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W.A. Dunstan · B. Dell · N. Malajczuk

The diversity of ectomycorrhizal fungi associated with introduced *Pinus* spp. in the Southern Hemisphere, with particular reference to Western Australia

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Abstract Although pines have been established in plantations in Western Australia for over 100 years, knowledge of the ectomycorrhizal fungal flora is incomplete, or lies in unpublished reports. A survey of ectomycorrhizal fungi associated with *Pinus* spp. was conducted throughout south-western Australia. Compared with other regions in the Southern Hemisphere where pines have been introduced, the ectomycorrhizal flora of pines in Western Australia is particularly depauperate, with only nine species of fungi identified from sporocarps and a further two taxa identified from mycorrhizas. Species identified from sporocarps (*Hebeloma crustuliniforme, Lactarius deliciosus, Paxillus involutus, Rhizopogon luteolus, R. roseolus, R. vulgaris, Suillus luteus, S. granulatus, Thelephora terrestris*) and *Cenococcum geophilum* are a subset of a larger pine mycorrhizal flora found in eastern Australia, and 8 of the 10 identified species are common to all regions in the Southern Hemisphere where pines have been introduced. These fungi are typically associated with trees, including pines, in the Northern Hemisphere and, apart from *Cenococcum geophilum* and *T. terrestris*, are not associated with indigenous vegetation in Western Australia. The mycorrhizal flora colonising roots in a plantation of *Pinus radiata* D. Don was also investigated, and compared with species identified as present by above-ground sporocarp production. Potential reasons for the limited ectomycorrhizal flora of pines in Western Australia are discussed.

Key words Ectomycorrhizal fungi · *Pinus* · Southern Hemisphere · Western Australia

W.A. Dunstan · B. Dell (✉) · N. Malajczuk
School of Biological Sciences, Murdoch University,
Perth, WA 6150,Australia
e-mail: dell@central.murdoch.edu.au, Fax: +61-8-9360-6303

Introduction

Extensive plantations of exotic pines, principally *Pinus radiata,* have been (and continue to be) established in the Southern Hemisphere since the latter half of the 19th century. For example, 1.5 million ha have been established in Chile, over 1 million ha in New Zealand, more than 700 000 ha in South Africa, and 800 000 ha in Australia (Anon. 1997). In Western Australia, large scale establishment of pines commenced in 1897 and 85 000 ha have been established, 70% as *Pinus radiata* D. Don and most of the remainder as *Pinus pinaster* Ait.. Plantings of pines are likely to increase in the southwest of Western Australia, particularly in the 400–600 mm rainfall zone, where the establishment of a further 150 000 ha of *Pinus pinaster* has been proposed (Anon. 1996). Although a large and diverse ectomycorrhizal (ECM) fungal flora is associated with eucalypts and other indigenous plant taxa in Australia (Bougher 1995; Castellano and Bougher 1994), the requirement for introduced ECM fungi compatible with pines was recognised soon after large-scale planting of pines in Western Australia began (Kessell 1926). Although comprehensive surveys of ectomycorrhizal fungi associated with introduced pines have been conducted elsewhere in the Southern Hemisphere (Chu-Chou 1979; Garrido 1986; van der Westhuizen and Eicker 1987), the fungal flora of Western Australia has not been surveyed, and regional floras of pine associated ECM fungi have not been compared.

Materials and methods

In addition to collections held at the CSIRO Herbarium, Floreat, Western Australia, ca. 250 collections and/or records of ectomycorrhizal fungi were made in plantations of *Pinus radiata* (ca. 5–60 years old) and *Pinus pinaster* (ca. 20–40 years old) and from individual trees or small plantings of other *Pinus* spp., throughout the southwest land division of Western Australia between May 1996 and December 1997.

