Mycorrhiza (2007) 17:519–526
DOI 10.1007/s00572-007-0124-7

ORIGINAL PAPER

Sporulation and diversity of arbuscular mycorrhizal fungi in Brazil Pine in the field and in the greenhouse

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Received: 19 October 2006 / Accepted: 6 February 2007 / Published online: 7 March 2007

Abstract The aim of this work was to assess the sporulation and diversity of arbuscular mycorrhizal fungi (AMF) at different forest sites with *Araucaria angustifolia* (Bert.) O. Ktze. (Brazil Pine). In addition, a greenhouse experiment was carried out to test the use of traditional trap plants (maize + peanut) or *A. angustifolia* to estimate the diversity of AMF at each site. Soil samples were taken in two State Parks at southwestern Brazil: Campos do Jordão (Parque Estadual de Campos do Jordão [PECJ]) and Apiaí (Parque Estadual Turístico do Alto Ribeira [PETAR]), São Paulo State, in sites of either native or replanted forest. In PECJ, an extra site of replanted forest that was impacted by accidental fire and is now in a state of recuperation was also sampled. The spore densities and their morphological identification were compiled at each site. In the greenhouse, soil samples from each site were used as inoculum to promote spore multiplication on maize + peanut or *A. angustifolia* grown on a sandy, low-fertility substrate. Plants were harvested, respectively, after 4 months or 1 year of growth and assessed for mycorrhizal root colonization. Spore counts and identification were also performed in the substrate, after the harvest of plants. Twenty-five taxa were identified considering all sites. Species richness and diversity were greater in native forest areas, being *Acaulospora*, the genus with the most species. Differences in number of spores, diversity, and richness were found at the different sites of each State Park. Differences were also found when maize + peanut or *A. angustifolia* were used as trap plants. The traditional methodology using trap plants seems to underestimate the diversity of the AMF. The use of *A. angustifolia* as trap plant showed similar species richness to the field in PECJ, but the identified species were not necessarily the same. Nevertheless, for PETAR, both *A. angustifolia* and maize + peanut underestimated the species richness. Because the AMF sporulation can be affected by many conditions, it is impossible to draw detailed conclusions from this kind of survey. More precise experiments have to be set up to isolate the different factors that modulate the ecophysiological interactions between host plant and endophyte.

Keywords AMF · Sporulation · *Araucaria* · Host-endophyte interaction

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Introduction

The characteristics and dynamics of occurrence of arbuscular mycorrhizal fungi (AMF) under natural conditions are important for the evaluation of the inoculum potential and root colonization in the process of understanding their behavior in the soil and determining their symbiotic efficiency (Bethlenfalvay and Linderman 1992). The presence and/or abundance of arbuscular mycorrhizal (AM) fungal spores show that these species may be active in the soil at a specific time, being capable of colonizing and multiplying themselves in the host's roots. However, other parameters and evaluations have to be carried out to check for the effectiveness of native AM fungal populations (Sieverding 1991).

AM fungi are not host-specific, i.e., each species does not colonize only one plant species (McGonigle and Fitter 1990), although there may be ecological specificity or selectiveness among symbionts (van der Heijden et al. 1998). Therefore, several AMF may colonize the host plant, and a specific fungus may be associated with different host plants. Nevertheless, the effect of a single-AM fungal species can differ in two different host plants. Recently, it has been shown that the growth rates (expressed as sporulation) of different AMF are host-specific, i.e., when inoculating a given AM fungal community onto different plant species, each host will selectively produce a differentiated spore composition. Moreover, host plants may produce positive or negative feedback on the endophyte, thereby also determining indirectly the diversity of the plant community (Bever et al. 1996; Bever 2002).

The traditional procedure to survey the presence of AMF in ecosystems relies on extraction of spores from soil by wet sieving techniques and taxonomic classification based on spore morphology. One problem is that low-sporulating species, as well as the ones whose sporulation is restricted to one season, would probably not be detected by this method (Moreira et al. 2006). It is also possible to multiply some of these AMF by inoculating mycorrhizal propagules into mycotrophic seedlings growing in the greenhouse (Brundrett et al. 1999). However, depending on the growth conditions, i.e., host plant (Bever et al. 1996; Carrenho et al. 2002) and environmental conditions in general, there may be a quantitative and qualitative change of the spore composition, which may not reflect the original composition in the field.

