REVIEW OF THE GLOBAL SITUATION OF PITCH CANKER

by

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Fusarium circinatum is the casual agent of the pine disease known as pitch canker. Pitch canker infections are characterised by the exudation of copious amounts of resin at the site of infection and can result in mortality of the tree, but most commonly suppress growth. The disease is present in a variety of locations globally and is of serious concern to the New Zealand *Pinus radiata* forestry industry. This report reviews the epidemiology and current situation of pitch canker worldwide.

***Fusarium*:**

Fusarium is a large genus that includes a variety of saprophytic and pathogenic fungal species, several of which have been associated with wilt, damping-off, root rot and canker diseases[1-3]. The fungus responsible for pitch canker in pines was originally identified as a *Fusarium* species and placed in the section *Liseola*[4]. However, the taxonomic designation of this fungus was hampered by problems with identification based on morphological characteristics. In 1949, the fungus was recognised as belonging to the species *F. lateritium* (Nees) and was assigned *F. lateritium* (Nees) emend. Synder and Hansen f. sp. *pini* Hepting, placing it in the section *Lateritium*[5]. Subsequently, the fungus was renamed *F. moniliforme* var. *subglutinans* (section *Liseola*), based on microconidia production in culture; isolates of the fungus were also reported to be cross-fertile with isolates of *Gibberella fujikuroi* var. *subglutinans*[6]. This name was then changed to *F. subglutinans* with the creation of a new species, which included *F. moniliforme* var. *subglutinans* amongst other synonyms[2].

Pathogenicity studies using *F. subglutinans* isolates from a variety of host plants resulted in the designation of those isolates pathogenic only to pines as *F. subglutinans* f. sp. *pini* Correll, Gordon, McCain, Fox, Koehler, Wood and Schultz[7]. The fungus was not listed as a separate species as previous mating studies by Kuhlman *et al.*[6] had found they produced fertile perithecia with *F. subglutinans* and instead, *F. subglutinans* f. sp. *pini* was designated as mating population H in the *G. fujikuroi* complex[8]. However, the pitch canker fungus was eventually described as *F.*

circinatum Nirenberg and O'Donnell (telomorph *G. circinata* Nirenberg and O'Donnell), based on the inability to reproduce the mating experiments[1] and molecular evidence[9-11]. Although *F. circinatum* has been shown to be interfertile with *F. subglutinans* isolated from *Zea mays* (teosinte), the pitch canker pathogen is recognised as a distinct biological species and this event is considered to be a hybridisation rather than evidence of outcrossing between these strains[12]. The final validation of collection and holotype specimen characterisation for this description was provided by Britz *et al.*[13].

Fungi; Ascomycota; Ascomycetes; Sordariomycetidae; Hypocreales; Nectriaceae; Fusarium circinatum.[14]

F. circinatum is characterised microscopically by: sterile coiled hyphae; branched conidiophores bearing polyphailides; abundant, mostly oboviod, non-septate microconidia ranging from 7.0-12.0 x 2.5-3.9 µm; and slender, cylindrical, multi-septate macroconidia ranging from 32.0-48.0 x 3.2-3.8 µm[1,13]. The telomorphic state, *G. circinata* is characterised by ovoidal to obpyriform, non-papillate, dark purple perithecia ranging from 325-453 µm high by 230-358 µm wide. Ascospores produced are ellipsoidal, ranging from 9.4-16.6 x 4.5-6.0 µm, pale brown and are septate, with additional septa developing after discharge[1,13]. In culture, *F. circinatum* on PDA medium produces aerial mycelium that is usually white to greyish-violet and most cultures also produce a grey to dark purple pigmentation[1,6].

Host species:

F. circinatum has been found to be pathogenic to, or reported on, over 60 species of pine, although, the degree of susceptibility is variable (Table 1). *Pinus radiata* and other members of the Attenuata Group, sub-section *Oocarpa* are considered the most susceptible species to pitch canker[15] and members of the sub-section *Pinaster*, especially *P. brutia*, the most resistant[16-18]. For some pines, such as *P. lambertiana* and *P. jeffreyi*, pathogenicity has been demonstrated in greenhouse experiments but not observed in the field[19]. 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 greenhouse susceptible species would be expected to display equivalent levels of disease in the field[20]. There are two varieties of *P. elliottii* in the southeastern USA, *P. elliottii* var. *densa,* distributed from Central to South Florida, and *P. elliottii* var. *elliottii,* host range extends from South Carolina to Central Florida and westward to Louisiana. Although significant differences in resistance between these varieties have been observed[21], for the remainder of this review both of these varieties will be

referred to as *P. elliottii*, as most studies do not distinguished the variety used when investigating the effects of pitch canker.

In addition to pines, *F. circinatum* has also been identified on *Pseudotsuga menziesii*. *Pseudotsuga menziesii* is the only species outside the pine genus that has been shown to be susceptible to pitch canker both in greenhouse experiments and in the field (Table 2). In general, most trees in the field are asymptomatic but those that do have active pitch canker infections do not usually display the characteristic symptoms, such as resinous, which can make detection difficult[22]. Low susceptibility to *F. circinatum* has been observed with pre- and post-emergence of *Cupressus macrocarpa* and *Eucalyptus regnans* seed and seedlings in greenhouse experiments[23] but these trees species are not considered susceptible to *F. circinatum*. The remainder of plant species tested have been found to be highly resistant to *F. circinatum*, these primarily have included tree or herbaceous plant species associated with pines in native forests or plantations[23-25]. Although gladiolus corms were originally shown to be susceptible to a pitch canker strain of *F. moniliforme* var., *subglutinans*[26], later re-examination of the isolates showed they were actually *F. proliferatum*[25], thus, gladiolus is not considered susceptible to *F. circinatum*.

Locations:

Pitch canker was first observed in 1945 on *P. virginiana* in North Carolina, USA[4]. Since its original sighting, the disease is now known to be throughout the southeastern USA occurring from Florida to as far north as Virginia, and westward to Texas[27,28]. Early reports also identified the pathogen in native *P. occidentalis* in Haiti [29,30]. In 1986, the disease was first observed in California in Santa Cruz[24]. Although pitch canker now occurs in the three mainland native *P. radiata* stands, on costal planted *P. radiata* and in many Christmas tree plantations throughout California[15,19], it has not spread northward up to Canada, as was expected. Unlike the disease distribution in the southeast, pitch canker in California appears constrained to near-coastal regions[15], with the exception of one site in the Sierra Nevada[31].

