DECAY POTENTIAL OF BASIDIOMYCETE FUNGI FROM *PINUS RADIATA*

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ABSTRACT

To study fungi causing decay of *Pinus radiata* in New Zealand, basidiomycetes were obtained from *P. radiata* wood, fruiting bodies on *P. radiata* and culture collections. Thirty-eight fungi were screened for decay potential using a wood block assay and a cellulase assay to identify those suitable for further study. Twenty isolates caused significant wood weight losses relative to uninoculated controls. Thirteen brown rot fungi produced the greatest weight losses (7.7-27.1%). Cellulolytic activity was exhibited by all fungi but was highest for six white rot fungi (0.18-0.35 μ moles glucose/min/ml). There was no correlation between wood weight loss and cellulolytic activity for the test fungi under these assay conditions.

Keywords: fungi, decay, cellulase, *Pinus radiata*.

INTRODUCTION

In New Zealand, there are 1.3 million hectares of *Pinus radiata* plantation forest (New Zealand Forest Owners Association 1996). Growth rates of *P. radiata* under New Zealand climate and soil conditions are the highest in the world (New Zealand Forest Industries Council 1992) which results in a greater sapwood depth than that of many Northern Hemisphere softwoods. The sapwood of *P. radiata* is highly susceptible to fungal decay with a life of less than two years in the ground and heartwood is also rated non-durable with a life of 5-10 years in the ground (Butcher and Drysdale 1991). Under suitable conditions, decay fungi can invade timber at any stage from felling and seasoning to processing and final use.

Wood decay fungi are predominantly from the subdivision Basidiomycotina. They invade wood cells and degrade cell wall components resulting in detrimental effects on strength and other wood properties (Rayner and Boddy 1988). There are two main types of decay fungi which are distinguished by the wood components that they degrade. White rot fungi have the ability to degrade cellulose, hemicellulose and lignin resulting in wood that is often fibrous with a bleached appearance. Brown rot fungi degrade only the polysaccharide components and residual wood is a brown lignin framework that is characterised by cubical shrinkage and wood collapse.

Economic losses due to wood decay are incurred through reductions in grade quality or losses of timber and the requirement to replace affected timber in structural use. Currently, *P. radiata* requires chemical preservative treatments for long-term durability. In the last 10-20 years, increasing environmental awareness and health concerns have prompted research on biological control to reduce or eliminate the use of fungicides (Freitag *et al.* 1991).

The first step in a biological control programme is to obtain a range of decay fungi from host plant material and develop methods to assess their decay potential under controlled conditions. Using this information, some of the fungi will be selected to challenge with potential biocontrol agents. This paper describes the isolation of basidiomycete fungi from a number of sources and reports on the results of two laboratory assays to determine decay potential.

MATERIALS AND METHODS

Collection of basidiomycete fungi

Wood and fruiting bodies were obtained from forestry sites in the central North Island, HortResearch trials and a national sapstain fungi survey being conducted by the University of Waikato. Basidiomycetes were isolated by plating fragments of wood or fruiting body onto benomyl malt agar (Adaskaveg *et al.* 1993). Standard aseptic laboratory procedures were used and all plates were incubated at 27°C. Emerging colonies were sub-cultured onto 2% malt agar (Difco) until pure cultures were obtained. Cultures possessing clamp connections or those identified by other features characteristic of basidiomycetes (Nobles 1965) were selected for screening.

Seven basidiomycete cultures were isolated from wood and 18 were isolated from fruiting bodies (Table 1). Eleven were identified prior to screening (in collaboration with Forest Research, Rotorua) and the remaining cultures have not been identified. Eleven fungi, isolated from *P. radiata*, were sourced from the Forest Research culture collection and two from Landcare Research New Zealand Limited (Auckland). All identified cultures were from the Order Aphyllophorales (Class Hymenomycetes).

Wood block assay

The assay was carried out in 500ml glass jars, each with a cottonwool insert in the lid to provide aeration. There were three replicate jars for each test fungus and six uninoculated control jars. A 9cm Whatman #2 filterpaper was placed on the bottom of each jar with 10ml of distilled water and a steel grid stand was positioned over the filterpaper. The jars were autoclaved at 121°C for 20 min. Fresh untreated *P. radiata* cubes (20mm) were equilibrated for two days at 25°C, labelled, and weighed prior to gamma irradiation of 27.7kGy (Mallinckrodt Veterinary Ltd, Lower Hutt). Internal and external fragments from irradiated cubes were plated onto 2% malt agar and showed effective sterilisation with no visible growth in 14 days. Three cubes were placed on the stand in each jar. Sterile distilled water (3 ml) was added to each cube prior to inoculation with a 14mm core of a 14 d fungal culture on 2% malt agar. Uninoculated control cubes were similarly treated but inoculated with a core of sterile agar. The jars were incubated at 25°C in the dark for 126 days. External mycelium on each cube was brushed off and weighed after drying at 100°C. Mean wood weight loss by each test fungus was evaluated on the basis of final cube dry weights relative to uninoculated controls. Examination of the cubes was used to categorise the test fungi into brown or white rot fungi (Nobles 1965).

