Decay fungi in decomposing post-harvest *Pinus radiata* root and branch debris

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Abstract Knowledge of fungal populations decomposing *Pinus radiata* debris following harvesting may reveal a basidiomycete able to compete with pathogenic *Armillaria* species or help explain variation in decomposition rates. Decay fungi were isolated after 2 or 3 years from buried root segments and branch segments placed on the soil surface at six sites in New Zealand. A large variety of decay fungi was obtained, different species being isolated at each site, as well as from branch versus root segments. Communities of decay fungi were more diverse in branches (one species per 1.01 segments) than root debris (one species per 5.53 segments). At the same site species tended to differ between spatially separated replicates for branch but not root segments. For root segments, identical fungi were frequently obtained at different depths in the same replicate. This implies that branch segments were colonised separately by air-borne spores, whereas root segments were exposed to mycelial growth through the soil. Few fungi were identified, but three, *Resinicium bicolor*, *Sistotrema* sp. and *Stereum sanguinolentum*, are common in *P. radiata* woody debris.

Keywords *Pinus radiata*, basidiomycete, decay fungi, woody debris.

INTRODUCTION

Plantations of *Pinus radiata* D. Don cover an area of 1.6 million ha over a wide range of sites throughout New Zealand, representing 90% of the total stocked production forestry estate (MAF 2010). Despite the importance of this species, much remains to be learned about its associated biota, particularly in relation to the organisms that colonise and decompose the woody residues left after stands have been harvested. This information is likely to be of value in a number of ways. For instance knowing the principal basidiomycete fungi that colonise woody debris is a logical first step to finding a potential biological control agent for armillaria root disease on sites where it is more severe. Such a fungus might act by competing with the *Armillaria* species present, reducing its inoculum potential by denying it access to the woody substrate (Hood et al. 2002, 2008a). Again, an awareness of the basidiomycete populations active in post-harvest residues is integral towards understanding the variation between regions in rates of woody debris decomposition and release of carbon into the atmosphere (Garrett et al. 2010). Variation in decomposition rates associated with different decay fungi has been demonstrated in indigenous forests (Beets et al. 2008), and the same may apply in exotic plantations.

Knowledge of the fungi affecting timber and wood products from commercial *P. radiata* plantations was summarised by Butcher & Drysdale (1991). Subsequent information on decomposer species was obtained during

research investigating the fungi colonising stems damaged in storms as well as those present in stumps formed during operational thinning (Hood & Gardner 2005; McCarthy et al. 2010; cf. Uzunovic et al. 2004). However, besides stumps, it is likely that armillaria disease centres may also arise from partially buried woody debris left after felling operations (Hood et al. 2002).

The present paper reports the results of a study to investigate basidiomycete species in decomposing woody residues by sampling within a larger trial set up to examine rates of decay of pine debris after harvest (Garrett et al. 2009). The purpose of the study was to determine the nature and diversity of the decomposer fungi present in branch and buried root material in order to see how they compared between different locations in both islands of New Zealand. Knowledge of the overall decomposer populations might help in selecting promising candidates for biocontrol of *Armillaria* for subsequent testing.

MATERIALS AND METHODS

The study was conducted at six of eight available sites in a larger trial, three in the North Island (Woodhill, 36° 33'S 174° 14'E; Rotoehu, 37° 56'S 176° 30'E; Kaingaroa, 38° 36'S 176° 25'E) and three in the South Island (Golden Downs, 41° 30'S 172° 53'E; Balmoral, 42° 47'S 172° 37'E; Rolleston, 43° 37'S 172° 20'E). All sites were on recently clear-felled and re-planted *P. radiata* production forest land, except for the Rolleston site which was ex-pasture and Rotoehu which was a cleared agro-forestry site. The parent trial was installed at the North Island sites during 2005 (between June and August) and in the South Island between December 2006 and April 2007. The design included replication, with material in each replicate (both branches and roots) being taken from the same tree, where possible, in order to minimise any effect of genetic variation. Suitable material was collected from the same locality (within 10 km at each site), with the exception of that for Balmoral which came from the Rolleston site. Roots and branches were cut into 300 mm long segments, and separated into small (10–50 mm) and large (50–100 mm) diameter classes (measured at the segment centre). Each replicate consisted of one large and one small diameter branch segment, and two subsets of root segments, each with four small and one large diameter root segment. Material for each replicate was installed horizontally in vertical tiers with the branch segments placed on the soil surface, and the two subsets of root segments buried 100 mm and 300 mm below the surface, respectively. All branch and root segments within one replicate were linked to each other and to an identification tag by tying with blue insulation wire to facilitate full recovery when harvested.

