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David J. Midgley · Lyndon A. Jordan · Jennifer A. Saleeba · Peter A. McGee

Utilisation of carbon substrates by orchid and ericoid mycorrhizal fungi from Australian dry sclerophyll forests

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Abstract The utilisation of a range of cell-wall-related and aromatic carbon substrates by multiple genotypes of three ericoid mycorrhizal fungal taxa was compared with two orchid mycorrhizal fungal taxa. Both groups of fungi catabolised most common substrates, though significant inter- and intraspecific variability was observed in the use of a few carbon substrates. Orchid mycorrhizal fungi had limited access to tannic acid as a carbon source and did not use phenylalanine, while the ericoid mycorrhizal fungi used both. Utilisation of tryptophan was limited to single genotypes of each of the orchid mycorrhizal fungi, and to only two of the three ericoid mycorrhizal fungi examined. Although broadly similar, some significant differences apparently exist in carbon catabolism of ericoid and orchid mycorrhizal fungi from the same habitat. Functional and ecological implications of these observations are discussed.

Keywords Orchidaceae · Ericaceae · Mycorrhiza · Carbon utilisation · Competition

Introduction

Mycorrhiza increase access to minerals in soil and are crucial to the success of the families Ericaceae and Orchidaceae globally (Read 1991; Smith and Read 1997; Cairney and Meharg 2003). Soils of Australian dry sclerophyll woodlands and heathland vegetation are of poor nutrient status. In particular, nitrogen and phosphorus are scarce and associated with the organic fraction of the soil (King and Buckney 2002). The capacity of mycorrhizal fungi to access mineral nutrients stored in organic matter may determine the survival of ericaceous plants and orchids in depauperate Australian soils.

D. J. Midgley (✉) · L. A. Jordan · J. A. Saleeba · P. A. McGee
School of Biological Sciences, Macleay Building (A12),
University of Sydney,
2006 NSW Sydney, Australia
e-mail: dmidgley@bio.usyd.edu.au
Tel.: +61-2-93518691
Fax: +61-2-93514471

Most information on functional aspects of ericoid mycorrhizal (ERM) fungi was derived from studies of single isolates of the northern hemisphere ERM fungus *Rhizoscyphus ericae* (DJ Read) WY Zhuang & Korf. ERM fungi improve access by host plants to nitrogen and phosphorus sources (Leake and Read 1997). ERM fungi express cellulytic, hemicellulytic and pectinolytic enzymes, along with phenol oxidase activities, which may facilitate host access to nitrogen and phosphorus within moribund plant material or from polyphenol complexes in soil (Perotto et al. 1993, 1997; Varma and Bonfante 1994; Bending and Read 1996a,b; Burke and Cairney 1997a, b; Cairney and Burke 1998; Piercey et al. 2002). The array of hydrolytic enzymes produced by ERM fungi may also enable limited saprotrophic growth in the absence of the host (Perotto et al. 1993, 1997). Australian ERM fungi are, by comparison, relatively poorly studied. Multiple genotypes of two ERM fungi from Australian epacrids utilise cellobiose, crystalline cellulose, glucose, galactose, mannose, starch and xylan as sole sources of carbon for growth in axenic culture, indicating that they have broadly similar saprotrophic capabilities to northern hemisphere ERM fungi (Midgley et al. 2004a). Australian terrestrial orchids associate with a range of diverse, primarily basidiomycetous, fungi (Warcup 1971, 1981; Bougoure et al. 2005). The largest assemblage includes taxa formerly assigned to the form genus *Rhizoctonia*, many of which have teleomorphs in *Tulasnella*, *Sebacina*, *Ceratobasidium* and *Thanatephorus* (Rasmussen 1995).

Studies based primarily on single isolates of predominately northern hemisphere orchid mycorrhizal (OM) fungi indicate utilisation of arabinose, cellobiose, cellulose, glucose, galactose, maltose, mannose, pectin, starch, tannic acid and xylan (Burgeff 1909; Holländer 1932; Hadley and Perombelon 1963; Smith 1966; Nieuwdorp 1972; Barroso et al. 1985; Rasmussen 1995; Zelmer et al. 1996). Our ability to interpret and extrapolate these data to OM fungi more generally is limited. A number of studies have cultured OM fungi on solid media supplemented with sterilised wood, leaf litter or paper. The use of such complex substrates as supplements prevents precise determination of the substrates used for growth. Additionally, OM fungi can produce significant biomass on water agar without supplementation

