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Association of the pitch canker pathogen Fusarium circinatum with grass hosts in commercial pine production areas of South Africa

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Association of the pitch canker pathogen *Fusarium circinatum* with grass hosts in commercial pine production areas of South Africa

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The pitch canker pathogen, *Fusarium circinatum*, has major impacts on production in pine nurseries and plantations in South Africa. Thus far, efforts to reduce local spread have focused on rouging of infected pines and sanitation to eliminate local sources of inoculum. Although the host range of *F. circinatum* was thought to be limited to pines and Douglas-fir, recent studies in California indicate that this fungus is capable of infecting grasses as a symptomless endophyte. Consequently, it is possible that grasses represent a reservoir of inoculum that influences the occurrence of disease in South African pine nurseries and plantations. The objectives of this study were to survey a wide range of grass species in both nurseries and plantations in South Africa for the presence of *F. circinatum.* In all, 22 species of grass were sampled at a nursery in Mpumulanga and in a plantation on the Western Cape. Isolates obtained from grasses were identified based on morphological criteria and DNA sequence data. *Fusarium circinatum* was recovered from vegetative tissues of four grass species including *Briza maxima*, *Ehrharta erecta* var. *erecta*, *Pentameris pallida* and one species that could not be identified. All isolates were pathogenic to pines and comparable in virulence to a known *F. circinatum* isolate that was included as a positive control. These studies indicate that grasses may constitute inoculum reservoirs that could facilitate persistence and dissemination of the pathogen in nurseries, and provide a means for the pathogen to move between widely separated pine stands, where grass hosts occur in intervening areas.

Keywords: alternate hosts, *Fusarium circinatum*, grasses, *Pinus*, pitch canker, Poaceae

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Introduction

Fusarium circinatum is one of the most destructive pathogens of pines worldwide, especially on certain highly susceptible species desirable in forestry, such as *Pinus radiata* and *P. patula* (Wingfield et al. 2008; Gordon 2012). This fungus has major impacts on pine production in many countries, including the USA, Chile, Spain and South Africa (Wingfield et al. 2008; Gordon 2012). In South Africa, *F. circinatum* is an established pathogen in nurseries and plantations of mature pines (Wingfield et al. 2008; Mitchell et al. 2011, 2012). In nurseries, it is responsible for seedling mortality, where it is primarily associated with girdling lesions at the root collar (Morris 2010; Mitchell et al. 2012). In plantations, it can severely reduce post-planting survival, often in association with cryptic infections in planting stock (Morris 2010; Mitchell et al. 2012). In addition, trees can suffer reduced growth and mortality from branch and trunk cankers, and in seed orchards infections can result in contaminated seed (Wingfield et al. 2008; Morris 2010).

Since its initial description in 1946, *F. circinatum* has been considered a specialised pathogen, with all known hosts being in the Pinaceae (*Pinus* species and Douglas-fir) (Hepting and Roth 1946; Dwinell et al. 1985; Gordon 2006). However, recent studies in native *P. radiata* and *P. muricata* forests in California have revealed that *F. circinatum* can also infect grasses (family Poaceae) within pitch-canker-infested stands (Swett and Gordon 2012). Grass-associated isolates were shown to be pathogenic on pines and somatically compatible with isolates obtained from pines (Swett and Gordon 2012; Swett et al. 2013). Studies using *Zea mays* (maize) as a model system have shown that *F. circinatum* can establish infections through both horizontal and vertical modes of transmission and is capable of infecting root, shoot and developing ear tissue (Swett and Gordon 2009). Corn plants show no symptoms or measurable reduction in biomass as a consequence of infection by *F. circinatum.*

In South Africa, known sources of inoculum in nurseries may include contaminated planting trays or irrigation water, airborne inoculum from other infected pines, and/or infested seed (Morris 2010). In plantations, other infected pines are considered the primary inoculum source. In both systems, grass-host reservoirs of *F. circinatum* could be significant contributors to disease development in pines.

The objectives of this study were to survey a wide range of grass species in both nurseries and plantations for the presence of *F. circinatum*, and confirm pathogenicity of grass-associated isolates in pines. These studies could

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provide foundational knowledge concerning the potential for grasses to serve as reservoirs for the pitch canker pathogen in the country.