Segments were harvested approximately 2 (South Island) or 3 years (North Island) after installation, two of several replicates uplifted at each site being used in this study. After washing, isolations were made from a 125 mm length cut from each end of each segment. Pieces were split asceptically and five small chips were taken from the centre of each end portion (10 chips per segment) and plated onto 2% malt agar supplemented with 100 ppm streptomycin sulphate and 10 ppm benomyl to discourage competition by bacteria and non-decay fungi, respectively. After incubation for periods of up to 6 weeks, emerging mycelia were subcultured in tubes of 2% malt agar. Bacterial cultures were mostly recorded but not isolated. Culturally identical isolates were sorted into groups and a record kept of those recognised as basidiomycetes (mainly those with clamp connections or occasionally clampless basidiomycetes of known identity). Remaining fungi (except yeasts, sporulating hyphomycetes and mucoraceous species) were examined with ∝-naphthol, and those testing laccase-positive, indicative of white rot behaviour, were also treated as basidiomycetes (Stalpers 1978). Non-basidiomycete fungi were not considered further. Culture descriptions were prepared of representative cultures of each basidiomycete species, with emphasis on their key distinguishing features, when grown on 2% malt agar plates for more than 6 weeks (Nobles 1965; Stalpers 1978). Each species was labelled B_x pending precise identification, where 'B' represents basidiomycete, and 'x' identifies the particular species.

RESULTS AND DISCUSSION

The results from this study indicated that there is a large variety of fungi responsible for the decomposition of branch and root debris remaining after harvesting operations in *P. radiata* plantations in New Zealand. Altogether, 41 species cultured from most decomposing segments at all sites were recognised as decay fungi (Tables 1 and 2; this excludes three laccase-positive isolates at Woodhill that *may* represent additional species, Table 1). Several trends were apparent. Apart from two species, the basidiomycete fungi obtained differed between sites. Species found at more than one site were B_H from branch segments at Kaingaroa and Golden Downs Forests, in the North and South Islands respectively; and B_S from branch segments at the Balmoral and Rolleston sites in Canterbury (it may be significant that material for these two sites came from the same location). It was also found that species colonising the above-ground branch segments differed from those in the buried root segments, with two exceptions. These were basidiomycetes B_J and B_R at the Rotoehu and Rolleston sites, respectively, which while common in root segments, were also isolated from branch segments of one replicate at each site (Tables 1 and 2).

Within sites, fungi also tended to differ between replicates, but more so for branch than root samples. For branch segments this effect applied to all but one site (Species B_S occupied branch segments in both replicates at Balmoral; Table 2). With root segments the tendency was less marked, and one species was shared between replicates at each of four sites. These were species B_G, B_J, B_Z and B_R at Kaingaroa, Rotoehu, Balmoral and Rolleston, respectively (Tables 1 and 2). With roots, moreover, the same species was commonly present in all or most segments at both depths. The only exceptions to this were in one replicate at each of the Rotoehu, Balmoral and Rolleston sites (even here, species B_J was still obtained from both depths in each replicate at Rotoehu). In most root (and many branch) segments, the same basidiomycete was isolated from both ends, indicating that each species had colonised the whole segment prior to harvest (Tables 1 and 2). Populations of decay fungi were therefore more diverse within branch than root segments. Basidiomycetes averaged one species per 1.01 branch segments, significantly different from one species per 5.53 segments for roots (conservatively including the three laccase-positive isolates in root segments at Woodhill as distinct species, Table 1; $P<0.01$; differences between sites and between replicates were not significant, $P>0.05$). Such a result would be expected if separate colonies are established by means of air-borne basidiospores in the material exposed on the soil surface. For roots, the absence of strong differences within replicates between and within soil depths regardless of diameter, suggests that subterranean colonisation occurs by means of vegetative mycelial growth through the soil between samples for distances of up to 200 mm. This is a reasonable supposition even if confirmation was not attempted by testing vegetative compatibilities between isolates. At two sites the mycelium of a particularly vigorous root decay fungus appears to have grown up through the soil reaching right to the branch segments at the surface (Species B_J and B_R at Rotoehu and Rolleston, respectively; Tables 1 and 2).

