



Revista Brasileira de Ciência do Solo

ISSN: 0100-0683

revista@sbcs.org.br

Sociedade Brasileira de Ciência do Solo
Brasil

ANTONIOLLI, Z. I.; FACELLI, E.; O'CONNOR, P.; MILLER, D.; OPHEL-KELLER, K.; SMITH, S. E.
SPORE COMMUNITIES OF ARBUSCULAR MYCORRHIZAL FUNGI AND MYCORRHIZAL
ASSOCIATIONS IN DIFFERENT ECOSYSTEMS, SOUTH AUSTRALIA

Revista Brasileira de Ciência do Solo, vol. 26, núm. 3, 2002, pp. 627-635

Sociedade Brasileira de Ciência do Solo

Viçosa, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=180218340007>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative

SPORE COMMUNITIES OF ARBUSCULAR MYCORRHIZAL FUNGI AND MYCORRHIZAL ASSOCIATIONS IN DIFFERENT ECOSYSTEMS, SOUTH AUSTRALIA⁽¹⁾

**Z. I. ANTONIOLLI⁽²⁾, E. FACELLI⁽³⁾, P. O'CONNOR⁽³⁾,
D. MILLER⁽³⁾, K. OPHEL-KELLER⁽⁴⁾ & S. E. SMITH⁽³⁾**

SUMMARY

Communities of arbuscular mycorrhizal fungi (AMF) were surveyed in different South Australian ecosystems. The soil was wet-sieved for spore extraction, followed by the determination of presence and abundance of AMF species as well as the percentage of root colonization. Mycorrhizal associations were common and there was substantial fungal diversity in different ecosystems. Spores were most abundant in the permanent pasture system and less abundant under continuous wheat. The incidence of mycorrhizal associations in different plant species and the occurrence of *Arum*- and *Paris*-type colonization generally conformed with previous information. Spores of seventeen AMF were verified throughout seasonal changes in 1996 and 1997 in the permanent pasture and on four host species (*Lolium perenne*, *Plantago lanceolata*, *Sorghum* sp. and *Trifolium subterraneum*), set up with the same soils under greenhouse conditions. *Glomus mosseae* was the dominant spore type at all sampling times and in all trap cultures. Mycorrhizal diversity was significantly affected by different sampling times in trap cultures but not in field-collected soil. *P. lanceolata*, *Sorghum* sp. and *T. subterraneum* as hosts for trap cultures showed no differences in richness and diversity of AMF spores that developed in association with their roots. Abundance and diversity were lowest, however, in association with *L. perenne*, particularly in December 1996. Results show that the combination of spore identification from field-collected soil and trap cultures is essential to study population and diversity of AMF. The study provides baseline data for ongoing monitoring of mycorrhizal populations using conventional methods and material for the determination of the symbiotic effectiveness of AMF key members.

Index terms: trap cultures, *Glomus*, *Gigaspora*.

⁽¹⁾ Received for publication on march of 2001 and approved on april of 2002.

⁽²⁾ Departamento de Solos, Universidade Federal de Santa Maria (RS). CEP 97105900 E-mail: zaida@ccr.ufsm.br

⁽³⁾ The Centre for Plant Root Symbioses and Department of Soil and Water, The University of Adelaide, PMB 1, Glen Osmond, South Australia, 5064, Australia. E-mail: sally.smith@adelaide.edu.au

⁽⁴⁾ South Australian Research and Development Institute, GPO Box 397, Adelaide, South Australia, 5001, Australia. E-mail: ophelkeller.kathy@wpo.pi.sa.gov.au

RESUMO: *COMUNIDADES DE ESPOROS DE FUNGOS MICORRÍZICOS ARBUSCULARES E ASSOCIAÇÃO MICORRÍZICA EM DIFERENTES ECOSSISTEMAS NO SUL DA AUSTRÁLIA*

Comunidades de fungos micorrízicos arbusculares (FMAs) foram estudadas em diferentes ecossistemas, no sul da Austrália. A extração dos esporos do solo foi efetuada pelo método de peneiramento úmido, para posterior quantificação e identificação das espécies de FMAs. A percentagem de raízes colonizadas também foi determinada. Associações micorrízicas foram comuns em todos os ecossistemas estudados. Os esporos foram mais abundantes no sistema de pastagem permanente e menor no sistema de plantio contínuo com trigo. Percebeu-se a incidência de associações micorrízicas nas diferentes espécies de planta e a ocorrência de Arum e Paris-tipo de colonização. Esporos de 17 espécies de FMAs foram identificados durante as variações sazonais de 1996 e 1997 no sistema de permanente pastagem e em quatro hospedeiros (Plantago lanceolata, Sorghum sp., Trifolium subterraneum, Lolium perenne) cultivados em casa de vegetação. Glomus mosseae foi a espécie dominante em todas as épocas de coleta e nos vasos de cultivo. A diversidade micorrízica não foi significativa nas diferentes épocas de amostragem no campo, mas esta foi significativa nas culturas armadilhas. P. lanceolata, Sorghum sp. e T. subterraneum como hospedeiras das culturas armadilhas não mostraram diferença no número e diversidade de esporos de FMAs associados ao sistema radicular. Entretanto, o número e a diversidade de espécies foram mais baixas com L. perenne (principalmente em dezembro de 1996). A identificação de esporos extraídos do solo do campo e de plantas armadilhas é essencial para o estudo das populações e diversidade dos FMAs. Este trabalho proporciona dados para monitoramento das populações usando técnicas convencionais e material para determinação da efetividade simbiótica de espécies chaves da comunidade de FMAs.