Materials and methods

Sampling

All collections were made between 11 March and 5 April 2012. Grasses were sampled from two sites: (1) a nursery in Ngodwana in the Mpumalanga province, which grows *Pinus* species (primarily *P. patula*) and *Eucalyptus* species and (2) a *Pinus radiata* plantation near Cape Town in the Western Cape province. In the Ngodwana nursery, grasses were sampled both beneath the raised benches on which seedlings were grown, and at the periphery of the shade cloth, within 3 m of areas with recent pitch canker contamination. Within the plantation, grasses were collected along roadsides and, when possible, beneath the canopy, within 3 m of symptomatic trees.

Grass specimens were collected only if floral structures were present (either actively flowering or recently senesced). All above-ground parts were collected, including flowers, stalks/stems and leaves, and all tissue collected was asymptomatic. Where possible, species of grasses were identified by staff of the Mercer Arboretum and Botanical Gardens, Pretoria. In total, 22 species of grass were collected: 12 at the Ngodwana nursery and 12 at the plantation on the Cape (two species occurred at both sites), with five to 10 plants of each species collected at each site, for a total of approximately 200 samples.

Isolation procedures

Samples were stored at 4 °C and processed within 2–12 d after collection. Seven to 10 stems, between 10 and 30 cm in length, were processed for each species. Leaves, flowers and nodes (or, in the absence of nodes, three to five internodal segments) were detached from each stem, cut into 5 cm segments and placed together in a polyvinal mesh bag. Tissue in bags was rinsed in 0.1% Tween 20, surface disinfested by immersion for 10 s in 70% EtOH followed by 30 s in 0.1% NaOCl, and aseptically transferred to paper towels to remove residual bleach. Tissue was divided into different plant parts (leaves, flowers and stem segments or nodes), aseptically placed on a *Fusarium* selective medium (FSM) (Aegerter and Gordon 2006), and incubated at 25 °C.

Morphological identification

Between five and 10 d after preparation, all sporulating colonies were examined under a light microscope at 100× magnification for the presence of polyphialides and spores in false heads, but not in chains, as described by Leslie et al. (2006). For all cultures meeting these criteria, a single hyphal tip from a recently germinated spore was transferred to 0.5% potassium chloride agar, on which spores in chains could readily be distinguished from false heads. Records were also taken for all other *Fusarium* species recovered from grasses, which could be putatively identified based on morphology. All isolates are maintained in the culture collection (CMWF) of the Forestry Agricultural Biotechnology Institute, University of Pretoria, South Africa.

DNA sequence analyses

The identities of all putative *Fusarium circinatum* isolates were confirmed by BLAST search analysis against the Fusarium-ID database (http://isolate.fusariumdb.org/index.php) (Geiser et al. 2004) and phylogenetic comparison with species representative of the American clade of the *Gibberella fujikuroi* species complex (Kvas et al. 2009). The data set also included *F. circinatum* mating type tester strains (CMWF497 and CMWF498) and the *F. circinatum* isolate for which a full genome sequence is available (Fsp34) (Wingfield et al. 2012). Fungal DNA was extracted from pure cultures using the PrepMan Ultra DNA extraction kit (Applied Biosystems, Foster City, CA, USA).

The TEF1-α region was amplified by PCR using primers EF1 and EF2 (O'Donnell et al. 1998), on an Applied Biosystems 2720 Thermal Cycler, with reaction mixtures containing c. 5 ng l^{-1} DNA, 0.3 M of each primer, 250 M dNTPs (Fermantas, Nunningen, Switzerland), 0.04 U l^{-1} *Taq* DNA polymerase (Roche Molecular Biochemicals, Manheim, Germany) and PCR buffer with $MgCl_2$ (Roche). Thermal cycler conditions were as follows: 5 min at 95 °C, followed by 35 cycles at 92 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplicons were sequenced on an ABI PRISM® 377 DNA sequencer (Applied Biosystems) in both directions with the original primers. Taxon identity was assigned based on a BLAST match of 98% or greater homology with one or more accessions in the database. Maximum likelihood analyses were performed in PhyML 2.4.3 (Guidon and Gascuel 2003) using the best-fit substitution model TIM2 with gamma correction (Tavare 1986) as determined by jModeltest (Posada 2008). Bootstrap confidence values were based on 1 000 replications.

