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Culture characteristics of *Antrodia xantha*, *Coniophora olivacea* and *C. puteana*

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Summary. – The effect of temperature and water potential on extension growth, and wood degradation capacities of the Aphyllophorales *Coniophora olivacea, C. puteana* (Coniophoraceae) and *Antrodia xantha* (Polyporaceae) were studied. Extension growth of these species was maximum at 25 °C and inhibited at 37 °C. Mycelial extension growth was also examined at seven NaCl and KCl mediated osmotic potentials. Growth was maximum at –0.5 and –1.0 MPa and decreased at reduced potentials. *A. xantha* was most sensitive, and *C. olivacea* least, to decreasing water potentials. Little growth occurred in the species at –3.0 MPa.

The decay capacity of cultured species was evaluated, based on absolute weight loss, on unprocessed *Eucalyptus grandis, Pinus patula* and *Pterocarpus angolensis* blocks using a modification of the soil/wood-block test method. Significant inter- and intra-host differences in the decay capacities of fungi were found. *P. angolensis* was degraded most after 12 weeks, by *C. olivacea.*

Résumé. – L'auteur a étudié l'effet de la température et de la teneur en eau sur la croissance radiale et la capacité à dégrader le bois par les champignons Aphyllophorales, *Coniophora olivacea, C. puteana* (Coniophoraceae) et *Antrodia xantha* (Polyporaceae). La croissance de ces espèces est maximale à 25 °C et inhibée à 37 °C. L'accroissement en diamètre du mycélium a aussi été étudié à sept valeurs de la pression osmotique obtenues par des concentrations différentes de NaCl et KCl. La croissance est maximale à –0.5 et –1.0 MPa, elle diminue à des valeurs plus basses. *A. xantha* est le plus sensible, *C. olivacea* le moins, à la diminution de la teneur en eau. On ne constate qu'une faible croissance à –3.0 MPa.

La capacité à dégrader le bois par les souches en culture de ces espèces a été évaluée en mesurant la perte absolue de poids de blocs non traités d'*Eucalyptus grandis, Pinus patula* et *Pterocarpus angolensis,* selon la technique modifiée du test sol/morceau de bois. Des différences significatives ont été trouvées, tant en comparant la capacité de dégradation des espèces sur un même hôte que celle des espèces étudiées sur les trois hôtes proposés. Le bois

le plus dégradé, après 12 semaines d'incubation, était celui de *P. angolensis* inoculé par le mycélium de *C. olivacea.*

Key words: Basidiomycetes, physiological studies, wood decay

Introduction

Temperature and moisture are the major environmental factors influencing wood decomposition (Gilbertson, 1980; Boddy, 1983). Temperature affects various enzymatic reactions and the observed response by a fungus is a summation of these effects (Rayner & Boddy, 1988). The effect of temperature on extension growth of Aphyllophorales in culture has been studied by many workers (Humphrey & Siggers, 1933; Cartwright & Findlay, 1934; Davidson & Lombard, 1953; Jansen 1969; Niemelä, 1977; Boddy, 1983; Lombard, 1990).

Though water acts as a solvent for metabolic processes and participates in some of them, relatively few studies have addressed the effect of water potential on the growth of Aphyllophorales (Wilson & Griffin, 1979; Griffin, 1981; Boddy, 1983; Koske & Tessier, 1986). In culture, the desired osmotic potentials can be obtained by the addition of solutes (Tresner & Hayes, 1971; Boddy, 1983; Rayner & Boddy, 1988). Matric potential is the predominant water potential component in wood (Griffin, 1981). Theoretically, fungi exhibit similar response patterns to osmotic and matric potential effects (Griffin, 1972).

Tests involving the growth of fungi on wood are widely used in studies of decay rates and in assaying toxicity of preservative fungicides (American Society for Testing and Materials (ASTM), 1986; European Committee for Standardisation (CEN), 1982).

Weight loss is the most common indicator of the rate of decay. Other relatively novel and specialised methods for determining weight loss involve the use of impaction resistance or loss of tensile strength (Rayner & Boddy, 1988).

The studies reported here were part of a broad project on the taxonomy (Masuka & Ryvarden, 1992), ecology (Masuka, 1992a) and biology of Aphyllophorales in *Pinus* and *Eucalyptus* plantations in Zimbabwe (Masuka, 1992b). This paper reports on the results of temperature, water potential and wood degradation studies of three common saprotrophs, *Antrodia xantha* (Fr.) Ryv.(syn. *Poria xantha*), *Coniophora olivacea* (Fr.:Fr.) Karst. and *C. puteana* (Fr.) Karst. (syn. *C. cerebella*), found on pines in Zimbabwe.

Materials and Methods

Experiments were based on seven-day old isolates from samples in Table 1, kept in the dark at 25 °C.

