SPECIES OF *FUSARIUM* ON *PINUS RADIATA* IN NEW ZEALAND

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ABSTRACT

The common diseases of pines caused by *Fusarium* species in New Zealand are damping-off and root rot disorders of seedlings in forest nurseries. The emergence of pine pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini*, as a serious threat to *Pinus radiata* plantations has increased the need for an awareness of the species associated with pines in New Zealand. Standard morphological procedures were used to identify *Fusarium* cultures isolated from diseased pines and from soil in forest nurseries. *Fusarium oxysporum* and *F. solani* were the most commonly found species in bare-root *P. radiata* nurseries, and were obtained from both seedlings and from soil. The species most frequently obtained from branches and needles of older trees in plantations were *F. avenaceum* and *F. sambucinum*, but these were not associated with serious disease.
Keywords: *Fusarium* spp., *Pinus radiata.*

INTRODUCTION

Species of *Fusarium* have a world-wide distribution and pathogenic species have been recorded from a large number of host plants. In conifers *Fusarium* spp. are mainly known as nursery pathogens, causing pre-emergence and post-emergence damping off, root rot of older seedlings and stunting. In plantations, only one species (*F. subglutinans* f. sp. *pini* Correll et al. = *F. circinatum* Nirenberg & O'Donnell nom. invalid. Arts. 37.1) is known to cause serious damage to *Pinus* spp.

The importance of pine pitch canker disease, caused by *F. subglutinans* f. sp. *pini*, as a potentially serious threat to *Pinus radiata* D. Don plantations has increased the need for a more comprehensive knowledge of the *Fusarium* species associated with *P. radiata* in New Zealand. *Fusarium subglutinans* f. sp. *pini* is known to be seed-borne (Storer et al. 1998) and seed is a likely pathway for introduction of the fungus into New Zealand and Australia. If the fungus were to enter the country and become established in a *P. radiata* nursery, the initial disease symptoms in young pine seedlings would be indistinguishable from symptoms of a number of other diseases and disorders, including those caused by other *Fusarium* spp. This was the case in South Africa in 1990 where losses in a *P. patula* nusery (Viljoen et al. 1994) were originally attributed to *F. oxysporum* rather than to pine pitch canker disease (M.J. Wingfield, pers. com.). For early detection of pine pitch canker it is essential to have a good knowledge of the species of *Fusarium* currently associated with *P. radiata* in New Zealand as well as the ability to identify *Fusarium* spp. quickly and accurately. This paper reports the species of *Fusarium* found associated with *P. radiata*, and previously unpublished in New Zealand, and provides a basis of knowledge for the future.

MATERIALS AND METHODS

Nursery soil survey 2001

During August/September 2001 fourteen pine growing nurseries were sampled, two from each of the following biological regions (Crosby et al. 1974), Northland (ND), Waikato (WO), Bay of Plenty (BP), Nelson (NN) and Southland (SL), and one each from Taupo (TO), Gisborne (GB), Hawke's Bay (HB) and Dunedin (DN). From two to seven soil samples, at least 50 m apart, were collected from each nursery. Each sample

comprised 100 g of soil taken from the seedling root zone. Each soil sample was shaken and 0.1 g of soil sparsely sprinkled over the surface of each of three 90 mm Petri dishes containing Nash Snyder + malachite green medium (Castellá et al. 1997). Dishes were kept at 25ºC in the dark for seven days.

New Zealand Forest Research Institute records

Species of *Fusarium* have been regularly isolated from pine specimens, and from soil in which pines are growing, during diagnostic procedures carried out at the Forest Health Reference Laboratory, Forest Research, Rotorua. Forest pest and disease diagnostic services have been provided by Forest Research since the 1950s as part of the national forest health surveillance programme (Flux et al. 1993), and have also been available to nurseries and forests independently of the surveillance programme.

Isolation methods for *Fusarium* spp.

Material from root systems was well washed under running water, dried and surface sterilised in 10 volumes of hydrogen peroxide. Discoloured tissue segments were placed on 3% malt extract agar (MEA) and amended Nash-Snyder media (Nash & Snyder 1962) in Petri dishes. Surface tissue was removed from above-ground plant material with a sterilised scalpel and discoloured tissue segments placed on 3% MEA in Petri dishes.