Pathogenicity tests

Pathogenicity tests were conducted between June and October 2012 on six-month-old *Pinus patula* seedlings, grown from seed obtained from a multiclonal, open-pollinated orchard. To prepare inoculum, fungal isolates identified as *F. circinatum*, as described above, were grown for a minimum of 14 d on potato dextrose agar (39 g DIFCO Bacto PDA, 1 l deionised H_2O), after which spores were suspended in 15% glycerol and the concentration was adjusted to 5×10^4 spores ml^{-1}.

Inoculation trials were arranged in a completely randomised design with 30 replicate trees per isolate. Each trial included trees inoculated with the known virulent isolate, FCC 3579, as a positive control. Seedlings were inoculated by removing shoot tips with sterile pruning shears, and placing a 1 ml droplet of spore suspension on the cut surfaces (Porter 2010). Following inoculation, plants were maintained under greenhouse conditions and watered daily. As a negative control, trees were wounded as described above but inoculated with 15% glycerol instead of a spore suspension.

Lesion length measurements were taken at 50 d post-inoculation to confirm pathogenicity on pines. Re-isolation of the pathogen was accomplished by detaching the leading margin on the stem, surface disinfesting the tissue in 0.5% NaClO and placing it on FSM. Resulting cultures were

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identified as *F. circinatum* by amplifying a diagnostic DNA sequence using specific primers CIRC 1A and CIRC 4A (Schweigkofler et al. 2004).

Results

In total, six isolates recovered from asymptomatic grasses were identified as *F. circinatum* based on morphological criteria and a TEF-1α sequence that was a 98–98.6% match (560–630 base pairs) with one *F. circinatum* isolate in the Fusarium-ID database (NRRL 26432). Phylogenetic placement of these isolates within the *Gibberella fujikuroi* species complex (GFSC) confirmed they are most closely related to *F. circinatum* (Figure 1). In addition, of the 20 isolates putatively identified as *F. circinatum*, based on morphology, 14 had a TEF-1α sequence that was most similar to other *Fusarium* species, including *F. anthophilum* (Fusarium-ID accession number: FD 01297) and an undescribed species in the GFSC (NRRL 25807). In addition to these species, there were many *Fusarium* isolates that, based on morphology, did not resemble *F. circinatum*, including species putatively identified as *F. oxysporum*, *F. solani*, *F. proliferatum* and *F. sporotrichoiodes*. The isolation frequency was not recorded for these isolates and identity was not further investigated.

All six isolates of *F. circinatum* originated from one of four grass species: *Briza maxima*, *Ehrharta erecta* var. *erecta*, *Pentameris pallida* and one unidentified species, all of which were collected at the Tokai plantation (Table 1). The fungus was found in association with all vegetative plant parts (leaves and stems), but was never recovered from floral tissue (Table 1). No isolates were recovered from samples collected at the nursery in the Mpumulanga province (Table 1).

Inoculations confirmed that all six isolates of *F. circinatum* from grasses (CMWF1232, CMWF1235, CMWF1243, CMWF1256, CMWF1294 and CMWF1295) were pathogenic to *P. patula* seedlings, with symptoms and similar lesion size to those caused by the positive control isolate FCC 3579, ranging from 39 to 49 mm across all isolates (Figures 2 and 3). No lesions developed in the non-inoculated controls. *Fusarium circinatum* was successfully recovered from lesions induced by each of the six isolates, based on identification using diagnostic PCR (Figure 4).