Growth response to temperature

The medium used throughout was 2% (w/v) malt extract agar (MEA) unless stated otherwise. Cubes of about 4 mm^3 colonised MEA were cut and transferred to the edges of Petri dishes. Four Petri dishes per isolate were incubated at 15, 20, 25, 30 and 37 °C. Radial growth was measured along two diameters at right angles to each other every second day for two weeks. Petri dishes with no visible growth after two weeks were re-incubated at 25 °C for the same period. Thereafter, absence of growth was taken as evidence that the isolate had been killed at the first temperature. Data were analysed using the Student's *t*-test (Sokal & Rohlf, 1981).

Isolates failing to grow at 37 °C were incubated in triplicate at that temperature for 3, 6, 9, 12 or 15 days before being transferred to 25 °C. The subcultures were examined daily for growth, for up to two weeks. The absence of visible growth from the cubes into the medium was taken as evidence that the isolate had been killed.

Growth response to varying osmotic potentials

Sodium chloride (NaCl) and potassium chloride (KCl) were used to obtain the desired osmotic potentials in the medium. The composition of the basal medium (BM) was 7 g malt extract, 1 g Difco Bacto peptone, 0.5 g Difco Bacto yeast extract and 15 g agar in 1 l distilled water. The amount of NaCl or KCl required to achieve a required osmotic potential (MPa) was calculated from Koske and Tessier's (1986) formula:

$$g \text{ of KCl } L^{-1} \text{ of BM} = (\text{water potential} - 0.14) * 1.595$$

The concentration of NaCl used was previously determined, based on the same formula, using a dew-point microvoltmeter (Masuka, 1992b).

Media ingredients were weighed precisely to 1 mg. The media were covered, autoclaved at 121 °C at a pressure of one bar for 20 min and left to cool to 20 °C before pouring plates. Mycelial cubes of isolates of *A. xantha, C. olivacea* and *C. puteana* were placed near the edges of Petri dishes. There were three replicates of each isolate and two isolates per species tested at –0.14, –0.5, –1.0, –1.5, –2.0, –2.5 and –3.0 MPa. Petri dishes were wrapped in parafilm and

Table 1. Origin of isolates

Species and isolate number	Country[1]	Locality[2]	Host[3]	Substrate
A. xantha				
(765/2)	N	Ås	Pa	log on the ground
(90027)	N	'Mt'	–	timber in a house
(AJM 203)	Z	Er	Pp	on burnt log
C. olivacea				
(AJM 426)	Z	Gw	Pp	log on the ground
(AJM 1001)	Z	St	Pp	log on the ground
C. puteana				
(76–77/2)	N	Ås	B	branch on the ground
(82–97/3)	N	Ås	Ps	log on the ground
(AJM 801)	Z	Ch	Pp	log on the ground

[1] N = Norway, Z = Zimbabwe; [2]Ås = Ås University of Agriculture, Ch = Chisengu; Er = Erin; Gw = Gwendingwe, 'Mt' = Mycoteam, St = Stapleford; [3] B = Betula sp., Pa = Picea abies, Pp = Pinus patula, Ps = Pinus sylvestris.

sealed in plastic bags before incubation at 25 °C in the dark for five days. Extension growth was measured along two diameters at right angles to each other after the incubation period. Student's *t*-tests (Sokal & Rohlf, 1981) were used to compare the growth of isolates of a species.

Wood degradation studies

The soil/wood-block test method (ASTM, 1986) was modified. Freshly cut branchwood-blocks (50 × 20–25 mm) of *Eucalyptus grandis* Hill ex Maid, *Pinus patula* Schiede & Deppe and *Pterocarpus angolensis* DC were dried in an oven at 105 °C (accurate to 2 °C) for 24 h and weighed (precise to 0.01 g) to determine the initial dry weights. The bark was not removed. The volumes of 10 blocks of each species were determined by the water displacement method (blocks wrapped in polythene film). Blocks were then immersed in water for four hours (and the 10 blocks of each species weighed) before being autoclaved at 121 °C for 40 min. The blocks were left to cool for three hours before each was separately placed on autoclaved 10–15 ml damp sand in 50 × 50 × 80 mm glass chambers. The chambers were previously sterilised in 2.5 % sodium hypochlorite for five minutes. Mycelial cubes of *A. xantha*, *C. olivacea* and *C. puteana* were inoculated onto the ends of blocks. There were 15 blocks of each fungus-host combination and an equal number of control blocks. *C. olivacea* and *C. puteana* were both inoculated, at opposite ends, on five *P. patula* blocks.

The chambers were partially wrapped in parafilm and incubated in the dark at 25 °C for 12 weeks. Every month five blocks of each treatment were cleaned of surface mycelium, dried at 105 °C for 24 h and weighed. Weight loss was calculated as the average of five replicates. Interspecies interaction test blocks were assessed after eight weeks. Data were analysed using the Kruskal-Wallis Test (Sokal & Rohlf, 1981).

Results

Growth response to temperature

The optimum temperature for growth was in the 20–30 °C range (Figure 1). Growth rates of the isolates were maximum at 25 °C. Generally, the effects of high temperatures (30–37 °C) on mycelial extension were more drastic than those of low temperatures (20–15 °C). Differences between isolates of a species (Student's *t*-test) were not significant.