The population of mycorrhizal roots was examined in a 22-year-old *Pinus radiata* plantation (2 ha) established on a pasture site near Margaret River (34°S, 115°E), Western Australia. PVC infill cores (1-cm mesh, 60 mm diam. × 180 mm, $n = 28$) were placed at random throughout a section of the plantation, 1 m from the tree-row centre-line and equidistant between adjacent trees in the same row. Cores were harvested after 9 months and the soil from the top 150 mm was gently washed through a 1-mm sieve. Roots were cut to 10–15 mm and subsampled where necessary after the method of Brundrett and McGonigle (1994). Mycorrhiza types in infill cores were identified by comparison with mycorrhizas directly beneath fruit bodies on the same site and from descriptions of *Pinus radiata* and *Pinus* spp. mycorrhizas by Chu-Chou and Grace (1983b), Ingleby et al. (1990), and Palm and Stewart (1984). Percentages of each type of mycorrhizal short root were calculated after examining and counting 100–250 short roots per sample under a stereomicroscope. Mycorrhizas that could not be readily identified were hand-sectioned and examined at higher magnification. Mycorrhizal fungi were surveyed 15 times at the field site at 2–6 week intervals between May and October 1996 and 1997.

Results

The ectomycorrhizal flora of *Pinus* spp. found in the southwest of Western Australia was compared with records of ECM fungi associated with *Pinus* spp. in Mediterranean and sub-humid regions elsewhere in the Southern Hemisphere, specifically South America (Chile, Argentina, southern Brazil), South Africa (Republic of South Africa), New Zealand, and eastern Australia (Table 1).

In the survey of the southwest of Western Australia, nine species of ECM fungi were recorded from sporocarps. The most commonly collected fungi were *Rhizopogon roseolus* and/or *R. vulgaris*, *Hebeloma crustuliniforme*, and *Suillus granulatus*. *Suillus luteus* fruiting was always limited and confined to limited areas within plantations in higher rainfall areas (>ca. 1000 mm). *Rhizopogon luteolus* was apparently absent from most sites; however, where it occurred, it often fruited in abundance, often with *R. roseolus/R. vulgaris*. *Thelephora terrestris* was also not observed at many sites, but when present often fruited extensively. Although there have been a few collections of *Paxillus involutus* in association with *Pinus* spp. in Western Australia (N. Malajczuk, unpublished data), all collections of *Paxillus involutus* in this survey were associated only with *Quercus* spp. and *Populus* sp.. In Western Australia, *Lactarius deliciosus* occurs at a single location associated with *Pinus radiata*, as the result of a deliberate introduction within the last 15–20 years (J Titze, personal communication). Apart from *S. luteus*, there were no apparent constraints on the distribution of specific fungi, such as soil type, competition from other ECM fungi, tree age or species. At several sites, all epigeous species (apart from *Paxillus involutus* and *L. deliciosus*) and one or more *Rhizopogon* spp. were present.

Percentage mycorrhizal short roots by type extracted from infill cores in the 22-year-old *Pinus radiata* plantation are included in Table 2. Mycorrhizas of *S. luteus* and *S. granulatus* were not found in infill cores, although both fungi fruited elsewhere in the plantation. Black mycorrhizas with surface cystidia ('Bcb' type), formed by an unidentified basidiomycete, were common in infill cores (Table 2) and in soil samples taken from the plantation for other purposes.

Hebeloma crustuliniforme produced sporocarps in both years and was the most common and widely distributed species of ECM fungus which produced fruit bodies in the 22-year-old plantation. *Suillus luteus* fruited in both years but was confined to a few areas of the plantation, and *S. granulatus* occurred more widely as scattered individual sporocarps throughout part of the plantation. Fruiting by *R. roseolus/R. vulgaris* occurred in both years but was restricted to a few areas, and over the entire site, only two collections of *Thelephora terrestris* were made. Sporocarps or mycorrhizas of *R. luteolus* or *Paxillus involutus* were not found in the plantation.