Representative cultures of nearly all 41 species were lodged in the Culture Collection of the New Zealand Forest Research Institute Ltd Reference Laboratory, Rotorua (NZFS), together with a brief description and culture code for each (NZFS 3466-3475, 3478-3516, 3518-3549; code definition based on Nobles (1965) and Stalpers (1978) as modified by Hood et al. (2008b)). Although cultures were morphologically distinctive, it was not possible to identify most species isolated formally by name. However, species B_G and B_H were recognised culturally as *Resinicium bicolor* (Alb. & Schwein.) Parmasto and *Sistotrema* sp., respectively, and it became clear that B_F and B_R were the same species, both equating to the somewhat culturally variable *Stereum sanguinolentum* (Alb. & Schwein.) Fr. (treated as such during analyses). Identification in earlier studies in native forests was achieved by comparing with cultures from authenticated fungal fruitbodies (Hood et al. 2008b), but fruitbodies appear less common in *P. radiata*

Table 1 Basidiomycete fungi cultured from surface branch and buried root segments at three North Island forest sites. Obtained from isolation attempts from each end of one small and one large branch segment, and one large and four small root segments at two depths, per replicate. A dash (-) denotes no basidiomycete isolated (from five attempts). B_J? indicates probably species B_J, B_{asid} represents an uncoded, laccase-+ve species, and B_A;B_B signifies both species B_A and B_B isolated from the same end. B_F, B_G and B_H represent *Stereum sanguinolentum*, *Resinicium bicolor* and *Sistotrema* sp., respectively.

Site, harvest date & replicate	Segment size	Branches		Buried roots			
				100 mm deep		300 mm deep	
		End 1	End 2	End 1	End 2	End 1	End 2
Woodhill, May 2008							
1	Small	B_A	B_A	B_C	B_C	B_C	B_C
				-	B_C	-	B_{asid}
				B_C	-	-	B_C?
				-	B_{asid}	B_C	B_C
	Large	-	B_A;B_B	-	B_{asid}	-	-
2	Small	B_D	-	B_E	B_E	-	-
				-	-	B_E	B_E
				B_E	B_E	-	-
				B_E	-	-	-
	Large	B_D	B_D	B_E	-	B_E	B_E
Kaingaroa, August 2008							
1	Small	B_F	B_F	B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
	Large	-	B_F	B_G	B_G	B_G	B_G
2	Small	-	B_H	B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
				B_G	B_G	B_G	B_G
	Large	B_I	B_I	B_C	B_C	B_G	B_G
Rotoehu, August 2008							
1	Small	-	B_L	B_J	B_J	-	-
				-	-	-	-
				B_J?	B_J	B_J	B_J
				B_J	B_J	B_J	B_J
	Large	B_J	B_K	B_J	B_J	B_J	B_J
2	Small	B_M	B_M	-	-	B_P	B_P
				-	-	B_P	B_P
				B_N;B_O	B_O	B_J	B_J
				-	B_N	B_J	B_J
	Large	-	-	B_J	B_J	B_P;B_{Gd}	B_{Gd}

Table 2 Basidiomycete fungi cultured from surface branch and buried root segments at three South Island forest sites. Obtained from isolation attempts from each end of one small and one large branch segment, and one large and four small root segments at two depths, per replicate. A dash (-) indicates no basidiomycete isolated (from five attempts) and a space a missing segment. B_S;B_{SS} signifies both species B_S and B_{SS} isolated from the same end. B_R and B_H represent *Stereum sanguinolentum* and *Sistotrema* sp., respectively.