Termos de indexação: plantas armadilhas, Glomus, Gigaspora.

INTRODUCTION

Symbiotic arbuscular mycorrhizal fungi (AMF) are often the most abundant fungi in soil, where the extraradical phase of the symbiosis is composed of fungal mycelium and spores. Spores are one of the means of survival of AMF in the absence of living roots and are also the developmental stage on which taxonomy of the group and hence identification of species' presence is currently based (Smith & Read, 1997). Agricultural practices may alter spore populations (Abbott & Robson, 1982; Allen & Boosalis, 1983; Dodd et al., 1990; Sieverding, 1991), and may be reflected in changes in the vegetative colonization of roots as well as in the aspect of the symbiosis involved in nutrient exchange between the partners. The productivity of plant communities will depend on the symbiotic effectiveness of the colonizing fungi and possibly also on the diversity of the fungal assemblage (van der Heijden et al., 1998). The functional significance of formation of the two main morphological AMF types (*Arum*-AMF and *Paris*-AMF) is not yet clear, but improved knowledge of their distribution in different plants, together with physiological investigations, will remedy this situation (Smith & Smith, 1997). Our aim was to investigate the effects of different crops on AMF

communities. The Waite Permanent Rotation trial, established in 1925, provides unique resources for an investigation of this type and was selected for this study in 1996, including a preliminary survey of permanent pasture, wheat-fallow, continuous wheat, and an adjacent arboretum, followed by a more detailed survey of the permanent pasture treatment over two years.

MATERIALS AND METHODS

Location and details of site

The Waite Permanent Rotation Trial is situated on the Waite Campus of the University of Adelaide, Urrbrae, South Australia (Grace et al., 1995). The climate is Mediterranean, with cool wet winters and hot dry summers and an average annual rainfall of ~500 mm. The soil is a fine sandy loam (red-brown earth; Stace et al., 1968), pH 5.9, negligible calcium carbonate, soil organic carbon between 0.67 and 1.28 % depending on rotation. The chosen rotations were permanent pasture (uncultivated), wheat-fallow and continuous wheat (conventionally cultivated). Samples were also taken from an adjacent arboretum.

The permanent pasture phase was selected for a more detailed study over two years in 1996 and 1997.

Sampling

A preliminary survey of one plot of the Waite Permanent Rotation Trial was realized in July 1996, followed by a more detailed investigation of the permanent pasture in July (winter) and December (summer) of 1996 and 1997. A range of pasture, crop and weed species were then growing freely on the trial plots. Plant species were identified as far as possible from vegetative material. The small number of plants of each species did not warrant an accurate statistical analysis and data can only be taken as an indication of colonization. Soil samples were collected from each plot by taking 43 cores (10 cm diameter x 20 cm deep) at random, including the root systems of plants growing in the plot. The soil was separated from the roots. The cores were pooled to produce one composite soil sample which was air-dried, mixed, and sieved through a 2 mm sieve before being used as inoculum for trap cultures and for enumeration of AMF spores. The samples were stored in sealed plastic bags at 4 °C until spores could be counted.

Enumeration of spores

For the preliminary survey, spores were recovered from triplicate 100 g soil samples by wet-sieving the soil (Gerdeemann & Nicolson, 1963), followed by soaking for 20 min. The remaining material on the sieves was washed into beakers. For the seasonal surveys of permanent pasture, spores were recovered from triplicate 25 g samples of the composite soil sample using a method recommended by Dr. C. Walker (personal communication). Each sample was dispersed in tap water and the suspension kept undisturbed for 30 min to allow soil particles to settle. The suspension was decanted through 710 and 25 µm sieves. More water was then added to the sample which was stirred to resuspend spores and left to stand for 15 sec, to allow sand-sized particles to settle. The resultant supernatant was then decanted through the two sieves. This was repeated four more times until the supernatant became clear. The material from the 25 µm sieve was then centrifuged in water, and the debris discarded. After centrifuging for 20 sec the supernatant spores on 75 % sucrose were washed with water. Material from the 710 µm sieve was observed without further treatment. Spores which appeared to be un-parasitized and cytoplasm-filled were counted under a dissecting microscope. For ease of comparison results are given as numbers of spores per 25 g soil.