Araucaria angustifolia (Bertoloni) Otto Kuntze (Brazil Pine) is a coniferous tree that is symbiotic only with AMF, and does not associate with ectomycorrhizal fungi. It has been shown that this tree responds well to the inoculation with AM fungi (Moreira-Souza and Cardoso 2002). Thus, the knowledge on the AM fungal diversity in *A. angustifolia* forests is important to help define strategies for management or even reforestation of such forests that are in danger of being extinct. Different locations and different ways of forest formation (e.g., natural or reforested [R]) may affect the diversity of AM fungal species in the soil in *A. angustifolia* forests (Moreira et al. 2006).

The objective of this work was to examine the AM fungal diversity in forests with *A. angustifolia* (natural or R) in two different State Parks in São Paulo, Southeastern Brazil by direct extraction and morphologic identification of AM fungal spores, and the AM fungal diversity at the same sites being assessed by means of previous spore multiplication in the greenhouse using either maize + peanut or *A. angustifolia* as trap plants.

Materials and methods

Experimental areas and sampling

Soil samples were collected in May 2002 from forests with *A. angustifolia* (Bert.) O. Ktze., either native or R, in two State Parks (conservation and environmental protection areas of São Paulo State): State Park of Campos do Jordão (Parque Estadual de Campos do Jordão [PECJ]; 22°44′S, 45°30′W) and Tourist State Park of Alto Ribeira (Parque Estadual Turístico do Alto Ribeira [PETAR]; 24°38′S, 48°50′W), respectively, located at Campos do Jordão and Apiaí municipalities, in the State São Paulo, Southeastern Brazil. The climate in both State Parks is classified as Cfb (Köppen), characterized as subtropical (upland), mesothermal, and humid.

The native forest (NF) sites consisted of adult trees approximately 100 years old, whereas the R sites were composed by 60-year-old trees. An extra R site, which had been affected by accidental fire (reforestation area affected by fire [RF]) in the winter of 2001, was also sampled in PECJ. Five *A. angustifolia* trees were randomly selected in each site (approximately 1,000 m^2), and three 300-g soil samples were taken in the root zone at equidistant points around each tree at 0–20 cm depth. Samples were stored in plastic bags for later determination of number of spores (Gerdemann and Nicolson 1963), and identification of the AM fungal species, according to Schenck and Pérez's Manual (Schenck and Pérez 1990) and descriptions supplied by INVAM (2003) (http://invam.caf.wvu.edu). All subsamples from each site (15) were pooled and submitted to chemical and physical analyses (Table 1).

Pot experiments in the greenhouse

The substrate for plant growth was obtained by mixing a sample of a sandy soil (Typic Quartzipsamment), collected at 0–20 cm depth, and coarse washed river sand (2:1 v/v),

Table 1 Chemical and physical soil characteristics of the experimental areas in Apiaí (PETAR), and Campos do Jordão (PECJ), SP, Brazil, and the soil mix used in greenhouse experiments

Sites		Chemical							Physical		
		pH $CaCl_2$	OM g dm^{-3}	P mg dm^{-3}	K mmolc dm^{-3}	Ca	Mg	H + Al	Sand Percentage	Silt	Clay
PETAR	NF	4.7	115	30	2.8	102	20	64	51	20	29
PETAR	R	3.4	95	13	2.5	14	7	281	26	25	49
PECJ	NF	3.7	128	13	3.7	19	17	228	54	4	42
PECJ	R	3.2	88	10	2.3	2	4	347	51	18	31
PECJ	RF	3.1	65	8	2.8	1	2	281	57	6	37
Soil mix		4.0	13	3	1.4	4	3	31	86	2	12

NF, native forest; *R*, reforestation; *RF*, reforestation affected by fire

followed by sterilization in an autoclave (121°C for 2 h). The objective was to obtain a sandy, low-fertility substrate (Table 1) without native AMF.

