adult trees, trees showing typical symptoms were heavily infested with the weevil, *Pissodes nemorensis* (Coutinho *et al*. 2007). This insect has previously been shown to be associated with *F. circinatum* in the SE United States where it creates wounds that may become infected by airborne spores of the fungus (Blakeslee *et al*. 1978). An association between this fungus and insect had been previously predicted (Gebeyehu and Wingfield 2003), but the nature of this association is poorly understood and requires further investigation.

In Spain, Romón *et al*. (2007) determined the association between several insects and the pitch canker fungus. They isolated *F. circinatum* spores from the root weevil *Brachyderes incanus*, as well as several of the bark beetles species. They also showed that the anti-aggregation pheromone verbenone could potentially be used as part of an integrated pest management strategy for controlling pitch canker as it significantly reduces aggregation of *Pityophthorus pubescens* and *Ips sexdentatus*, both of which frequently harbour *F. circinatum*. However, aggregation of other insects carrying *F. circinatum* in Spain at significant frequencies (e.g. *Brachyderes incanus*, *Hylurgops palliatus*, *Hypothenemus eruditus*, *Hylastes attenuatus* and *Orthotomicus erosus*) were not affected.

Heavy levels of *F. circinatum* are found in most *P. radiata* nurseries in Chile, where the fungus infects both seedlings and clonal hedge plants (Wingfield *et al*. 2002*b*). Infections are also found on the roots and root collars of young trees, in the first year after establishment. Where roots of these trees have been infested by the scolytine bark beetle *Hylastes ater*, *F. circinatum* has occasionally been isolated from damage associated with this

insect (M. J. Wingfield, unpubl. data). The pine shoot moth, *Ryocionia bouliana*, an insect known to be associated with *F. circinatum* in the United States (Matthews 1962; Runion and Bruck 1985) causes serious damage to *P. radiata* in Chilean plantations. While the insect and pathogen have not been found in association in Chile, this could develop in the future and lead to the appearance of pitch canker on adult trees.

Control

Cultural control

At present there is no absolute means of controlling pitch canker in nurseries and forest plantations. An integrated management approach can, however, reduce the economic impact of the disease. Integrated management can include adequate quarantine measures, appropriate nursery and silvicultural management, and genetic selection for clones of species that are less susceptible to the pitch canker pathogen.

Quarantine measures should be in place to exclude the fungus from areas that are currently free of pitch canker and to prevent the establishment of new strains of *F. circinatum* in areas where the pathogen is already present. In New Zealand, stringent regulations have been established to limit importation of plant material including a complete restriction on the importation of conifer seed or live plant tissue from countries known to have pitch canker. These measures can be effective in excluding *F. circinatum* propagules from New Zealand, but great concern exists about the accidental introduction of the fungus via wood products such as packaging material and contaminated insects and plant material harboured in second-hand vehicles, used logging equipment or in camping equipment (Dick 1998; Gadgil *et al*. 2003).

Little research has been conducted on the control of *F. circinatum* in pine seedling nurseries. The most important means to prevent new infections in nurseries is the use of disease-free seed. However, where a pathogen is already established in a nursery, sound nursery practices and the highest levels of hygiene are of great importance in preventing disease outbreaks. The use of pathogen-free irrigation water, sterile growth media and containers as well as roguing diseased plants can reduce the population size of the pathogen within a nursery. The control of vectors and wounding agents is also economically feasible in nurseries (Hurley *et al*. 2007*a*, 2007*b*).