Identification of spores

Preparation of spores for identification was carried out according to Morton (1986). Species

identification was based on spore color, size, surface ornamentation and wall structure. Permanent slide vouchers were made of all fungi and some fungi were established in single species cultures and retained in the Soil and Water Department, University of Adelaide. The spores were genus or species identified according to the keys of Schenck & Pérez (1990), Hall & Fish (1979), INVAM Web (<http://invam.caf.wvu.edu/>) and CD-ROM Demo Version - The BEG - Expert System Arbuscular Mycorrhizal Fungi by Rosendahl and Dodd.

Trap cultures

The plants used were *L. perenne*, *T. subterraneum*, *P. lanceolata* and, present at the field site, and *Sorghum* sp since it is frequently recommended and used for maintenance of pot cultures (Morton et al., 1993). Seeds were surface sterilized with 0.5 % NaOCl for 10 min, rinsed in sterile distilled water and germinated on moist filter paper at 25 °C. After 7 days seedlings were transplanted into 1 kg pots containing a mixture of unsterilized field-soil from the permanent pasture and autoclaved sand (1:1 w:w). Seedlings of *T. subterraneum* were inoculated with 1 mL of *Rhizobium trifolii* Dangeard, suspended in a 1 % sucrose solution at transplanting. All plants were grown in a growth room for five months under the following conditions: 12 h, photoperiod, 18-25 °C temperature, 60-75 % relative humidity, 240 µE m⁻² s⁻¹ irradiance, and received weekly 15 mL of Long Ashton nutrient solution without phosphate (Smith & Smith, 1986).

Assessment of spore production and mycorrhizal colonization

Four cores (1.5 cm diameter x 12 cm deep) were taken from each pot culture 120 days after germination. The soil was used to assess spore populations and the roots were used to determine the percentage of roots colonized by AMF. The roots were washed thoroughly with water, cut into 1.0 cm long pieces, fixed in ethanol (50 % v/v) and stored until they could be processed. Fixed roots were cleared in 10 % KOH, acidified with 0.1 N HCl and stained with trypan blue (0.01 % in lactoglycerol), a modification of the method of Phillips & Hayman (1970). Roots that remained dark after clearing were bleached with H₂O₂ before staining. Stained roots were rinsed in tap water and stored in 1:1 (v/v) glycerol:water. The stained roots were examined microscopically at a magnification between 10 and 100 x to observe the AMF structures and determine AMF type of colonization using the criteria given in table 1 (Gallaud, 1905; Smith & Smith, 1997). Percentage colonization was assessed using the grid line intersect method (Giovannetti & Mosse, 1980).

Community indices

Abundance (total number of spores), species richness (number of species) and diversity of

Table 1. Features of colonization in plant species with *Arum*- and *Paris*-type patterns of fungal development

Mycorrhizal type	Mycorrhizal features				
	Intercellular hyphae	cell to cell spread of hyphae	Arbuscules subtended by	Coils in hypodermal cells	coils in cortical cells (± arbuscules)
<i>Arum</i>	Present	Absent	Intercellular hyphae	Present	Absent
<i>Paris</i>	Usually absent	Present	Intracellular coils	Present	Present

Source: Gallaud (1905); Smith & Smith (1997).

sporulating AMF spores in the samples were determined. Diversity was calculated as Simpson's index (Begon et al., 1990) according to the following equation:

$$D = \frac{1}{\sum_{i=1}^S P_i^2} \quad (1)$$

where: D is the diversity, S the sum of species, P_i the proportion of the total spore number in the i^{th} species

Statistical analysis

The spore population data of each plot were subjected to analysis of variance (ANOVA) to determine if there were significant differences between plots. Abundance, richness and diversity in soils collected from the permanent pasture at four times were compared by one-way ANOVA, followed by Tukey's (HSD) test ($P < 0.05$). An analysis of variance by GLM (General Linear Models Procedure, SAS (1986) was used to investigate the effects of sampling time and host plants on richness, diversity, production of spores and percentage colonization in trap cultures because factor combinations had different numbers of host plants (four sampling times, three host plants for July 1996 and four host plants for December 1996, July 1997 and December 1997). The data were tested for normal distribution and transformed to improve normality when necessary. The Tukey (HSD) test was used to compare means (SAS, 1986).