Discussion

ECM fungi associated with pines in Western Australia

In Western Australia, Hilton (1982) recorded *R. roseolus* [≡ *R. rubescens* (Tul. & C. Tul.)Tul. & C. Tul., Pegler et al. 1993] as one of two *Rhizopogon* spp. present; however, Grgurinovic (1997) described an early collection from Western Australia as *R. vulgaris* and a few more recent collections of *R. vulgaris* have been made (JM Trappe, unpublished work). Based on spore size, the principle discriminating character between *R. roseolus* and *R. vulgaris* (Grgurinovic 1997; Pegler et al. 1993; JM Trappe, personal communication), 80% percent of a representative selection of *R. roseolus/R. vulgaris* collections made during this study could be ascribed to *R. vulgaris*. *Rhizopogon roseolus* and/or *R. vulgaris* produced large numbers of sporocarps with both *Pinus radiata* and *Pinus pinaster* of all age classes. Chu-Chou and Grace (1984) found that *R. roseolus* (cited as *R. rubescens*) was the most common fungus isolated from mycorrhizas of *Pinus radiata* in conventional forests in New Zealand but was replaced by other fungi in plantations established on ex-pasture sites of higher fertility (Chu-Chou and Grace 1987). In Western Australia, *R. luteolus* was found much less frequently than other *Rhizopogon* spp. and appeared to be absent from some plantations but fruited abundantly in other plantations in close proximity established on similar soil types.

Hebeloma crustuliniforme is common and widely distributed in the southwest of Western Australia in association with pines, oak, birch and poplar. *H. crustuliniforme* was observed under pines up to 60+ years old in Western Australia, but in New Zealand is apparently replaced quickly by *Amanita muscaria, Scleroderma verrucosum,* and *Rhizopogon* spp. (Chu-Chou 1979).

Table 1 Ectomycorrhizal fungi associated with introduced *Pinus* spp. in the Southern Hemisphere[54]

Species	South America	South Africa	New Zealand	Australia (Eastern)	Western Australia	Remarks
Basidomycetes						
Amanita excelsa (Fr.) P. Kumm.		+ [17,18,40,41]				
A. gemmata (L.: Fr.) Gillet	+ [9]					
A. muscaria (L.: Fr.) Hook.	+ [9,32]	+ [17–19,40,41]	+ [2,6,36]	+ [7,11,29]		
A. pantherina (D. C.: Fr.) P. Kumm.	+ [10]	+ [18,40,41]				
A. phalloides (Fr.) Link	+ [32*]	+ [18,40,41]	+ [36*,30**]	+ [31**,29**]		
A. rubescens (Pers.: Fr.) Gray	+ [9]	+ [17,18,40,41]		+ [7**,11*]		
Amanita sp. 1			+ [30]			[30] hosts possibly including *Pinus* spp.
Amanita sp. 2		+ [17,18]				
Boletus edulis Bull.: Fr.	+ [9,33]	+ [17,18,20,41]	+ [39**]	+ [50*]		[20] *Suillus* sp. (?) illustrated as *B. edulis*
Boletus sp.		+ [17–19]				
Chalciporus piperatus (Bull.: Fr.) Bat.	+ [33]	+ [40,41]	+ [2,21,35]	+ [49,50]		
Chroogomphus vinicolor (Peck) O. K. Mill.	+ [33]					[33] reported as *Gomphidius vinicolor* Peck
C. superiorensis (Kauffman & A. H. Sm.) Singer	+ [33]					[33] reported as *G. superiorensis* Kauffman
C. rutilus (Schaeff.: Fr.) O. K. Mill.	+ [33]					[33] reported as *G. rutilus* (Schaeff.: Fr.) S. Lundell & Nannf.
Cortinarius spp.	+ [9]					
Hebeloma crustuliniforme (Bull.: Fr.) Quél.	+ [9]	+ [40]	+ [2]	+ [11]	+ [12,49]	
H. cylindrosporum Romagn.		+ [41]				
H. longicaudum (Fr.) sensu Lange	+ [9]					
H. mesophaeum (Pers.) Quél.	+ [27]			+ [11,31,48]		
H. testaceum (Batsch: Fr.) Quél.				+ [11]		
Inocybe eutheles (Berk. & Broome) Quél.		+ [41]				
Inocybe aff. *geophylla* (Sowerby: Fr.) Kummer	+ [9]					
I. lacera (Fr.) Kummer			+ [4]			
I. lanuginella (J. Schröt.) Konrad & Maubl.		+ [40]				
I. patouillardii Bres.				+ [31]		
Inocybe sp. 1			+ [2]			
Inocybe sp. 2 (subgen. Inocybe)	+ [9]					
Inocybe sp. 3	+ [9]					
Laccaria amethystina Cooke	+ [10]					[10] reported as *L. amethystea* (Bull.) Murrill
L. bicolor (Maire) Orton	+ [10]					
L. fraterna (Cooke & Massee: Sacc.) Pegler					+ [11,50]	T. W. May in[11]; reported as *L. lateritia* Malençon
L. laccata (Scop.: Fr.) Cooke	+ [9]	+ [17,40,41]	+ [2,37]	+ [11**,49*]		T. W. May in[11]; limited collections
L. montana Singer	+ [10]					
L. ohiensis (Mont.) Singer	+ [32]		+ [23]			[23,32] reported as *L. tetraspora* Singer
L. proxima (Boud.) Pat.			+ [23]	+ [11,50]		T. W. May in[11]
L. pumila Fayod	+ [10]					[10] reported as *L. altaica* Singer
L. tortilis (Bolton) Cooke			+ [37*]	+ [11**]		T. W. May in[11]
Laccaria sp.		+ [17]				