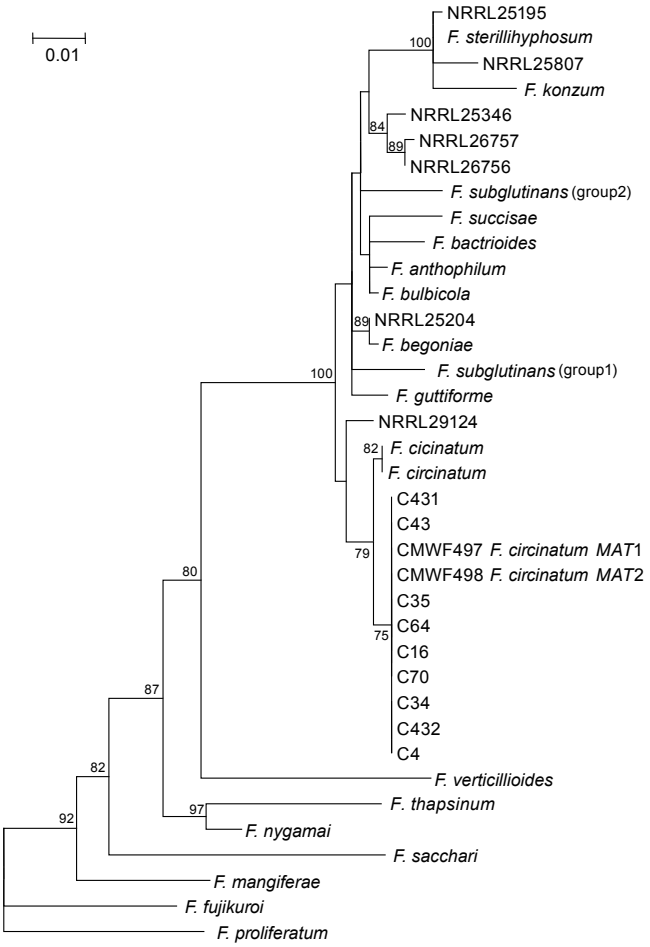

Figure 1: Maximum likelihood tree derived from analysis of a partial *Gibberella fujikuroi* TEF 1α data set, which included *F. circinatum* mating type tester strains (CMWF497 and CMWF498), the *F. circinatum* isolate for which the genome sequence is available (Fsp34) (Wingfield et al. 2012) and species representative of the American clade of the *Gibberella fujikuroi* species complex. The bootstrap values above 75% are indicated at nodes

Discussion

The results of this study have shown for the first time that grass species in South Africa can be infected by *F. circinatum.* These findings support the earlier discovery that asymptomatic grasses collected below infected pines in California can be infected with the pathogen (Swett and Gordon 2012), and that *F. circinatum* can colonise *Zea mays* (maize) as an asymptomatic endophyte (Swett and Gordon 2009). The fact that *F. circinatum* can colonise grasses is perhaps not surprising, given that it is a close relative of other *Fusarium* spp. that are well-known commensal and pathogenic associates of corn, wheat and other species in the grass family (Kuldau and Yates 2000; Desjardins 2003). An important result of the present study is that some of the *Fusarium* spp. isolated from grasses in pine plantations cannot be distinguished from *F. circinatum* based only on morphology. It is thus imperative that identifications of these fungi are based on careful DNA sequence comparisons. Given the importance of making rapid and accurate identifications, a simple and reliable PCR test should be developed and verified for this purpose.

It was interesting that only grasses collected from a plantation in the Western Cape and not those from the nursery in Mpumulanga were infected with *F. circinatum*. This is possibly due to the fact that inoculum of this fungus would be more abundant in the plantation than in the nursery environment. For example, a recent study (Fourie et al. 2014) has shown that air-borne inoculum in the nursery where the present study was conducted is sparse and strongly localised.

More extensive surveys are needed to establish the geographic range over which colonisation of grasses can occur in South Africa. Furthermore, it would be useful to know the time during the year when infections become