Since pitch canker was first observed in the USA, it has also been found to occur on pine trees in a variety of locations worldwide (Figure 1). Other countries known to have pitch canker include: Chile[32], Japan[33,34], Mexico[35-37], South Africa[11,38], and Spain[39]. It has also been speculated that the pathogen occurs in several other countries, such as Italy, Iraq and South Korea, but these are considered unconfirmed in the absence of supporting or unambiguous reports. The centre of origin for *F. circinatum* is currently unknown. Originally, the pathogen was thought to have

been introduced to the USA from Haiti, where it was considered abundant and endemic[29]. However, more recently Mexico has been considered another potential centre of origin based on the wide distribution but low disease levels; this has also been supported by results from molecular analyses[36,40]. The pathogen has been identified on 19 pine species in 13 states across central Mexico[37].

In Japan, pitch canker was first discovered in 1987 in native *P. luchensis* on Amamiooshima and the Okinawa Islands[33]. The disease causes branch and shoot dieback and resinous stem cankers but is not considered to be problematic[41]. Pitch canker is known to be present in nurseries and plantations of *P. radiata* in Spain, where it is causing severe. Although the fungus was originally reported in the Basque region, it is now reported to be spreading through other regions of Spain[42]. However, as the Spanish government does not acknowledge that pitch canker is present, there are few reports on the disease status in this region. *F. circinatum* has also been found in containerised stock and nurseries in Chile[32] and South Africa[38], and is believed to have been introduced from contaminated seed stock[43,44]. Although the fungus is considered to be well established in both countries, the pathogen has not yet been discovered in the field[32,45]. It is unknown why the disease has not spread to plantations, especially so in South Africa where the disease has been present since the early 1990's and occurs in all pine growing areas[38,43].

Symptoms:

Pitch canker disease in pines is characterised by exudation of large amounts of resin in response to an infection. All tissue of susceptible hosts: needles, shoots, branches, male and female strobili, seeds, stems and roots, can be infect by *F. circinatum,* although resin soaking is not associated with seed infection[46,47]. The first symptom of pitch canker is usually the wilting and discolouration of needles, which eventually turn red and fall off the tree, and subsequently, branch dieback[15,48]. Dieback occurs from the tip of the branch to the lesion, due to obstruction of water flow caused by resinous cankers that form at the site of infection and completely girdle the branch[15]. However, as the fungus does not grow far proximally from the site of infection on branches it is unlikely to reach the bole of the tree and thus, damage proximal from the lesion is minimal[15,49]. All branches can become infected and at any point along on a branch, although succulent, current-year growth tends to be more susceptible than woody tissue[7,15,49]. Individual branch infections are unlikely to kill a tree but multiple infections can cause extensive dieback in the canopy and this may potentially lead to mortality[50]. In addition to branch cankers, cankers can also develop on the main stem and exposed roots[51]. Although cankers that form on the boles of larger trees or on large branches do not generally girdle the tree, they can severely weaken the

tree and are indicative of an advanced diseased state[48,52]. Nonetheless, tree mortality can be caused directly from girdling bole cankers and extensive crown dieback, however, in many cases death can also be attributed to secondary factors such as bark beetles, which are attracted to stressed trees[22,52,53].

The site of a bole or branch canker in pines is usually sunken and is associated with extensive resin flow[4,7]. The resin produced soaks the wood beneath the canker resulting in a honey-coloured wood[28], which is characteristic of pitch canker. The canker does not develop callous tissue or swellings[28]. *F. circinatum* disseminates predominantly via pink-coloured, asexual spores, which are produced at the needle fascicles but are usually not associated with bole cankers[4,54-57]. The sexual state of the fungus has rarely been observed in the field[44,54,58]. In contrast to pines, pitch canker infections in *Pseudotsuga menziesii* do not appear to be associated with resin production[19]. The fungus has not been isolated from resin streaming on the bole, although branch dieback has been observed[22]. However, infection sites tend to be covered with callous tissue[19] rather than resin.

Pitch canker also affects seed and seedlings, although identification and diagnoses can be problematic, as the symptoms (rot root, wilting, damping-off), either separately or together, of pitch canker in young plants are similar to those caused by other fungal diseases[15,38]. *F. circinatum* is a seedborne pathogen and infection can result in the visible deterioration of the seed, however, infected seed frequently display no symptoms until the seed germinates[47,59]. In some cases, infected seed can germinate and produce symptomless seedlings from which the fungus can be isolated; it is unknown whether such seedlings would eventually show pitch canker disease symptoms[59]. The fungus can be present externally in the seed coat or internally within the seed[15,59]. In addition, *F. circinatum* can also survive in the soil, thus, infected seeds that germinate or seedlings growing in infested soil can develop pitch canker associated root rot or damping-off[15,38,46]. Root rot is characterised by necrotic and undeveloped roots, and damping off, by collapsing, withered stems or rotting of the germinating seedling[38]. Both pre- and post-emergence mortality is common[38]. In older seedlings, stem cankers can develop from airborne spores or, at the soil level, from infested soil[15]. Like the cankers that develop in larger trees, these lesions are associated with resin flow[15]. A single basal infection can completely girdle the stem causing severe wilting and can eventually kill the seedling[15].

Inoculum dispersal, survival and spore load:

Spores are produced in sporodochia, usually on the branches of their pine host near the needle fascicle, and are released after rain softens the sporodochia. The spores can be dispersed either by wind or in water splash and maximum dispersal has been found to occur during rain accompanied by turbulent air [60]. There has been no evidence of diurnal patterns of dispersal[60]. *F. circinatum* spores can be recovered from the air throughout the year near infected trees[7,61,62], although the highest frequencies of airborne spores in both the southeast USA and California has been found to occur during the autumn/winter months[61,63]. Spores have also been found to survive longer during autumn/winter than during the spring/summer months[64]. This correlates with observational data on the incidence of pitch canker infections in the southeast USA in *P. taeda*; infections have been observed to be more widespread and artificial inoculations are more successful during the autumn/winter months than those in spring/summer[62]. As the majority of rainfall in the southeast USA occurs between May to October, with almost no rain from January to April, this is not surprising[21]. The first symptoms of pitch canker can occur at any time of the year however, dieback associated with pitch canker was found to be more prevalent during the summer months[62]. Likewise, branch tip dieback in California on *P. radiata* was found to progress fastest during spring and slowest during winter, although the time of inoculation was not significant[7].

