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ORIGINAL PAPER

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Glomalean mycorrhizal fungi from tropical Australia

I. Comparison of the effectiveness and specificity of different isolation procedures

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Abstract A comparison of different methods for isolation of vesicular-arbuscular mycorrhizal (VAM) fungi into open-pot cultures was undertaken as part of a study of the diversity of these fungi. Four different isolation techniques using spores separated from soil, soil trap cultures, root samples, or transplanted seedlings grown in intact soil cores were used to obtain as many fungi as possible from each site. Isolation methods were compared using paired samples from the same locations within natural (savanna, rocky hill, wetland, rainforest) and disturbed (minesite) habitats in a seasonally dry tropical region in the Northern Territory of Australia. There were large differences in (i) the efficiency (rate of increase in mycorrhizal colonisation), (ii) the proportion of successful cultures, (iii) fungal diversity (number of fungal species in each culture) and (iv) specificity (identity of species isolated) between these four procedures. However, the less-efficient procedures generally resulted in a higher proportion of cultures of one fungus, which could be used without further isolation steps. Most species of *Scutellospora*, *Acaulospora* and *Gigaspora* were obtained primarily from field-collected spores, but only 50% of these culture attempts were successful. Spores from these initial cultures produced mycorrhizas much more rapidly and successfully when used to start second-generation cultures. Several species of fungi, rarely recovered as living spores from field soils, were dominant in many trap cultures started from soil or roots. Most of these fungi were *Glomus* species, that were first distinguished by colonisation patterns in roots and eventually identified after sporulation in second- or third-generation trap cultures. These experiments demonstrated that glomalean fungi in the habitats sampled belonged to two functional categories, based on whether or not spores were important propagules. The "non-sporulating" fungi were dominant in many trap cultures, which suggests that these fungi had higher total inoculum levels in soils than other fungi. Pot-culturing methods provided additional information on fungal diversity which complemented spore occurrence data obtained using the same soil samples and provided valuable new information about the biology of these fungi.

Key words Vesicular-arbuscular mycorrhizas · Glomales · Isolation and propagation · Tropical ecology · Fungal diversity

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Introduction

Propagation of cultures of vesicular-arbuscular mycorrhizas (VAM, arbuscular mycorrhizas) formed by glomalean fungi requires growth in association with a living plant. Cultures of these fungi are necessary to provide living fungal and mycorrhizal root material for research and practical applications. These cultures are also essential for taxonomic research, where they may provide sufficient healthy spores of fungi from field-collected soils (Walker 1992; Morton 1992). Researchers generally make "pot cultures" by growing an inoculated host plant in a sandy soil supplemented with a nutrient solution with low levels of phosphorus (Menge 1984; Jarstfer and Sylvia 1993). Glomalean fungi can also be propagated by growing plants in aeroponics, or other semi-hydroponic systems, or in root organ cultures (Plenchette et al. 1982; Bécard and Piché 1992; Jarstfer and Sylvia 1993), but these methods are not practical for isolating new fungi from field soils. Initiation and maintenance of living cultures of VAM fungi is difficult and time consuming and consequently is a

key factor limiting both research activities and practical uses of these fungi.

Despite the fundamental importance of pot-culture isolation methods, there have been few reports of new methods or comparisons of the relative efficiencies of different methods for initiation of these cultures. The procedures most often used are isolation from spores – which have been separated from a field soil by a sieving procedure, or trap cultures – where a bait plant is grown in diluted soil from a field site (Mosse 1956; Gilmore 1968; Menge 1984; Morton et al. 1993; Jarstfer and Sylvia 1993; Brundrett et al. 1996c). Isolation from spores has the advantage that these may belong to an identified fungus and result in a single-species isolate. However, not all VAM fungi produce sufficient quantities of spores in field soils to allow isolation or identification, so trap cultures can reveal species not observed to sporulate in soils (Miller et al. 1985; An et al. 1990; Stutz and Morton 1996; Watson and Milner 1996). In natural habitats VAM fungi also have hyphal networks, old root fragments, etc. which function as propagules (McGee 1989; Brundrett 1991). Spores present in soils may fail to germinate if they are old or parasitised (Lee and Koske 1994). Trap culturing methods often produce more healthy spores than the soils from which they were started, but usually result in a mixture of species which changes with subsequent culture generations (Morton et al. 1993; Bever et al. 1996). Trap cultures based on soil-dilution methods result in the isolation of more species than other methods (An et al. 1990; Watson and Milner 1996).

The work described in this paper is part of a study which aimed to characterise the role of mycorrhizal associations in undisturbed and disturbed habitats in the Alligator Rivers Region (ARR) of the Northern Territory of Australia. Pot-culture isolation techniques were used to provide isolates of glomalean fungi for research and practical use and to help establish their diversity in soils from different habitats in this region. Four isolation methods utilising different types of propagules produced by the fungi in soils were applied in an attempt to obtain as many species of fungi as possible. The main objectives of these culture experiments were to allow the efficiency and specificity of different isolation methods to be compared and to determine which VAM fungi have the most abundant propagules in ARR soils.