Table 1 Details of isolates used in the present study

Isolate code	Isolate name	Host/reference	Source
RP2	Orchid mycorrhizal *Rhizoctonia solani* (AG12) genotype I	*Pterostylis accuminata* (Pope 2001)	AJ Perkins
RP5	Orchid mycorrhizal *Rhizoctonia solani* (AG12) genotype II	*Pterostylis accuminata* (Pope 2001)	AJ Perkins
RP7	Orchid mycorrhizal *Rhizoctonia solani* (AG12) genotype III	*Pterostylis accuminata* (Pope 2001)	AJ Perkins
CP1	Orchid mycorrhizal *Ceratorhiza* sp. genotype I	*Pterostylis nutans* (unpublished)	AJ Perkins
CP3	Orchid mycorrhizal *Ceratorhiza* sp. genotype II	*Pterostylis longifolia* (unpublished)	AJ Perkins
CP13	Orchid mycorrhizal *Ceratorhiza* sp. genotype III	*Pterostylis bicolor* (unpublished)	AJ Perkins
T1G3	Epacrid mycorrhizal fungus MG110 genotype III	*Woollsia pungens* (Midgley et al. 2002)	DJ Midgley
T1G4	Epacrid mycorrhizal fungus MG110 genotype IV	*Woollsia pungens* (Midgley et al. 2002)	DJ Midgley
T1G5	Epacrid mycorrhizal fungus MG110 genotype V	*Woollsia pungens* (Midgley et al. 2002)	DJ Midgley
T6G9	*Woollsia* mycorrhizal fungus VI genotype 3	*Woollsia pungens* (Midgley et al. 2004c)	DJ Midgley
T6G11	*Woollsia* mycorrhizal fungus VI genotype 5	*Leucopogon parviflorus* (Midgley et al. 2004c)	DJ Midgley
T6G12	*Woollsia* mycorrhizal fungus VI genotype 6	*Leucopogon parviflorus* (Midgley et al. 2004c)	DJ Midgley
Re	*Rhizoscyphus ericae* 101	*Calluna vulgaris* (Leake and Read 1990)	DJ Read

(DJ Midgley, JA Saleeba, PA McGee, unpublished data), making observations on growth on solid media difficult to interpret. Moreover, some older studies include proteinaceous nitrogen, a source of both carbon and nitrogen, in their growth medium, preventing quantification of biomass yields directly due to the added carbon source. Finally, ERM and ectomycorrhizal fungi have intraspecific and interspecific variation in their use of nitrogen, phosphorus and carbon sources (Anderson et al. 1999; Cairney 1999; Cairney et al. 2000; Sawyer et al. 2003; Midgley et al. 2004a,b; Guidot et al. 2005). Intra- and interspecific variation in nutrient use has not yet been investigated among OM fungi.

Orchids and ericoid plants co-occur in heathland and woodland communities. If host capacity to access nutrients from organic substrates depends on the abilities of its associated fungi, then ERM and OM fungi may have differing suites of enzymes, enabling use of different resources in soil. Alternatively, ERM and OM fungi may compete for the same resources. Ericoid and ectomycorrhizal fungi from woodland communities differ in their use of nitrogen (Whittaker and Cairney 2001; Sawyer et al. 2003; Midgley et al. 2004b). Whether or not this also applies to the use of carbon by ericoid and orchid mycorrhizal fungi remains unclear. Thus, to further explore the physiological potential of ERM and OM fungi, the growth of multiple genotypes was compared on a range of cell-wall-related and aromatic carbon substrates.

Materials and methods

The fungi included three genotypes of epacrid mycorrhizal fungus MG110 (Midgley et al. 2002) from *Woollsia pungens* (Cav.) F. Muell. and three genotypes of *Woollsia* mycorrhizal fungus VI (Midgley et al. 2004c) isolated from *W. pungens* and *Leucopogon parviflorus* (Andrews) Lindl (Table 1). The OM fungi included three isolates of *Rhizoctonia solani* Kühn (teleomorph, *Thanatephorus cucumeris* (Frank) Donk) anastomosis group (AG) 12, from *Pterostylis accuminata* R. Br (Perkins, unpublished, Table 1, RP), and three of *Ceratorhiza* sp. from *Pterostylis nutans* R.Br at Gymea, *Pterostylis longifolia* at Putty and *Pterostylis bicolour* R.Br. at Vineyard, respectively (Perkins, unpublished, Table 1, CP). An isolate of the well-studied ERM fungus *R. ericae* (DJ Read; 101) was included for comparison. The growth characteristics and abilities of Australian ERM fungi to utilise cellobiose, carboxymethylcellulose (CMC) and xylan for growth have been reported (Midgley et al. 2004a).