Table 1: Summary of grass species collected and *F. circinatum* recovery data

	Grass species sampled	Locations collected[a]	*F. circinatum* recovered (isolate no.)	Plant part[b]
1	*Avena* sp.	CT	(−)	na
2	*Briza maxima*	CT	(+) (CMWF1235)	L
3	*Chloris pycnothrix*	Ng	(−)	na
4	*Cynosurus echinatus*	CT	(−)	na
5	*Digitaria sanguinalis*	CT	(−)	na
6	*Digitaria ternata*	Ng	(−)	na
7	*Digitaria* unknown sp.	Ng	(−)	na
8	*Ehrharta erecta* var. *erecta*	CT	(+) (CMWF1243, CMWF1256)	L, SN
9	*Ehrharta rehmannii* subsp. *subspicata*	CT	(−)	na
10	*Eleusine coracana* subsp. *africana*	Ng	(−)	na
11	*Eragrostis biflora*	CT	(−)	na
12	*Eragrostis curvula*	CT	(−)	na
13	*Eragrostis mexicana* subsp. *virescens*	Ng	(−)	na
14	*Eragrostis pilosa*	Ng	(−)	na
15	*Eragrostis trichophora*	Ng	(−)	na
16	*Melinis repens* subsp. *repens*	Ng	(−)	na
17	*Panicum maximum*	Ng	(−)	na
18	*Paspalum dilatatum*	Ng, CT	(−)	na
19	*Pennisetum clandestinum*	CT	(−)	na
20	*Pentameris pallida*	CT	(+) (CMWF1294)	L
21	*Sporobolus africanus*	Ng, CT	(−)	na
22	Unknown	CT	(+) (CMWF1232, CMWF1295)	L, SN

[a] Locations: Ng = nursery in Ngodwona, CT= plantation in Cape Town

[b] Plant part from which *F. circinatum* was recovered: L = leaves, SN = stem nodes

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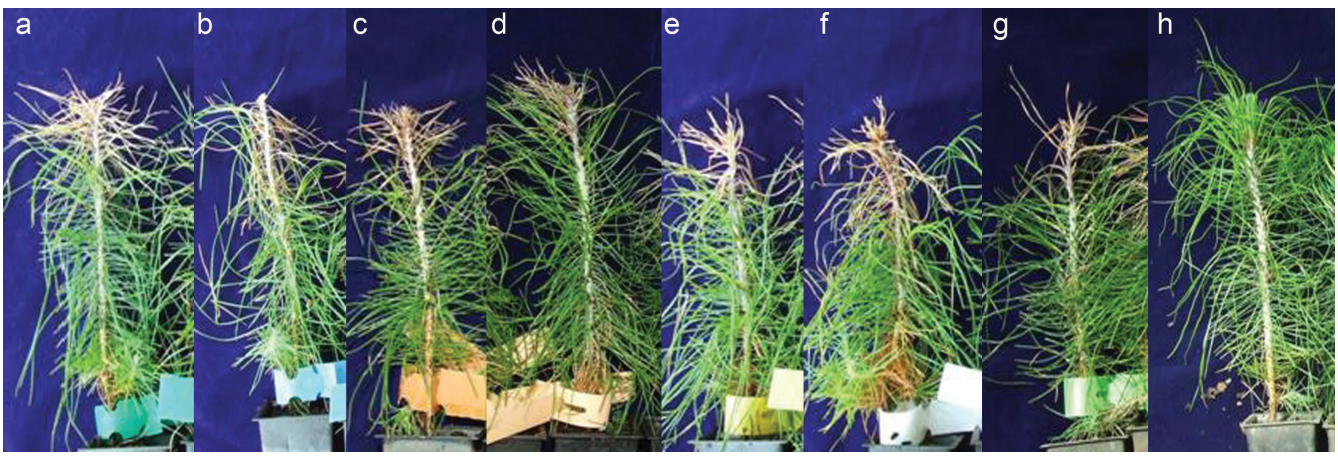

Figure 2: Representative symptoms on *Pinus patula* seedlings 50 d after inoculation by *Fusarium circinatum* isolates CMWF1232 (a), CMWF1235 (b), CMWF1243 (c), CMWF1294 (d), CMWF1256 (e) and CMWF1295 (f) obtained from grasses, and isolate FCC 3579, known to be virulent on pines (g), as well as a non-inoculated control (h)

established and to link these to an understanding of the epidemiology of pitch canker. The low recovery of *F. circinatum* from the plantation site (recovered from up to 2/10 samples per species) and absence of the pathogen from samples collected at the nursery in Ngodwona indicate that more intensive sampling may be needed to establish infection frequencies. In addition, greenhouse trials with common native and introduced grass species are required to better characterise the host range of *F. circinatum* within the grass family. Such information will help to guide efforts to manage potential inoculum sources in nurseries and plantations. Further studies are needed to determine if grasses and pines support genetically distinct populations of *F. circinatum*, and whether or not sexual reproduction can occur on a grass host.