Table 1 (continued)

Species	South America	South Africa	New Zealand	Australia (Eastern)	Western Australia	Remarks
Lactarius deliciosus (L.: Fr.) Gray	+ (9,31)	+ (18,40,41)	? (28)	+ (31,48)	+ (51)	(28) introduction attempted, current status not known.
L. fragilis (Burl.) Hester & A. H. Sm.	+ (10)					
L. hepaticus Plow. apud Boud.		+ (41)				
L. pipertatus (Scop.: Fr.) Gray	+ (9**)					
L. rufus (Fr.) Fr.	+ (10)					(10) including *L. rufus* var. *parvus* Hesler & A. H. Sm.
Lactarius sp.		+ (18)				
Lyophillum sp.			+ (2)			
Paxillus involutus (Batsch: Fr.) Fr.	+ (27*,33*)	+ (41)	+ (22**)	+ (11,47)	+ (12,49**)	(41) uncommon in association with *Pinus*
Pisolithus tinctorius (Pers.) Coker & Couch				+ (14)		(14) isolated from nursery pine seedling roots
Rhizopogon honoratoi Speg.	+ (9)					
R. luteolus Fr. apud Fr. & Nordh.	+ (9)	+ (17,40,41)	+ (2,3)	+ (1,11)	+ (13,49)	
R. nigrescens Coker & Couch	+ (10)					
R. roseolus (Corda) Th. Fr.	+ (9)	+ (38,41)	+ (2,3)	+ (1,11)	+ (8,13,49)	(2,3,8,38,41) reported as *R. rubescens* Tul. & C. Tul.
R. vulgaris (Vittad.) M. Lange		+ (38)		+ (11)	+ (11,49)	
Rhizopogon sp. 1		+ (17)				
Rhizopogon sp. 2	+ (9)					
Russula caerulea (Pers.) Fr.		+ (40,41)				
R. capensis A. Pearson		+ (18,40,41)				described from South Africa
R. cyanoxantha (Schaeff.: Secr.) Fr.		+ (18)				
R. pectinata (Bull.) Fr.		+ (17)				
R. sardonia Fr. em. Romell	+ (9)	+ (18,40,41)				
E. sororia (Fr.) Romell		+ (18,41)		+ (49**)		(25) Angiosperm hosts only
Russula sp. 1		+ (18)				
Russula sp. 2				+ (15)		
Scleroderma bovista Fr.	+ (10)		+ (3,28)			
S. cepa (Vaill.) Pers.		+ (17,41*)				
S. citrinum Pers.	+ (9,26*)	+ (40,41)	+ (2,3)			
S. floridanum Guzmán	+ (10)					
S. fuscum (Corda) E. Fisch.	+ (10,26*)					
S. uraguayense (Guzmán) Guzmán	+ (10)					
Scleroderma verrucosum Vaill.: Pers.		+ (17,40,41**)	+ (2,3)			
Scleroderma sp.	+ (9,26**)					
Setchelliogaster tenuipes (Setch.) Pouzar	+ (16)					(16) associated with eucalypts and pines; (25) specific for *Eucalyptus* spp.
Suillus bellini (Inzenga) Watling	+ (40,41)					
Suillus bovinus (L.: Fr.) Kuntze		+ (18,40,41)		+ (46)		
S. brevipes (Peck) Kuntze			+ (21)	+ (11,46)		
S. cothurnatus Singer	+ (10)					
S. granulatus (L.: Fr.) Kuntze	+ (9,10,32)	+ (17–19,40,41)	+ (2,21,35)	+ (11,46)	+ (12,46,49)	
S. luteus (L.: Fr.) Gray	+ (9,32,33)	+ (40,41)	+ (2,21,35)	+ (11,46)	+ (12,46,49)	
S. punctatipes (Snell & E. A. Dick) A. H. Sm. & Thiers				+ (46)		(46) limited collections; variant of *S. granulatus*?