A preliminary experiment examined the growth characteristics of OM fungus isolates on low carbon–nitrogen modified Melin Norkrans (low CN MMN) liquid medium (Marx and Bryan 1975) containing (l^{-1}) glucose, 5 g; KH_2PO_4, 0.30 g; $(NH_4)_2HPO_4$, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.14 g; $CaCl_2$, 50 mg; NaCl, 25 mg; $ZnSO_4$, 3 mg; ferric EDTA ($C_{10}H_{12}FeN_2NaO_8$), 12.5 mg; and thiamine, 0.13 mg (pH adjusted to 5.0–5.5 prior to adding ferric EDTA and autoclaving). Cultures were grown in a non-shaking incubator in the dark at 25°C and harvested every 4 days for 19 days. At harvest, mycelia were removed manually from the nutrient solution, blotted dry on paper, dried overnight at 80°C, and then weighed to determine the biomass. Growth curves were then constructed in Microsoft Excel X to determine the time required to reach 25 mg of biomass and, thus, to compensate for intrinsically different rates of growth.

Inoculum consisted of two plugs (5 mm in diameter) of actively growing fungus excised from the leading edge of colonies growing on low CN MMN agar in all treatments.

Table 2 Median biomass (mg dry wt) on carbon-free controls for all isolates, produced after 4 (RP isolates), 8 (CP isolates) or 10 (all ericoid mycorrhizal isolates) days in culture at 25°C

Isolate	RP2	RP5	RP7	CP1	CP3	CP13	T1G3	T1G4	T1G5	T6G9	T6G11	T6G12	Re
Biomass	1.8	3.6	2.5	2.3	2.9	2.8	3.9	3.5	3.6	3.9	5.3	3.7	2.9
(±IQR)	(0.3)	(0.4)	(0.3)	(0.3)	(0.4)	(0.5)	(0.4)	(0.4)	(0.2)	(0.5)	(0.2)	(0.6)	(0.4)

IQR Interquartile range

Five replicate 9-cm-diameter Petri dishes containing 25 ml of glucose-free low N MMN liquid medium for each isolate were supplemented with either beechwood xylan (Sigma), *d*-cellobiose (Sigma), *l*-arabinose (Fluka), pectin from citrus fruit (Sigma), sodium carboxymethylcellulose (Fluka), tannic acid (Sigma) along with the amino acids,