The inoculum representing each site was obtained by pooling the three soil samples collected at the root zone around each *A. angustifolia* tree. Thus, five samples of 150 g of soil collected at each site were used as inoculum. The average spore density in each inoculum is shown in Tables 2 and 3.

The treatments consisted of five inocula sources, one from each site, which were incorporated into the substrate and either sown with maize (*Zea mays* L.) + peanut (*Arachis hypogaea* (L.) Moench), growing together in the same pot, or planted with *A. angustifolia* seedlings used as trap plants, with five replications. Maize + peanut seeds were previously surface-sterilized in 70% ethanol for 5 min, followed by 2% hypochlorine for 3 min, and rinsed in distilled water. *A. angustifolia* seeds were surface-sterilized in the same way, scarified to accelerate germination, and pregerminated in trays containing autoclaved sand for 40 days (Moreira-Souza and Cardoso 2003). Uniform seedlings of about 7 cm height were transplanted to the pots. Six liter pots were used for maize + peanut plants and 20-l pots for *A. angustifolia* plants. Each tree sampled in the field represented one replicate in the pot experiment for each trap plant. In addition, when *A. angustifolia* was used as trap plant, an extra control without mycorrhizal inoculum was added to evaluate the effect of the inoculum on the growth of *A. angustifolia*. Each kind of host plant was conducted and later analyzed as a separate experiment. Plants received 50 ml of P-free Hoagland's nutrient solution monthly and were watered with distilled water whenever necessary.

Maize + peanut plants were cultivated up to the end of the plant cycle (i.e., 120 days). After this, pots were no longer watered for another 20 days, to stimulate AM fungal sporulation. *A. angustifolia* plants were conducted for 1 year after transplanting (with 20 days without irrigation before harvest), because the sporulation of AMF in the pots was very scarce after 4 months. After the water stress, spores were extracted and classified. Spore densities and diversity were determined for all pots. For Araucaria pots, this procedure had also been performed previously at 180 days. To determine the root percentage colonization, roots were cleared with 10% KOH (and 10% H_2O_2 for *A. angustifolia* roots), and stained with pen blue ink and acetic acid (Vierheilig et al. 1998). For maize and peanut roots, the percent root colonization was assessed by the gridline method, whereas, for the coarser roots of *A. angustifolia*, root segments of about 1 cm were assembled on eight slides and observed under the microscope (Giovannetti and Mosse 1980). The dry biomass of *A. angustifolia* was determined, to calculate the mycorrhizal efficiency of the inocula (ratio between the biomass production of mycorrhizal and non-mycorrhizal plants; Moreira-Souza and Cardoso 2002). Data from the greenhouse experiments were submitted to one-way analysis of variance and means compared by the Duncan's multiple range test ($P<0.05$) after an entirely randomized experimental design.

Results

Field sites

Considering the five sites in the two State Parks, 25 AM fungal taxa were identified in the *A. angustifolia* root zone, belonging to the following genera: *Acaulospora* (11 species), *Scutellospora* (6), *Glomus* (4), *Entrophospora* (2), and *Gigaspora* (2). The greater diversity was observed in the NF in PETAR (17) compared to the NF in PECJ (15). Considering all sites in the different State Parks, 21 species were found in PECJ and 20 in PETAR. Among these species, 16 were common to both State Parks.

Table 2 Number of spores for each arbuscular mycorrhizal (AM) fungal species found at three sites (NF, R, and RF) at the State Park of Campos do Jordão (PECJ), SP, Brazil, and in the greenhouse using soil from the same sites as inoculum source, in different host plants