In pine plantations and seed orchards it can be possible, in some instances, to avoid or reduce the impact of disease outbreaks with appropriate silvicultural practices. Environmental stress increases the susceptibility of *Pinus* spp. to the pitch canker pathogen. Planting sites, therefore, should be suited for pine production, planting density should not be inordinately high, trees should be thinned to appropriate stocking levels, and the site should have adequate drainage to avoid waterlogging (Blakeslee *et al*. 1982; Runion and Bruck 1986; Blakeslee *et al*. 1999). California's protracted drought from 1987 to 1992 is believed to have contributed to the Californian pitch canker epidemic, especially since high mortality stands were situated on soils with poor water holding capacity (Owen and Adams 2001). Heavy levels of fertilisation also increase the susceptibility of pines to pitch canker (Fisher *et al*. 1981; Fraedrich and Witcher 1982; Blakeslee *et al*. 1999) and nutrient supplementation should, therefore, be carefully managed. Severe outbreaks of pitch canker have also been associated with high levels of nitrogen emissions from air-conditioned chicken houses (Lopez-Zamora *et al*. 2007). Although first reported in Florida, a similar phenomenon has been observed in many other south-eastern states and it is believed that outbreaks of pitch canker will become more prevalent as nitrogen loads increase (Meeker *et al*. 2005). Because *F. circinatum* is a wound-infecting pathogen, wounding of trees should be avoided during cone harvesting and other forest management practices (Dwinell *et al*. 1985). Branch tip cankers have been pruned out of landscape trees in California, but this practice has not been successful in slowing the progress of the disease or in increasing the lifespan of a tree (Gordon *et al*. 2001).

Long-term management of pitch canker of *Pinus* spp. grown in plantations can only be achieved by planting resistant selections or species in conjunction with appropriate silvicultural management practices. Strong genetic heritability has been observed in several SE United States pines (Rockwood *et al*. 1988; Kayihan *et al*. 2005) and a wide variation in resistance has been observed in native *P. radiata* forests (Gordon *et al*. 2001). Therefore, it is feasible to develop resistant planting stock, which can be used for commercial forestry and for replanting of native forests. Planting of resistant *Pinus* spp. can be considered as an alternative to improving the existing planting stock, which is currently utilised by commercial forestry (Wingfield *et al*. 2002*a*). In this regard, prior screening of *Pinus* spp. and families can be used to reduce the likelihood of disease in plantations (Hodge and Dvorak 2000; Matheson *et al*. 2006; Roux *et al*. 2007).

In addition to inherent genetic resistance, evidence of induced resistance has been reported in stands of *P. radiata* in California (Gordon 2006). Repeated inoculation of trees and seedlings has resulted in reduced lesion expansion both in greenhouse trials and in the field (Storer *et al*. 1999*a*; Bonello *et al*. 2001*a*) and this may provide opportunities to enhance the resistance of planting stock in the future. The long-term applicability of induced resistance to disease management in plantation forestry is, however, unknown and it deserves further study. Key issues to be explored include: (1) the duration of elevated resistance following an induction event, (2) the specificity-generality of induced resistance, and (3) potential decreases in productivity resulting from differential allocations of carbon to defence rather than growth.

Chemical and biological control

A variety of control methods have been investigated for preventing or reducing the effects of pitch canker in pines. Thiabendazole is a systemic and residual fungicide that has been tested in various concentrations *in vitro* and *in vivo* with varying results. Initial studies showed that this fungicide limited growth of *F. circinatum* and that it prevented pitch canker symptom expression (Runion and Bruck 1988*a*, 1988*b*), although, the reduction in disease incidence observed was not considered sufficient to warrant its use (Runion *et al*. 1993). The application of thiabendazole in paint on pruning wounds has been found to prevent artificial infection of *F. circinatum.* However, it has been reported that relatively high levels of resistance to benomyl, a thiabendazole derivative, exist in populations of the *G. fujikuroi* species complex (Yan and Dickman 1993), making the use of thiabendazole and its derivatives

problematic. Likewise, the use of such chemicals is strongly discouraged in many countries and they are unlikely to provide solutions for nursery infestations.

Various biological control strategies have been evaluated for the control of the pitch canker fungus involving antagonistic fungi or bacteria. Dumroese *et al*. (1988) noted the benefits of *Trichoderma* spp. on the seed coats of conifer seed to control seedling diseases caused by *Fusarium* spp. However, later studies have found *Trichoderma* spp. to be ineffective against *F. circinatum* (Mitchell *et al*. 2004). Barrows-Broaddus and Kerr (1981) found that several *Arthrobacter* spp. (common soilborne bacteria) that were recovered during isolations of *F. circinatum* were effective at inhibiting the pitch canker pathogen in culture. The ability of these bacteria to influence disease progress on *P. elliottii* was subsequently investigated. Although several of the *Arthrobacter* isolates were able to reduce the number of *F. circinatum* conidia present at the wound site in comparison to the control, none were effective at reducing canker size or preventing infection by *F. circinatum* (Barrows-Broaddus and Dwinell 1985*b*).