In addition to airborne and water splash dispersal, *F. circinatum* spores are capable of surviving in soil, needle litter and wood debris[15,38,65]. In soil, studies have shown that the pathogen can survive for several months in wet soil and at least up to one year in dry soil[66], although it has been reported that isolates of *F. circinatum* have still been viable after three years in soil under refrigeration[67]. The survival of *F. circinatum* in needle litter is unknown, however, based on the survival of spores in soils, it is expected that the pathogen will be viable for at least one year. *F. circinatum* spores have been isolated from the wood of infected trees that have been removed and also from slash piles near infected sites. In addition, they have been found to be extremely tolerant of drying[66]. Studies of the survival of *F. circinatum* in wood chips and branches in California found that the pathogen was still viable after year and was even successfully recovered from three-year old branches[68]. It is unknown whether the isolation of this pathogen in this study was from viable mycelium or the presence of spores. Composting has been found to be effective at eliminating the pathogen from wood debris, as is exposure to high temperatures, 50^{o}C or greater, for at least ten days[66]. The length of time that *F. circinatum* spores can survive on insect is currently unknown.

The effect of inoculum load of *F. circinatum* spores on the development of disease has been investigated in both the southeast USA and California. In both regions, varying results on the influence of spore load has been reported. In greenhouse experiments on *P. taeda*, a significant difference in dieback was reported with differing spore loads; the significant difference varied along the gradient of spore loads tested, $10^2 - 10^7$ conidia/ml[69]. Likewise, in a study on pitch canker lesion lengths in native stands of *P. radiata*, Storer *et al*. found a significant difference between spore loads (25 spores produced significantly smaller lesion size than 125 spores or greater) and betweens trees, and a significant interaction between these two factors[70]. In contrast, both Gordon *et al*[20] (25 to 2,500 spores) and Hodge and Dvorak[18] (50, 000 and 100, 000 spores/ml) found no significant difference in greenhouse experiments between lesion lengths and spore loads for a variety of different pine species tested. Similarly, no significant difference in lesion size was detected for greenhouse and field inoculated *P. radiata* using spore loads of 50 and 250 spores[17]. It would seem unlikely that spore dose would influence pitch canker infections as the number of spores carried by vectors, such as insects, is likely to be low; preliminary studies on twig beetles in California have estimated less than 10 spores per beetle[17]. Although the effects of spore load on the incidence of infection is unclear, it is possible that other factors, such as temperature and humidity or wound site conditions may be more influential on the success of spores causing infections rather than the actual number of spores present.

Infection vectors and wounding agents:

F. circinatum can be disseminated vertically, through infected seed, or horizontally by spores that can be vectored by a variety of different agents, such as wind, rain, animals, insects or soil, to the host tree. With vertical transmission, as mentioned above, the pathogen can be carried externally, either on or within the seed coat, or internally, within the seed, and can result in deterioration of the seed itself or mortality of the germinated seedling[15,47,59]. For horizontal transmission of *F. circinatum*, although spores can be disseminated by a range of factors, successful infection occurs when vectoring of the spores is coupled with wounds or openings on the tree; intact tissue is not vulnerable to invasion by the fungus[20,69]. In general, pitch canker has been associated with wounds created by insects, weather or mechanical damage, however, the importance of specific vectors and wounding agents has varied between locations that have pitch canker. The foremost difference has been between the southeastern USA, where the disease is mainly thought to occur from weather and mechanical damage rather than through insects, and California, where pitch canker infections are almost solely associated with insects.

In the southeastern USA, pitch canker infections have predominantly been found to be 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[28,56,71,72]. Spores are believed to be vectored to the wounds by wind or rain splash[28,60,62]. Outbreaks of pitch canker have been correlated with severe damage caused from hurricanes and the subsequent decline in disease over the following years has been attributed to the lack of fresh wounds[56]. Greenhouse studies on *P. elliottii* and *P. taeda* have shown that fresh wounds are significantly more susceptible than older wounds[69]. Likewise infections have been found to occur in seed orchards where the mechanical equipment used is attached to the main stem and at wounds created from removal of cones or branches from the trees[28,71]. Infections on wounds caused by cattle hooves or the bending of branches by ravens have also been observed[21]. Although it has been widely purported that insects are not a significant factor in spreading the disease, several reports have shown that there is an important association between insects and disease occurrence. For example, pitch canker infections have been found to be coupled with insect wounds in several pine species[30,54,64,73,74] and the application of carbofuran, a systemic insecticide, resulted in a reduction of both insect shoot damage and pitch canker infections, suggesting a direct relationship between the two factors[75]. The insects that have been implicated in the spread of pitch canker in the southeast USA include *Pissodes nemorensis* (deodar weevil), *Rhyacionia frustrana* (Nantucket pine tip moth) and other *Rhyacionia* spp., although, the frequency that 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 [73,76]. A positive correlation between seedling terminals damaged by *Rhyacionia* spp. and the incidence of pitch canker infections has also been reported[75]. Likewise, spores have also been isolated from *Pissodes nemorensis* in Florida and feeding wounds were found to be correlated with pitch canker infections in plantations[64,74]. Greenhouse inoculations using contaminated weevils have resulted in successful infections of pine seedlings where as trees with feeding wounds from *F. circinatum*-free weevils remained disease free until artificially inoculated[74]. No association of the pathogen with *Ips* spp. (bark beetles), has been demonstrated[50].