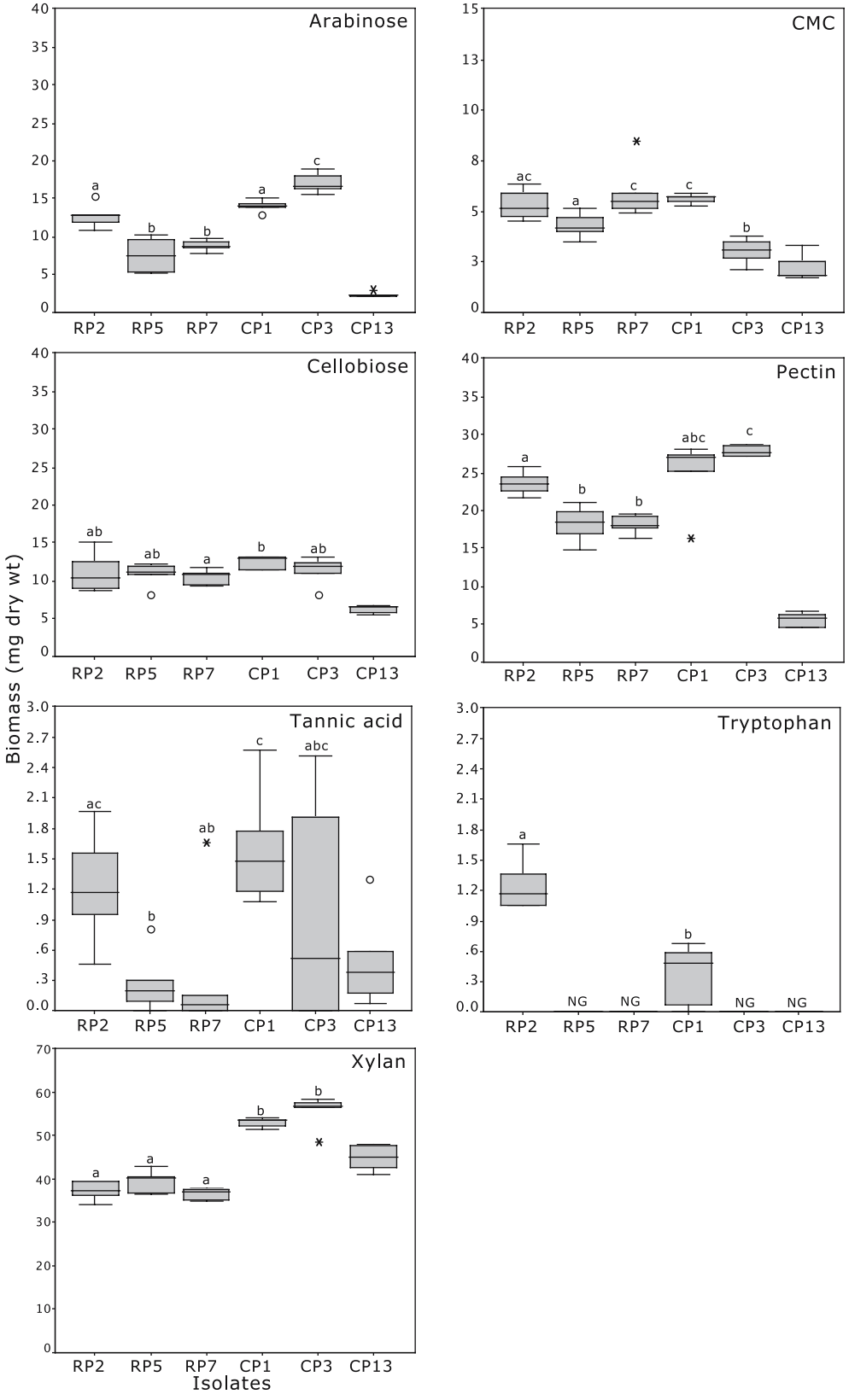

Fig. 1 Boxplots of biomass yields by three genotypes of *R. solani* (RP2, RP5 and RP7) and three genotypes of *Ceratorhiza* sp. (CP1, CP3 and CP13) in liquid media supplemented with arabinose, carboxymethylcellulose (CMC), cellobiose, pectin, tannic acid, tryptophan or xylan. *Boxes* represent interquartile range (IQR); *dark centre bar* represents the median biomass yield. *Whiskers* indicate the maximum and minimum yields. *Open circles* indicate outliers 1.5×IQR; *asterisks* indicate outliers 3×IQR. *Lowercase letters* indicate significant differences ($P<0.05$) between isolates as determined by the Kruskal Wallis test and subsequent Mann–Whitney *U*-tests. Scaling of *Y*-axis differs between substrates. *NG* No growth

l-phenylalanine (Sigma), or *l*-tryptophan (Sigma) to a final concentration of 2 g C l^{-1}. The abilities of orchid and ericoid mycorrhizal fungi to utilise two insoluble carbon sources, chitin from crab shells (<710-μm particle size, Sigma) and Sigmacell crystalline cellulose (20 μm-particle size, Sigma) for growth were also examined. As in other treatments, inoculum was added to five replicate 25-ml screw-capped tubes containing carbon-free MMN supplemented with either particulate chitin or crystalline cellulose to a concentration of 2 g C l^{-1} and growth was determined by visual comparison to carbon-free controls. For those carbon substrates that also contained nitrogen (chitin, phenylalanine and tryptophan), inorganic sources of nitrogen were omitted.

The length of incubation time was adjusted to ensure that all treatments were still in the exponential phase of growth at time of harvest which was standardised to ca 25 mg fungal biomass on low CN MMN containing glucose as the carbon source. Thus, after 4 (RP isolates), 8 (CP isolates) or 10 days (ericoid isolates) in culture in the dark at 25°C, isolates were harvested and weighed. Raw biomass yields were corrected for growth from carbon stored in inoculum and plugs by the subtraction of the median biomass yield in the absence of exogenous carbon from the raw data for each treatment. Data were analysed using SPSS v.11 (SPSS, Chicago). All data were tested for normality and some were found to be non-parametric. As transformations failed to normalise the data, data were analysed using the Kruskal–Wallis test and, where significant differences ($P<0.05$) were observed, the Mann–Whitney *U*-test procedure was used to identify significantly different pairs ($P<0.05$). Differences between mean pooled biomass yields were determined using an independent two-sample *t*-test.

Results

Growth kinetics of OM fungi

The general growth characteristics of the OM fungi were similar to those observed for other fungi in batch culture (Meletiadis et al. 2001). CP13, however, grew poorly in low CN MMN, reaching only 15 mg biomass after the time course had been completed and was, therefore, excluded from statistical comparison of genotypes. The three genotypes of *R. solani* all reached 25 mg biomass in ca 4 days, and CP1 and CP3 in 8 and 10 days for ERM fungi (Midgley et al. 2004a). These incubation times were used in subsequent experiments.

Growth in the absence of carbon

Median biomass yield without exogenous carbon ranged from 1.8 to 5.3 mg total dry weight (Table 2). Mean biomass yield (±standard error) of pooled ERM fungal taxa in the carbon-free treatment was 3.87±0.13 mg dry weight (DW), and was significantly higher than that of pooled OM fungal taxa (2.77±0.11 mg DW) in the same treatment. Isolate T6G11 consistently produced greater biomass ($P<0.05$) on carbon-free controls than any other isolate. Conversely, biomass yields by RP2 on carbon-free controls were lower ($P< 0.05$) than all other isolates except CP1.