Seed is a potential source of inoculum and a vehicle for dispersal of the pitch canker fungus. Levels of contamination of seed can be tested by embryo dissection, crushing seeds on *Fusarium* selective medium (Nelson *et al*. 1983) or crushing seeds on blotting paper and subsequently examining them for fungal growth (Anderson 1986). On potentially contaminated seed, several seed treatments have been tested for the control of *F. circinatum* seedling disease. Runion and Bruck (1988*a*) found that thiabendazole suspended in 10% dimethyl sulfoxide was an effective seed treatment for the control of the pitch canker fungus. Treating seed before stratification with sodium hypochloride, after stratification with sodium peroxide or ethanol, or after stratification in hot water significantly reduced seedborne *Fusarium* spp. (Dumroese *et al*. 1988). In contrast, Storer *et al*. (1998*a*) found that sodium hypochloride was ineffective in reducing *F. circinatum* contamination of *P. radiata* seed due to high levels of internal seed contamination by the pathogen. Dwinell and Fraedrich (1999) found that internal seed contamination by *F. circinatum* could be reduced by soaking *P. radiata* seed for 15 min in a 30% hydrogen peroxide solution. Hydrogen peroxide can penetrate the seed coat, thereby scarifying the seed, which increases the germination rate. However, due to the endophytic nature of *F. circinatum* in seeds, no absolute means of controlling the fungus has been found (Storer *et al*. 1998*a*).

Summary

Since its initial discovery in 1946, pitch canker has become one of the most important diseases of pines in the world. There is little doubt that the disease seriously threatens plantation forestry worldwide, especially where there is a strong reliance on highly susceptible species such as *P. radiata* and *P. patula*. The appearance of *F. circinatum* in plantations in Spain and South Africa, along with its presence in nurseries in Chile, substantially enhances this threat.

Pitch canker is a varied and complex disease of *Pinus* spp. Our understanding of the susceptibility of pine species and the epidemiology of this pathogen has improved over the past few decades. However, despite a substantial increase in the knowledge of pitch canker and its causal agent, there are many questions that remain to be answered. Although pitch canker is established in many countries on a variety of hosts, predicting how this fungus will behave in a plantation situation is difficult. For instance, the reason why the pathogen has remained restricted to nurseries in South Africa for at least one and a half decades is an enigma. Whether it will remain restricted to nurseries in Chile is likewise unknown and it is a cause for considerable concern. It is possible that the absence of certain insects has influenced this situation, although in both these countries insects similar to those associated with the disease in various parts of the United States are present. Furthermore, the disease can occur on large trees in the absence of insect damage. This would suggest a strong environmental effect also contributes to disease development. It is possible that the drier climate in South Africa and northern Chile, where *F. circinatum* is present, may not be conducive for disease establishment or spread.

The recent appearance of *F. circinatum* and pitch canker in a variety of new areas worldwide is of great concern to both plantation forestry as well as the protection of native stands of *Pinus*. Efforts to exclude this pathogen have thus far not been very effective, despite knowledge on how it is disseminated and the strict quarantine measures that have been applied to prevent its introduction into new areas. In many cases, swift and stringent eradication of the fungus could certainly have prevented both the establishment and spread of this disease in new regions.

While new outbreaks of pitch canker in various parts of the world are of concern, there are also positive signs regarding this disease. Reports of success in lowering or maintaining the level of pitch canker through management of pine plantations in the SE United States and the apparent remission of pitch canker in California are encouraging. However, it is unknown whether these control methods would be effective in intensively managed monocultures of *Pinus* spp. such as those found in many southern hemisphere countries. Problems in controlling this devastating pine disease and the associated economic losses to the forestry sector highlight the importance of promoting an understanding of the ecology and epidemiology of the pathogen. Pitch canker is clearly a pine disease of growing global importance and every effort must be made to manage this threat to pine forest ecosystems and plantations worldwide.

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