Table 1 (continued)

Species	South America	South Africa	New Zealand	Australia (Eastern)	Western Australia	Remarks
S. subacerbus McNabb			+[21]			[21] related to *S. acerbus* A. H. Sm. and Thiers and *S. pungens* Thiers and A. H. Sm.
S. subaureus (Peck) Snell	+[33]					[33] with *P. radiata*; [39] host range *Populus* spp. and *Quercus* spp.
S. variegatus (Sw.: Fr.) Kuntze		+[40]				
Tricholoma albobrunneum (Pers.: Fr.) Kummer		+[41]				
T. fagnani Singer	+[9]					[9] indigenous to Chile
T. flavovirens (Pers.: Fr.) N. Lund	+[32*]					
T. myomyces (Pers.: Fr.) Lange	+[9]					
T. pessundatum (Fr.) Quél.	+[9]		+[6,43]			
T. saponaceum (Fr.: Fr.) Kummer		+[18,41]		+[50]		
T. scalpturatum (Fr.) Quél.	+[9]					
T. terreum (Schaeff.: Fr.) Kummer			+[43]	+[31]		
T. virgatum (Fr.: Fr.) Kummer				+[50]		
Tricholoma sp. 1			+[28]			
Tricholoma sp. 2	+[9]					
Thelephora terrestris Ehrh.: Fr.	+[9]	+[17–19,40,41]	+[2]	+[15,31]	+[12,49]	
Xerocomus chrysenteron (Bull.: St.-Amans) Quél.	+[9,33**]		+[21**]	+[49**]		
Ascomycetes						
Cenococcum geophilum (Sowerby) Ferd. & Winge	+[9]	+[40]	+[24]	+[15]	+[49]	
Elaphomyces granulatus Fr.	+[9]					[9] reported as *Elaphomyces cervinus* (L.) Schltdl.
Tuber rapaeodorum Tul. & C. Tul.		+[20]				
Tuber sp.			+[3–5]			[52] possibly *Tuber maculatum* Vittad.
Zygomycetes						
Endogone flamicorona Trappe & Gerd.			+[3–5]	+[45]		
Densospora tubaeformis (P. A. Tandy) McGee				+[44]		[44] ectomycorrhiza formation with *Pinus kesiya* seedlings; reported as *Glomus tubiforme* P. A. Tandy
n[52]	52–55	35–36	30–32	32–34	10	