RESULTS

Survey of AMF spores

Spore densities ranged from 10-170 spores 25 g⁻¹ of soil, with the highest count occurring in the permanent pasture (Table 2). The number of recovered fungal genera was high in uncultivated soils, with *Gigaspora*, *Acaulospora* and *Glomus* being found in both arboretum and permanent pasture soils. *Scutellospora* was also present in the permanent pasture. Colonization was generally low

Table 2. Number of spores and genera of fungi present in different ecosystems in South Australia, May 1996

Site	Spores/25 g	Genera of fungi
Arboretum	38 bc	<i>Acaulospora</i> , <i>Glomus</i> , <i>Gigaspora</i>
Permanent pasture	170 a	<i>Acaulospora</i> , <i>Glomus</i> , <i>Gigaspora</i> , <i>Scutellospora</i>
Wheat-fallow	80 b	<i>Glomus</i>
Continuous wheat	10 c	<i>Glomus</i> , <i>Scutellospora</i>

Means followed by same letters are not significantly different ($P > 0.05$).

and variable with the highest level of root colonization (56.5 % in *Trifolium* sp.) observed in the pasture treatment (Table 3).

Data relating to colonization patterns in different species were combined for all treatments (Table 4). 'Fine endophyte' presumed to be *Glomus tenue*, Greennall Hall was observed in almost all roots samples, together with 'coarse hyphae', more typical of other mycorrhizal species.

Colonization patterns (*Arum*- or *Paris*-types) were determined on infection units formed by coarse endophytes only (Table 4). Unequivocal *Arum*-type colonization was observed in *Trifolium* sp., *Plantago lanceolata* and *Dactylis glomerata* L. A second member of the Poaceae (*Triticum* sp.) showed characteristics of both types, and *Hypochoeris radicata* L. (Asteraceae) also had 'mixed' colonization patterns.

Seasonal variations in the AMF assemblage in permanent pasture

Sixteen species of AMF (mainly *Glomus* species), plus one unidentified spore type, were found in the permanent pasture (Table 5), with the composition of the fungal assemblage differing between field-

Table 3. Percentage of root length infected by AMF of volunteer plants sampled from four different sites at Waite Campus, in July, 1996

Site	Species	Family	AMF-colonization
Arboretum	<i>Danthonia tenuis</i>	Poaceae	+ (5.8) ⁽¹⁾
	<i>Hypochaeris radicata</i>	Asteraceae	-
	<i>Trifolium</i> sp.	Fabaceae	+ (18.9)
	<i>Triticum aestivum</i> L.	Poaceae	+ (7.4)
Permanent pasture	<i>Dactylis glomerata</i>	Poaceae	+ (8.7)
	<i>Trifolium</i> sp.	Fabaceae	+ (56.5)
	<i>Plantago lanceolata</i>	Plantaginaceae	+ (21.4)
	<i>Rumex</i> sp.	Polygonaceae	+ (11.5)
Wheat-fallow	<i>Labiata</i> sp.	Labiatae	+ (6.3)
	<i>Polygonum aviculare</i>	Polygonaceae	-
Continuous wheat	<i>Trifolium</i> sp.	Fabaceae	+ (14.0)
	<i>Polygonum aviculare</i>	Polygonaceae	+ (27.8)

⁽¹⁾ Percent root length colonized. + present, - absent.

Table 4. Incidence of *Arum* (A) and *Paris* (P)-type mycorrhizas in different species in the Waite Permanent Rotation Trial

Species	Intercellular hyphae	Cell-cell hyphae	Hyphal coils in cortex	Hyphae coils in hypodermal cells	Arbuscules	A or P
Asteraceae						
<i>Hypochaeris radicata</i>	Variable	Occasional	Rare	Yes	Small	Intermediate/ <i>Arum</i>
Poaceae						
<i>Dactylis glomerata</i>	Yes	No	No	No	Yes	<i>Arum</i>
<i>Triticum</i> sp.	Variable	Rare	Yes	Yes	Yes	<i>Paris</i>
Fabaceae						
<i>Trifolium</i> sp.	Yes	No	Rare	Yes	Yes	<i>Arum</i>
Polygonaceae						
<i>Rumex</i> sp.	No	Yes	No	Yes	No	<i>Paris</i>
Plantaginaceae						
<i>Plantago lanceolata</i>	Yes	No	No	Yes	Yes	<i>Arum</i>

collected soil and trap cultures. Nine species were the same in both. However, *Acaulospora* sp., *Glomus aggregatum* Schenck & Smith emend. Koske and *G. macrocarpum* Tul. & Tul. were only recovered in field-collected soil and *Entrophospora* sp., *Glomus clarum* Nicol. & Schenk, *G. coronatum* Giovannetti and *G. etunicatum* Beck. & Gerd. only in trap cultures.