Substrate utilisation

All isolates of OM fungi produced measurable biomass on arabinose, CMC, cellobiose, pectin, tannic acid and xylan (Fig. 1). For all OM fungal isolates, significantly greater biomass yields were produced on xylan ($P<0.05$) than for all other substrates (Table 3). All orchid mycorrhizal fungi produced measurable biomass on pectin as the sole carbon source; indeed yields on this substrate were, with the exception of CP13, the second largest ($P<0.05$). Biomass yields on tannic acid were lowest for all OM fungi, except for CP1 and RP2 where yields on tryptophan were significantly lower or not significantly different from those obtained on tannic acid, respectively (Table 3). Biomass produced on particulate cellulose for all genotypes of both *R. solani* and *Ceratorhiza* sp. were vastly greater than mycelia present on carbon-free controls and some clarification of the growth medium, presumably due to digestion of particulate cellulose, was observed. All isolates of ERM fungi produced measurable biomass on arabinose, pectin, tannic acid and phenylalanine as sole carbon sources (Fig. 2). Biomass yields for ERM were generally greater on pectin, tannic acid or arabinose and lower on phenylalanine or tryptophan, though this varied between genotypes (Table 4).

Intraspecific variation

Intraspecific variation was apparent within both *R. solani* and *Ceratorhiza* sp. in use of arabinose, CMC, pectin, tannic acid and tryptophan (Fig. 1). This variation, however, did not exceed an order of magnitude. In the tryptophan treatment,

Table 3 Differences in biomass yields of orchid mycorrhizal fungus *R. solani* AG12 (RP2, RP5 and RP7) and *Ceratorhiza* spp. (CP1, CP3 and CP13) on different substrates

Substrate	Genotypes					
	RP2	RP5	RP7	CP1	CP3	CP13
Arabinose	III	III	III	IV	IV	II
Cellobiose	III	IV	IV	IV	III	III
CMC	II	II	II	III	II	II
Pectin	IV	V	V	V	V	III
Tannic acid	I	I	I	II	I	I
Xylan	V	VI	VI	VI	VI	IV
Phenylalanine	–	–	–	–	–	–
Tryptophan	I	–	–	I	–	–

Significantly different yields ($P<0.05$) within a single genotype are designated with different Roman numerals as determined by the Kruskal–Wallis test and subsequent Mann–Whitney *U*-tests. Roman numerals are in ascending order from lowest to greatest biomass yield