Materials and methods

The soils used in this experiment were collected from 32 sites in undisturbed and disturbed habitats in the Alligator Rivers Region during field trips in 1991 and 1992 (Brundrett et al. 1996a, b). This region includes Kakadu National Park and adjacent parts of Arnhem Land and consists of lowland eucalypt savanna woodland, upland, wetland, rainforest and estuarine vegetation on lateritic and sandstone-derived soils (Wilson et al. 1990). The disturbed sites sampled were minesite waste-rock habitats with varying amounts of vegetation cover (Brundrett et al. 1996b). The climate is tropical with a long dry season and a shorter wet season with periods of heavy monsoonal rainfall (Dunlop and Webb 1991).

Samples (1–3 kg) from the top 10 cm of the soil profile were collected from three locations along a transect at each site. Soils were collected in April–May 1991 and February–March 1992 at the end of the wet season, when root activity would be declining for most plants. The same soil samples from each site were used to start trap cultures and spore-based cultures after separation of spores from soil. Soil was sampled next to intact soil cores used to grow seedlings for bioassays at the same transect locations (see Brundrett et al. 1996a, b) and extra seedlings from these bioassays were also used as trap plants to start pot cultures. Soil samples were allowed to dry at air temperature (30–40 °C), before transport to Perth, then stored at room temperature (10–30 °C) for 3–6 months before being used to start pot cultures. A limited number of root samples were also collected to start cultures. These were paired with trap cultures started using soil from the same locations.

Specific details of methods used for experiments are provided here and more detailed instructions for pot culturing VAM fungi are available elsewhere (Morton et al. 1993; Brundrett et al. 1996c). Clover [*Trifolium subterraneum* L. cv. Seaton Park inoculated with *Rhizobium leguminosarum* bv. *trifolii* (TA1)] and forage sorghum [*Sorghum bicolor* (L.) Moench × *sudense* (Piper) Stapf cv. Sudax] were used as host plants for pot cultures. Five pre-germinated seeds were planted in each pot then thinned to 2 plants after germination. They were grown in non-draining pots filled with an extremely infertile sandy soil (approximately 1 ppm available P). Pots with soil were watered to field capacity using a complete nutrient solution with a P level giving 60% of maximal growth (see Table 3.3 in Brundrett et al. 1996c). Pots were watered to field capacity by weight every 1–2 days with deionised water and nitrogen was supplied every 2 weeks. Pots were maintained on open benches in a glasshouse where a clean environment was maintained by frequent washing of the floor with water.

To assess mycorrhiza formation and sporulation in living pot cultures, 1-cm diameter × 10-cm deep (30 g) soil samples were taken approximately every 2 months using a small soil corer. Steamed sand without nutrients was used to fill sampling holes. Soil from cores was washed through a coarse (1-mm mesh) sieve to collect root samples. A finer (40-μm mesh) sieve was used to collect spores which were further separated by centrifugation in 50% sucrose. Spores were mounted on microscope slides and examined to identify fungi. The diameter of unmounted spores was measured with an eyepiece scale. Roots were gently washed free of sand and examined under a dissecting microscope to observe external hyphae, immature spores and other structures. These roots were cleared and stained and the total length of mycorrhizal and non-mycorrhizal roots was quantified using the gridline intersection method (Giovannetti and Mosse 1980). Randomly selected segments of roots from each sample were mounted on slides and viewed with a compound microscope to identify fungi within roots by morphological patterns (Abbott 1982; Brundrett et al. 1996c). Pot cultures without any mycorrhizas were discarded after 4 months and the rest allowed to grow for 6–8 months.

Pot cultures from spores

Spores were separated from 6 soil samples from each site by wet-sieving and centrifugation with 50% sucrose using 50–200 g of soil from each sample. Larger soil samples (500–1000 g) were sometimes used to obtain spores from disturbed sites. Spores were examined under a dissecting microscope, sorted into categories based on size, colour and other features. Those considered to be different species were assigned numbers within a genera (Table 1). Spores from each category were mounted on slides in a polyvinyl alcohol-lactic acid- glycerol based mountant with Melzer's reagent, crushed under the cover slip and observed with a compound microscope to confirm that each putative species was morphologically different. Healthy spores of uniform appearance from each category were transferred to an individual filter paper

Table 1 Results of isolation experiments using spores, soil, roots, or transplanted seedlings from natural habitats and disturbed minesites (*DS*). Numbers of successful cultures are shown for each fungus. Note that cultures are listed by dominant fungus, but most trap cultures also contained other fungi. Fungi were identified by spores (A, B), or root morphology patterns (C–F). Summary data showing the average and maximal number of species present in cultures are also shown for each isolation procedure