The risk posed by infected grasses to pine plantation forestry in South Africa and elsewhere will depend on the extent to which *F. circinatum* can produce inoculum on grass hosts. Preliminary studies have shown that *F. circinatum* will sporulate on senescing grass tissue under controlled conditions (Swett et al. 2013). If this occurs under natural conditions, grasses could facilitate infection of pines in

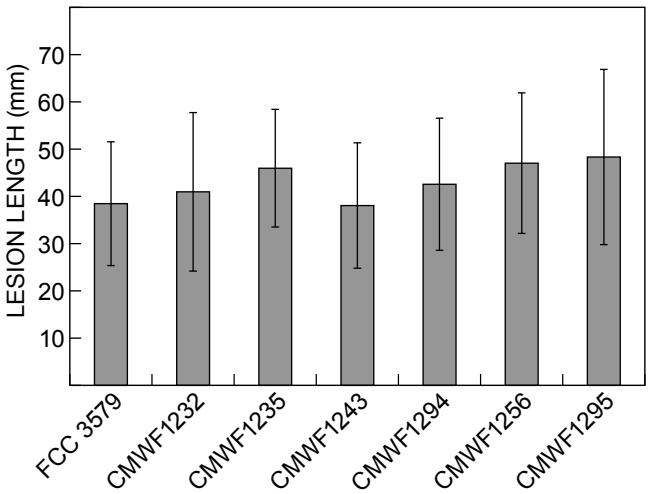

Figure 3. Mean lesion length 50 d after inoculation with the positive control (FCC 3579) and six *F. circinatum* isolates from grasses (CMWF1232, CMWF1235, CMWF1243, CMWF1294, CMWF1256 and CMWF1295)

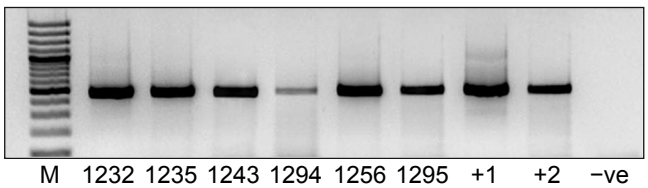

Figure 4: Amplicons obtained from DNA extracts of fungi recovered from symptomatic pine tissue, using *F. circinatum* specific primers CIRC 1A and CIRC 4A. M = 100 bp ladder; 1232–1295 = *Fusarium circinatum* isolates from grasses (CMWF isolate numbers); +1 (FCC 3579) and +2 (FCC 3580) = known *F. circinatum*; -ve = negative control (ddH_2O instead of DNA template)

nurseries and plantations. In addition, grass savannas, which are a dominant ecosystem in many areas where pines are grown, could provide a bridge for spread of *F. circinatum* from infested to uninfested stands. It is important to also recognise that the potential for *F. circinatum* to infect species in the grass family may offer opportunities for global movement of this pathogen in association with agronomic crops as well as ornamental taxa. This will be of particular concern if vertical transmission occurs, allowing grain crops to serve as carriers of *F. circinatum*. Further studies are warranted to better characterise the full extent of the host range of *F. circinatum* and to determine how this may influence the efficacy of regulatory measures intended to limit the risk of introductions to uninfested areas.

Conclusions

Pitch canker has major impacts on pine production in South Africa and many other countries, including Chile, Spain and the USA. Quarantine efforts have been focused on regulating movement of conifers, which were the only hosts known to be susceptible to *F. circinatum*. However, recent findings indicate that this fungus also has a cryptic association with grasses. This study extends previous findings of grass associations in native pine forests in the USA to the pine nurseries and plantations in South Africa. Recovery from diverse grass species in South Africa and confirmation of virulence in pines suggests that populations in grasses may constitute a previously unrecognised and unregulated source of inoculum in South African pine production systems, where grasslands are a dominant ecosystem. Further studies to evaluate the reproductive biology in grass populations will provide insight into the relative importance of this association in different regions and potential strategies for management.