C. puteana isolates did not grow at 25 °C following incubation at 37 °C. *A. xantha* and *C. olivacea* failed to grow at 25 °C after 15 days initial incubation at 37 °C (Figure 2). Longer incubation periods resulted in prolonged recovery.

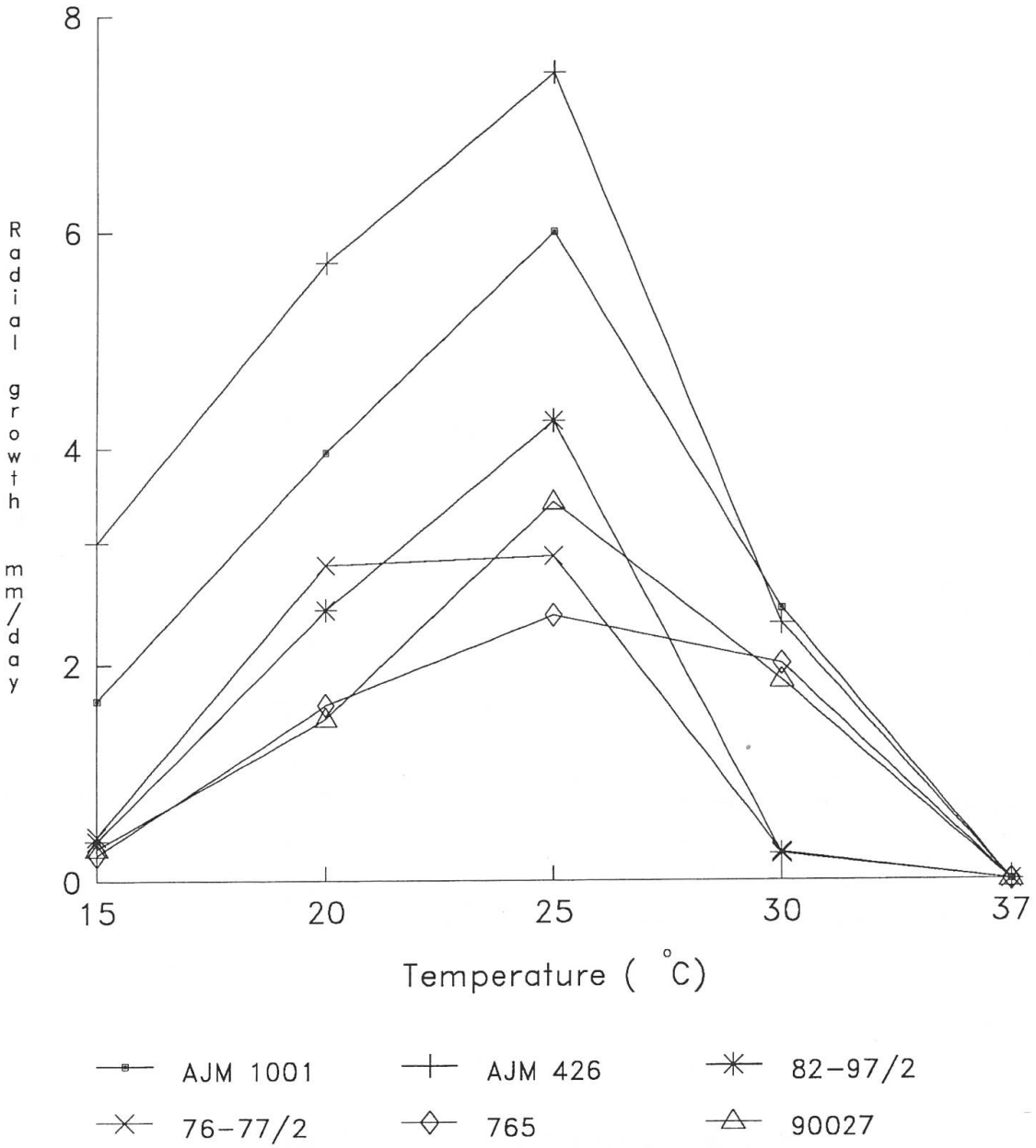

Figure 1. Daily linear growth rates of species at various temperatures; 765 and 90027 are Antrodia xantha, *AJM 426 and AJM 1001 are* Coniophora olivacea, *76–77/2 and 82–97/3 are* Coniophora puteana.