Species		A Spores	B Spores (DS)	C Soil trap	D Soil trap (DS)	E Roots (DS)	F Transplant
Scutellospora							
	1	1	2		1		
	2	8	2		1		
	3	10	1				
	4	4	1				
	5	0					
	6	5					
	7	1		3			1
Gigaspora sp.		6	1	1			
Acaulospora							
	1	7	2				
	2	1					
	3	2					
	4	0	1				
	5	2	3				
	6			5			1
Glomus							
	1			6	5	6	3
	2	1		1			4
	3		4		2	1	
	4			5	5	1	3
	5			2	1		
	6			2	5	5	5
	7			1			
	8			5	1	1	2
	9		3				
No. successful		48	20	42	21	14	19
No. attempted		110	36	70	30	15	220
Average No. spp.		1.11	1.39	1.87	3.25	1.92	1.18
Maximal No. spp.		2	2	4	5	4	2

triangle (approximately 1×2 cm). Spores on paper triangles were kept on damp filter paper and used to start pot cultures within 1–2 days of separation from soil. A standard procedure (Mosse 1956; Gazey et al. 1992; Brundrett et al. 1996c) was used to initiate pot cultures whenever sufficient spores of a fungus could be found, in an attempt to isolate as many fungi from each habitat as possible. 5–100 large or 50–500 small spores were used for each attempt. Spores on the filter-paper triangles were washed into a wide, 3-cm deep hole in pots of steamed sand watered to field capacity with nutrient solution. These spores were then covered with sand and incubated for 1 week before sorghum seeds were planted over them. A total of 110 pot cultures was attempted with spores from 22 undisturbed habitats and 36 pot cultures with spores from 10 disturbed habitats (Table 1).

Trap cultures using soil

Field soils mixed with sterile sandy soil were used to isolate VAM fungi (Menge 1984; Morton et al. 1993; Jarstfer and Sylvia 1993). Each trap culture was started with 100 g from undisturbed sites or 250 g from disturbed sites of mixed and sieved soil (particles <2 mm). This soil was placed between two layers of steamed sand in 1 l plastic pots, resulting in a total of 1.5 kg of dry soil (see Brundrett et al. 1996c). Clover or sorghum seedlings were sown in these pots several days after first watering them to field capacity. There were 66 trap cultures started using soil from 22 undisturbed habitats and 30 trap cultures using soil from 10 disturbed sites (Table 1).

Trap cultures using roots

A limited number of trap cultures were also initiated using root inoculum (Gilmore 1968; Liberta et al. 1983). Roots of 6 *Acacia* species (*A. difficilis, A. dimidiata, A. holosericea, A. oncinocarpa, A. plectocarpa* and *A. tropica*), from disturbed mine sites and *Bambusa arnhemica* and *Pandanus spiralis* collected from natural wetland habitats were used. Each culture was started with 1–3 g of roots, which had been air dried for transport and stored at room temperature for several months. Replicate root samples were cleared and stained to measure VAM colonisation, which was high in all cases. Roots were layered within 1.5 kg of steamed sand planted with sorghum as described above. A total of 15 pot cultures was started using root samples (Table 1).

Pot cultures from transplanted seedlings

Clover seedlings grown in intact cores of soil from field sites for 4 weeks were obtained after a bioassay experiment (Brundrett et al. 1996a). These putatively mycorrhizal seedlings were each transplanted into a 100-ml conical pot containing 150 g of steamed sand to which nutrients were added at the same concentrations used for other cultures. These small pots allowed many mycorrhizal clover plants to be grown in a small space. Plants growing in these free-draining pots were maintained by regular watering with deionised water. Ten seedlings from soil cores taken from each of 22 natural habitats were used to start 220 cultures (Table 1). Two

pots from each site were randomly selected and harvested after 2, 3 and 4 months to quantify VAM formation and spore production. Pots were given a second application of nutrient solution after 3 months. After 4 months, pots were sampled by removing the bottom 1/4 of their soil and roots to quantify root colonisation by VAM fungi and spore formation, before allowing the rest of the soil to dry.

Second- and third-generation pot cultures

In this paper, a generation is defined as one growth cycle by mycorrhizal fungi in host roots initiated from quiescent propagules, either collected from the field, or resulting from an earlier pot culture. At the end of a generation, cultures were air dried for 2–4 months in a glasshouse by withholding water until weight loss ceased. Then dead shoot material was removed and pot culture soil was sealed in airtight containers and stored at room temperature for 6 months or longer before use. This storage period was to ensure that propagules were able to germinate (Tommerup 1992). New pot cultures were started using 10–500 healthy looking spores from a spore-based culture, or 130–259 g of mixed inoculum from a trap culture (sand, roots and spores). The amount of inoculum (spores or soil) was similar to that used to start first-generation cultures and it was applied in the same way.

Results

Pot cultures from spores

Soils from the plant communities sampled contained many healthy-looking VAM spores that could be used to initiate cultures, when sampled at the end of the growing season. It appeared that VAM fungus sporulation peaked at this time (as the root activity of plants was declining). The use of spores of individual fungi found in soil from undisturbed habitats resulted in 44 successful first generation pot cultures from 110 attempts (Table 1). The number of culture attempts for each spore type varied from 25 to 2 pots depending on their frequency and abundance in soil samples. Isolation success rates were relatively low for all VAM spore types and varied from 0 to over 50% of culture attempts. However, the low success rate was compensated by the value of resulting cultures, since almost all contained a single fungus species (Table 1). The number of spores used to start cultures varied considerably, due to variation in soil spore density, but was not correlated with the success of cultures. The relatively high failure rate for spore cultures suggests that most spores from field soils did not germinate (this was confirmed by germination tests (M. Brundrett, unpublished data). The isolation of VAM fungi with spores from disturbed sites was somewhat more successful, with 20 isolates resulting from 36 attempts (Table 1). For most fungi, VAM colonisation levels in roots took a long time to increase in successful first-generation pot cultures (Fig. 1A), again presumably because of the low germination rate of field-collected spores.