Cellulase assay

Cellulolytic activity was determined by measuring reducing sugars using a p-hydroxybenzoic acid hydrazide (PAHBAH, Sigma) method (Lever 1973). Tryptone-yeast extract nutrient medium (Rainey 1992) was used with carboxymethylcellulose (CMC, Aldrich) as a carbon source. There were duplicate flasks of 100 ml medium for each test fungus. Flasks were inoculated with 5ml of culture grown for 3 d at 25°C in yeast malt broth (2g yeast, Difco; 15g malt). The flasks were incubated at 25°C at 140 rpm. Samples of culture supernatant (1 ml) were taken at 1, 2, 4 and 8 days after inoculation. The highest cellulase activity over the five sampling times was used for comparative purposes.

Cellulase activity was measured in a reaction mixture containing 0.02-0.10 ml of culture supernatant and 0.30 ml of 1% (w/v) CMC in 100mM citrate buffer, pH 6. The mixture was incubated at 50°C for 15 min before the reaction was stopped by addition of 1 ml PAHBAH reagent and boiling for 6 min. Absorbance of the reaction mixture was read at 420 nm. Cellulase activity was expressed as carboxymethylcellulase units (U) which are defined as $\mu\text{moles glucose/min/ml}$ against reagent blanks and in relation to glucose standards.

Mycelial biomass accumulation in liquid culture was determined at eight days by vacuum-extraction onto pre-dried (at 80°C) and weighed Whatman #2 filterpapers.

Assay analyses

Regression analysis was used to determine any correlation between the wood block and the cellulase assays. For the wood block assay, the final dry weight of each cube was adjusted using the initial equilibrium weight as a covariate to take account

of initial variation between cubes due to differences in size and density. Dunnett's test (Dunnett 1955) was used to compare the mean wood weight loss by each test fungus with the uninoculated control.

RESULTS

Wood weight loss caused by the test fungi ranged from -0.1-27.1%, relative to uninoculated controls (Table 1). Twenty isolates caused significant weight losses of 6.2% or greater ($P < 0.05$). The 13 isolates causing the highest weight losses (7.7-27.1%) were all brown rot fungi and included cultures of *Gloeophyllum sepiarium*, *Antrodia serialis*, *Oligoporus leucomallellus*, *Poria* sp., *Coniophora olivacea* and other unidentified isolates. Mean wood weight loss by the brown rot fungi (13%) was significantly higher (SED 1.86) than the white rot fungi (3.8%) but the variation was much greater for the brown rot fungi. The 18 isolates which did not cause significant weight losses included cultures of the white rot fungi *Schizophyllum commune*, *Resinicium bicolor*, *Pycnoporus coccineus* and other unidentified isolates.

TABLE 1: Source of basidiomycete fungi, wood weight loss and cellulase activity.

Strain No.	Name	Source ¹	Origin ²	Location	Rot ³	Wood weight loss (%)	Cellulase activity (U)
1	<i>Antrodia serialis</i>	LR	wood	Waipoua Forest	BR	15.9	0.06
19	<i>Coniophora olivacea</i>	FR	wood	Kaingaroa Forest	BR	7.7	0.03
123	<i>Ganoderma</i> sp.	FR	fb	Te Wera Forest	WR	6.6	0.02
4	<i>Gloeophyllum sepiarium</i>	AAC	fb	HortResearch trial	BR	19.3	0.17
14	<i>Gloeophyllum sepiarium</i>	AAC	fb	HortResearch trial	BR	20.1	0.04
5	<i>Gloeophyllum sepiarium</i>	AAC	fb	HortResearch trial	BR	20.9	0.10
15	<i>Gloeophyllum sepiarium</i>	AAC	fb	HortResearch trial	BR	22.7	0.04
122	<i>Hapalopilus nidulans</i>	FR	wood	Parengarenga	WR	3.8	0.00
121	<i>Junghuhnia vineta</i>	FR	wood	Matahina	WR	6.2	0.16
117	<i>Oligoporus leucomallellus</i>	FR	wood	Omataroa	BR	14.3	0.08
124	<i>Pycnoporus coccineus</i>	LR	wood	Waipoua Forest	WR	4.2	0.23
101	<i>Pycnoporus coccineus</i>	AAC	fb	East Coast	WR	5.1	0.12
103	<i>Phlebiopsis gigantea</i>	AAC	fb	Kinleith Mill	WR	4.6	0.13
130	<i>Phlebiopsis gigantea</i>	AAC	wood	Kinleith Forest	WR	6.2	0.09
119	<i>Phlebiopsis gigantea</i>	FR	wood	Berwick Forest	WR	6.9	0.23
104	<i>Phlebiopsis gigantea</i>	AAC	fb	Waipa Mill	WR	7.0	0.10