Site, harvest date & replicate	Segment size	Branches		Buried roots			
				100 mm deep		300 mm deep	
		End 1	End 2	End 1	End 2	End 1	End 2
Balmoral, February 2009							
1	Small	B_S;B_{AN}	B_S	B_Y	B_Y	B_Z	B_Z
				-	-	B_Z	-
				-	B_Y	B_Z	-
				-	B_Y	-	B_Z
	Large	B_S	B_S	B_Y	B_Y	-	-
2	Small	-	B_{AL}			B_{AJ}	B_{AO}
				-	B_{AJ}	-	B_Z
				B_{AJ}	B_{AJ}	B_Z	B_Z
				B_{AJ}	-	B_Z	-
	Large	B_S	B_S;B_{SS};B_{AM}	B_Z	B_Z;B_{AJ}		
Rolleston, February 2009							
1	Small	B_R	B_R	B_R	B_R	B_R	B_R
				B_R	B_R	B_R	B_R
				B_R	B_R	B_R	B_R
				B_R	B_R	B_R	B_R
	Large	B_S	B_S;B_R	B_R	B_R	B_R	B_R
2	Small	B_T	-	-	B_R	B_U	B_U
				-	-	B_U	B_W;B_V
				B_{VV}	B_{VV}	-	B_V
				-	B_R	B_V	B_V
	Large	B_{TT}	B_{TT}	B_R	B_R	B_U	B_U
Golden Downs, February 2009							
1	Small	B_{AA}	B_{AA}	B_{AD}	-	B_{AD}	B_{AD}
				B_{AD}	B_{AD}	-	-
				B_{AD}	B_{AD}	-	B_{AE}
				B_{AD}	B_{AD}	B_{AD}	B_{AD}
	Large	B_{AB};B_{AC}	B_{AB}	B_{AD}	B_{AD}	B_{AD}	B_{AD}
2	Small	B_{AP}	B_H	B_{AI}	B_{AI}	-	B_{AI}
				B_{AI}	-	B_{AI}	-
				-	B_{AI}	B_{AI}	B_{AI}
				B_{AI}	B_{AI}	B_{AI}	B_{AI}
	Large	B_{AG};B_{AH}	B_{AG};B_{AH}	B_{AI}	B_{AI}	B_{AI}	B_{AI}

debris, although they do occur. It may be possible to identify some isolates by molecular means, but it is not known how many belong to exotic or cosmopolitan species that may be represented on international databases and how many may be indigenous and not yet investigated molecularly. Failure to identify species by name is frustrating, but trends and patterns were still apparent even if it was necessary to refer to fungi simply by labels.

The range of fungi found in this study contrasts with findings from work with coarse woody debris in indigenous forests, where smaller numbers of basidiomycete species were obtained in greater frequencies. This is presumably because of successful competition by specific, more aggressive fungal colonisers within fewer substrate units of very much larger volume than in this study with radiata pine segments (Heilmann-Clausen & Christensen 2004; Hood et al. 2008b). However, results compare with the higher levels of biodiversity reported on stumps of *Picea abies* (L.) H. Karst. in Sweden (within its natural range; Vasiliauskas et al. 2005a) and possibly *Picea sitchensis* (Bong.) Carrière in the United Kingdom (outside its natural range; Woodward 2003). While three species identified in this study, *R. bicolor*, *S. sanguinolentum* and *Sistotrema* sp., are known as common decay agents of *P. radiata* in New Zealand (Cunningham 1959; Butcher 1967, 1968; Ah Chee et al. 1998; Hood & Gardner 2005; McCarthy et al. 2010), as in other regions, most species isolated were not recognisable from previous work with this host, for instance in an earlier study with *P. radiata* thinning stumps (Hood & Gardner 2005). A notable omission is *Phlebiopsis gigantea* (Fr.) Jülich, which is common on above-ground coarse woody debris in pine plantations. This species was only isolated within the first 15 months during the earlier study (Hood & Gardner 2005), and if able to colonise small branches may possibly have been replaced by other fungi early in the present investigation (Vasiliauskas et al. 2005b).

Although comprehensive, this study provides only a glimpse of the decay fungi present in decomposing woody roots and branches in *P. radiata* plantations. Populations were varied, but may be even more diverse than indicated here, and to understand the patterns and trends fully will require more widespread sampling both regionally and over time. Some decay species may be more common than indicated and certain fungi may succeed others as decomposition proceeds. It is also possible that the season when the trees fell or were uprooted may influence which species may colonise the material at different sites. Likewise, the fungi that initially colonise roots collected from uprooted trees exposed to air spora may differ from those where the roots remain buried after felling. Either way, the more aggressive species will dominate in an environment conducive to vigorous mycelial growth in and between roots within the moist soil. Despite these uncertainties, this study has revealed the diversity that exists among the decomposer basidiomycetes present in decaying pine debris throughout New Zealand. It has also been beneficial in providing an assortment of wood colonising fungi, a selection of which will be tested for their effectiveness as potential contenders for controlling armillaria root disease.

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