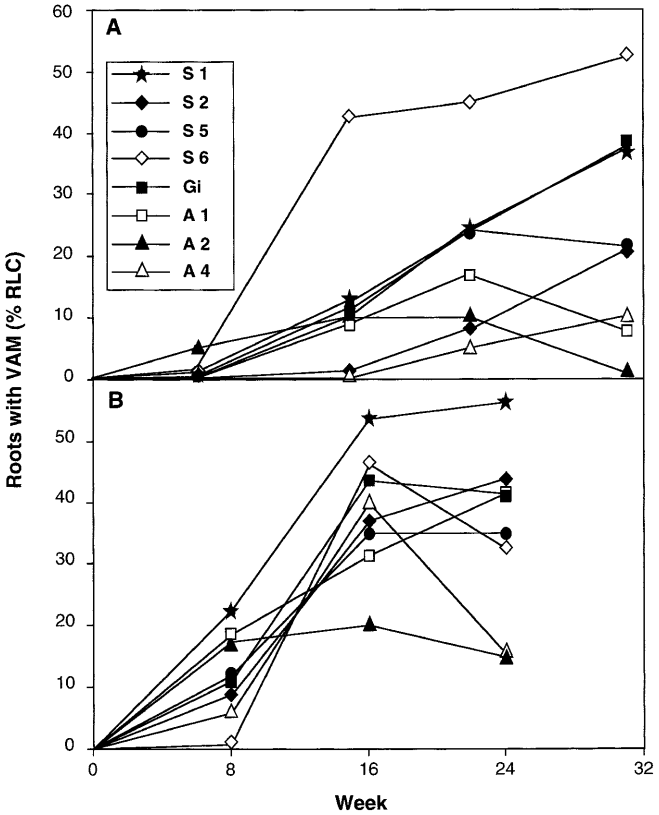

Fig. 1A,B Average values for the time-course of VAM development in successful sorghum pot cultures of the 8 glomalean fungi most commonly isolated from spores. **A** First-generation cultures from field-collected spores. **B** Second-generation cultures using spores from existing cultures. *RLC* root length colonised, *S Scutellospora, A Acaulospora*, *G Gigaspora*

The diversity in spore-based cultures could be easily assessed by identifying spores sieved from cultures, as all the first-generation spore cultures containing substantial amounts of mycorrhizal roots also contained spores. Cleared and stained roots were also examined to exclude additional non-sporulating fungi. The majority of spore-based cultures contained a single fungus species (Table 1), but a few had two species (started with two types of spores similar in size and colour). Isolates of *Scutellospora, Gigaspora* and *Acaulospora* were most often obtained (Table 1), because spores of these fungi occurred in sufficient numbers to start pot cultures in most soil samples. Living *Glomus* spores, were rare in most soils (most were devoid of contents and with holes in their walls), except in samples from disturbed or wetland habitats – which were used to isolate several *Glomus* species (Table 1). A species of *Scutellospora* (6) which was common in soil samples, consistently failed to become established in pot cultures (Table 1). Spores of this fungus appeared healthy, but failed to form mycorrhizas (six attempts). An *Acaulospora* species, which was less common, also failed to establish in culture (two attempts). All remaining species detected as spores in soil were established in culture using a single soil type as the growing medium.

Trap cultures using soil

Mycorrhizal colonisation levels typically built up more rapidly in trap cultures started from soil than in those started from spores (Fig. 2), suggesting higher initial propagule numbers in the former. The overall success rate was higher than for spore-based cultures, but there were more species of fungi in soil trap cultures than in those started by other means (Table 1). Consequently, it was more difficult to identify all the fungi present in each culture. Cultures were evaluated by sieving soil for spores and by examining cleared and stained root samples for VAM, but only mycorrhizal morphology patterns in roots could be used to identify dominant fungi as most fungi did not sporulate. It is possible that diversity in first-generation cultures was underestimated because some rare fungi were overlooked.

The majority of soil trap cultures started using soil from natural or disturbed habitats were dominated by *Glomus* species, along with one species of *Acaulospora* and one of *Scutellospora* (Table 1). These latter fungi were different from the *Acaulospora* and *Scutellospora* species isolated using spores from the same soil samples. Thus species not detected as living spores in soil were dominant initially in most trap cultures and must have become established from other propagules. Five common types of *Glomus* colonisation, with different vesicle and hyphal structures, were identified morphologically in trap culture roots. These were eventually linked to different fungal species after sporulation in second- or third-generation cultures.