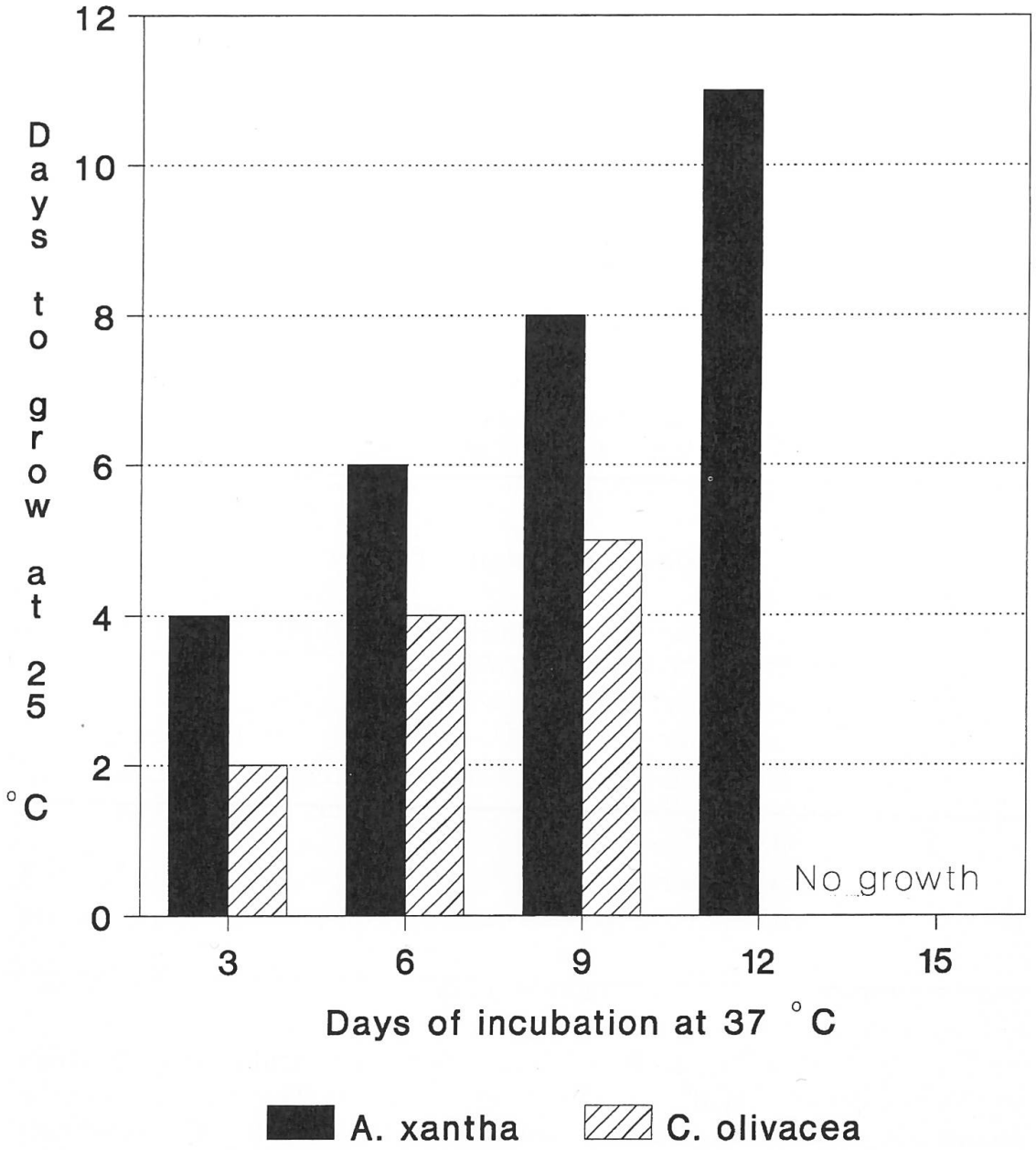

Figure 2. Growth of isolates at 25 °C following incubation at 37 °C.

Effect of osmotic potential on growth

The growth of *A. xantha*, *C. olivacea* and *C. puteana* at seven NaCl and KCl mediated osmotic potentials is shown in Figure 3 (a and b). *A. xantha* was most sensitive to low potentials, ceasing growth at –1.5 to –2.5 MPa. *C. olivacea* and *C. puteana* (76–77/2) still showed considerable growth at –3.0 MPa. The general response of isolates was similar on NaCl and KCl amended media. There was an initial stimulation of growth up to –1.0 MPa, except in *A. xantha* (765), on NaCl amended media. On KCl amended media no stimulation occurred in *A. xantha* (765) and *C. puteana* (82–97/3). There was a decrease in growth in both *C. olivacea* isolates from –0.14 to –0.5 MPa then a stimulation to –1.0 MPa followed by a gradual decline in growth at lower osmotic potentials on KCl amended media. Comparisons (Student's *t*-tests) of growth of each isolate on the two media and isolates of a species on either media showed no significant differences.

Rates and amounts of decay

Variation among wood blocks of a species, with respect to recorded parameters, is evident from the standard errors (Table 2).

Table 3 shows a summary of the states of decay (weight loss) of *E. grandis*, *P. angolensis* and *P. patula* after 4, 8 and 12 weeks. Within-isolate variation, even on the same host, was large. The highest weight loss occurred in *P. angolensis* blocks inoculated with *C. olivacea*. *C. olivacea* consistently degraded wood most and *C. puteana* least.