In contrast to the southeastern USA, 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[15,22,68,77,78], however, establishing the exact interaction between the insects and pitch canker infections has been 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 feedings activities, that can result in pitch canker infections[7,68,77,79]. These insects predominantly feed in the crown of *P. radiata*[80,81] and are believed to initiate the majority of branch canker infections observed. Repeated infections by these insects leads to intensification of the disease and can severely weaken the tree. Similarly, several *Ips* spp. (bark beetles), are also known to carry the pathogen and are responsible for causing infections on large branches or the main bole, as this is where they establish galleries[78]. *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[78,80,82,83]. In addition, several other insects have been identified that potentially have a role in vectoring or creating wounds. 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[7]; the importance of these insects in the epidemiology of the disease is unknown. Conversely, *Aphrophora canadensis* (spittlebug) has been implicated as a wounding agent for pitch canker infections but vectoring of the spores to the wounds is thought to occur from airborne spores that get trapped in the spittle masses, rather than directly from *A. canadensis* [15]. The transmission of pitch canker to *Pseudotsuga menziesii* has not been established, however, it is possible that insect species that have a large host range, such as *Pityophthorus* spp., whose host range includes other pine species that coexist with *Pseudotsuga menziesii*, may be responsible for initiating infections[84].

In addition to insect-mediated infections, pitch canker infections in California can also occur in wounds created mechanically or from natural physical damage, as occurs in the southeastern USA. However, the frequency 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 branches also did not become infected, unless artificially inoculated[7,20]. Spore trapping studies have shown a high density of *F. circinatum* spores where the disease is present, suggesting that the lack of infection is not due to inoculum[7,63]. It has been speculated that if windblown or rain-splashed spores do access natural or mechanical injuries, that the wound dries out before a successful infection can occur[22]. Although *P. radiata* occurs in coastal areas of California that are subject to fog, high humidity and heavy rainfalls are not as common as in the southeast USA. Another example of how climate may influence the occurrence of successful pitch canker infections is the limited spread of the disease up the west coast. Initially, pitch canker was expected to spread inland

from California up the coast to Canada but currently, the distribution is still limited to the Californian coast[15], with the exception of one successful inland establishment in the Sierra Nevada[31]. In the laboratory *F. circinatum* was unable to grow at temperatures below 10^{o}C[85], thus it is possible that the colder temperatures and higher elevation may not support growth of the pathogen outside this region. The upper temperature range for growth of *F. circinatum* has not been determined.

Although the moisture levels in California are probably not sufficient for infection of natural or weather related wounds, temperature and humidity are likely to play an important role in disease progression. For instance, in California pitch canker has progressed significantly faster in the areas adjacent to the coast that are frequently covered in fog than in coastal, inland populations of *P. radiata*[51]. The coastal region exposed to this fog belt does not extend far inland, approximately 1.5 kilometres, and severe infection levels have been recorded in coastal, inland populations[51]. However, it is possible that the additional moisture that this band of fog provides is adequate for more efficient infection by *F. circinatum*. Current studies underway on the effects of moisture and humidity have found a wide variation in the efficiency of infections under different temperature and humidity conditions, although the presence of a water droplet during the inoculation significantly increased the frequency of infection[66]. In contrast to weather conditions, the type of wound was found to significantly effect infection by the pathogen, specifically, infection frequency increased with the use of a 1.6 mm drill bit than a dissecting needle[66]. This difference has been attributed to the depth of the wound and it has been speculated that the drill can access the plant's own moisture from the deeper penetration. Likewise, in *P. taeda,* pinholes and slit wounds were more likely to be infected than wounds created from branch or needle removal[69] and in both *P. taeda* and *P. elliottii*, the most successful infections occurred with wounding that imitated both the depth and location of those produced by feeding insects[21]. It has been proposed that the difference in inoculation efficiency observed is due to low resin production (insects that feed tend to use areas of the tree that do not produce large quantities of resin), so the wound cannot be sealed off and the creation of a suitable wound site that provided enough moisture for infection and will not dry out[21]. However, for more shallow wounds, it is likely that infection is more dependent on temperature and high humidity conditions.

For countries such as Chile and South Africa, where pitch canker occurs only in the nurseries[32,45], the lack of infection in the field has been attributed to the low frequency of insect wounding agents and/or climate conditions[43,45]. As *F. circinatum* spores can be vectored by a

variety of insects species and transmitted in the air or by rain-splash, it is unlikely that vectors of this disease are not present, and similarly, wounds created from natural physical damage are known to occur[45]. Instead, the lack of infections in the plantations may be due to a lack of pine-associated insects. The pine species planted in both Chile and South Africa are exotic, in contrast to California and the southeast USA, where the majority of pines are native and occur with their respective coevolved insect communities. This theory has also been supported by correlative data from California where the exotic species *P. canariensis, P. pinea,* and *P. halepensis* have been observed to have considerably less infections in the field than the native pine species, *P. radiata*[20]. However, *Pissodes nemorensis* and *Rhyacionia* spp.*,* which have been associated with pitch canker in the USA, are present in South Africa and Chile, respectively and, along with some other pine-specific insects, are known to feed on the pines[43,86,87]. Thus, it is possible that the low levels of pine-specific insects in conjunction with inadequate weather conditions for pathogen growth may be the limiting factors for pitch canker infections to occur. For example, if colder climates are not able to support *F. circinatum* outside of California, despite the presence of natural insect populations, then it is feasible that the climate conditions in Chile and South Africa may also not be amenable. Likewise, if the rainfall and humidity in California is sufficiently different from that in the southeast USA, then conditions in Chile and South Africa may also not permit pitch canker infections from occurring in natural injuries. The agents involved in wounding and vectoring the pathogen in Mexico, Spain and Japan, where the disease is present in the field, have not yet been established[58] .