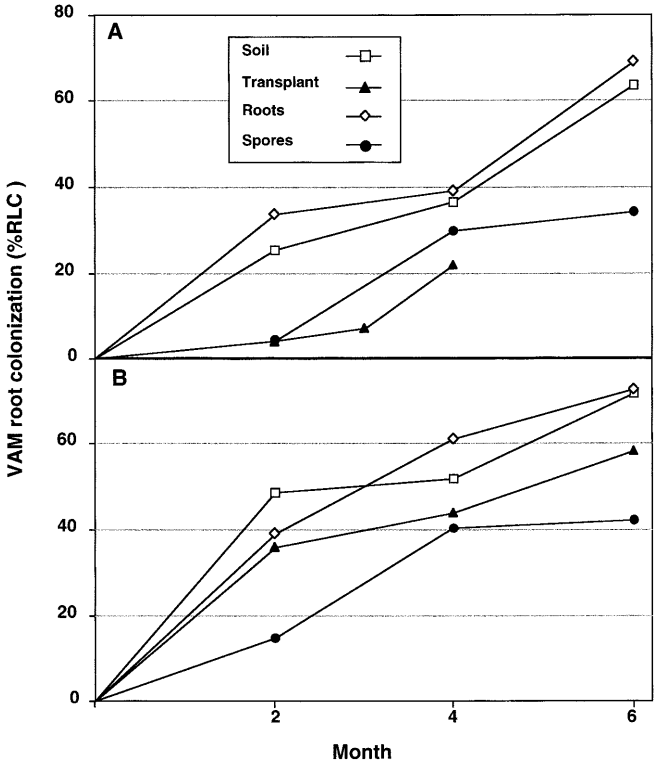

Fig. 2 The development of mycorrhizal colonisation in (**A**) first- or (**B**) second-generation pot cultures started using spores, soil, transplanted seedlings, or root inoculum. Values are averages for all cultures (*RLC* root length colonised)

Trap cultures using roots

A limited number of trap cultures using dried roots were started to allow comparison with trap cultures using soil or spores from the same locations. Roots were an efficient way of initiating cultures of some fungi associated with particular plants, as mycorrhizal colonisation levels in pots increased more rapidly than in cultures started with other inoculum forms (Fig. 2). However, the diversity of fungi isolated by this method was relatively low. Most fungi did not sporulate in first-generation cultures, but *Glomus* species were observed to be dominant, with 1–4 fungi recognised by root morphology patterns in each culture (Table 1).

Pot cultures from transplanted seedlings

Transplanting mycorrhizal clover bait plants from bioassay experiments into pots resulted in the establishment of some VAM fungi into pot cultures, but overall success rates were poor (Table 1). The diversity of fungi in the resulting cultures was very low, as almost all the successful pots contained one species of *Glomus* (Table 1). *Scutellospora* or *Acaulospora* isolates were obtained only once by this means. The build-up of mycorrhizal colonisation levels was much slower than for other isolation techniques (Fig. 2). However, examination of roots showed that most of the successful cultures contained a single fungus, which did not require further purification. Examination of seedlings grown as replicates of those used to start cultures confirmed that most had been colonised by VAM fungi (Brundrett et al. 1996a). A second experiment using larger (1-l) pots and two host plants produced similar results (not presented) and showed that the low diversity of fungi was not due to the small volume (100 ml) of pots used in the first experiment.

Second-generation cultures

Mycorrhiza formation and spore production in second-generation cultures started by different methods is compared in Figures 1–4. Differences in the rate of mycorrhizal colonisation likely resulted because higher amounts of inoculum were used to start soil- and root-based cultures than spore-based cultures. However, the average rate of sporulation in spore-based cultures was higher than in root or transplant cultures, which typically had higher levels of VAM (Fig. 3). Spore-based cultures were started using isolates of *Scutellospora, Gigaspora* and *Acaulospora* and several *Glomus* species

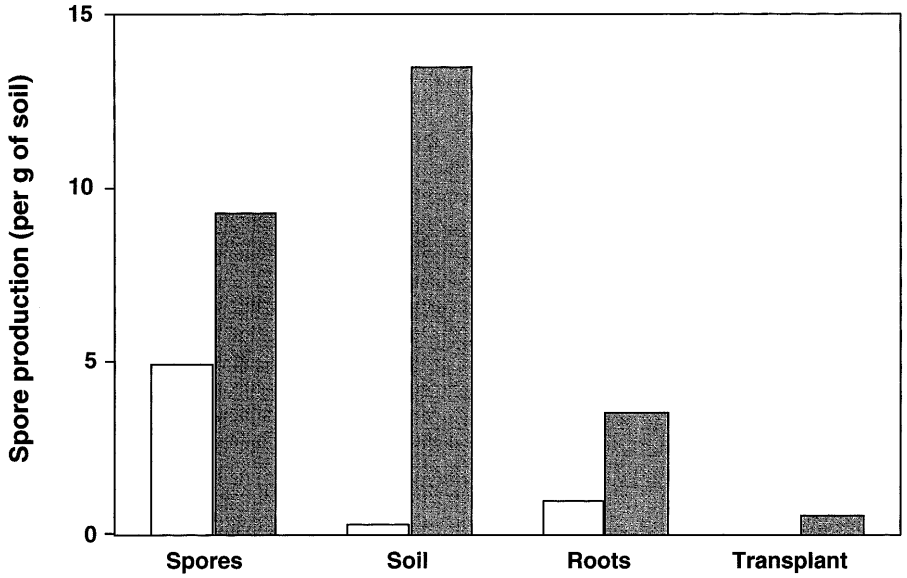

Fig. 3 Average spore production values for all first- (□) and second-generation (■) pot cultures started from spores, soil, roots or transplanted seedlings. Cultures were grown for 6–7 months using similar inoculum levels and growing conditions

which were present as spores in many soil samples from the field. These same fungi sporulated in all cultures where there was substantial mycorrhizal development. Increased sporulation in second-generation cultures likely resulted because mycorrhizal colonisation levels built up more rapidly (Fig. 3).