[1] Beaton et al. (1984); [2] Chu-Chou (1979); [3] Chu-Chou and Grace (1983a); [4] Chu-Chou and Grace (1983b); [5] Chu-Chou and Grace (1984); [6] Chu-Chou and Grace (1990); [7] Cleland (1924); [8] Cunningham (1944); [9] Garrido (1986); [10] Giachini and Oliveira (1997); [11] Grgurinovic (1997); [12] Hilton (1982); [13] Hilton (1988); [14] Lamb (1979); [15] Lamb and Richards (1970); [16] Lazo (1972); [17] Lundquist (1986); [18] Lundquist (1987a); [19] Lundquist (1987b); [20] Marais and Kotzé (1977); [21] McNabb (1968); [22] McNabb (1969); [23] McNabb (1972); [24] Mejstrik (1970); [25] Molina et al. (1992); [26] Raithelhuber (1987); [27] Raithelhuber (1988); [28] Rawlings (1958); [29] Reid 1979; [30] Ridley (1991); [31] Shepherd and Totterdell (1988); [32] Singer (1953); [33] Singer (1964); [34] Singer and Digilio (1957); [35] Stevenson (1961); [36] Stevenson (1962); [37] Stevenson (1964); [38] Theron (1991); [39] Thiers (1975); [40] van der Westhuizen and Eicker (1987); [41] van der Westhuizen and Eicker (1996); [42] Wang et al. (1995); [43] Wang et al. (1997); [44] Warcup (1985); [45] Warcup (1990); [46] Watling and Gregory (1989); [47] Watling and Gregory (1991); [48] Willis (1950); [49] this paper, Dunstan WA, personal observation; [50] Fuhrer BA, personal communication; [51] Titze J, personal communication; [52] Hall IR, personal communication; [53] lower estimate excludes taxa that (a) may not be mycorrhizal with *Pinus,* and (b) were identified to genus only, but which may be conspecific with fungi identified to species by other authors. [54] South of the Tropic of Capricorn including Mediterranean, Humid Sub-tropical, and Marine West Coast climatic zones

* fungal species known to be mycorrhizal with *Pinus* spp., but not clear whether collection/s were associated with *Pinus*

** species known to be mycorrhizal with *Pinus* spp., but all collection/s associated with genera other than *Pinus*

Table 2 Ectomycorrhizas, by type and percent of mycorrhizal short roots, from a 22-year-old *Pinus radiata* plantation

Mycorrhiza type	Percent infected short roots[a]
Thelephora terrestris	34.6 (7.8)
'Bcb' type[b]	25.3 (6.0)
Hebeloma crustuliniforme	23.6 (6.5)
Rhizopogon roseolus/vulgaris	1.8 (0.8)
Cenococcum geophilum	0.6 (0.3)
undefined[c]	10.1 (3.7)

[a] Bracketed figure equals one standard error
[b] Black cystidiate ectomycorrhizas, mycobiont unidentified basidiomycete
[c] proably early stage of infection by *Hebeloma crustuliniforme* and/or *Thelephora terrestris*

In Western Australia, *S. granulatus* is the more common and widespread of the two *Suillus* spp. associated with pines. *S. luteus* appears to be more abundant in areas of higher rainfall, and this is similar to observations by Garrido (1986) and Stevenson (1961), where *S. luteus* is replaced in warmer areas by *S. granulatus* and *A. muscaria* in Chile and New Zealand, respectively. There is variation in macroscopic characters between some collections of *S. granulatus* from Western Australia, which was also noted in New Zealand collections by McNabb (1968). There may be several species closely related to *S. granulatus* present in Western Australia (NL Bougher, personal communication), as there may be in eastern Australia (Watling and Gregory 1989).

There were few collections of *T. terrestris* in pine plantations in this study; however, where fruiting occurred the fungus was quite common, often forming 'fairy rings' in mineral soil under trees 4–30 years old. *T. terrestris* is the common contaminant of pine seedlings grown in sterile or partly sterile soil (Marx et al. 1970), but Guinberteau et al. (1989) state that *T. terrestris* may be replaced under field conditions by more competitive ECM fungi. This contrasts with our observations of fruiting by *T. terrestris* under mature trees and the high proportion of *T. terrestris* mycorrhizas in root cores (Table 2), which suggests that *T. terrestris* is a successful competitor of other ECM fungi of pines under Western Australian conditions.