Spore abundance in field soil was higher in July 1996, when plants were growing and decreased at the end of the growth season in December (Table 6). At all four sampling times the predominant

recovered fungal species was *G. mosseae*, followed by an unidentified *Glomus* sp., *G. microaggregatum* Koske, Gemma & Olexi, *Glomus invermaium* Hall and *Gigaspora* sp. Spores of *Glomus microaggregatum* were usually found inside *Gigaspora*, *Scutellospora* and *Glomus* spores. *Gigaspora margarita* was the most abundant member of the Gigasporineae. Species richness was much higher in July 1996 than in December 1996, 1997 or July 1997. Simpson's index of diversity was between 2-3, and did not differ significantly between sampling times.

Table 5. Mycorrhizal fungal species present in soil collected from Permanent Pasture or obtained in trap cultures

AMF fungi species	Field collected soil	Trap culture
<i>Acaulospora</i> sp.	+	-
<i>Entrophospora</i> sp.	-	+
<i>Gigaspora margarita</i>	+	+
<i>Gigaspora</i> sp.	+	+
<i>Glomus aggregatum</i>	+	-
<i>Glomus clarum</i>	-	+
<i>Glomus constrictum</i>	+	+
<i>Glomus coronatum</i>	-	+
<i>Glomus etunicatum</i>	-	+
<i>Glomus</i> sp.	+	+
<i>Glomus macrocarpum</i>	+	-
<i>Glomus microaggregatum</i>	+	+
<i>Glomus mosseae</i>	+	+
<i>Glomus</i> sp.	+	+
<i>Scutellospora</i> sp.	+	+
<i>Scutellospora heterogama</i>	+	+

+ present, - absent.

Table 6. Number of spores of AMF species recovered from field-collected soil of the Permanent Pasture. Species are listed in order of spore abundance

Species of AMF	Jul. 1996	Dec. 1996	Jul. 1997	Dec. 1997
Number of spores/25 g soil				
<i>Acaulospora</i> sp.	0	1	1	0
<i>Gigaspora margarita</i>	6	0	1	1
<i>Gigaspora</i> sp.	6	0	2	1
<i>Glomus aggregatum</i>	4	0	0	0
<i>Glomus constrictum</i>	2	0	0	0
<i>Glomus microaggregatum</i>	0	0	7	8
<i>Glomus mosseae</i>	53	20	28	23
<i>Glomus</i> sp.	7	5	8	1
<i>Glomus</i> sp.	8	1	3	2
<i>Scutellospora heterogama</i>	1	1	0	0
<i>Scutellospora</i> sp.	5	0	1	1
Total	92	28	51	37
Community indices				
Richness	9.67 a	5.33 b	6.33 b	4.67 b
Diversity	3.04 a	2.04 a	3.02 a	2.17 a
Abundance	133 a	47 c	81 b	68 c

Means for community indices followed by the same letter are not different ($P < 0.05$).

Data from trap cultures also indicated that *G. mosseae* was the predominant fungal species, followed by *G. invernaium* and *Glomus* sp. (Table 7). In general, all trap culture hosts produced more *G. mosseae* spores from the field-collected soil samples

in 1997 than 1996. Spores of *G. etunicatum* were recovered only with *L. perenne* and *Sorghum* sp. as hosts; *Entrophospora* sp. and *G. constrictum* were recovered with *T. subterraneum*; and *Scutellospora heterogama* (Nicol & Gerd.) Walker & Sanders and *G. coronatum* with *Sorghum* sp. However, the frequency of recovery and abundance were low for all these fungal species.

Community indices in the trap cultures changed with sampling time and host (Table 7). *T. subterraneum* grown on soil sampled in July 1997 and in December 1996 and *Sorghum* sp. grown in soil sampled in July 1997 produced the highest abundance of spores ($p \leq 0.0001$). Most of those spores were immature. Species richness was variable between hosts and sampling time, with no clear emerging pattern. Simpson's diversity index ranged from 1.5 to 3.5 and was significantly higher in July of both years (data for all hosts). In general, *L. perenne* grown in soil collected in December 1996 showed the lowest species richness, diversity and abundance of associated fungi.

DISCUSSION

Despite relatively low spore densities in some treatments of the trial, volunteer weeds and pasture species became colonized, indicating that infective propagules were present. Most of the plant species that have been reported to be mycorrhizal (Harley & Harley, 1987) were colonized and the incidence of *Arum*- and *Paris*-type colonization in general agreed with previous records (Smith & Smith, 1997). The data provide a new record of the occurrence of *Arum*-type colonization in *Plantago lanceolata* (Plantaginaceae). *Hypochaeris radicata*, in the normally mycorrhizal Asteraceae, was not colonized in the arboretum, but we have found it to be mycorrhizal at other sites, with intermediate characteristics between *Arum*- and *Paris*-type. Variable colonization is most likely due to patchy distribution of inoculum. The two members of sampled Poaceae, *Dactylis glomerata* and *Triticum* sp., had *Arum*- and *Paris*-type colonization, respectively. Both patterns have been previously recorded in this large and diverse family, and it may be that, as with the Liliaceae, more data for a range of species in different subfamilies will show whether these have characteristically different AMF morphologies to clarify the picture (Smith & Smith, 1997). Although the morphological type of AMF can generally be predicted from the taxonomic position of the plant (that is, the plant identity appears to "control" the type of AMF formed), recent work with *Lycopersicon esculentum* Mill has shown that the identity of the fungus can also be important in some plants (Cavagnaro et al., 2001). In *L. esculentum* (which is a member of the Solanaceae in which *Arum*- and