Table 1 continued over

Table 1 continued

Strain No.	Name	Source ¹	Origin ²	Location	Rot ³	Wood weight loss (%)	Cellulase activity (U)
118	<i>Gloeocystidiella socratum</i>	FR	wood	Velva Std	WR	4.3	0.02
17	<i>Poria</i> sp.	FR	wood	Wenita Forest	BR	12.7	0.06
120	<i>Resinicium bicolor</i>	FR	wood	Kaingaroa Forest	WR	3.4	0.04
108	<i>Resinicium bicolor</i>	AAC	fb	Kinleith Forest	WR	3.6	0.03
3	<i>Schizophyllum commune</i>	AAC	fb	Waipa Mill	WR	0.5	0.35
2	<i>Schizophyllum commune</i>	AAC	fb	Waipa Mill	WR	0.7	0.34
18	<i>Sistotrema brinkmannii</i>	FR	wood	Kinleith Forest	WR	1.7	0.04
116	<i>Stereum sanguinolentum</i>	FR	wood	Port Charles	WR	6.4	0.07
111	Unidentified	AAC	wood	Kinleith Forest	BR	2.2	0.10
106	Unidentified	AAC	fb	Kinleith Forest	BR	2.7	0.07
107	Unidentified	AAC	wood	Kinleith Forest	BR	3.7	0.12
11	Unidentified	AAC	fb	HortResearch trial	BR	4.1	0.03
109	Unidentified	AAC	fb	Kinleith Forest	BR	4.2	0.17
110	Unidentified	AAC	wood	Kinleith Forest	BR	6.9	0.04
8	Unidentified	AAC	fb	East Coast	BR	9.5	0.11
112	Unidentified	AAC	fb	HortResearch trial	BR	13.6	0.08
10	Unidentified	AAC	fb	Mamaku Forest	BR	19.1	0.15
9	Unidentified	AAC	fb	HortResearch trial	BR	19.5	0.04
12	Unidentified	AAC	wood	HortResearch trial	BR	27.1	0.13
6	Unidentified	AAC	wood	HortResearch trial	WR	-0.1	0.18
7	Unidentified	AAC	fb	Kinleith Forest	WR	0.7	0.07
113	Unidentified	AAC	wood	unknown	WR	1.2	0.23

¹ AAC- A. Ah Chee; FR- Forest Research; LRNZL- Landcare Research

² Wood or fruiting body (fb) isolation material

³ BR- brown rot fungi; WR- white rot fungi

Cellulase activity for the test fungi ranged from 0-0.35 U (Table 1). The six highest producers of cellulase (0.18-0.35U) were all white rot fungi (*S. commune* isolates 2 and 3, *Phlebiopsis gigantea* 119, *P. coccineus* 124 and unidentified isolates 113 and 6). Mean cellulase activity and mycelial biomass of the brown rot fungi (0.08U and 56mg) was lower than the white rot fungi (0.13 U and 65 mg) but this was not significant in either case (SED 0.027 and 9.9, respectively) and the variation was much greater for the white rot fungi.

Regression of wood weight loss versus cellulolytic activity for all of the test fungi did not show any correlation. The same regressions applied to brown rot and white rot fungi groupings also showed no correlations. The 13 brown rot isolates causing the highest wood weight losses exhibited cellulase activity of only 0.04-0.17 U. Of the six white rot isolates producing the highest cellulase activity, five caused statistically

insignificant wood weight losses (-0.1-4.2%) and the other isolate, *P. gigantea* 119, produced a weight loss of only 6.9%.

DISCUSSION

In this study, there was no correlation between the wood block assay and the cellulase assay. Although this implies that the potential of the test fungi to cause weight loss in wood was not related to their ability to produce cellulase in liquid culture, both assays were carried out under only one set of conditions which may not have been optimal for each test fungus. White and brown rot fungi are reported to have different requirements for wood decay (Hedley and Foster 1972) and this may also be the case for cellulase production in liquid culture.

In the wood block assay, conditions favoured decay by the brown rot fungi. Conditions conducive to decay by white rot fungi may include relatively high wood moisture content and nutrient supplementation (Highley and Scheffer 1970). In this study, conditions were closer to those likely to be encountered in the field compared to the standard American Society for Testing and Materials method (ASTM 1986) where soil is used, as a moisture retaining substrate and a nutrient source, and wood cubes are steam-sterilised. As a consequence, the wood weight losses here were less than those commonly reported. For example, Hedley and Foster (1972) reported weight losses of 12%, 16% and 60% for *P. coccineus*, *C. olivacea* and *Gloeophyllum trabeum*, respectively whereas in this study there were losses of 4-5%, 8% and 19-23% (for the related *G. sepiarium*), respectively.

In the cellulase assay, the ability of the test fungi to grow in liquid culture and the medium employed may have influenced enzyme production and accumulation. Conditions appeared to favor cellulolytic activity and growth by the white rot fungi. Previous fungal cellulase research has used different methods and organisms so comparisons of results from this study were not possible.

The wood block assay most resembled the field situation so it was primarily on the basis of this assay that the following fungi were chosen for further study: *G. sepiarium* 5, *P. gigantea* 104 and *S. commune* 2. These species are commonly reported on *P. radiata* (Butcher 1968). In future, the 18 week incubation period of the wood block assay may be reduced to 70-84 days by incorporating aspects of the ASTM method and using smaller wood blocks. Further studies with the chosen fungi will include characterisation and comparison of growth and decay on agar and wood followed by biological control investigations.

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