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References

Aegerter BJ, Gordon TR 2006. Rates of pitch canker induced seedling mortality among *Pinus radiata* families varying in levels of genetic resistance to *Gibberella circinata* (anamorph *Fusarium circinatum*). *Forest Ecology and Management* 235: 14–17.

Desjardins AE. 2003. *Gibberella* from A (venaceae) to Z (eae). *Annual Review of Phytopathology* 41: 177–198.

Dwinell LD, Barrows-Broaddus JB, Kuhlman EG. 1985. Pitch canker – a disease complex of southern pines. *Plant Disease* 69: 270–276.

Fourie G, Wingfield MJ, Wingfield BD, Jones NB, Morris AR, Steenkamp ET. 2013. Culture-independent detection and quantification of *Fusarium circinatum* in a pine-producing seedling nursery. *Southern Forests* 76: 131–143.

Geiser DM, Jimenez-Gasco MM, Kang S, Makalowski I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O' Donnell K. 2004. FUSARIUM-ID v.1.0: a DNA sequence database for identifying *Fusarium. European Journal of Plant Pathology* 110: 473–479.

Gordon TR. 2006. Pitch canker disease of pines. *Phytopathology* 96: 657–659.

Gordon TR. 2012. Biology and management of *Gibberella circinata*, the cause of pitch canker in pines. In: Alves-Santos FM, Diez J (eds), *Control of* Fusarium *diseases*. Trivandrum: Research Signpost. pp 195–208.

Guidon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52**:** 696–704.

Hepting GH, Roth ER. 1946. Pitch canker, a new disease of some southern pines. *Journal of Forestry* 55: 742–744.

Kuldau GA, Yates IE, 2000. Evidence for *Fusarium* endophytes in cultivated and wild plants. In: Bacon CW, White JF (eds), *Microbial endophytes*. New York: Marcel Dekker. pp 85–120.

Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp ET. 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. *Fungal Diversity* 34: 1–21.

Leslie JF, Summerell BA, Bullock S. 2006. *The* Fusarium *laboratory manual*. Oxford: Blackwell Publishing.

Mitchell RG, Coutinho T., Steenkamp E, Herbert M, Wingfield MJ. 2012. Future outlook for *Pinus patula* in South Africa in

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the presence of the pitch canker fungus (*Fusarium circinatum*). *Southern Forests* 74: 203–210.

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

Morris A. 2010. A review of pitch canker fungus (*Fusarium circinatum*) as it relates to plantation forestry in South Africa. Howick: Shaw Research Centre, Sappi.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from the nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Science of the USA* 95: 2044–2049.

Porter B. 2010. Pathogenicity and competition studies on *Fusarium circinatum*, a pathogen of pine trees. MSc thesis, University of Pretoria, South Africa.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.

Schweigkofler W, O'Donnell K, Garbelotto M. 2004 Detection and quantification of *Fusarium circinatum*, the casual agent of pine pitch canker, from two California sites by using a real-time PCR approach combined with a simple spore trapping method. *Applied and Environmental Microbiology* 70: 3512–3520.

Swett CL, Gordon TR. 2009. Colonization of corn (*Zea mays*) by the pitch canker pathogen, *Fusarium circinatum*: insights into the evolutionary history of a pine pathogen. *Phytopathology* 99: S126–S127.

Swett CL, Gordon TR. 2012. First report of grass species (Poaceae) as naturally occurring hosts of the pine pathogen *Gibberella circinata*. *Plant Disease* 96: 908–908.

Swett CL, Huang M, Begovic A, Steenkamp ET, Wingfield MJ, Gordon TR. 2013. A new dimension to pitch canker epidemiology: biology of *Fusarium circinatum* as a grass colonist in native and managed pine systems. In: Browning J, Palacios P (compilers), *Proceedings of the 60th Annual Western International Forest Disease Work Conference*, *8–12 October 2012, Tahoe City, California*. s.l.: WIFDWC. pp 113–116.

Tavare S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.

Wingfield BD, Steenkamp ET, Santana QC, Coetzee MPA, Bam S, Barnes I, Beukes CW, Chan WY, de Vos L, Fourie G et al. 2012 First fungal genome sequence from Africa: a preliminary analysis. *South African Journal of Science* 108(1/2): Art. #537, 9 pages.

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

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