OCCURRENCE AND DISTRIBUTION OF VESICULAR–ARBUSCULAR MYCORRHIZAL FUNGI IN COFFEE (*Coffea arabica* L.) PLANTATIONS IN CENTRAL SAO PAULO STATE, BRAZIL[1]

*E. S. LOPES**
*E. OLIVEIRA**
*R. DIAS**
*N. C. SCHENCK***

Resumen

Durante el verano se colectó muestras de suelo de la rizosfera y raíces de plantas de café en 27 fincas de la región central del Estado de Sao Paulo, para estudiar la presencia de hongos en micorriza vesicular-arbuscular (VAM). La mayoría de las fincas fueron fertilizadas anualmente, las muestras de suelo de la rizosfera presentó un pH mayor que 5.4, más 30 ppm de P y no se encontró toxicidad de Al^{3+}.

Se logró identificar un total de 22 especies de hongos VAM y se observó 20 especies no descritas. El género Acaulospora, *incluyendo especies no descritas con esporas pequeñas, de color amarillo-oro y paredes laminadas se encontró en todas las muestras,* Glomus *spp. se presentó en el 81% de los sitios y* Gigaspora *y* Sclerocystis *spp. se encontraron en el 60% y el 40% de las muestras, respectivamente.* Gigaspora margarita, *descrita como muy eficiente para el desarrollo de plántulas de café no pudo encontrarse en ninguna muestra. La colonización de raíces por hongos VAM osciló entre el 4 y el 46%.*

Introduction

The vesicular-arbuscular mycorrhizal (VAM) fungi promote an increased nutrient uptake by plants they colonize and thus benefit their growth. The majority of wild and cultivated plant species establish mycorrhizal associations (7).

As early as in 1897 Janse (5) reported that coffee plants are normally colonized by VAM fungi. It was shown recently (8) that some species of VAM fungi promote enhancement of coffee seedling growth and phosphorus uptake. *Gigaspora margarita* Becker and Hall was a very effective species, while *G. heterogama* Gerdemann & Trappe colonized the roots without stimulating growth. Coffee plants usually are grown in containers in a nursery before being introduced into the field. If artificial inoculation is confirmed to be advantageous, this could encourage the development of large scale nursery inoculation with VAM fungi. Although few inoculation experiments with VAM fungi on coffee have been carried out in the field, in most of them beneficial effects were observed.

This survey was conducted with the objectives of determining the species of VAM fungi associated with coffee roots in one important coffee region of Sao Paulo State, and to relate their distribution and percentage of root colonization with soil chemical analysis data.

Materials and methods

All samples were collected from Feb. 2 to 9, 1981, at the locations indicated in Table 1, situated in the area within 21-22°S and 47-50°W (Figure 1). They were collected at 0-15 cm depth, under the trees. For each sample there were three replicates, collected within an area of maximum 5 000 m^2. Each replicate consisted of composited soil and root subsamples from five non adjacent neighbour trees. Each replicate of composited subsamples were placed in plastic bags and maintained in a styrofoam box during transport. For each sample, crop age, management, and origin of the seedlings, where known, were recorded.

1 Received for publication in October 12, 1982.
This research was partially funded by IICA/EMBRAPA/CNPq.

* Instituto Agronômico de Campinas, S.P., Brazil.

** University of Florida, Gainesville, Fla. USA.

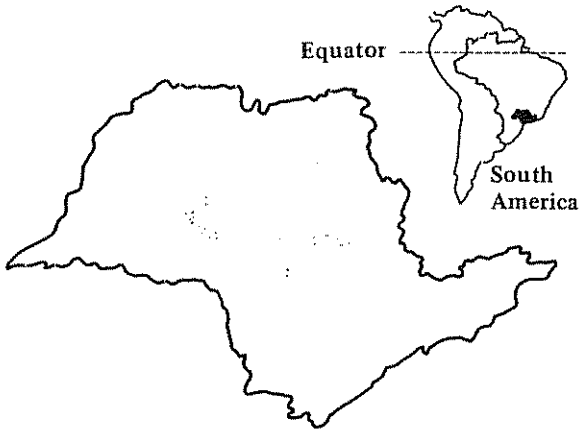

Fig. 1. Sample collecting sites for Vesicular-arbuscular Mycorrhizal fungi associated with coffee survey.