For cultures started from soil, roots, or transplanted seedlings, a second generation of pot cultures (started using soil from the first culture) was usually required for spores to be produced, so that the identity of fungi could be confirmed. Limited sporulation in first-generation cultures was correlated with the dominance of some *Glomus* species (Table 1). These fungi were designated as slow or infrequent sporulators, because they did not form spores in young pot cultures with relatively high mycorrhizal colonisation levels, and because healthy spores of these species were rare in soil samples from the field. The lack of sporulation in first- and second-generation cultures from transplanted seedlings was correlated with the slow build up of mycorrhizal colonisation in these cultures (Fig. 2).

Almost all second-generation spore cultures, started using spores from first-generation isolates, were successful (only 2 out of 42 failed). Thus spores produced in pot cultures were much more viable than those obtained from the field. Germination trials (data not presented) confirmed that most spores from dried pot cultures were capable of rapid germination (usually within 3 days). Mycorrhizal colonisation levels in roots increased rapidly in second-generation cultures, often reaching a peak after 16 weeks (Fig. 1B). This contrasted with the slower development of mycorrhizal colonisation in first-generation cultures (Fig. 1A). When compared to cultures started using soil or roots, mycorrhizal colonisation levels were lower in second-generation cultures started from spores (Fig. 2).

Information on spore production and mycorrhizal colonisation levels in second-generation spore-based pot cultures was obtained for different fungal isolates. These measurements revealed substantial variation in average spore density in pot cultures between different species and isolates of fungi (Fig. 4), with the *Acaulospora* isolates sporulating more abundantly than those of other genera. There was no clear overall relationship between spore production and mycorrhiza formation in pot cultures (Fig. 5). Mycorrhizal colonisation levels were relatively low in many *Acaulospora* cultures that sporulated abundantly, while the reverse was true for *Glomus* species. However, there was a strong (log-linear) inverse correlation between the average diameter of spores of different fungi and their production in pot-culture soils (Fig. 6). Thus, small-spored fungi, such as *Acaulospora* and *Glomus*, produced many more spores in the cultures examined than large-spored *Scutellospora* and *Gigaspora* species.

Pot cultures found to contain a single VAM fungus were given isolate numbers. Most of these pure isolates were originally started from spores. However, some cultures started by transplanting mycorrhizal seedlings also contained a single fungus. Spores from second- or third-generation soil or root trap cultures were used to start single-isolate cultures of additional species. The use of non-draining pots which were watered to weight ensured that there was no cross-contamination between fungal cultures. Uninoculated control pots occasionally became contaminated by a fine endophyte which apparently survived steam sterilisation of the soil or was spread by dust. There was no evidence of contamination by other VAM fungi. Small core samples allowed the quality of cultures to be assessed periodically with minimal damage to plant root systems.

Discussion

This study provided the first comprehensive comparison of the efficiency and specificity of different meth-

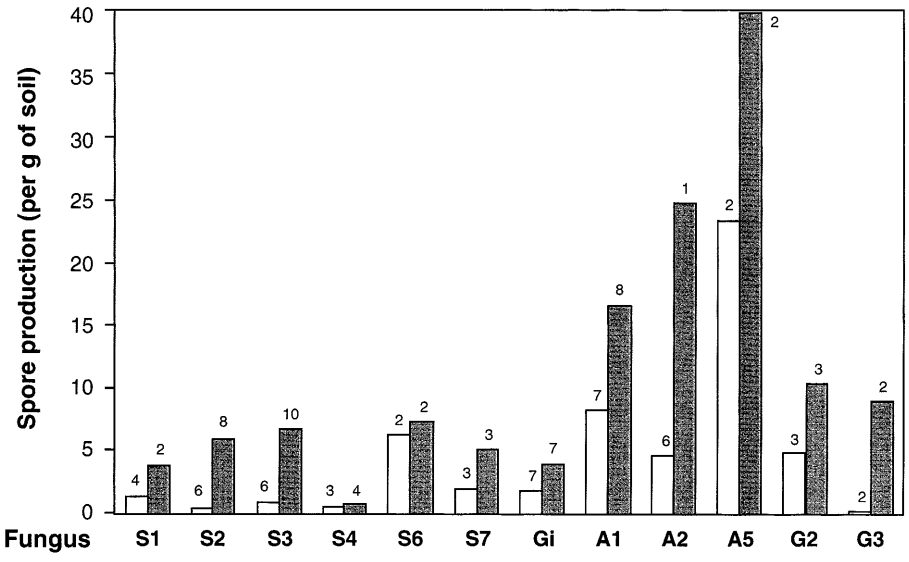

Fig. 4 Average spore production for different species of Glomalean fungi in first- (□) and second-generation (■) pot cultures started from spores. Fungus species within genera are represented by numbers. Numbers of isolates are shown above bars *A Acaulospora*, *G Gigaspora*, *S Scutellospora*