Table 7. Influence of *Lolium perenne* (L), *Plantago lanceolata* (P), *Sorghum sp.* (S) and *Trifolium subterraneum* (T) as hosts for trap cultures on populations of mycorrhizal fungi and on mycorrhizal fungal community indices

Species of AMF	July/1996			December/1996				July/1997				December/1997			
	L	S	T	L	P	S	T	L	P	S	T	L	P	S	T
Spore number per 25 g soil															
<i>G. mosseae</i>	7	3	11	4	7	6	8	20	11	17	20	22	17	12	18
<i>G. invernatum</i>	3	0	2	0	1	0	1	10	9	9	13	4	4	4	8
<i>Glomus spp.</i>	1	5	5	1	2	3	2	3	1	4	4	0	2	2	3
<i>G. microaggregatum</i>	2	0	8	0	0	0	6	2	0	2	3	0	0	1	1
<i>Gl. margarita</i>	3	0	1	0	1	0	1	1	2	4	3	1	0	1	1
<i>Gigaspora sp.</i>	1	1	0	0	0	1	2	1	1	0	1	3	1	1	2
<i>Scutellospora sp.</i>	1	0	1	0	1	0	0	2	0	2	2	1	3	0	0
<i>G. etunicatum</i>	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Entrophospora sp.</i>	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
<i>S. heterogama</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>G. coronatum</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>G. constrictum</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Community indices															
Richness	5.0 ab	3.7 bc	6.7 a	1.7 c	3.3 bc	3.3 bc	5.3 ab	6.3 a	4.3 ab	5.3 ab	5.7 ab	5.3 ab	3.7 bc	4.7 ab	4.3 ab
Diversity	3.5 ab	2.8 abc	4.2 a	1.5 c	2.4 bc	2.5 bc	3.5 ab	3.0 abc	2.8 abc	3.4 ab	3.2 ab	2.2 bc	2.1 bc	2.6 bc	2.6 bc
Abundance	22.3 ed	22.7 ed	34.3 bcd	11.7 e	31.0 bcd	27.0 cde	63.0 a	44.7 b	44.7 b	69.0 a	67.7 a	37.0 bcd	35.3 bcd	20.1 ed	40.0 bc
Colonization (%)	59.0 cd	83.7 a	75.0 ab	43.9 ef	78.4 ab	83.7 a	81.0 a	20.3 g	56.9 ed	79.9 a	83.3 a	32.3 fg	64.8 bcd	70.9 abc	79.8 a

Means for community indices followed by the same letter are not different ($P < 0.05$).

Paris-AMF have been recorded). *G. coronatum*, *Scutellospora calospora* and *Gigaspora margarita* formed *Paris*-AMF, whereas *G. mosseae*, *G. intraradices* and *G. versiforme* formed *Arum*-AMF. Experimental work with other "variable" host taxon is needed to determine whether fungal control of AMF morphology is more widespread than currently thought and if it can explain some of the variations observed in the field, where numerous AMF are present.

Colonization in *Rumex brasnis* L. (Polygonaceae) was of the *Paris*-type and lacked arbuscules. *Rumex* species are sometimes recorded as non mycorrhizal (Read et al., 1976), although Sanders and Fitter (1992) showed that the presence of *R. acetosa* influenced spore populations in soil. More work is required on this genus, to determine if arbuscules are formed at any stage and whether colonization has any influence on growth or health of the plants. The other member of the Polygonaceae sampled (*Polygonum aviculare*) was not colonized.

The mycorrhizal assemblage

All data showed that *G. mosseae* was the most abundant spore type in the permanent pasture. Single species dominated in other situations: *G. mosseae* in a pasture on a former mallee site in South Australia (E. Facelli, unpublished data), *Acaulospora laevis* Gerd. & Trappe in both virgin and agricultural soils in Western Australia and New Zealand (Abbott & Robson, 1977) and *Gigaspora* spp. in a continuous soybean crop and in soybean rotations with other species (Hendrix et al., 1995). These fungi may have

preferential or particularly effective mycorrhizal associations with plant hosts present in the different systems, leading to high spore production. Alternatively, antagonism or competition between fungal species could also be involved (Gemma et al., 1989). *Glomus mosseae* was among those fungi Stutz & Morton (1996) found to inhibit the sporulation of other species in trap cultures. More research is needed to distinguish between these possibilities.