AM fungal species	*Araucaria angustifolia*						Peanut + maize		
	Field			Greenhouse			Greenhouse		
	NF	R	RF	NF	R	RF	NF	R	RF
Acaulospora bireticulata Rothwell and Trappe	2	2	–	–	20	10	–	6	2
A. foveata Trappe and Janos	4	2	8	11	12	8	–	–	–
A. gerdemannii Schenck and Nicol.	2	4	12	–	–	–	–	–	–
A. koskei Blaszkowski	–	–	–	10	29	38	6	6	6
A. laevis Gerd. and Trappe	–	18	4	18	12	15	–	2	2
A. mellea Spain and Schenck	–	26	–	26	28	39	6	6	6
A. morrowiae Spain and Schenck	–	–	–	10	–	–	–	–	–
A. scrobiculata Trappe	–	8	4	4	–	–	–	–	–
A. spinosa Walker and Trappe	8	–	–	–	–	–	–	–	–
Acaulospora sp. 1	6	–	–	25	–	–	4	–	–
Acaulospora sp. 2	6	–	–	–	–	–	–	–	–
Acaulospora sp. 3	4	6	10	–	–	–	–	–	–
Archeospora leptoticha (Schenck and Smith) Morton and Redecker	–	–	–	–	18	43	–	6	10
A. trappei (Ames and Linderman) Morton and Redecker	–	–	–	6	–	–	–	–	–
Entrophospora colombiana Spain and Schenck	24	4	8	16	2	18	–	–	–
Gigaspora decipiens Hall and Abott	–	–	–	–	20	21	–	–	4
G. margarita Becker and Hall	–	24	10	–	22	20	–	–	–
Gigaspora sp.1	–	–	–	11	–	–	2	2	–
Glomus diaphanum Morton and Walker	–	–	6	–	–	–	–	–	–
G. etunicatum Becker and Gerd.	18	20	30	–	–	–	–	–	–
G. macrocarpum Tul. and Tul.	14	84	100	–	–	–	–	–	–
G. microcarpum Tul. and Tul.	–	–	–	–	14	4	–	–	–
G. sinuosum (Gerd. and Bakshi) Almeida and Schenck	–	–	–	–	–	–	2	–	–
Glomus sp.1	4	6	18	–	–	–	2	2	–
G. rubiforrme (Gerd. and Trappe) Almeida and Schenck	–	–	–	26	10	12	2	2	4
Scutellospora calospora (Nicol. and Gerd.) Walker and Sanders	8	–	–	–	–	–	–	–	–
S. cerradensis Spain and Miranda	–	–	–	–	–	–	–	–	2
S. heterogama Nicol. and Gerd.	2	–	–	–	–	–	–	–	–
S. pellucida (Nicol. and Schenck) Walker and Sanders	–	–	–	16	20	–	–	–	2
Scutellospora sp.1	22	–	2	10	–	–	–	–	–
Scutellospora sp.2	–	6	6	–	–	–	–	–	–
Scutellospora sp.3	2	–	–	–	–	–	–	–	–
Total number of spores	126	210	218	189	197	228	24	32	38
Richness (total no. of species)	15	13	13	12	12	11	7	8	9

Sampling sites: *NF*, native forest; *R*, reforestation; *RF*, reforestation affected by fire

The most frequent species in the NF in PECJ were *Entrophospora colombiana* (19%) and Scutellospora sp.1 (17%), whereas, in PETAR, the most frequent ones were *Glomus* macrocarpum (48%) and *Acaulospora* scrobiculata (16%). The most frequent species in the R area in PECJ were *G. macrocarpum* (40%) and *Gigaspora* margarita (11%), whereas, in the R area previously affected by fire, the most frequent species were *G. macrocarpum* (46%) and *Glomus etunicatum* (14%). In the R area in PETAR, the species *G. macrocarpum* (44%) and *Acaulospora* gerdemannii (11%) were the most frequent ones (Tables 2 and 3).

Greenhouse

When using maize + peanuts as host plants, the total number of spores was higher in PETAR than in PECJ, considering all sites; however, the diversity was higher in PECJ (13 species) than in PETAR (11 species), with only one species, *Glomus* sp.1, common in both forests. When *A. angustifolia* was the host, there were more spores in pots containing inoculum from PECJ than from PETAR, independently of the site. Species richness was still greater in PECJ (18 species) than in PETAR (7 species), and five species were common to both State Parks (Tables 2 and 3).