P. patula blocks inoculated with *A. xantha* or *C. olivacea* were softened and perforated at the ends, after 12 weeks. Within-host differences in decay among fungi were not significant in *P. patula* at the first assessment (Table 4). There were significant among-host differences in decay capacities after eight weeks following inoculation with *A. xantha*.

There was greater weight loss in blocks inoculated with both species than in blocks inoculated with only one species (Table 5). The differences in the amounts of decay among the three subsets were subjected to a Kruskal-Wallis test and found to be significant ($P = 0.05$, $H = 5.791$).

Discussion

Temperature and osmotic potential studies

The extension growth rate of *C. puteana* and *A. xantha* at various temperatures were in a range previously reported for these species (Humphrey & Siggers, 1933; Loman, 1962; Davidson & Lombard, 1953; Rayner & Boddy 1988).

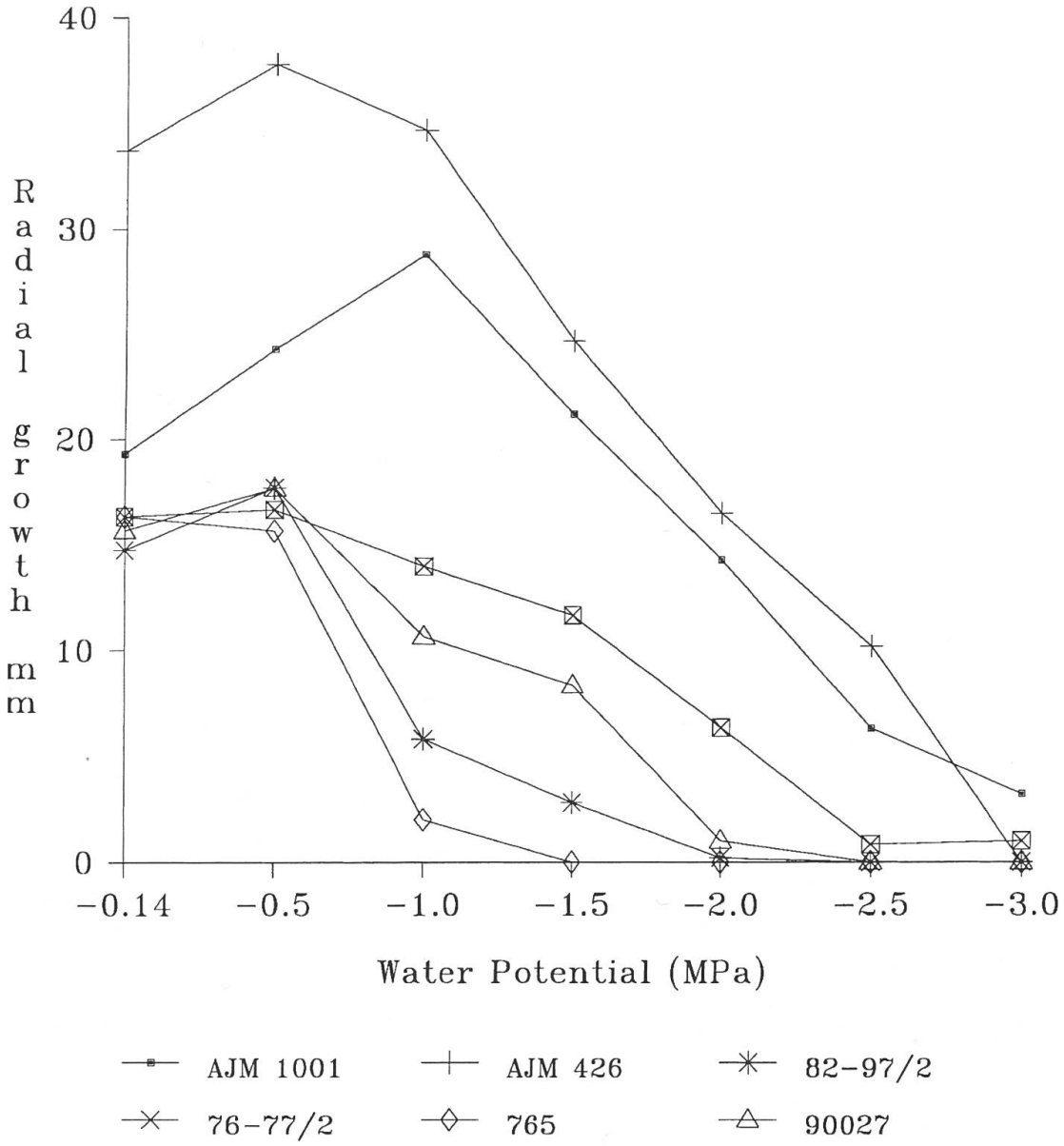

Figure 3a. Effect of water potential on growth; a) NaCl amended media, b) KCl amended media. Isolates are: 765 and 90027 are Antrodia xantha; *AJM 426 and AJM 1001 are* Coniophora olivacea; *and 76–77/2 and 82–97/3 are* C. puteana