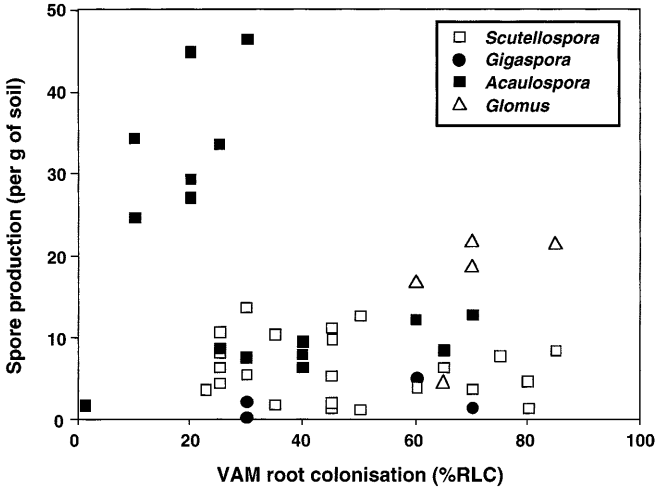

Fig. 5 Comparison of mycorrhiza formation for different fungus isolates within genera and spore production values in pot cultures. Each point is one second-generation isolate. There is more than one isolate of each species (*RLC* root length colonised)

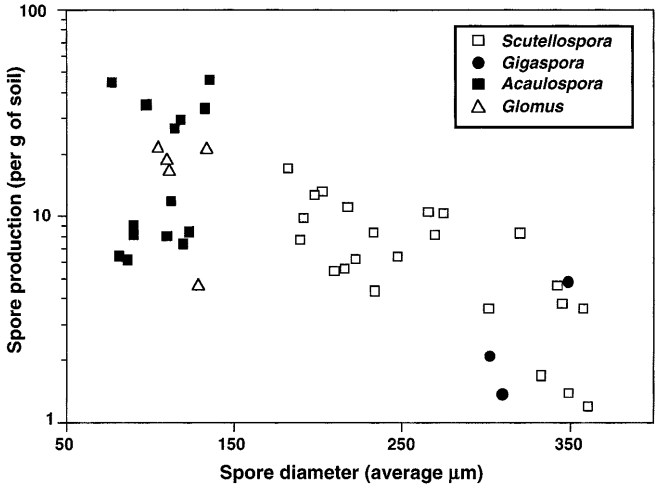

Fig. 6 The relationship between the average diameter of spores for different fungal isolates within genera and spore production values (on a log scale) in pot cultures started from spores. Each point is one second-generation isolate. There is more than one isolate of each species

ods for isolating VAM fungi. The four different techniques used to initiate pot cultures of VAM fungi were found to complement each other very well, because they often produced cultures dominated by different species from the same soil samples. Healthy spores of *Acaulospora, Gigaspora* and *Scutellospora* species were often common enough in soils (at the end of the growing season) to allow direct isolation of these fungi. However, the success rate of spore pot culture initiation attempts was fairly low, so it would be advisable to start a large number of them. Fungi which produced many spores in soils were less likely to be isolated than other fungi when the same soil samples were used to start trap cultures. In particular, several *Glomus* species, one *Acaulospora* and one *Scutellospora* species were dominant in many trap cultures started using soil or roots taken from soils devoid of living spores of these fungi. For these species, inoculum levels in soils were not related to numbers of spores. The soil-based trap culture isolation procedure apparently selected the most competitive VAM fungi present in a soil, or those that could best adapt to the growth conditions used. However, other fungi present in these cultures may become more important in subsequent generations of cultures (Stutz and Morton 1996). A trap culture technique using serial dilution of soil (An et al. 1990) apparently can result in the isolation of more fungi in trap cultures, but would not allow as many soils to be used. Another method which uses pregerminated spores to start single-spore cultures should also be considered if sufficient healthy spores of fungi can be obtained directly from soils (Brundrett and Juniper 1995).

Care must be taken in interpreting results obtained using any one culture initiation method in population studies, as the diversity of fungi present in soils may be under-represented. It is recommended that spore-based isolation procedures be used, along with a soil or root-based trap culture method, to obtain as wide a diversity of fungi as possible from a soil. When comparing the isolation methods used, there was a trade-off between the number of fungus species in cultures and the efficiency of fungal inoculum propagation. The more efficient methods (trap cultures from bulk roots and soil) used inoculum sources which would have contained many more propagules than the less-efficient methods (selected spores, transplanted seedlings). However, cultures produced by the slower methods were usually pure enough to be used for taxonomic studies after only one generation. Trap cultures amplify fungal biomass in soils, but can not be used directly in experiments because fungal communities within cultures would be likely to change with time and this could lead to inconsistent results. The relatively low probability of success and slow development of mycorrhizas resulting from the two slower isolation methods suggests that the resulting cultures were initiated by very few (perhaps only one) viable propagules. Thus the genetic diversity of cultured fungi may be lower than it was in the soil and this may reduce the variability of some taxonomic or physiological properties of fungi.