Table 3 Number of spores for each AM fungal species found at two sites (NF and R) at the Tourist State Park of Alto Ribeira (PETAR), SP, Brazil, and in the greenhouse using soil from the same sites as inoculum source, in different host plants

AM fungal species	*Araucaria angustifolia*				Peanut + maize	
	Field		Greenhouse		Greenhouse	
	NF	R	NF	R	NF	R
Acaulospora bireticulata Rothwell and Trappe	2	6	–	–	–	–
A. foveata Trappe and Janos	12	12	–	–	48	–
A. gerdemannii Schenck and Nicol.	10	28	–	–	–	8
A. laevis Gerd. and Trappe	–	–	–	15	–	–
A. mellea Spain and Schenck	–	24	–	–	–	–
A. morrowiae Spain and Schenck	–	4	–	15	–	–
A. scrobiculata Trappe	60	14	40	–	48	24
A. spinosa Walker and Trappe	8	10	12	–	–	4
Acaulospora sp. 1	10	8	25	–	16	10
Acaulospora sp. 2	12	–	–	–	20	–
Acaulospora sp. 3	8	14	–	–	10	6
Entrophospora colombiana Spain and Schenck	2	2	11	32	4	2
E. kentinensis Wu and Liu	6	–	–	–	–	–
Gigaspora decipiens Hall and Abott	–	6	–	–	–	–
G. margarita Becker and Hall	34	4	–	–	–	–
Glomus diaphanum Morton and Walker	–	–	–	–	–	10
G. etunicatum Becker and Gerd.	20	2	–	–	–	–
G. macrocarpum Tul. and Tul.	182	116	–	–	–	–
G. microcarpum Tul. and Tul.	–	–	4	2	–	–
Glomus sp. 1	4	–	–	–	4	44
Glomus sp. 2	–	–	–	–	–	8
Scutellospora heterogama Nicol. and Gerd.	2	–	–	–	–	–
S. pellucida (Nicol. and Schenck) Walker and Sanders	6	16	–	–	–	–
Scutellospora sp. 1	4	–	–	–	–	–
Total number of spores	382	266	92	64	150	116
Richness (total no. of species)	17	15	5	4	7	9

Sampling sites: *NF*, native forest; *R*, reforestation

With maize + peanut as trap plants, the mycorrhizal root colonization did not differ, when considering the different inoculum sources. The root colonization ranged from 39 to 67% in maize roots and from 17 to 48% in peanut roots. However, the root colonization levels in maize were always higher than in peanut plants (data not shown).

When considering *A. angustifolia* as host plant, the levels of root colonization after 1 year of plant growth was the lowest with inoculum from the R site that had been accidentally affected by fire at PECJ (Table 4). In relation to dry biomass, plants grown in the control pots showed the lowest biomass production and very poor growth. Plants

Table 4 Root colonization, total plant dry weight, and mycorrhizal efficiency in *A. angustifolia* after 1 year of growth when soil samples from different sites at two State Parks (PETAR and PECJ) were used as inoculum, and number of spores of AM fungi in the substrate 180 days after plant growth (n=5)

Treatments		Root colonization (%)	Total dry weight (g)	Mycorrhizal efficiency (%)	Spores in 100 g of substrate
Control		–	13.6 c	–	–
PETAR	NF	36.7 a	71.7 a	427	150 a
PETAR	R	34.0 a	69.9 a	414	54 b
PECJ	NF	37.1 a	54.4 a	300	107 a
PECJ	R	33.8 a	36.5 b	168	20 b
PECJ	RF	21.7 b	55.9 a	311	27 b

Means followed by the same letter do not differ from one another by Duncan's test ($P \leq 0.05$). *NF*, native forest; *R*, reforestation; *RF*, reforestation affected by fire.

that grew in the substrate inoculated with soil from the R site at PECJ showed the second lower dry biomass, whereas plants grown in the substrate inoculated with soil from the other sites presented the highest biomasses, with no difference between them. As a result, the mycorrhizal efficiency was high, ranging from 168 to 427%. Finally, the spore density at 180 days was higher in the substrate when the inoculum source came from the NFs of both State Parks than when the inoculum came from the R sites.

Discussion

The data on species diversity, considering all sites in both State Parks, showed that, although some AMF species are shared, the diversity is quite variable among them. Among the 25 species found in the two State Parks, only 16 occurred in both. Many factors may have lead to such differences, for example, differences in soil chemical properties and the different floral composition in each State Park. Although the native and the R areas are composed predominantly by *A. angustifolia*, other plant species are also found in the understory (Moreira et al. 2007a,b).