Cultural Practices:

Cultural practices such as fertilisation, irrigation and stand density have been found to influence the incidence and severity of pitch infections in plantations. For instance, in the southeast USA fertilised trees have been found to have significantly longer pitch canker lesion lengths and a higher rate of disease incidence than unfertilised trees[88-90]. In *P. elliottii* the effect of fertiliser has been attributed to a combination of high nitrogen (N) and phosphorus (P), as a low level of both or either nutrient (Figure 2) had no lower effects on disease severity[21,90]. Other nutrients were not found to have a significant effect[90]. However, Blakeslee *et al.* found that fertilisation of *P. taeda* with fertiliser consisting of potassium (K) plus other nutrients also resulted in intensification of pitch canker infections in plantations[89]. Similar results with fertilisers have been demonstrated in greenhouse experiments on *P. elliottii, P. taeda* and *P. virginiana.* For all tree species, seedlings exposed to a higher N content in fertiliser showed more symptoms of pitch canker and greater lesion size than seedlings with lower levels of N or control plants [88,91]. Other nutrients, including P and K, were found to have an additive effect but alone were not

influential in increasing disease severity[88]. It has been proposed that the enhanced disease severity observed from the higher N content is a result of a direct increased availability of N to the pathogen rather than indirectly via host metabolism. This is based on greenhouse experiments where disease severity was greater in seedlings with excised shoot tops (removes host's N sink, therefore greater N content in stem) and high N content, in comparison to excised seedlings with low N or intact seedlings[91]. However, it is possible that the stress of excision could also have a significant effect on disease severity rather than the increased level of N in the stems alone. The majority of studies on the effects of fertilisers have been completed on *P. elliottii* and *P. taeda*. For both tree species, canker length and the incidence of infection differed among families and also between years for field studies[89,91]. In California, pitch canker initially spread through *P. radiata* planted in urban regions and on golf courses, both of which are often subjected to high levels of fertilisers and nutrients[51]. It is possible that the high level of mortality, to some extent, could be attributed to fertilisation.

In the southeastern USA, fertilisation of a stand can result from the application of commercial fertilisers or from the proximity to a chicken house. In 1977, a correlation between pitch canker related mortality and the presence of chicken farms was reported; stands within 75 metres from chicken houses were found to have extremely high incidences of pitch canker, which was attributed to increased levels of nitrogen from the chicken houses[92]. In the 1970's chicken houses were ventilated by the lifting of large flaps on the sides of the houses, nowadays, air-conditioning is required in all chicken houses, which has resulted in the addition of large fans[93]. A recent study has found that these fans are responsible can deposit of substantial quantities of nitrogen on the areas adjacent to the fans and when bordered by pine plantations, this can result in severe devastation of the stand[93,94]. Specifically, the levels of nitrogen in the foliage and litter were significantly increased[94]. Although first reported in Florida, similar cases have also been reported in Mississippi and Louisiana and with the large number of plantations bordering chicken houses in many southeastern USA states, it is believed that the disease will become more prevalent[93,95].

In addition to fertilisation, pitch canker has been observed to occur in overstocked or densely packed stands in both *P. elliottii* and *P. taeda* plantations in the southeastern USA[71,89,96,97], and the incidence of disease was also found to be positively correlated with tree spacing[96]. In view of this, the effect of stand density, both alone and in conjunction with fertilisation, has been investigated. Blakeslee *et al.* found that in *P. taeda* thinning could significantly reduce the incidence and severity of pitch canker infections[89]. However, thinning in combination with

fertilisation resulted in a high level of disease, equivalent to that observed with fertilisation alone[89], supporting the correlation between fertilisation and pitch canker. Although thinning can result in wounding of adjacent trees, no thinning-wound related pitch canker infections were observed in this study[89]. In *P. elliottii*, thinning was not only found to reduce the incidence of pitch canker infections but also enhanced the growth and recovery of previously infected trees[97,98]. It is also possible that stand density has been influential in *P. radiata* stands in California, where the suppression of fire has resulted in the overstocking of native stands. The lower levels of pitch canker infections and the apparent recovery of infected trees in the past few years has been attributed to induced resistance responses but could also be a function of reduced stress levels from the reduction in stand density, caused from pitch canker related mortality.

The low frequency of infections in thinned or less densely stocked stands could be attributed to a reduction in the local spore inoculum levels through the removal of infected material. However, as there is a high level of airborne inoculum year round in most stands, the effect of this is likely to be low. Instead, the lower levels of disease severity and incidence is more likely to be due to an alleviation of moisture stress, a factor know to influence pitch canker infections [99]. In *P. elliottii* seedlings, moisture stress induced by reduced irrigation resulted in an increase in pitch canker induced shoot dieback in comparison to seedlings that were not subjected to water stress. An association between droughts and pitch canker incidence has also been observed in *P. elliottii* in Florida[100] and it has been speculated that the four year drought, 1991-1994, in California may have been partially responsible for the rapid spread of the disease[53,101].

Disease development and impact:

Observations of the development of pitch canker within individual trees can help predict the likelihood of infections occurring and can also facilitate the understanding of disease progression. In California, it has been observed that the level of disease intensity increases in a stand only after many trees have become infected[52]. A significant interaction between tree crown height and disease severity was reported; trees that had larger crown heights were less likely to have disease than those with small crown heights[51] and the majority of infections have been found to occur in the upper third of the crown[52]. However, no significant interaction between disease severity and tree trunk size (DBH) was determined[51]. Trees that displayed branch tip symptoms were more likely to have stem cankers, especially so if the number of branch tips infected was greater than ten and regardless the level of disease severity within the stand[52]. Due to the high level of insect involvement in California, it is likely that these observations are correlated with the selection of trees/branches and the vectoring of the pathogen by insects. In

the southeastern USA, pitch canker in *P. elliottii* has been observed to occur on the of the leader or bole of young trees and the predominant area of the crown infect was initially the lower crown, although as the disease progressed this shifted to the higher crown classes[98]. Like observed in California, no interaction between disease levels and tree diameter was detected[57,98]. The variations between the two regions is likely to be due to the different insect populations involved in the disease in each location.