In this study, most species of *Acaulospora, Gigaspora* and *Scutellospora* observed in roots failed to survive when transplanted seedlings or dry roots were used to initiate pot cultures. The transplant procedure apparently was only successful when roots contained high colonisation levels of *Glomus* species with intraradical vesicles, but was still valuable because the resulting cultures often contained a pure fungal isolate. Bever et al. (1996) also observed that the diversity of fungi isolated by a transplant method was substantially lower than in trap cultures. Biermann and Linderman (1983) compared the capacity of root fragments colonised by different VAM fungi to act as propagules and observed that fungi without vesicles could not initiate mycorrhizas from roots. However, Tommerup and Abbott (1981) found that *Glomus* species with vesicles and a *Gigaspora* species without them could both initiate mycorrhizas from root fragments. It is possible that root-based isolation procedures can be improved if future research considers the age and species of transplanted seedlings or the qualities of roots and storage conditions used.

In a study of two *Acaulospora* species, Gazey et al. (1992) observed that sporulation began only after root colonisation levels built up to threshold values. In the present study, the relationship between levels of mycorrhizal colonisation and the onset of sporulation was more complex. Most isolates of *Acaulospora, Scutellospora* and *Gigaspora* produced spores in all successful cultures, even if mycorrhizal colonisation levels were very low. In contrast, many *Glomus* isolates did not sporulate in first-generation trap cultures despite relatively high levels of mycorrhizal colonisation. The slow sporulation by these *Glomus* species made it necessary to examine mycorrhizal morphology in cleared and stained root samples to assess fungal diversity in cultures. This technique was an efficient way to distinguish colonisation by different genera of VAM fungi in trap cultures and also allowed different categories of *Glomus* mycorrhizas to be distinguished. These *Glomus* categories were found to correspond to different species once sporulation occurred in second-generation cultures. For quality control assessment of pot cultures, it is important to examine root morphology in order to identify non-sporulating fungi.

In pot cultures, variation in spore production could not be explained by mycorrhizal colonisation levels, but there was a strong inverse correlation between the size of spores of different fungi and the numbers produced. This relationship has also been observed for many other soil organisms (Chuang and Ko 1981). The reason why some VAM fungi produce many small spores and others only a few large ones is unclear, as single germinated spores of small-spored species have a similar capacity to establish mycorrhizas as those of large-spored fungi (Brundrett and Juniper 1995). If sporulation is used as a criterion for evaluating fungi, it should be noted that fungi with relatively small spores are likely to produce many more of them than those with large spores.

In this study, *Glomus* species which only sporulated after several generations of cultures were often observed to produce spores concentrated in a few large aggregations (sporocarps). These fungi may have to build up large amounts of fungal biomass before sporulation can occur. Sporulation by *Glomus* species with aggregated spores is likely to be primarily as a means of dispersal, if they are consumed by small animals (McGee and Baczocha 1994; Janos et al. 1995). These fungi probably rely primarily on other propagules which are more widely dispersed through the soil for their survival and spread. The lack of viable spores of these species in most soil samples may result from only occasional sporulation or its restriction to certain locations in soil. It should be noted that other *Glomus* species readily produced spores in young pot cultures and these spores were not aggregated. The contrasting sporulation trends observed provide evidence of substantial differences in life history strategies both between and within genera of glomalean fungi.

Fungi in the tropical soils studied here could be separated into two major functional categories, based on whether or not they produced spores as a major propagule in soils. One consequence of these two contrasting life cycles is that pot culture methods should be used in conjunction with spore-based taxonomic surveys of fungi, as the spore-based method only works with fungi that sporulate readily, while the trap methods can detect non-sporulating fungi that may be an important component of soils. Non-sporulating fungi have often

been reported to be important in soils from ecosystems (Miller et al. 1985; Liberta et al. 1983; McGee 1989; Alexander et al. 1992; Brundrett and Abbott 1995; Stutz and Morton 1996; Merryweather and Fitter 1998).

The number of species (especially *Glomus*) isolated into pot cultures always exceeded those identified from field-collected spores, suggesting that fungal surveys based solely on spore observations are inaccurate, at least in these habitats. There have been many spore-based surveys in the past and results of these should only be compared to other surveys using similar methods. The fungi identified from pot cultures, but not as spores, included some of the most important fungi present in soils, as shown by their aggressive colonisation of host roots in bioassay and trap culture experiments.

Differences between groups of VAM fungi in their isolation and sporulation in pot cultures may reflect major differences in their life-history strategies. In particular, it seems that spores are of minor importance as propagules for some *Glomus* species, but much more important for most fungi in the genera *Acaulospora, Gigaspora* and *Scutellospora.* In the past, a limited number of careful studies of fungal biology have suggested substantial differences between species of VAM fungi in their life-history characteristics (McGee 1989; Abbott et al. 1992; Merryweather and Fitter 1998). Further research is required to determine whether these characteristics are correlated with the capacity of fungi to survive and spread in soils. We still have much to learn about the basic biology of glomalean fungi. Detailed observations of pot cultures can provide valuable information about the structure and function of structures produced by different fungal species in roots and soil and provide reliable information for taxonomic studies.

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