– No growth

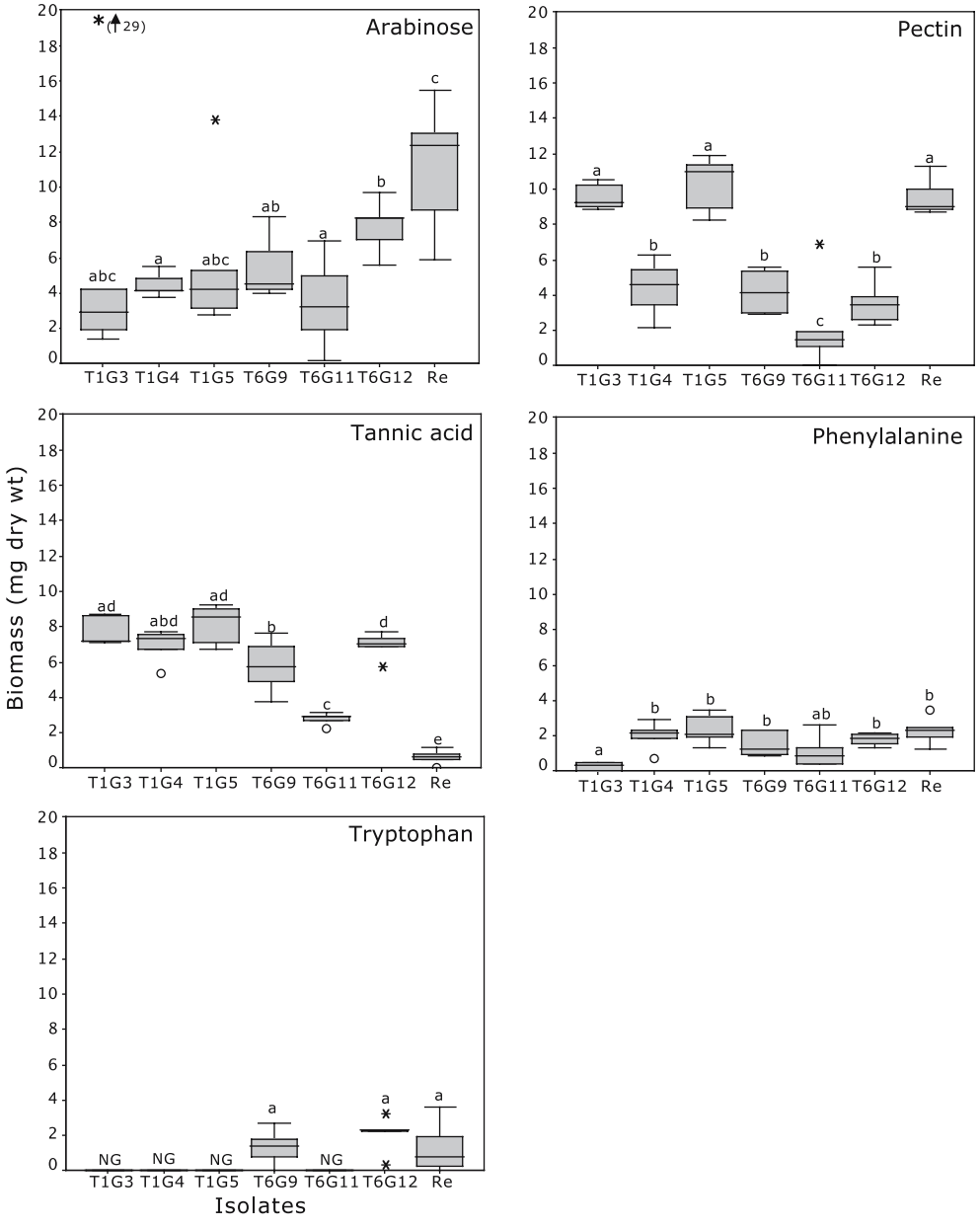

Fig. 2 Boxplots of biomass yields by three genotypes of Epacrid mycorrhizal fungus MG110 (T1G3, T1G4 and T1G5), three genotypes of *Woollsia* mycorrhizal fungus VI (T6G9, T6G11 and T6G12) and *R. ericae* 101 in liquid media supplemented with arabinose, pectin, tannic acid, phenylalanine or tryptophan. *Boxes* represent interquartile range (IQR); *dark centre bar* represents the median biomass yield. *Whiskers* indicate the maximum and minimum yields. *Open circles* indicate outliers 1.5×IQR; *asterisks* indicate outliers 3×IQR. *Lowercase letters* indicate significant differences ($P<0.05$) between isolates as determined by the Kruskal Wallis test and subsequent Mann–Whitney *U*-tests. *NG* No growth

Table 4 Differences in biomass yields of ericoid mycorrhizal genotypes of epacrid mycorrhizal fungus MG110 (T1G3, T1G4 and T1G5), *Woollsia* mycorrhizal fungus VI (T6G9, T6G11 and T6G12) and *R. ericae* 101 on different substrates

Substrate	Genotypes						
	T1G3	T1G4	T1G5	T6G9	T6G11	T6G12	*R. ericae* 101
Arabinose	II/III	II	II	II	I/II	III	III
Pectin	III	II	II	II	I	II	III
Tannic acid	II	III	II	II	II	III	I
Phenylalanine	I	I	I	I	I	I	II
Tryptophan	–	–	–	I	–	I	I/II

Significantly different yields ($P<0.05$) within a single genotype are designated with different roman numerals as determined by the Kruskal–Wallis test and subsequent Mann–Whitney *U*-tests. Roman numerals are in ascending order from lowest to greatest biomass yield
– No growth

Table 5 Differences between mean biomass yields (±SE) for orchid mycorrhizal and Australian ericoid mycorrhizal isolates derived from pooled data

Treatment	Orchid mycorrhizal fungi	Ericoid mycorrhizal fungi	Significance (*t*-test)
Arabinose	10.45 (±0.9)	6.4 (±1.0)	<0.01
Pectin	19.69 (±1.4)	6.7 (±0.7)	<0.01
Tannic acid	0.83 (±0.15)	7.5 (±0.4)	<0.01

only one genotype of each taxon, RP2 and CP1, produced measurable yields. Significant intraspecific variation in biomass yields ($P< 0.05$) were also observed in both epacrid mycorrhizal fungus MG110 on pectin and phenylalanine and *Woollsia* mycorrhizal fungus VI on pectin, tannic acid and tryptophan (Fig. 2). Genotypes were, however, broadly similar and intraspecific variation within both Australian ERM taxa did not exceed an order of magnitude. Amongst isolates of *Woollsia* mycorrhizal fungus VI, utilisation of tryptophan for production of measurable biomass was limited to isolates T6G9 and T6G12.