Losses from pitch canker in stands or plantations can include reduced lumber quality, reduced growth and tree mortality. In the southeast USA, mortality of up to 24% from pitch canker in infected *P. elliottii* stands has been reported, although it would be expected that the average loss per year would be lower than this[28]. In a study on the effects of pitch canker on volume growth in *P. elliottii*, Arvanitis *et al.*[102] found that mortality and growth suppression was predicted to account for a 4.5% decrease in the anticipated total volume growth for one year and Blakeslee and Oak[50] estimated a 21% reduction in harvest yield. Growth suppression alone has been reported to account for 60-80% of the wood volume lost per year and in seed orchards a reduction of 28% in cone yield has been reported[28,102]. These figures do not necessarily include losses from reduced lumber quality. In California, the frequency of mortality is unclear as many trees are removed before they have died if landowners consider they pose a safety risk or are ascetically unpleasing[15,51]. Figures for reduction in tree growth are unknown as *P. radiata* is generally found in native stands or as planted ornamentals. However, in view of the high level of susceptibility of *P. radiata* to *F. circinatum*, it would be expected that the rate of mortality and suppression of growth would be at least that observed in the southeast USA. Mortality or deformation of *P. radiata* trees in Christmas tree plantations in California has also been problematic. In 1992, it was reported that 5% of *P. radiata* Christmas trees died due to pitch canker, although the number rendered unusable is unknown[48]. In nurseries, losses can be extremely high from either pre- or post-emergence mortality from contaminated seed or soil, or the mortality of older seedlings from airborne inoculum[28,38]. In general, estimates of yield and economic losses for Christmas tree plantations and nurseries are unclear.

Chemical and biological controls:

A variety of control methods have been investigated for preventing and/or reducing the effects of pitch canker in pines planted in urban settings, plantations or native stands. Once the disease is established, the most common form of control in the southeast USA and California has been to prune initial branch infections when first detected and to remove heavily infected or dying trees[15,19,52,103]. Pruning of infected branches is unlikely to completely control the disease, as

mortality or severe disease levels are usually a result of multiple infections causing extensive crown dieback rather than one canker that girdles the trees, but can help delay the development of pitch canker[19,104]. However, it is possible that pruning could create wounds suitable for infection by *F. circinatum*, unless the wounds are treated to prevent infections from occurring. For instance, application of thiabendazole (a systemic and residual fungicide) in paint on pruning wounds has been found prevent infection by artificial inoculation of the pathogen[103]. The removal of heavily infected trees can potentially help reduce inoculum levels, insect populations or alleviate moisture stress for adjacent trees, but is frequently done for aesthetic or safety purposes[15,103]. A variety of chemical controls have been investigated but most have had limited effectiveness. In *P. taeda*, treatment of seedlings, which had been prior inoculated with the pitch canker pathogen, with thiabendazole resulted in a reduction of disease symptoms and re-isolation of *F. circinatum*[105]. Likewise, multiple applications of thiabendazole to mature *P. taeda* trees also resulted in a reduced number of pitch canker infections[75]. Thiabendazole also was effective at inhibiting growth of *F. circinatum* in culture[105]. In contrast, fungisol injections in mature *P. radiata* were not effective at reducing disease severity in trees that were already infected or at preventing new infections from occurring[104]. It has also been proposed that insecticides could reduce the levels of pitch canker by minimising the spread of the disease to healthy plants through insects. In *P. taeda* the insecticide carbofuran was effective at reducing the incidence of insects damage and also reduced the number of pitch canker infections[75]. The use of biological controls has also been evaluated. Barrows-Broaddus and Kerr 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[67]. 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 prevent infection by *F. circinatum*[106].

In addition to control of pitch canker in seedlings and mature trees, control for contamination of seeds, both externally and internally, has also been investigated. Seed contamination is one of the most problematic areas of the disease cycle as it results in high levels of pre- and post-emergence mortality and, as well, contributes to the dissemination of the pathogen. Several methods have been found to be effective at reducing external contamination but internal infections can be more difficult to treat without killing the seed. Soaking *P. palustris* seed in thiabendazole-dimethyl sulfoxide was effective at increasing germination and also lowered the recovery of *F. circinatum* from seedlings and seed that did not germinate in comparison to the

control[107]. Likewise, treatment of *P. radiata* seed with sodium hypochlorite or hydrogen peroxide was found to reduce the incidence of *F. circinatum*[59,108]. Although fungicides, insecticides and other biological controls could be effective in preventing or minimising the impact of *F. circinatum* in nursery settings or from seeds, the application of such chemicals at sufficient quantities on mature trees and in plantations would probably not be feasible.

Host resistance and pathogen virulence:

Genetic resistance to pitch canker has been demonstrated in all pine species tested[18,45,56,109], however, the mechanism(s) behind this resistance are currently unknown. The level of resistance has been found to vary between pine species and it has been speculated that this is mostly likely a function of long-term exposure to the pathogen. For instance, most *Pinus* species, which occur in Central America and the southeastern USA where pitch canker is thought to have originated from or been present for many years, have shown a high level of resistance[18]. In contrast, the level of resistance in *P. radiata* and other species in the 'Attenuata Group', which are generally located in the California region and were only recently introduced to pitch canker, has been lower[18,45,110]. However, *P. patula,* as well as some other *Pinus* species which are located in the pathogen's range in Mexico and would be assumed to have some level of resistance to pitch canker, are highly susceptible to the pathogen, suggesting that other factors maybe involved[18,36,45]. In some cases, it is possible that the variation observed in the level of resistance between species may be due to the inoculation procedures used rather than differences in genetic resistance. However, Morse et al. found that different species of pine responded differentially to infection by *F. circinatum,* in a study using a strain of the pathogen that had been transformed with the green fluorescent protein (GFP)[111]. In *P. oocarpa,* which is relatively resistant to the pathogen, fungal biomass remained localised to the wound site after 8 days whereas, the fungus was spread across the stem in the susceptible *P. radiata*[111]. A basic rating of resistance, based on field observations and greenhouse inoculation experiments, is listed in Table 1.