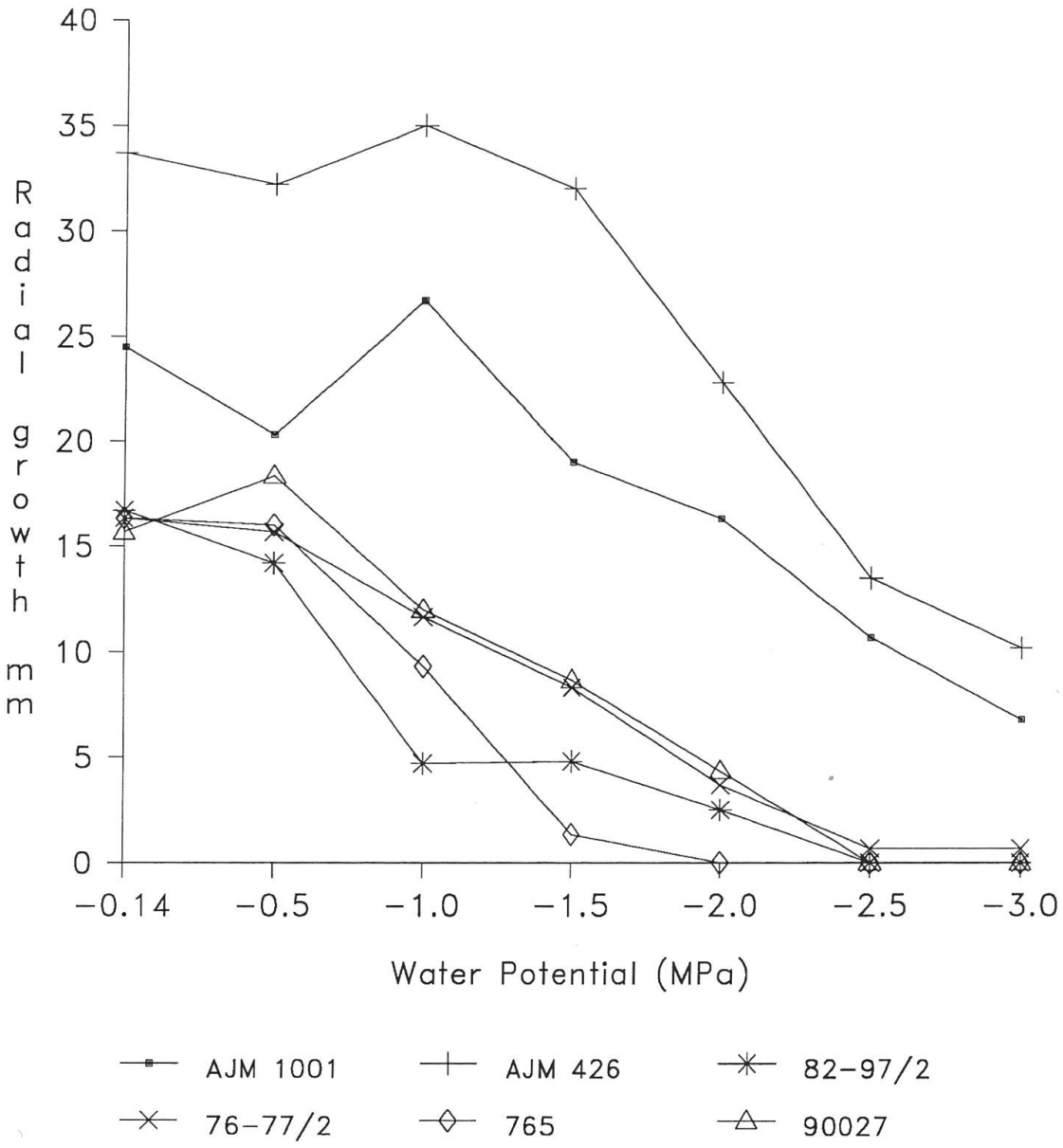
40
35
30
25
20
15
10
5
0
Radial growth mm
−0.14
−0.5
−1.0
−1.5
−2.0
−2.5
−3.0
Water Potential (MPa)
AJM 1001
AJM 426
82−97/2
76−77/2
765
90027

Figure 3b.

Table 2. Pre-treatment data for *E. grandis, P. angolensis* and *P. patula*.

Parameter	Species		
	E. grandis	*P. angolensis*	*P. patula*
Dry weight (g)			
mean	10.123	8.086	10.138
SE	0.431	0.416	0.684
Weight after immersion in water (g)			
mean	13.446	9.424	13.632
SE	0.544	0.444	0.927
Volume (cm)			
mean	19.750	17.250	17.250
SE	1.042	0.757	0.724
Density (g/cm)			
mean	0.515	0.469	0.584
SE	0.008	0.015	0.019
Moisture content (w/w)			
mean	0.346	0.168	0.347
SE	0.074	0.010	0.039

Table 3. Mean decay, adjusted for the control, after 4, 8 and 12 weeks.