Samples were sieved on 2 mm screen, a 50 g subsample removed, and placed in a refrigerator (5°C) until spore extraction. Root samples were washed and a portion fixed in formalin acetic acid and another portion was added to sterile soil in 15-cm pots and planted with coffee and siratro (*Macroptilium atropurpureus*) to isolate VAM fungi colonizing the roots as described by Gerdemann and Trappe (3). Chemical analysis of a soil sample from each location consisting of composited subsamples from the three replicates were made at the laboratories of the Section of Soil Fertility, Instituto Agronômico. The following extractors were used: KCl 1N for Al^{+++}, Ca^{++}, and Mg^{++}; H_2SO_4 0.05 N for K and P; carbon was oxidized with $K_2Cr_2O_7$ 4N, in presence of H_2SO_4; potentiometric determination of pH was in 1:2.5 aqueous suspension.

Spore extractions were made by the wet sieving method of Gerdemann and Nicolson (2) using sieves with 720, 250, 105 and 53 μm openings. Fractions retained in each sieve were centrifuged (3 500 rpm) for 2 minutes in water, and then for 1.5 minutes in a 45% sucrose solution. Spores observed in each fraction were counted and separated for identification after mounting in lactophenol on glass slides.

Root samples of each replicate (circa 0.5 g) were cleared and stained as described by Philips and Hayman (9). Root colonization by VAM fungi was then evaluated by the grid plate method of Giovannetti and Mosse (4).

Results

Most of the soils of the studied area are classified as Podzolized (Lins and Marilia variations) great groups. They are sandy loam soils of medium fertility which were put into cultivation, starting with coffee, some 50-70 years ago. Coffee is still the major crop in the area. Results of chemical analysis of soil samples are presented in Table 1. Sample numbers 3, 4, 5, 7, 8 and 27 were from Latosolic soils. With exception of sites number 27 and 22, which had not received any fertilizer since planting (6 year old crop), all the others had received variable annual amounts of fertilizers and different management, which could explain the variation in pH and nutrient content among locations.

Spores of species of VAM fungi in the genus *Acaulospora* were recovered from all the soil samples (Table 2). Species of *Glomus* were found in 81 percent of the samples while *Gigaspora* and *Sclerocystis* were observed in 60 and 40 percent of the samples, respectively. As many as 20 types of spores, with characteristics differing from presently described species, were found in this survey. Attempts to isolate these fungi are under way. One spore type, with an unusual thick (43 μm), long subtending hyphae, and yellow-gold reticulated contents (possibly a new genus) was also observed in one sample site.

Although the genus *Glomus* was common throughout the area, the most frequently recovered species, *G. fasciculatum,* occurred only in 44 percent of the sampled sites, and thus was not as common as an undescribed *Acaulospora* sp. (A–1), recovered from 92 percent of the samples (Table 2).

Table 3 indicates the relative occurrence, and number of spores of all described and undescribed species which occurred in at least 10 percent of the sample locations. The five mostly found undescribed species indicated in Table 3 could be briefly characterized as follows:

Acaulospora sp. A–1 Small (50-150 μm), globose, golden yellow to honey colored spores, with 3-8 μm thick, 2-3 layered all, with non reticulated contents.

Acaulospora sp. A-2 Spherical or elongate small spores (50-110 x 75-150 μm) with irregular reticulate contents and a thin (0.5 - 9.0 μm) hyaline wall.

Acaulospora sp. A-3 Small (80-120 μm) red brown spherical spores, with a thick wall, having a sinous pattern on it.

Sclerocystis sp. S-1 Small elongate (20-32 x 26-38 μm) yellow-brown spores with 5-7.5 μm wall. Sporocarps are small, with peridium formed of extremely thin hyphae.

Table 1. Chemical characteristics of the soils sampled for VA mycorrhizae under coffee trees in 27 locations in Central São Paulo State, Brazil.