Identification technique

Isolates of *Fusarium* were grown on potato dextrose agar (PDA), carnation leaf agar (CLA) (Nelson et al. 1983) and synthetic nutrient agar (SNA) (Nirenberg & O'Donnell 1998). Petri dishes were kept at room temperature under near-ultra violet light. Replicate PDA plates were held at 25ºC in the dark. Cultures were examined over a four-week period and morphological characters noted as prescribed by Nelson et al. (1983).

Isolation results are recorded in the Forest Health Database (FHDB) and representative samples and cultures are placed in the Mycological Herbarium (NZFRI-M) and Culture Collection (NZFS).

RESULTS

Nursery soil survey 2001

Results from nursery soils are presented in Table 1. *Fusarium oxysporum* Schlect. emend. Snyder & Hansen was the most frequently isolated species. Only one other species, *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen, was obtained, and from only two samples. No species of *Fusarium* were isolated from the two Southland nurseries.

TABLE 1: *Fusarium* species isolated from nursery soil where bare rooted *P. radiata* stock was growing.

Bioregion[1]	No. samples	*Fusarium* species isolated	Number of samples containing *Fusarium* spp.
ND	7	*F. oxysporum*	1
WO	6	*F. oxysporum*	2
		F. solani	1
BP	6	*F. oxysporum*	4
TO	7	*F. oxysporum*	3
GB	3	*F. oxysporum*	2
HB	5	*F. oxysporum*	4
NN	6	*F. oxysporum*	3
		F. solani	1
DN	3	*F. oxysporum*	2
SL	6	-	0
TOTAL	49		23

[1]Bioregions are defined in the methods section.

Forest Health Database - nursery records

The database, dating back to 1964, contains 147 records of *Fusarium* spp. isolated from diseased pine plants in nurseries. *Fusarium oxysporum* was the most common species isolated, with *F. solani* recorded less often. A further 4 species (Table 2) have been infrequently identified. For 110 records the identifications have not been to species level.

TABLE 2: Forest Health Database records for *Fusarium* spp. associated with *P. radiata* in nurseries.

	Bioregion[1]								
Species	BP	GB	HB	NC[2]	ND	NN	TO	WI[2]	TOTAL
F. lateritium						1			1
F. moniliforme			1						1
F. oxysporum	13	2		2	1	3	2	1	24
F. sambucinum						1			1
F. subglutinans			1						1
F. solani	1		2		1	5			9
TOTAL	14	2	4	2	2	10	2	1	37

[1]Bioregions are defined in the methods section.
[2]Additional bioregions: NC = North Canterbury, WI = Wanganui.

Forest Health Database - plantation records

Six species of *Fusarium* were found in association with a variety of conditions of *Pinus radiata* (Table 3). In most cases where a *Fusarium* sp. was isolated from lesions or dieback of needles, shoots, leaders or branches there was evidence of physical injury and the infection could be assumed to be secondary in nature, i.e. a weak pathogen or opportunistic fungus invades the host plant through wounded tissue. Both *F. avenaceum* (Fries) Saccardo and *F. sambucinum* Fuckel were regularly found in this association, with *F. acuminatum* Ellis & Everhardt, *F. equiseti* (Corda) Saccardo and *F. oxysporum* being found less frequently. Several of these species were often isolated from dying roots. Pathogenicity testing with New Zealand isolates of *Fusarium* spp. obtained from branches and needles of *P. radiata* have not been carried out and their role in the dieback condition remains uncertain.

TABLE 3: *Fusarium* spp. isolated from *P. radiata* in plantations.

Species	No. records	Condition of plants (no. of records)
F. acuminatum	3	Dieback (2). Root rot (1).
F. avenaceum	15	Dieback associated with physical injury (10). Dying needles (3). Dying shoots (2).
F. equiseti	4	Dieback associated with physical injury.
F. oxysporum	12	Dieback associated with physical injury (5). Root rot (7).
F sambucinum	12	Dieback (8). Root rot (4).
F. solani	1	Root decline due to water-logging.

DISCUSSION

A number of publications contain reference to *Fusarium* spp. associated with pines in New Zealand without providing identification to species level (Birch 1937; Gilmour 1966; Hutchinson & Reid 1984). The only published reports of named species are of *F. acuminatum* (as *Gibberella acuminata*) and *F. equiseti* (as *G. intricans*) (Anon. 1999).