Species	Assessment (weeks)	Amount of decay (g)		
		E. grandis	*P. angolensis*	*P. patula*
Control	4			
mean		0.092	0.075	0.059
SE		0.022	0.036	0.020
	8			
mean		0.079	0.067	0.047
SE		0.042	0.015	0.006
	12			
mean		0.141	0.144	0.156
SE		0.026	0.098	0.033
A. xantha	4			
mean		0.286	0.190	0.376
SE		0.046	0.061	0.079
	8			
mean		1.493	1.289	1.657
SE		0.103	0.212	0.109
	12			
mean		1.980	1.793	2.408
SE		0.299	0.253	0.146
C. olivacea	4			
mean		0.741	0.651	0.549
SE		0.122	0.083	0.107
	8			
mean		1.605	2.015	1.109
SE		0.162	0.438	0.368
	12			
mean		2.442	3.152	2.650
SE		0.450	0.507	0.607
C. puteana	4			
mean		0.293	0.161	0.313
SE		0.059	0.013	0.144
	8			
mean		0.339	0.478	0.340
SE		0.064	0.087	0.188
	12			
mean		0.770	0.486	0.583
SE		0.307	0.180	0.144

Table 4. Kruskal-Wallis analysis of decay capacities of fungi on different hosts.

Parameter	H value for assessment times		
	4 weeks	**8 weeks**	**12 weeks**
E. grandis	5.7179*	9.4200***	6.7400**
P. angolensis	9.3800***	5.9500**	7.0342**
P. patula	1.6200$^{n.s.}$	7.5800**	5.5029*
A. xantha	3.7800$^{n.s.}$	8.2900***	1.6429$^{n.s.}$
C. olivacea	1.9714$^{n.s.}$	1.8200$^{n.s.}$	1.6200$^{n.s.}$
C. puteana	2.3079$^{n.s.}$	3.6200$^{n.s.}$	0.5429$^{n.s.}$
Control	3.6200$^{n.s.}$	0.9150$^{n.s.}$	1.9630$^{n.s.}$

$^{n.s.}$ not significant at $P \leq 0.10$; * significant at $P \leq 0.10$; ** significant at $P \leq 0.05$; and *** significant at $P \leq 0.01$.

Table 5. Weight loss in double-inoculated *P. patula* blocks

Species	Weight loss	SE
C. olivacea	1.104	0.588
C. puteana	0.336	0.058
C. olivacea vs. *C. puteana*	1.238	0.207
Control	0.047	0.006

Previous studies of the response of *C. olivacea* could not be found in the literature. The species tested, like most basidiomycetes, are mesothermic (Rayner & Boddy, 1988).

The inability of *C. olivacea* to grow at 25 °C following initial incubation at 37 °C for 12 and 15 days clearly indicates that mycelium of the fungus does not survive periods of heat stress.

Basidiomycetes, generally, show low tolerance to decreasing osmotic potentials (Griffin, 1981; Boddy, 1983; Koske & Tessier, 1986) compared to other fungi (Hocking & Pitt, 1979). Of nine Basidiomycetes tested by Koske and Tessier (1986) only one grew at –4.0 MPa (about 25–30 % wood moisture content). The cessation of growth at low osmotic potentials might be due to a combination of reduced water availability and solute toxic effects.

The ecological significance of present studies is limited because laboratory studies cannot simulate field conditions though in some studies (Rayner & Todd, 1979) apposite patterns have been observed both in culture and nature.

Assessment, rate and amount of decay

The fungi tested in this study were selected for various reasons. *C. olivacea* was widespread in pine plantations. *C. puteana* was not as common but was collected on timber in use and at field timber depots. *A. xantha* was included as a reference species, being found on both pines and eucalypts. *P. angolensis* is indigenous, but not endemic, to Zimbabwe. *P. patula* is native to Mexico while *E. grandis* is native to Australasia. None of the test fungi have been recorded on *P. angolensis*. It was thought worthwhile to ascertain whether growth and decay by the fungi would be inhibited under optimal growth conditions.

Previous work on wood degradation with *C. puteana* (ASTM, 1986; CEN, 1982) was aimed at determining the toxic effects of preservative chemicals and was conducted on processed wood blocks with a large inoculum presence. There are no previous studies employing unprocessed host and non-host wood blocks. Unprocessed wood is ideal for tests aimed at ascertaining the potential decay capacities of fungi in nature and in screening species for biological control of decay fungi.

The four-hour soaking of wood blocks proved to be adequate, and a reserve moisture supply in the sand provided an ideal moisture content regime for wood degradation for the duration of the experiment. A major disadvantage, if the experiment is prolonged, might be the build up of unfavourable carbon dioxide:oxygen ratios in the chambers. In future studies changes in the moisture contents of sand and blocks should be monitored as they certainly affect the rate of decay.