The association between pines and *C. geophilum* (identified from ECM and sclerotia) was predicted given the broad host range and distribution of the fungus (Trappe 1964) and that *C. geophilum* is frequently associated with *Eucalyptus* spp. in Australia (Chilvers 1968; Malajczuk and Hingston 1981). In this study, mycorrhizas of *C. geophilum* occurred at a very low frequency in root samples (Table 2). Ingleby et al. (1990) also noted that mycorrhizas of *C. geophilum*, although ubiquitous on seedlings and mature trees, are usually found in small numbers.

Although *Paxillus involutus* is present in Western Australia, has a broad host range including pines (Molina et al. 1992; Trappe 1962), and has formed ECM with both *Pinus radiata* and *Pinus pinaster* in pure culture (Malajczuk et al. 1982; Pera and Alvarez 1995), only two collections of this species in association with pines have been made in Western Australia (N. Malajczuk, unpublished work). In their reviews of ECM fungi of *Pinus radiata* and *Pinus pinaster* Malajczuk et al. (1982) and Pera and Alvarez (1995) do not cite *Paxillus involutus* as having been found associated with either species in the field. Given that Fries (1985) found different intersterility groups of *Paxillus involutus* to have clear preferences for conifer or deciduous hosts, and that most collections in Western Australia and all collections of *Paxillus involutus* by McNabb (1968) in New Zealand and Garrido (1986) in Chile were associated with broad-leaf hosts, then most of the isolates of *Paxillus involutus* introduced into the Southern Hemisphere are possibly not pine compatible, or compete very poorly with other species of pine ECM fungi.

The black cystidiate mycorrhizas ('Bcb' type) found in this study with *Pinus radiata,* and incidentally with *Pseudotsuga menziesii,* are very similar to those formed between *Pinus radiata* and an unidentified basidiomycete (*Pinus radiata* + B218), and with *Pseudotsuga menziesii,* described from New Zealand (Chu-Chou and Grace 1983b,c). An unidentified basidiomycete with dark brown mycelium was readily isolated from 'Bcb' type mycorrhizas of *Pinus radiata* using the methods and a modification to the isolation medium (5 mg l^{-1} benomyl, 30 mg l^{-1} chlortetracycline, 10 mg l^{-1} trimethoprim) described by Erland and Söderström (1990). In eastern Australia, Lamb (1979) and Lamb and Richards (1970) also isolated unidentified basidiomycetes from pine roots which were used to resynthesise mycorrhizas with pine seedlings. This suggests there is potential for some indigenous fungi to form ECM associations with introduced pines.

In the plantation examined in more detail, root sampling was limited to a single harvest and above-ground assessment of fungi was essentially limited to the presence/absence of sporocarps. However, the total absence of fruiting structures which could be associated with 'Bcb' type mycorrhizas, together with the few records of *Thelephora terrestris* fruiting above ground compared with the proportion of ECM below-ground, strongly suggests that sporocarp production is a poor measure of the relative importance of specific fungi. In a study of ECM fungi associated with *Pinus muricata*, Gardes and Bruns (1996) also found that some common mycorrhiza types were poorly or unrepresented in above-ground sporocarp production. More sites need to be investigated before conclusions can be made regarding above- and below-ground relationships of pine ECM fungi in Western Australia.