It is quite difficult to correlate general soil characteristics with the occurrence of different AMF species and number of spores. *G. macrocarpum* and *A. scrobiculata* showed high frequency especially in the NF in PETAR. On the other hand, the most frequent species in PECJ were *E. colombiana* and *Scutellospora* sp.1. Although both sites presented similar organic matter (OM) content, they differed in pH, P availability, Ca, H + Al, and clay–silt proportions (Table 1). The soil pH is a factor that generally acts on the AM fungal species composition (Clark 1997). In this study, the soil pH at all sites was acidic, ranging from 3.1 to 4.7. Although *A. scrobiculata* prevailed in soils with higher OM content and lower pH, other results obtained elsewhere suggested that increase in soil OM tended to be unfavorable for occurrence of *A. scrobiculata*, whereas the increase in soil pH was favorable (Saggin-Júnior and Siqueira 1996).

G. macrocarpum and *G. etunicatum* showed high frequency of occurrence in all subareas studied. Again, opposite results were obtained elsewhere, because an increase in soil OM appeared to affect *A. scrobiculata* negatively, whereas the increase in soil pH was favorable. On the other hand, *Acaulospora morrowiae*, *Acaulospora mellea*, and *E. colombiana* prevailed in acid soils (Saggin-Júnior and Siqueira 1996).

Different factors can influence the AMF life cycle, such as temperature, luminosity, dynamics of plant species, rainfall, soil fertility, root exudations, and competition with other microorganisms and possible interactions with them. Therefore, it is very difficult to establish an AMF distribution pattern. In this study, it was observed that *Acaulosporaceae* presented the highest number of species in all treatments, in both areas (Tables 2 and 3). Generally, species of the family *Acaulosporaceae* are found in Brazil in soils with low pH (Trufem 1990, 1995; Gomes and Trufem 1998; Stürmer 1999; Moreira-Souza et al. 2003), as it is the case in our study.

When using *Araucaria* as host plant in pots, low numbers of spores were observed on the 120th day in all treatments, with an increase during the second spore collection, six months later, and an even higher increase after a year. Thus, experiments on AMF sporulation in *Araucaria* (a perennial plant) should be evaluated considering longer periods of time than experiments using short-cycle plants. When observing the *Araucaria* plants after a year of growth in pots, the difference in development between mycorrhizal and nonmycorrhizal plants was highly visible. Most control plants were small and starving, and presented brownish discoloration on many twigs. The inoculated ones, however, grew well and vigorously, independently on the AMF inoculum composition.

There was a great variation in sporulation among AMF species, some appearing in all subareas in PECJ and others only in PETAR. In addition, some AMF species (*Acaulospora koskei, A. mellea, Archaeospora leptoticha, Glomus rubiformis, Glomus sinuosum, Scutellospora cerradensis,* and *Scutellospora pellucida)*, which had not been identified in any of the subareas under field conditions, had their sporulation favored under greenhouse conditions for treatments representing the subareas in PECJ. Furthermore, some other AM fungal species (*Acaulospora foveata*, *A. gerdemannii*, *A. scrobiculata*, *Acaulospora spinosa*, *Acaulospora* sp.2, *Acaulospora* sp.3, *E. colombiana*, *Glomus diaphanum*, and *Glomus* sp.2) only sporulated in pots representing the subareas from PETAR (Tables 2 and 3).

It might be expected that the cultivation of two host plants together, a gramineous and a leguminous species, in the same pot, would stimulate the sporulation of a higher number of AMF species. However, such expectation was not confirmed at all in our research. In addition, some contradicting results were found in the literature: Howeler et al. (1987) reported that AMF produced more spores in leguminous than in gramineous species, whereas Simpson and Daft (1990) observed the opposite, i.e., greater sporulation of *Glomus clarum* in sorghum and in millet than in peanut plants. Thus, each host–endophyte combination, in addition to the environment, seems to have its own outcome.

When trying to analyze Tables 2 and 3 in greater detail, it became evident that the multiplication of the AMF (evaluated as presence or absence of their spores) was completely unforeseeable and irregular, varying among

each combination of host plant, soil type, and ecosystem, without any defined pattern.