It was unexpected that non-host wood would be degraded to the same or greater extent as host wood. The results may indicate the adaptational capacity and hence versatility of the biochemistry of the enzyme systems for wood degradation. Some caution in the interpretation of the data is required as decay in blocks inoculated with both *C. olivacea* and *C. puteana* was not additive, and culture studies cannot provide absolute evidence of the behaviour of fungi in nature. Interaction with other fungi and environmental factors possibly play a more significant role in determining the type, rate and amount of decay.

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References

American Society for Testing and Materials (ASTM) (1986). Standard method of testing wood preservatives by laboratory soil block cultures. *ASTM Committee on Wood*, D 1413–76. pp. 240–246.

Blumenfeld, S. N. (1984). Studies on the degradation of pine wood blocks by Aphyllophorales. Materials und Organismen 19, 153–262.

Boddy, L. (1983). Effect of temperature and water potential on growth rate of wood-rotting Basidiomycetes. *Transactions of the British Mycological Society* **80** (1), 141–149.

Cartwright, K. St G. & Findlay, W. P. K. (1934). Studies in the physiology of wood destroying fungi II. Temperature and rate of growth. *Annals of Botany* **48**, 481–496.

Cartwright, K. St G. & Findlay, W. P. K. (1958). Decay of Timber and its Prevention. London: Her Majesty's Stationery Office.

Davidson, R. W. & Lombard, F. F. (1953). Large-spored brown house-rot fungi in the United States. *Mycologia* **45**(2), 88–100.

European Committee for Standardisation (CEN) (1982). Wood preservatives – Determination of the toxic values against wood destroying Basidiomycetes cultured on an agar medium. *European Standard EN 113*. 15 pp.

Gilbertson, R. L. (1980). Wood-rotting fungi of North America. *Mycologia* **72**(1), 1–42.

Griffin, D. M. (1972). Ecology of soil fungi. Chapman and Hall: London, UK.

Griffin, D. M. (1981). Water and Microbial Stress. Advances in Microbial Ecology **5**, 91–136.

Hocking, A. D. & Pitt, J. I. (1979). Water potential relations of some *Penicillium* species at 25 °C. *Transactions of the British Mycological Society* **73**, 141–145.

Humphrey, L. J. & Siggers, P. V. (1933). Temperature relations of wood-destroying fungi. *Journal of Agricultural Research* **42**(12), 997–1008.

Jansen, F. K. (1969). Effect of constant and fluctuating temperature on the growth of four wood-decaying fungi. *Phytopathology* **59**, 645–647.

Koske, R.E. & Tessier, B. (1986). Growth of some wood and litter-decay Basidiomycetes at reduced water potential. *Transactions of the British Mycological Society* **86**(1), 156–158.

Loman, A. (1962). The influence of temperature on the location and development of decay fungi in Lodgepole pine logging slash. *Canadian Journal of Botany* **40**, 1545–1559.

Lombard, F. F. (1990). A cultural study of several species of *Antrodia* (Polyporaceae, Aphyllophorales). *Mycologia* **82**(2), 185–191.

Masuka, A. J. (1992a). Aspects of the ecology of Aphyllophorales in pine and eucalypt plantations in Zimbabwe. *Kirkia* **14**(1), 125–144.

Masuka, A. J. (1992b). Taxonomy of Aphyllophorales and their role in wood degradation in pines and eucalypts in Zimbabwe. D.Phil. thesis. University of Zimbabwe.

Masuka, A. J. and Ryvarden, L. (1992). Aphyllophorales on *Pinus* and *Eucalyptus* in Zimbabwe. Mycotaxon **44**(1), 243–250.

Niemelä, T. (1977). The effect of temperature on the two culture types of *Phellinus tremulae* (Fungi, Hymenochaetaceae). *Annals of Botany Fennici* **14**, 21–24.

Rayner, A.D.M. & Boddy, L. (1988). *Fungal Decomposition of Wood. Its Ecology and Biology.* John Wiley and Sons: Chichester, UK.

Rayner, A. D. M. & Todd, N. K. (1979). Population and community structure and dynamics of fungi in decaying wood. *Advances in Botanical Research*, **7**, 333–420.

Sokal, R. L. & Rohlf, F. J. (1981). *Biometry.* 2nd. Ed. W. H. Freeman and Company: New York, USA.

Thompson, W. & Rayner, A. D. M. (1983). Extent, development and function of mycelial cord systems in soil. Transactions of the British Mycological Society, 81(2) 337–345.

Tresner, H. D. & Hayes, J. A. (1971). Sodium chloride tolerance of terrestrial fungi. *Applied Microbiology* **22**, 210–213.

Trinci, A. P. J. (1969). A kinetic study of Aspergillus nidulans and other fungi. Journal of General Microbiology **57**, 11–24.

Wilson, J. M. & Griffin, D. M. (1979). The effect of water potential on the growth of some soil Basidiomycetes. *Soil Biology and Biochemistry* **11**, 211–212.