Interspecific variation

Interspecific variation between *R. solani* and *Ceratorhiza* sp. was minimal, the two species being broadly similar in their abilities to utilise arabinose, CMC, cellobiose, pectin, xylan and tannic acid for biomass production (Fig. 1). Both *R. solani* and *Ceratorhiza* sp. were unable to produce biomass on phenylalanine. In the chitin treatment, particulate chitin was bound to the hyphae in an adhesive matrix and therefore biomass was not quantified. More mycelia of *R. solani* genotypes were apparent in the chitin treatment than in carbon-free controls, and no *Ceratorhiza* sp. genotypes grew on chitin.

The three ericoid mycorrhizal taxa were similar in their abilities to utilise arabinose, pectin, tannic acid and phenylalanine. The single isolate of *R. ericae* yielded lower biomass ($P< 0.05$) on tannic acid than either epacrid mycorrhizal fungus MG110 or *Woollsia* mycorrhizal fungus VI (Fig. 2). Epacrid mycorrhizal fungus MG110 appears to be unable to utilise tryptophan for growth, while both *Woollsia* mycorrhizal fungus VI, with the exception of isolate T6G11, and *R. ericae* were able to produce measurable biomass on this substrate.

Ericoid and orchid mycorrhizal fungi

The pooled mean biomass obtained from all isolates of OM fungi was greater ($P<0.01$) than the pooled mean biomass for Australian ERM fungi on arabinose and pectin (Table 5). Indeed, the orchid mycorrhizal fungi produced more than 1.5 times the biomass on arabinose and almost three times the biomass on pectin as that produced by Australian ericoid isolates. Conversely, Australian ERM fungi produced nine times as much biomass as the OM fungi on tannic acid (Table 5). Additionally, all ERM, and no OM fungi, yielded measurable biomass on phenylalanine.

Discussion

ERM and OM fungi utilise a diverse array of carbon substrates in axenic culture. Both groups can degrade cellulose, cellobiose and CMC. This suggests the production of cellobiohydrolases, endoglucanases and β-glucosidases enabling the complete hydrolysis of cellulose to glucose. The complete use of cellulose is unsurprising, as these enzymes are presumably required for host cell penetration. Additionally, all orchids are mycoheterotrophic during germination (Smith and Read 1997), and, with the exception of those fungal symbionts that form ectomycorrhizas with other plants (e.g. Taylor and Bruns 1999), the endophytic fungi associated with orchid protocorms are presumably obtaining carbon from the breakdown of cellulosic materials in the soil. It is likely therefore that OM fungi express cellulytic enzymes in soil, at least in the early stages of orchid development.

Like cellulose, the potential of mycorrhizal fungi to degrade pectin is presumably important during penetration of the root of the host. Northern hemisphere ERM and OM fungi are known to produce polygalacturonases in culture (Hadley and Perombelon 1963; Nieuwdorp 1972; Perotto et al. 1993, 1997). Polygalacturonase activity is low in some ERM fungi that grew well on pectin (Perotto et al. 1997), suggesting the production of further pectinolytic enzymes. The present study indicates that Australian ERM and OM fungi also degrade pectin and utilise it as a substrate for growth, though further work is required to establish which enzymes are involved.

In *R. ericae*, growth on xylan is mediated by the production of at least one xylanase and a range of accessory enzymes (Burke and Cairney 1997a,b). Growth in and clarification of the insoluble fraction of the xylan suspension by OM fungi in the present study indicates the presence of similar enzyme activities in these fungi. Oddly, biomass of OM fungi produced on the polysaccharide xylan exceeded that on the pentose monosaccaride arabinose (Table 3). Beechwood xylan is a glucuronoxylan and is mainly composed of glucuronic acid and xylose units, though it may also contain some arabinose side chains. The utilisation of arabinose in higher fungi is via the pentose-phosphate shunt with L-arabinose being converted to D-xylulose 5-phosphate via a number of intermediates (Jennings 1995). D-Xylose, the most prevalent monomer of beechwood xylan, is catabolised via the same pentose-phosphate pathway but is less energetically demanding to process (Richard et al. 2001). Previous studies on an unidentified *Coprinus* species and *Stereum gauspatum* (Fr.) yielded biomasses 56 and 43% lower, respectively, than those obtained on glucose (Johnson and Jones 1941; Herrick 1940 in Jennings 1995), suggesting that arabinose is less easily, or more slowly, assimilated than hexose sug-

ars in some basidiomycetes. Data presented here indicate that this also applies to basidiomycetous OM fungi.