Spore abundance varied with time of sampling, that is, winter (July) samples had significantly more spores than summer (December) samples (Table 6). This might be explained by the presence of actively growing host plants, able to support the production of new spores. In this highly seasonal environment plant growth ceases as rainfall declines in summer. No more spores were produced and numbers dropped, maybe due to predation and hyperparasitism (Fitter & Garbaye, 1994; Bakhtiar et al., 2001). This factor might also have contributed to the lower abundance of spores in July 1997, when particularly high numbers of nematodes, collembolans and mites were observed in the field soil compared with July 1996.

Species richness was also higher in July than December, probably reflecting the high abundance of spores, rather than real seasonal differences. This interpretation is supported by data from the pot cultures, which also showed high spore abundance generally associated with high species richness and diversity (Table 7). However under growth-room conditions, the most abundant spore populations developed in 1997 rather than in 1996. Similar

increases in abundance and species richness have also been observed in three cycles of successive trap cultures associated with *Prosopis* spp. (Stutz & Morton, 1996). Species diversity was generally high in the field (average index of 2.7), but did not differ significantly between sampling times. There are no other published values of Simpson's index for AMF spore communities with which to compare these findings, but communities can be diverse with 19 species found in the experimental field plots used by Johnson et al. (1992).

Field sampling of spores did not detect all present fungal species. *G. tenue* contributed to the colonization in most root samples obtained from the field, but could not be detected in the spore populations. Trap cultures recovered four species that were not found as spores in field soil, *Entrophospora* sp., *G. clarum*, *G. coronatum* and *G. etunicatum*, but were presumably present in root fragments as mycelia or vesicles, or as spores in such low numbers that they were not detectable by the sampling procedure. Trap cultures may also have increased the opportunity for development of certain species by providing suitable hosts or growth conditions. Differential responses in spore production of AMF in relation to host species have also been reported by Sanders & Fitter (1992), where the number of spores of *G. constrictum* increased with *P. lanceolata* and spores of *A. laevis* increased with *Holcus lanatus* or *Rumex acetosa*.

Differences in spore production of the varied fungal species were observed between the different plant species used for trap cultures. These differences could reflect some degree of specificity in root colonization and spore production, but this seems unlikely since there were no differences in species richness or diversity of AMF spores when different plant species were used as hosts for trap cultures (with the exception of *L. perenne* in December 1996).

In conclusion, the arbuscular mycorrhizal assemblage (as spores) in permanent pasture is dominated by *G. mosseae*. Two unidentified *Glomus* species and one unidentified spore type were the only other AMF present in all field samples. *Gigaspora margarita* and *Scutellospora* sp. were found at three of the four sampling times and in pot cultures set up with soil from all sampling times. Four fungi were found only in trap cultures and two other fungi only at one sampling time. Differences in species diversity at the different sampling times most probably reflect differences in total abundance and hence sensitivity of the sampling methods. It is now possible to target some questions about the fungal assemblage of particular species in the permanent pasture. For example: Is *G. mosseae* the most abundant member of the vegetative community as well as the spore community?; 'Do species that are less abundant in spore form (e.g. *G. margarita*) or

rarely present (e.g. *G. constrictum*), significantly contribute to root colonization?'; 'What is the relative effectiveness of these fungi in plant nutrition and growth?'. The establishment of single spore pot cultures and development of specific and quantitative DNA-based probes for *G. margarita* and *G. mosseae* will allow some of these problems to be addressed. Furthermore, the data from the pasture provide a sound basis from which to investigate the effects of different crops and management regimes in the Permanent Rotation Trial on AMF populations and activity.

ACKNOWLEDGEMENTS

We wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and The Federal University of Santa Maria, Rio Grande do Sul, Brazil for the scholarship and study leave awarded to Z.I. Antoniolli and the Cooperative Research Centre for Soil and Land Management which provided financial support for the project.