Six species of *Fusarium* are recorded in the Forest Research Forest Health database associated with *P. radiata* in forest nurseries but only two, *F. oxysporum* and *F. solani*, have been frequently isolated. This parallels the results described in this paper when only *F. oxysporum* and *F. solani* were isolated from the limited collection of nursery soils from around the country in 2001. These two species are often recorded around the world as causing disease of seedling pines (e.g. Mirabolfathy & Ershad 1996; Bloomberg 1981; Vaartaja & Bumbieris 1967).

No species of *Fusarium* were isolated from Southland soil during this survey, which corresponds with the very low numbers of *Fusarium* spp. from Southland recorded in the database. The Bay of Plenty and Nelson bioregions yielded the highest return of *Fusarium* in this study, also paralleling the FHDB records. However, database records do not represent a structured sampling programme throughout the country and these observations must be viewed cautiously.

Apart from the abundant literature on pine pitch canker disease there are relatively few reports of *Fusarium* species attacking pines outside the nursery. *Fusarium* species are often found in association with dead and dying tissue of plants but are generally regarded as being present saprophytically. Parasitic attack has only been recognised in the following five published examples.

- *F. avenaceum was* isolated from 4-year-old *P. sylvestris* L. (Huppel & Klingstrom 1964).
- *F. equiseti* was isolated from a *P. taeda* L. branch and caused slight stem lesion on inoculated seedlings (Solel et al. 1988).
- *F. oxysporum* caused root rot of 3-year-old *P. caribaea* Morelet var. *hondurensis* in Venezuela (Mohali 1996).
- *F. pseudocircinatum* O'Donnell & Nirenberg is a pantropical species known from many hosts, including *Pinus kesiya* Royle ex Gord. from the Phillipines (Nirenberg & O'Donnell 1998).
- *F. solani* caused root rot of 3-year old *P. caribaea* var. *hondurensis* in Venezuela (Mohali 1996).

The records from pines in New Zealand, showing that *F. avenaceum, F. equiseti, F. oxysporum* and *F. solani* are associated with diseased conditions of pine trees, are thus consistent with those from other countries. An association of *F. sambucinum* with dieback of pines, as reported here for *P. radiata,* has not previously been made. Pathogenicity testing would be required to determine whether any of the New Zealand isolates have parasitic capability on *P. radiata* trees.

Many species of *Fusarium* exhibit considerable variation in morphological characteristics, hence separation of species using traditional methods can be difficult. Within many of these species "forme speciales" have been assigned. These are host-specific but morphologically identical strains. This feature of the genus has ensured the rapid application and development of DNA fingerprinting to delineate species. Most species of *Fusarium* currently recorded from pines in New Zealand can be readily distinguished from *F. subglutinans* f. sp. *pini* based on a range of morphological criteria. This is with the exception of *F. subglutinans,* since morphologically it is virtually indistinguishable from *F. subglutinans* f. sp. *pini*. The morphological characteristics that separate some other species from *F. subglutinans* f. sp. *pini* may take up to 4 weeks to develop. Hence Forest Research, with reference collections of *F. subglutinans* f. sp. *pini* from California, and DNA from representative isolates of *F. subglutinans* f. sp. *pini* from the eastern USA, Mexico and South Africa, has developed a molecular method for the rapid separation of isolates of these closely related species. Eighty two isolates of *Fusarium* from New Zealand, comprising *F. anthophilum* (A. Braun) Wollenw. (1), *F. lateritium* (14), *F. moniliforme* (15), *F. oxysporum* (16), *F. proliferatum (*Matsushima) Nirenberg (6), *F. sacchari* (Butler) Gams (1), *F. subglutinans* (19), *F. sublunatum* Reinking (2) and 8 isolates identified only to species, have been used to refine and validate the method. The usefulness of this system was demonstrated in 2001 when there was a suspected outbreak of pine pitch canker in Australia. Within 3 days Forest Research was able to confirm that the suspect *Fusarium* sp. was not the pine pitch canker pathogen.

In summary, nine species of *Fusarium* are reported to be associated with *P. radiata* in New Zealand. In many cases where *Fusarium* spp. have been isolated the contribution of the fungus to the diseased condition is uncertain. Although species found in association with dieback of *P. radiata* in New Zealand are generally considered to be secondary colonisers of injured tissue, pathogenicity testing would be required to validate this view.

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