Species richness: comparison with regional floras

The number of taxa of ECM fungi of introduced pines found in different regions (Table 1) is no doubt related to such factors as the time since European colonisation commenced, trade sources and frequency of visitation, proximity to potential sources of pines and associated fungi in the Northern Hemisphere, and attributes of the fungi. The species richness of ECM fungi found in Western Australia associated with pines (11 species) is very low compared with other regions where pines have been introduced to the Southern Hemisphere (29–55 species; Table 1). Species richness is also low relative to the number of ECM fungi found in association or with the potential to form ECM associations with *Pinus pinaster* (ca. 69 species, Pera and Alvarez 1995) and *Pinus radiata* (ca. 50 species, Alvarez et al. 1993; Garrido 1986; Pera and Alvarez 1995), which are the principal pine species in plantations in Western Australia. Approximately 80% of the fungi identified to species in Table 1, including all fungi identified from sporocarps in Western Australia, have a natural distribution which includes Europe and North America (Arora 1986; Guzmán 1970; Lincoff 1981; Moser 1983; Mueller 1992; Pegler et al. 1993; Philips 1991; Smith and Zeller 1966; Thiers 1975). Therefore, precise conclusions cannot be made about the origins of fungi found in Western Australia. In contrast, ca. 20% of introductions to South America are of North American origin (Guzmán 1970; Hesler and Smith 1979; Miller 1964; Smith and Zeller 1966; Thiers 1975), and two species found in South Africa (*Suillus bellini* and *Russula pectinata*) are recorded in Europe but not North America (Moser 1983). The total numbers of pine ECM fungi reported from eastern Australia and New Zealand are similar and, although there are some differences between floras, there are also common elements (*Laccaria proxima, L. tortilis, Suillus brevipes, Tricholoma terreum* and *Endogone flamicorona*) which have not been reported from other regions considered in this study (Table 1).

Within the group of fungi common to both Europe and North America, there is a core group of eight species now recorded from all regions included in Table 1, and a further five species are common to all other regions except Western Australia. At least six of the core group of eight species could be regarded as ‘pioneer’ species, producing sporocarps with nursery seedlings (Chu-Chou and Grace 1990; Garrido 1986).

Introduction of ECM fungi with pines to Western Australia

Pryor (1958) recorded the introduction to Australia of an unnamed mycorrhizal fungus with potted *Picea abies* as late as the middle of this century, and at least some ECM fungi of pines could have been introduced into Australia with the first recorded introductions of *Pinus radiata* to Australia, possibly as potted plants via England in 1857 (Fielding 1957). Later stringent quarantine regulations, particularly limits on the importation of soil, probably limited the number of introductions.

In Western Australia, the first attempt to establish pine plantations on a large scale was in the winter of 1897 (Ednie-Brown 1897). Some early plantings, as direct sown seed or nursery-raised seedlings, were complete failures but results of other plantings using stock from the single State nursery established in 1898 at Hamel were extremely promising (Richardson 1907). Later attempts to establish new nurseries were initially unsuccessful, at least in part because of the absence of suitable mycorrhizal fungi (Kessell 1926, 1927). Deficiencies in compatible ECM fungi were remedied by inoculating soil in new nurseries with soil from an established pine nursery (probably Hamel; Kessell 1926), or by the later practice of inoculation with spores of *R. luteolus* (Kessell and Stoate 1938). Large numbers of seedlings from Hamel were distributed throughout the state from ca.1898 and, given the later practice of using soil as inoculum for mycorrhizal fungi in nurseries, then most if not all of the ECM fungal flora of pines in Western Australian plantations is derived and was rapidly disseminated from the ECM fungal flora initially established at Hamel.

Although Australian quarantine restrictions (international and interstate) are comprehensive, there is potential for future accidental introductions of pine ECM fungi into Western Australia. For example, we have recently observed two types of unidentified mycorrhizas, in addition to those of *L. laccata,* on *Pinus radiata* seedlings grown in a glasshouse where the soil substrate included pasteurised European sphagnum peat. Pryor (1958) also suggested that spore-infected seeds were a source of mycorrhizal fungi and, during the course of this study, we obtained evidence to support this hypothesis: *Suillus lakei* was found for the first time in the south-west of Western Australia in association with *Pseudotsuga menziesii,* which we believe to have been grown locally from imported seed.

Because of the low diversity of ECM fungi associated with pines in Western Australia, and provided further introductions of undesirable fungi do not occur, in particular *Amanita muscaria* and *A. phalloides* from eastern Australia, then the potential for successful introduction of selected multipurpose ECM fungi remains high.

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