The use of tannic acid as a sole carbon source differed significantly between ericoid and orchid mycorrhizal fungi. While both groups of fungi utilised tannic acid for growth, the ERM fungi, with the exception of the single isolate of *R. ericae*, produced more biomass than the OM fungi. Indeed, one ERM isolate (T1G4) produced significantly more biomass on tannic acid than on any other substrate. Single isolates of OM fungi have been previously suggested to grow on tannic acid concentrations up to 1–2% as a sole carbon source (Wolff 1933). Holländer (1932) and Burgeff (1936), however, were unable to grow OM fungi at concentrations above 0.05% tannin. In the present study, the concentration of tannic acid was 0.373%. OM fungi thus appear to have limited ability to utilise tannic acid as a carbon source. It was somewhat surprising that *R. ericae* 101 also grew poorly on tannic acid, despite being isolated from hair roots growing in heathland soils rich in humus (Leake and Read 1990). *R. ericae* 101 has previously been demonstrated to be unaffected by up to 500 mg l^{-1} tannic acid (Bending and Read 1996a), however, the concentration in the present study (3.73 g l^{-1}) was ca 7.4 times greater than that used by Bending and Read (1996a). *R. ericae* 101 may be less tolerant of tannic acid than Australian ERM fungi. It is noteworthy, however, that *R. ericae* 101 has been in culture since 1985 and may not be a meaningful representative of this taxon.

R. ericae can facilitate host plant access to nitrogen in chitin (Kerley and Read 1995). In the present study, all ERM and *R. solani* genotypes appeared to grow on chitin. Differences were observed between the RP and CP genotypes in their ability to utilise chitin. If OM fungi translocate nitrogen to the host, then the ability of *R. solani* genotypes to access chitinous nitrogen, in addition to carbon, may significantly benefit the host.

Utilisation of aromatic amino acids varied in the present study. All ERM isolates utilised phenylalanine, while no OM fungus yielded measurable biomass on the substrate. Only two genotypes of OM fungi, RP2 and CP1, were able to grow on tryptophan. Similarly, epacrid mycorrhizal fungus MG110 and one genotype of *Woollsia* mycorrhizal fungus VI were unable to utilise this substrate for growth. Tryptophan is a complex amino acid and may require specific transporters that are presumably absent or inactive in the fungi examined.

Differences in biomass between and within taxa of ERM and OM fungi on carbon-free controls may reflect differing strategies for storing and or recycling carbon sequestered within either the hyphae or the agar plug co-inoculated to the treatment. Alternatively, the variability may also be due to the removal of differing quantities of carbon in plugs under the growing layer of mycelium in the inoculum or a combination of the above factors.

Niche differentiation has been proposed for Australian ectomycorrhizal and ERM fungi (Whittaker and Cairney 2001) based on the differential use of various nitrogen substrates. Tannic acid has been demonstrated to inhibit growth of some northern hemisphere ectomycorrhizal fungi (Bending and Read 1996a) and, additionally, some ectomycorrhizal fungal species cannot utilise phenylalanine (Guidot et al. 2005) or histidine (Anderson et al. 1999; Sawyer et al. 2003). Australian ERM fungi can use histidine (Midgley et al. 2004b), phenylalanine and tannic acid for growth. Assuming that these laboratory data indicate patterns of substrate use of the fungi in their natural habitat, niche differentiation might also be proposed for ericaceous and orchideaceous plants in heathland and woodland communities where they co-occur. Ericaceous plants may thus gain access to nutrients from sclerophyllous plant litter and polyphenolics in soil, through fungal degradation of aromatic compounds such as tannins and complex amino acids. In contrast, orchid mycorrhizal fungi appear to have limited access to aromatic compounds, and if these observations apply to their growth in the field, they may not compete for use of these substrates with ericoid mycorrhizal fungi. The role of mycorrhiza in the nutrition of adult photosynthetic orchids is poorly understood, however, photosynthetic orchids may differ from other mycorrhizal plants in that the host may gain both carbon and mineral nutrients from the fungal symbiont. Data from the present study indicates that OM fungi can degrade a range of cellulosic substrates from which carbon may then be translocated to the host. Our preliminary observations of growth on chitin by *R. solani*, but not *Ceratorhiza* sp., may, if nitrogen is translocated to the plant by OM *R. solani*, give such hosts an advantage in soils deficient in mineral nitrogen and warrant further investigation.

The current study has demonstrated that Australian OM and ERM fungi can use an array of cell-wall-related carbon substrates for growth in axenic culture. Intra- and interspecific variation was observed both within and between orchid and ericoid mycorrhizal taxa in their abilities to access a range of carbon substrates. Additionally, Australian ERM fungi use both phenylalanine and tannic acid for growth, and appear more tolerant of high concentrations of the latter compound than *R. ericae* 101. The present study also extends the proposed niche differentiation of ectomycorrhizal and ERM fungi (Whittaker and Cairney 2001) and provides some evidence of a similar niche partitioning between ericoid and orchid mycorrhizal fungi from dry sclerophyll forests.

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