Sample No.	Location			e. mg/100 g soil			ppm	
		% C	pH	Al^{+3}	Ca^{+2}	Mg^{+2}	K	P
1	Rio Claro	5.5	5.4	0.1	5.6	1.1	336	180
2	Rio Claro	3.3	6.4	–	4.4	1.4	292	204
3	Rio Claro	1.7	5.9	0.1	3.3	0.5	128	91
4	Itirapina	2.2	5.3	0.4	1.0	0.3	152	30
5	Brotas	4.0	5.6	–	2.8	1.5	400	27
6	Brotas	2.2	5.6	0.7	1.1	0.4	136	37
7	Jaù	2.2	6.6	–	2.6	1.6	204	55
8	Jaú	2.5	6.2	–	5.3	1.5	132	40
9	Bauru	3.2	5.8	–	2.7	1.7	176	52
10	Bauru	1.1	5.0	0.3	0.8	0.2	68	78
11	Cafelandia	0.9	5.6	0.1	0.7	0.3	132	36
12	Lins	2.2	6.1	0.1	1.2	0.6	340	14
13	Lins	3.1	5.3	0.3	1.4	0.4	160	25
14	Marilia	1.7	5.7	–	3.6	0.4	76	217
15	Marilia	1.3	5.0	0.4	1.1	0.3	44	58
16	Marilia	1.4	5.0	0.2	2.4	0.5	44	133
17	Marilia	3.2	5.8	–	10.2	1.2	60	140
18	Marilia	2.7	6.8	–	7.6	2.4	312	122
19	Vera Cruz	1.3	6.0	–	1.6	0.6	400	81
20	Vera Cruz	1.5	6.4	–	1.8	1.1	112	18
21	Garca	1.7	4.7	1.1	0.5	0.2	52	14
22	Garca	1.2	5.5	0.1	2.5	0.4	120	12
23	Galia	2.3	6.1	–	1.9	1.1	236	37
24	Galia	2.1	6.1	–	2.2	1.3	192	34
25	Fermo Dias	1.2	5.2	0.4	0.7	0.3	72	20
26	São Manuel	2.9	4.9	1.0	0.6	0.2	764	11
27	São Manuel	1.8	5.4	0.1	1.6	0.5	102	4

Table 2. Occurrence of VA mycorrhizal fungi in coffee (*Coffea arabica* L.) plantations of the central region of São Paulo State. Data refers to average percentage of tree replicates for 27 locations.

Genus	Total species identified*	Relative occurrence of the genus (%)	Relative occurrence of most common species (%)
Acaulospora	5 (10)	100.0	92.6
Glomus	8 (4)	81.5	44.4
Gigaspora	6 (2)	59.2	29.6
Sclerocystis	3 (4)	40.7	18.5
Total	22 (20)	–	–

* Numbers in parenthesis refer to spore types which did not fit in any described species of the particular genus. Data on relative occurrence included spore types

Glomus sp. G-1 Globose or elongate small red to brown small spores (80-100 x 100-130 μm), with thick red wall (7.0-12 μm) and thick subtending hyphae.

Less frequently observed described species included *Acaulospora faveata* Trappe & Janos, *A. trappei* Ames & Linderman, *Glomus geosporum* (Nicol. and Gerd.) Walker, *Glomus trappei* James & Linder-

man, *Glomus claroideum* Schenck & Smith, and *Glomus occultum* Walker. Besides the spore types listed in Table 3, seven more in the genus *Acaulospora*, three in *Glomus*, two in *Gigaspora*, and three in *Sclerocystis* were recovered in less than 10 percent of the sites.

Counts of spores were made, but the occurrence of spore clusters or sporocarps in the genus *Glomus* and *Sclerocystis* made it difficult to obtain comparable counts within these genera. In any case, these two genera had low numbers of spores in this survey. Usually one to three spores or small clusters of those two genera were found per sample. The individualized nature of spores of *Gigaspora* spp. and *Acaulospora* spp. permitted an easier and more reliable count. However, there was great variation among replicates in those counts. The most commonly observed species of *Acaulospora* (A-1, a possible new species) also had the highest number of spores of any species, between 2 and 41 spores/50 ml of soil, with an average of 12.3 spores/50 ml of soil (Table 3). In general *Gigaspora* species had a much lower number of spores recovered. The most commonly found species of this genus, *G. pellucida*, was recovered in only 8 out of 27 locations and had an average of only 2.8 spores/50 ml of soil.