LITERATURE CITED

- ABBOTT, L.K. & ROBSON, A.D. Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils. *Aust. J. Agric. Res.*, 33:1049-1059, 1982.
- ALLEN, M.F. & BOOSALIS, M.G. Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytol.*, 93:67-76, 1983.
- BAKHITIAR, Y.; MILLER, D.; CAVAGNARO, T.R. & SMITH, S.E. Interactions between two arbuscular mycorrhizal fungi and fungivorous nematodes and control of the nematode with fenamiphos. *Appl. Soil Ecol.*, 17:107-117, 2001.
- BEGON, M.; HARPER, J. & TOWNSEND, C. *Ecology individuals, populations and communities*. Carlton, Blackwell Scientific Publications, 1990. 945p.
- CAVAGNARO, T.R.; GAO, L-L.; SMITH, F.A. & SMITH, S.E. Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytol.*, 151:469-475, 2001.
- DODD, J.C.; ARIAS, I.; KOOMEN, I. & HAYMAN, D.S. The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem. II. The effects of pre-crops on the spore populations of native and introduced VAM-fungi. *Plant Soil*, 122:241-247, 1990.
- FITTER, A. & GARBAYE, J. Interactions between mycorrhizal fungi and other soil organisms. In: ROBSON, A.; ABBOTT, L. & MALAJCZUK, N., eds. *Management of mycorrhizas in agriculture, horticulture and forestry*. Netherlands, Kluwer Academic Publishers, 1994. p.123-132.
- GALLAUD, I. Etudes sur les mycorrhizes endotrophs. *Rev. Generale Bot.*, 17:5-48; 66-83; 123-135; 223-239; 313-325; 425-433; 479-500, 1905.

- GEMMA, J.; CARRIERO, T. & KOSKE, R. Seasonal dynamics of selected species of VA mycorrhizal fungi in a sand dune. *Mycol. Res.*, 92:317-321, 1989.
- GERDEMANN, J.W. & NICOLSON, T.H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.*, 46:235-244, 1963.
- GIOVANNETTI, M. & MOSSE, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.*, 84:489-500, 1980.
- GRACE, P.R.; OADES, J.M.; KEITH, H. & HANCOCK, T.W. Trends in wheat yields and soil organic carbon in the Permanent Rotation Trial at the Waite Agricultural Research Institute, South Australia. *Aust. J. Exp. Agric.*, 35:857-864, 1995.
- HALL, I.R. & FISH, B.J. A key to the Endogonaceae. *Trans. Brit. Mycol. Soc.*, 73:261-270, 1979.
- HARLEY, J.L. & HARLEY, E.L. A check-list of mycorrhiza in the British Flora. *New Phytol.*, 105:1-102, 1987.
- HENDRIX, J.; GUO, B. & NA, Z. Divergence of mycorrhizal fungal communities in crop production systems. *Plant Soil*, 170:131-140, 1995.
- JOHNSON, N.C.; TILMAN, D. & WEDIN, D. Plant and soil controls on mycorrhizal fungal communities. *Ecology*, 73:2034-2042, 1992.
- MORTON, J.; BENTIVENGA, S. & WHEELER, W. Germ plasm in the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation and storage. *Mycotaxon*, 48:491-528, 1993.
- MORTON, J. Effects of mountants and fixatives on wall structure and Melzer's reaction in spores of two *Acaulospora* species (Endogonaceae). *Mycologia*, 78:787-794, 1986.
- PHILLIPS, J.M. & HAYMAN, D.S. Improved procedures for clearing and staining parasitic and vesicular -arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.*, 55:158-161, 1970.
- READ, D.J.; KOUCHEKI, H.K. & HODDGSON, J. Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. *New Phytol.*, 77:641-653, 1976.
- SANDERS, I.R. & FITTER, A.H. Evidence for differential responses between host-fungus combinations of vesicular-arbuscular mycorrhizas from a grassland. *Mycol. Res.*, 96:415-419, 1992.
- SAS INSTITUTE. SAS User's Guide: Statistics. Cary, 1986.
- SCHENCK, N.C. & PÉREZ, Y. Manual for the identification of VA mycorrhizal fungi. Gainesville, Synergistic Publications, 1990. 139p.
- SIEVERDING, E. Vesicular-Arbuscular Mycorrhiza management in tropical agrosystems. Eschborn, Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, 1991. 375p.
- SMITH, F.A & SMITH, S.E. Structural diversity in (vesicular)-arbuscular mycorrhizal fungi. *New Phytol.*, 137:373-388, 1997.
- SMITH, F.A & SMITH, S.E. Movement across membranes: Physiol. and Biochem. In: GIANINAZZI-PEARSON, V. & GIANINAZZI, S., eds. Physiological and genetical aspects of mycorrhizae., Paris, INRA, 1986. p.75-84.
- SMITH, S.E. & READ, D.J. Mycorrhizal symbiosis 2.ed. London, Academic Press, 1997. 605p.
- STACE, H.; HUBBLE, G.; BREWER, R.; NORTHOCTE, K.; SLEEMAN, J.; MULCALY, M. & HALLSWORTH, E. Glenside, Handbook of Australian Soils, 1968. 125p.
- STUTZ, J. & MORTON, J. Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Can. J. Bot.*, 74:1883-1889, 1996.
- van der HEIJDEN, M.G.A.; KLIRONOMOST, J.N.; URSIC, M.; MOUTOGLIS, P.; STREITWOLF-ENGEL, R.; BOLLER, T.; WIEMKEN, A. & SANDERS, I.R. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396:69-72, 1998.