In the native *Araucaria* forest (NF) in PECJ (Table 2), seven species of *Acaulospora* could be identified; in the R area, there were also seven species from which only four were identical to the ones found in NF. In the RF, only five species of this genus were found, all of them identical to R, but only three of which had been found in NF.

In the experiment with multiplication pots in the greenhouse, the plant substrate was a sandy soil somewhat different from the soil types of the forest (Table 1). When planted with *Araucaria* and inoculated with soil from the three areas from PECJ (Table 2), the results were as follows: with inoculum from NF, seven species of the genus *Acaulospora* were retrieved. Only two of them corresponded to the same species found in the NF field, whereas five species had not been identified there. Therefore, these figures lead to the conclusion that even the five species that had not been found in NF obviously could only have come from the NF inoculum, which increases to 12, the number of *Acaulospora* species present in NF.

However, when the host plants in the multiplication pots were peanuts and maize, only very few *Acaulospora* species did multiply, respectively, 3, 4, and 4, for the NF, R or RF inocula. In addition, two out of the three species found in NF inoculum were also different from the ones retrieved from the NF field.

Considering the same approach for the genus *Glomus*, one would find that, although only three species were identified in the NF field, at least five species had to be present, for they were all retrieved from the pots with NF inoculum. At PETAR (Table 3), eight *Acaulospora* species were found in the NF. However, only three of them sporulated in the greenhouse on *Araucaria*, and five on peanut and maize.

It would be possible to go on pointing out similar discrepancies also for the other genera and other areas. Such results lead us to conclude that the usual procedures for AMF surveys are very precarious at the best, especially in forest ecosystems, and it may be very common to underestimate the richness and/or diversity of AM fungi in such surveys.

In *Araucaria* ecosystems, it has also been demonstrated that sporulation is influenced by a strong seasonal effect (Moreira-Souza et al. 2003). Some authors reported different AMF sporulation patterns when associated with annuals or with perennial plants (Gemma and Koske 1988; Baylis 1969). In perennials, plant phenology may also have a profound influence on the sporulation pattern (Escudero and Mendonza 2005).

More recently, Bever et al. (1996) and Bever (2002) reported positive or negative feedback of the host species on the sporulation of different AMF, i.e., certain AMF species produced more spores on a certain host plant, although others did not sporulate or did so very sparingly. Therefore, the composition of the AMF community will be defined by the host plant, which may indirectly interfere with the coexistence or not of other plant species. These reports add even greater weight to the evidence obtained by van der Heijden (1998) that the composition of the AMF community will determine the composition and productivity of the plant community.

It will be necessary to design more detailed and specific experiments to be able to get some valid conclusions about the ecophysiological interaction between host plant and endophyte. So far, more questions have been posed than answers could be found. In spite of this great variability, some general inferences can be drawn, especially for the areas in PETAR: The richness of (sporulated) AMF species was much greater in the field (normally with the predominance of the NF). In the greenhouse, a much smaller number of species was retrieved. When changing the host plant from *Araucaria* to peanut + maize, sporulation was much poorer yet.

Acknowledgements Thanks are due to "Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)" for the postdoctoral grant to M. Moreira and financial support (process no. 01/05146-6). We also thank to the State Park of Campos do Jordão, the State Tourist Park of Alto Ribeira, and the Environmental Department of São Paulo State for allowing the samplings (Process no. 40405/98 COTEC 079/98). Special thanks are due to Denise L.C. Mescolotti and Luis F. Baldesin for technical assistance, and to Dr. Andrew Smith and the two anonymous referees for their helpful comments on the manuscript. E.J.B.N. Cardoso, S.M. Tsai, and M.A. Nogueira are CNPq Scholars.