P. radiata is the mostly widely planted commercial pine species in the world and also considered the most susceptible to pitch canker[18,43,112]. In view of this, the majority of resistance studies have been performed in this species and estimates of resistance have been based on field observations and greenhouse inoculation experiments[18,45]. Greenhouse inoculations have been shown to be predictive of resistance under field conditions for *P. radiata* as well as other *Pinus* species[17,20]. Specifically, lesion length is frequently used as an indicator of susceptibility as resistance responses based on lesion length have been shown to be independent of location suggesting genetic inheritance[17,20]. In California, the number of symptomless trees, taken to

represent natural resistance, in infected stands has been very low, ranging from 2.0% to 15% [15,52] and likewise, the level of resistance reported in greenhouse experiments has also been low[17,18,45]. Hodge and Dvorak reported the levels of resistance in commercial *P. radiata* populations from Chile and New Zealand to be extremely low, between 2.1% to 0.3%, this was in keeping with results from seed stock from the native Monterey, Cambria and Año Nuevo populations where resistance ranged from 0% to 2.5%[18]. A more recent study has shown similar levels of resistance among the native populations of *P. radiata* and families from New Zealand, Australia and Chile[113]. In addition, heritability was found to be significantly greater than zero, suggesting that there would be useful genetic gains from selection[113]. A wide variation in resistance among susceptible *P. radiata* clonal lineages has also been demonstrated. For instance, observations of infections in the field have shown that disease severity differs both among trees as well as over time and that trees with pitch canker infections will not necessarily develop extensive infection [48,51-53]. However, greenhouse studies on the resistance of germinating seed have indicated that this resistance expressed in older seedlings and trees is not functional in the emerging seedling[114]. It is possible that other environmental factors, such as insect feeding preferences or site conditions are also involved. Strong genetic heritability has also been observed in *P. virginiana, P. elliottii* and *P. taeda* in the southeast USA[56,109,115-117]. In the southeast USA, the decline in disease since the worst outbreaks in the early 1970's has been attributed to planting of more resistant pine material. Specifically, highly susceptible material from Georgia (*P. elliottii* var. *elliottii*) was planted in Florida and sustained high levels of infections, whereas the levels of infection in native *P. elliottii* var. *densa* from Florida, in adjacent stands, were substantially lower [21].

In addition to genetic resistance it has also been proposed that there are alternative mechanisms for resistance, such as induced resistance responses. In California a decline in the number of outbreaks and apparent remission of the trees has been observed[15,28,53,118]. Although this decline could be due to the natural selection of more resistant material, or a reduction in suitable wounds or conditions for infection, research has shown that repeated inoculation of *P. radiata* with *F. circinatum* results in a decrease of lesion length within two years[70,119] and in some heavily infected stands, up to 30% of the infected trees showed signs of remission of the disease[15]. Greenhouse inoculation trials using clonal material also resulted in a decrease in lesion size for trees predisposed to the pathogen, in comparison to control trees, over a six week period[119]. Overall, these results are indicative of a systemic induced resistance response. The efficiency or persistence of the induced resistance response is unclear at this stage. Whether this decline is due in part to induced resistance reactions and whether the disease will maintain this low level of

disease severity is unknown. In California, the first glimpse of induced resistance responses were observed about 10 years after the first reports of disease outbreaks in both landscape and native forest settings[70,119], plots are currently being monitored to determine the long-term effectiveness of this resistance mechanism. Nevertheless, in view of these results, it is possible that other forms of induce resistance are functional in mediating resistance against the pitch canker pathogen and may provide additive effects in the overall host resistance. Currently, studies are underway to investigate the molecular and biochemical pathways stimulated by pathogen infection. Thus far, chitinases have been found to be induced in both *P. oocarpa* and *P. radiata* after challenge with the pathogen, although there was no apparent induction of lignin or callose[111]. A cDNA array of potential pathogen-regulated genes from *P. elliottii* and *P. taeda* have been constructed[120], the identity, function and products produced by these cDNAs are presently being determined.

Genetic resistance of pines to pitch canker is intrinsically linked with pathogen virulence. The level of virulence of *F. circinatum* worldwide has been evaluated and in general, only limited variation in virulence has been observed among populations[45]. In South Africa, no significant differences in pathogenicity were found among eight isolates tested, with the exception of one isolate that was more virulent on *P. patula* than the other pine species tested, *P. radiata* and *P. elliottii*[45]. Likewise, minor variation in virulence has been demonstrated in isolates from the southeast USA on *P. taeda,* and *P. virginiana*[121] although in one study, no difference in pathogenicity was detected[56]. In California, the population of *F. circinatum* displays a wide variation in virulence and falls into two distinct virulence groups, the isolates within these groups did not interact differentially with the *P. radiata* clonal material tested[15,17]. However, a *P. radiata* genotype that was resistant to all of the California strains was susceptible to isolates from Florida, Mexico and South Africa[15], suggesting that the overall level of virulence in the Californian population is low. Changes in the virulence of a pathogen population can result from sexual reproduction generating novel pathotypes or from the introduction of strains with different virulence levels. The low level of virulence in the Californian population maybe due to the low level of genetic diversity combined with the predominance of clonal propagation of *F. circinatum*, in contrast to the southeast USA, South Africa and Mexico where numerous genotypes exist and sexual reproduction is thought to occur[9,40,122,123]. Thus, the durability of genetic resistance in *P. radiata* in California maybe dependent on a genetically static pathogen population.

Genetic diversity of *F. circinatum*:

F. circinatum is a heterothallic fungus. However, sexual reproduction has not been observed to occur in nature and thus, the fungus is thought to propagate clonally[15,124]. It is possible that outcrossing does occur, as both mating types are present among the populations worldwide and successful crosses have been achieved with strains both within and between populations in the laboratory[124-126]. Early studies of the genetic diversity of *F. circinatum* examined the variation in vegetative compatibility groups (VCGs) and found that VCGs in the southeast USA, Mexico and South Africa were high, indicating a well-established, outcrossing population, whereas the diversity of VCGs in California and Japan were low, suggesting a recently introduced population with limited, if any, outcrossing[9,40,122,123]. VCGs are loci in the fungal genome that are involved in the formation of vegetative heterokaryons between isolates of the same species. Isolates that have the same VCGs are referred to as vegetatively compatibile, as they are able to recognise each other as "self" and form heterokaryons[127]. This contrasts isolates that differ at one or more VCGs and are considered vegetatively incompatible, as they recognise each other as "non-self" and do not form heterokaryons[127]. VCGs can be used to measure the diversity in a population as they provide a natural way to estimate the number of genetically distinct individuals. The number of VCGs in a population can be influenced by factors such as the number of loci that control vegetative compatibility, the number of individuals in the founding population and the frequency of sexual reproduction; sexual reproduction results in the recombination of the loci that affect vegetative compatibility[127].