Attempts were made to relate genus and species incidence with the soil characteristics presented in Table 1, but no clear relationships were apparent. Table 4 summarizes one such attempt showing relative occurrence of the four genera, expressed as percent of locations in which they occurred, in relation to soil pH classes. The incidence of *Acaulospora*, and *Sclerocystis* was not affected by soil pH, on the range of 4.7 to 6.9. *Gigaspora* was not recovered from soils with pH higher than 6.5, and *Glomus* was less commonly found in locations with pH lower than 5.0.

Samples 14, 15, 16, and 17 were all from the same farm, and the crop had received basically the same management from planting. They differed in plant age, the younger one (No. 14) being only 6 months from transplant. In the other three sites (sample numbers 15, 16, and 17) the plants were 1, 2, and 5 years old, respectively. A tendency of decreasing root colonization with tree age was observed; the percentage of root colonization were 22.0, 23.4, 19.1, and 12.1 for samples 14, 15, 16, and 17, respectively.

Table 3. Relative incidence of species of VA mycorrhizal fungi recovered under coffee trees in Central São Paulo State, Brazil. Only species occurring at more than 10% of the sample sites are included.

Species	% Occurence	No. of spores per 50 ml of soil	
		range	average*
Acaulospora sp. (A-1)	92.3	2–41	12.3
Glomus fasciculatus (Thax.) Gerd. & Trappe	44.4	–	–
Acaulospora scrobiculata Trappe	40.7	1–15	4.7
Acaulospora laevis Gerd. & Trappe	37.0	1–5	2.0
Gigaspora pellucida Nicol. & Schenck	29.6	1–6	2.8
Acaulospora sp. (A–2)	25.9	2	2.0
Gigaspora gigantea (Nicol. & Gerd.) Gerd & Trappe	22.2	1–3	1.8
Acaulospora spinosa Walker & Trappe	18.5	1	1.0
Acaulospora sp. (A–3)	18.5	1–8	2.8
Glomus macrocarpus Tul. & Tul.	18.5	–	–
Glomus microcarpus Tul. & Tul.	18.5	–	–
Gigaspora gilmorei Trappe & Gerd.	18.5	1–2	1.6
Sclerocystis clavisporus (Trappe)	18.5	–	–
Sclerocystis coremioides B&B	18.5	–	–
Sclerocystis sp. (S–1)	14.8	–	–
Glomus etunicatus Becker & Gerdeman	14.8	–	–
Glomus sp. (G–1)	14.8	–	–
Gigaspora heterogama (Nicol. & Gerd.) Gerd. & Trappe	14.8	1–3	1.5
Gigaspora rosea Nicol. & Schenck	11.1	1–2	1.3
Sclerocystis sinuosa Gerd. & Bakshi	11.1	–	–

* For the average value only the sites in which the species occurred were considered.

Table 4. Relative incidence of four genera of Endogonaceae in rhizosphere of coffee trees of central São Paulo State, as a function of soil pH.

Genus	Soil pH				
	4.6–5.0	5.4–5.5	5.6–6.0	6.1–6.5	6.6–7.0
	Incidence %	Incidence %	Incidence %	Incidence %	Incidence %
Acaulospora	100	100	100	100	100
Gigaspora	80	50	62	50	0
Glomus	40	100	75	100	100
Sclerocystis	60	50	12	33	50
Number of samples in each pH range	5	6	8	6	2

Spores of *Acaulospora* were found in each of the four samples, but *Gigaspora* spores were not recovered from the two samples from older plants, in this particular farm. Individual data for root colonization are not shown, but root colonization in the soils which had received no P fertilizer (samples 21 and 22) were among the highest (38 and 43 percent, respectively) in this survey.