References

Baylis GTS (1969) Host treatment and spore production by Endogone. NZ J Bot 7:155–160

Bethlenfalvay GJ, Linderman RG (1992) Mycorrhizae in Sustainable Agriculture, Madison. ASA Spec Publ, Madison, WI, p 135

Bever J (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244:281–290

Bever J, Morton J, Antonovics J, Schultz P (1996) Host-dependent sporulation and diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82

Brundrett MC, Abbott LK, Jasper DA (1999) Glomalean mycorrhizal fungi from tropical Australia. I. Comparison of the effectiveness and specificity of different isolation procedures. Mycorrhiza 8:305–314

Carrenho R, Trufem SFB, Bononi VLR (2002) Effects of using different host plants on the detected biodiversity of arbuscular mycorrhizal fungi from an agroecosystem. Rev Bras Bot 25:93–101

Clark RB (1997) Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. Plant Soil 192:15–22

Escudero V, Mendonza R (2005) Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. Mycorrhiza 15:291–299

Gemma JK, Koske RE (1988) Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. Mycologia 80:211–216

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by sieving and decanting. Trans Br Mycol Soc 46:235–246

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Gomes SP, Trufem SFB (1998) Fungos micorrízicos arbusculares (*Glomales*, *Zygomycota*) na Ilha dos Eucaliptos, represa do Guarapiranga, São Paulo, SP. Acta Bot Bras 12:395–401

Howeler RH, Sieverding E, Saif SR (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant Soil 100:249–283

INVAM (2003) International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi. Species Description. Morgantown, West Virginia Agriculture and Forestry Experimental Station. Home page. http://invam.caf.wvu.edu

McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol Res 94:120–122

Moreira-Souza M, Cardoso EJBN (2002) Dependência micorrízica de *Araucaria angustifolia* (Bert.) O. Ktze. sob doses de fósforo. Rev Bras Cienc Solo 26:905–912

Moreira-Souza M, Cardoso EJBN (2003) Practical Method for Germination of *Araucaria angustifolia* \(Bert.) O. Ktze. Seeds. Sci Agric 60:389–391

Moreira-Souza M, Trufem SFB, Gomes-da-Costa SM, Cardoso EJBN (2003) Arbuscular mycorrhizal fungi associated with *Araucaria angustifolia* (Bert.) O. Ktze. Mycorrhiza 13:211–215

Moreira M, Baretta D, Tsai SM, Cardoso EJBN (2006) Spore density and root colonization by arbuscular mycorrhizal fungi in preserved or disturbed *Araucaria angustifolia* (Bert.) O. Ktze. ecosystems. Sci Agric 63:380–385

Moreira M, Baretta D, Tsai SM, Gomes-da Costa S, Cardoso EJBN (2007a) Arbuscular mycorrizal fungi in native and in reforested *Araucaria angustifolia* forest impacted or not by anthropogenic action in Brazil. Applied Soil Ecology (in press)

Moreira M, Baretta D, Tsai SM, Gomes-da Costa S, Cardoso EJBN (2007b) Biodiversity and distribution of arbuscular mycorrhizal fungi in *Araucaria angustifolia* (Bert.) O. Kuntze forest in Apiaí. Sci Agric (in press)

Saggin-Júnior OJ, Siqueira JO (1996) Micorrizas arbusculares em cafeeiro. In: JO Siqueira (ed). Avanços em Fundamentos e Aplicação de Micorrizas. Lavras, Brasil, p. 203–254

Schenck NC, Pérez Y (1990) Manual for the identification of VA mycorrhizal fungi, 3rd edn. Synergistic, Gainesville, FL, USA

Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. GTZ, Eschborn, Germany, p 371

Simpson D, Daft MJ (1990) Interactions between water stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum. Plant Soil 121:179–186

Stürmer SL (1999) Evolução, Classificação e Filogenia dos Fungos Micorrízicos Arbusculares. In Inter-relação Fertilidade, Biologia do Solo e Nutrição de Plantas. In: Siqueira JO, Moreira FMS, Lopes AS, Guilherme LRG, Faquin V, Furtini Neto AE, Carvalho JG. Sociedade Brasileira de Ciência do Solo, Lavras. pp 797–817

Trufem SB (1990) Aspectos ecológicos de fungos micorrízicos vesículo-arbusculares da mata tropical úmida da Ilha do Cardoso, SP, Brasil. Acta Bot Bras 4:31–45

Trufem SB (1995) Aspectos ecológicos de fungos micorrízicos arbusculares na rizosfera de plantas de restinga da Ilha do Cardoso, SP, Brasil. Rev Bras Bot 18:51–60

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 369:69–72

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007