A high number of VCGs was reported in Florida, with 45 VCGs[9]. The population was not dominated by a single VCG; the most common VCG, F6, only represented 11% of the population sampled[9]. This indicates that there is large, established, outcrossing population of *F. circinatum* in the southeast USA. Likewise, numerous VCGs have also been reported for South Africa and both mating types are known to exist[122,126]. The South African population was assigned to 23 VCGs and is dominated by four VCGs, SA1-4, that individually accounted for less than 13% of the total diversity in the sample population[122]. The high percentage of VCGs and presence of both mating types would indicate that that sexual reproduction is also occurring in South Africa. However, as pitch canker infections are restricted to nurseries and as the introduction of *F. circinatum* has only been recent, the high number of VCGs could also be due to a large founding population. The initial identification of the fungus was from one nursery in South Africa, where it has subsequently spread to other nurseries suggesting one introduction event of many individuals rather than multiple introductions[38,43,58]. An initial assessment of the genotypic diversity of the Mexican population of *F. circinatum,* revealed a high level of diversity

of VCGs; 7 VCGs from 10 samples[40]. However, as the sample size was so low, further testing is required to obtain a better understand of the diversity of VCGs in this population[40].

In contrast to the high number of VCGs reported for the southeast USA, South Africa and Mexico, only five distinct VCGs (C1-5) were initially identified in California in the late 1980's and specifically, the population was dominated by one VCG, C1, which represented 70% of the population sampled[9]. Since then, the number of VCGs has only increased to eight and the region is still dominated by C1, although the frequency of C3 had increased representing a third of the population sampled[123]. Laboratory experiments have shown that both mating types are also present in the Californian population of *F. circinatum*[124]. However, the low number of individuals in this region suggests low diversity within the founding population, coupled with a predominance of clonal propagation and limited sexual reproduction. Molecular data has also supported clonal propagation of the pathogen population in California, as isolates in the same VCG were found to be identical at seven loci[124]. Similarly, the diversity of VCGs in Japan was very low. Only one VCG, C7, which is a VCG also found in California, was identified[40]. This would suggest that the introduction of *F. circinatum* in Japan mostly likely originated from California. However, as the sample size assessed was small (5 isolates) further sampling would be required to determine whether other VCGs groups are present. With the exception of Japan, there was no overlap in the VCGs between California, southeast USA, South Africa and Mexico[9,40,122].

More recently, the genotypic diversity of the pitch canker pathogen has been determined using genomic polymorphic markers. Analysis of a selection of *F. circinatum* isolates from California, southeast USA, South Africa, Mexico and Japan, with known but different VCGs, showed an overlap of genetic diversity worldwide using these markers. Specifically, California, southeast USA and Japan were separated only by short genetic distances, D_{ARC} = 0.39-0.49 and D_{LR} = 0.26-0.196 and shared common genotypes despite isolates being assigned to different VCGs [40]. There were no shared genotypes between the USA /Japanese isolates and the South African/Mexican isolates, although South Africa and Mexico shared one common genotype. The South African and Mexican populations were, genetically, moderately related, D_{ARC} = 0.56 and D_{LR} = 0.126 and also shared a unique allele, not present in any other population, at a high frequency [40]. The sharing of alleles between unique genotypes is suggestive of sexual recombination. In view of these results, it has been speculated that pitch canker mostly likely spread from the southeast USA to California rather than from Mexico as was previously thought; the disease may have got to Japan either directly from the southeast USA or indirectly through

California, as the disease was discovered in Japan after it had been reported in California. This work also supports the hypothesise that *F. circinatum* was introduced to South Africa from contaminated seed stock from Mexico; further sampling of the Mexican population may reveal more common genotypes and alleles with South African isolates and also isolates from the USA. The variation in results between the studies using VCGs versus polymorphic markers gives an estimate of the specificity of these techniques. For instance, the sharing of identical genotypes among isolates from different VCGs suggests that the new VCGs may have been created from mutations within a clonal lineage rather than from sexual recombination. In view of this, the loci responsible for vegetative compatibility may be more likely to undergo mutations than other regions of the genome and as a result, population diversity measures based on VCGs are likely to be more sensitive than from the polymorphic markers.

In addition to the VCG- and polymorphic marker-based estimates of genetic diversity, other molecular techniques have been used for the identification of *F. circinatum* from other *Fusarium* species, which in turn, can also give a measure the diversity of the pathogen population. Phylogenetic trees have been produced from the nuclear rDNA subunit, mitochondrial small subunit and β-tubulin gene for the identification of *Fusarium* species[10,63,128]. Although the ITS region could not be used as a reliable identification technique, as two different copies of the ITS2 region were identified from the same isolate[10,128], the IGS region has been successfully used for detection and quantification of *F. circinatum*[63]. Specific primers for the *MAT1-1* and *MAT1-2* loci have been developed and can be used to determine the mating type of individual strains, which can give an estimate of population structure[129,130]. The histone H3 gene has been sequenced from *F. circinatum* and was found to contain two introns and was conserved in all the isolates studied[131]. This sequence has been used to distinguish *F. circinatum* from other *Fusarium* species and could also be used for phylogenetic studies of closely related organisms[32,36,131,132]. The gene encoding endopolygalacturonase (endoPG) in *F. circinatum* has been cloned and characterised[133]. This gene can be used to distinguish *F. circinatum* from some other *Fusarium* species but also could be used to elucidate the pathway of infection by *F. circinatum*, as endoPGs are known to be involved in digestion of the plant cell walls during infection[133,134]. RAPD and RFLP profiles of genomic DNA and RFLP profiles of mitochondrial DNA have also been used to distinguish *F. circinatum* from other *Fusarium* species, as well as to investigate the genetic diversity of the pitch canker pathogen population[9,126,135]. More recently, a DNA-based diagnostic system to detect *F. circinatum* infected material has been developed from sequences obtained from RAPD bands[135,136]. The test can distinguish *F. circinatum* from other

Fusarium species and was successfully used to detect the infected *Pseudotsuga menziesii* scion sent to New Zealand from California[31,136].

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