Discussion

This represents an extensive and systematic survey on occurrence of VA mycorrhizal fungi associated with coffee. After the initial reference of Janse (5) of mycorrhizal colonization of coffee roots, only two other recent published reports were found. Cardoso (1) observed nursery plants and found only well developed seedlings to be infected. Lopes *et al.* (8) observed that growth response of coffee to mycorrhizal fungi varied with the species of endophyte.

Miller *et al.* (6) observed variable degrees of infection (4 up to 74 percent) in forage grasses and legumes, in Nova Odessa, São Paulo State. From their description of spore types, apparently no *Gigaspora* species were recovered. The absence of *Gigaspora margarita* in this survey is of interest, since this species was highly effective in promoting growth of coffee seedlings (8). A survey which is underway in Cerrado soil of São Paulo revealed that *Gigaspora* spp. is fairly common in those acid soils (Penteado, V., Instituto de Botanica, S.P., personal communication).

The occurrence of mycorrhizal species observed in this survey are much higher than the ones observed in the survey of agricultural crops in Florida (10), which involved 30 different hosts, and higher soil variations. It is not known if the widespread occurrence, and abundant sporulation of *Acaulospora* spp., particularly the undescribed (A-1) golden yellow spore type might be associated with the coffee crop. Very likely this spore type is adapted to a wide range of conditions, since it has been observed in different areas and crops in the states of Para and Minas Gerais by one of the authors (E. Oliveira).

Summary

Coffee rhizosphere soil and root samples were collected during the summer, from 27 farms in the central region of Sao Paulo State, and were assayed for presence of vesicular-arbuscular mycorrhizal (VAM) fungi. Most farms had received annual fertilizer applications. Most rhizosphere soil samples had a pH higher than 5.4, over 30 ppm P and no Al^{3+} toxicity.

A total of 22 species of VAM fungi were identified and 20 other possibly undescribed species were observed. The genus *Acaulospora,* including a nondescribed species with small, golden-yellow spores having laminated walls, was found in all sample sites. *Glomus* spp. occurred in 81 percent of the sites. *Gigaspora* and *Sclerocystis* spp. were found in 60 percent and 40 percent of the samples, respectively. *Gigaspora margarita* which has been reported as highly effective in enhancing coffee seedling development was not detected. Root colonization by VAM fungi varied from 4 to 46 percent.

Literature cited

1. CARDOSO, E. J. N. Ocorrência de micorriza em café. Summa Phytopathologica 4:136-137. 1978.

2. GERDEMANN, J. W. and NICOLSON, T. H. Spores of mycorrhizal *Endogone* species

extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46:235-244. 1963.

3. GERDEMANN, J. W. and TRAPPE, J. M. The Endogonaceae in the Pacific Northwest. New York Botanical Garden and Mycological Society American. 1974. 74 p. (Mycologia Memoir 51).

4. GIOVANNETTI, M. and MOSSE, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytology 84:489-500. 1980.

5. JANSE, J. M. Les endophytes radicaux de quelques plantes javanaises. Annual Jard. Bot. Buitenzorg 24:53-201. 1897.

6. MILLER, R. H., CARDOSO, E. J. B. N. and CARDOSO, C. O. N. Some observations on mycorrhizal infection of tropical forage legumes and grasses in Brazil. Summa Phytopathologica 5:168-172. 1979.

7. MOSSE, B. Advances in the study of vesicular-arbuscular mycorrhizal. Annual Review of Phytopathology 11:171-196. 1973.

8. LOPEZ, E. S., OLIVEIRA, E., NEPTUNE, A. M. L. and MORAES, F.P. Efeito da inoculaçao do cafeeiro (*Coffea arabica* L.) com diferentes espécies de Fungos micorrízicos. Revista Brasileira de Ciencia do Solo 6 (in press). 1981.

9. PHILLIPS, J. M. and HAYMAN, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the Bristish Mycological Society 55:158-161. 1970.

10. SCHENCK, N. C and SMITH G. S. Distribution and occurrence of vesicular-arbuscular mycorrizal fungi on Florida agricultural crops. Crop Science Society of Florida. 40:171-175. 1981.