Journal of Applied Botany and Food Quality 87, 243 - 248 (2014), DOI:10.5073/JABFQ.2014.087.034

[1]Departamento de Biología, Facultad de Ciencias, Universidad de la Amazonía, Florencia, Caquetá, Colombia
[2]Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart-Hohenheim, Germany

Arbuscular mycorrhiza in Colombian coffee plantations fertilized with coffee pulps as organic manure

Raúl Hernando Posada[1], Ewald Sieverding[2]*

(Received December 23, 2013)

Summary

The distribution of arbuscular mycorrhizal (AM) fungal structures in roots, in soil surrounding roots and in amended coffee pulps (CP) was investigated in 12 coffee plantations in Colombia. Fresh CP had been added to plants 6-10 months before sampling. The questions were whether soil chemical and physical parameter and soil depth had an effect on mycorrhiza. Root colonization rates with AM increased in CP amended-plants ($F = 7.75$, $P < 0.001$) as compared to a non-amended control. Significantly more roots, CP and AM root colonization were found in the upper soil layer ($F = 41.24$, 9.54, 6.60 respectively, $P < 0.001$), while root external mycelium and CP colonization with AM were not affected by soil depth ($F = 14.82$, $P > 0.05$). External mycelium length differed between locations ($F = 5.89$, $P < 0.001$) and was inversely correlated with soil water content ($r = -0.655$, $P = 0.02$). External mycelium length per AM colonized root was higher in the lower soil layer ($F = 14.82$, $P < 0.05$). Soil aeration seemed to be an important physical characteristic for mycorrhiza development in and around coffee roots. Higher mycorrhiza colonization in CP amended-plants might be an adaptive strategy for nutrients acquisition, and AM external mycelium that colonizes CP might take up nutrients directly during CP decomposition.

Introduction

Coffee (*Coffea arabica* L.) is one of the most important cash crops in tropical countries of Central and South America, as well as of tropical Africa and Asia (IICA and PROMECAFE, 1997). Coffee is often grown in nutrient-deficient soils, where its establishment, development and production are limited by low levels of available phosphate (P) (GAUR and ADHOLEYA, 2002). The response curves to P fertilization indicate that coffee is an obligate mycotrophic plant depending on AM for its growth (OSORIO et al., 2002; DE ALMEIDA et al., 2003) and field trials have shown that arbuscular mycorrhizal fungi (AMF) can increase coffee growth and productivity up to 62 % (SIQUEIRA et al., 1998) via improved uptake of potassium and P (SIQUEIRA et al., 1994). In coffee seedlings propagated *in vitro*, VAAST et al. (1996) found that AM enhanced root and shoot growth and plant P status, resulting in a lower root-to-shoot ratio compared to non-mycorrhizal plants. In adult coffee plants, mycorrhizal root colonization rates varied from 4 to 80 %, indicating the existence of environmental factors that influence AM under field conditions (SAGGIN-JUNIOR and SIQUEIRA, 1996). The consistent effects of AM on plant development and productivity are higher in young seedlings and are diminished with crop age.

In order to conserve soil fertility, it is recommended to add organic amendments to coffee plantations, including fresh organic manure, compost and coffee pulp (FEDECAFE, 2003; VAIDYA et al., 2008). The coffee pulp (CP) is the main byproduct in the wet separation of coffee beans from fresh berries. The pulp consists of both the exocarp and mesocarp of berries. For every ton of coffee beans, about two tons of CP are produced (HOFMANN and BAIER, 2003). Organic matter content of dry CP is > 90 %; CP is rich in nitrogen (N, about 1-2 %) and low in P (< 0.1 %), while the C:N ratio is about 30 (BLANDÓN et al., 1998). Fresh CP is superficially incorporated into the upper soil layer around growing coffee plants. The slow decomposition of CP releases nutrients, which in turn are taken up by microorganisms or plants. Organic amendments, like CP, can increase water-holding capacity and reduce leaching of mineralized nutrients from soils. Latter is especially important in hilly landscapes with coffee plants (ARELLANO et al., 2000). Positive effects of such amendments are also increased soil aggregation, decreased soil compaction, and increased microbial activity (CELIK et al., 2004; ZELEKE et al., 2004; GRYNDLER et al., 2006). The general consensus is that organic amendments have beneficial effects on development of AM (GRYNDLER et al., 2006; VAIDYA et al., 2008).

It was reported that the mycelium of AMF could colonize rotten residues and organic materials in diverse ecosystems (ARISTIZABAL et al., 2004, POSADA et al., 2012), but its distribution in decomposing CP has not been evaluated. Superficial CP incorporation in coffee plantations implies more abundant nutritional supply to the topsoil. Although soil depth effects on AMF communities and root colonization were investigated with many plants in the past (SIEVERDING, 1991; OEHL et al., 2005; SMITH and READ, 2008), root external mycelium was seldom investigated at different soil depths (STEINAKER and WILSON, 2008). The root external mycelium may not only provide an increased surface area for interactions between plants and soil particles and between organic matter and their decomposing microorganisms, but also provide an important pathway for the translocation of energy-rich plant assimilates (products of photosynthesis) to microorganisms in deeper soil zones (FINLAY, 2008). On the other hand, soil parameter such as water content (STEVENS and PETERSON, 1996; SCHACK-KIRCHNER et al., 2000), pH (HEIJNE et al., 1996; ÁLVAREZ-SÁNCHEZ et al., 2011), organic carbon (C) content (ZHU and MILLER, 2003), and interactions between soil parameter (POSADA et al., 2008) may affect AM mycelium development, spore production and root colonization.

This study aimed to answer the following questions: 1) Do CP amendments influence the occurrence and distribution of AM in roots, rhizosphere (soil around roots) and CP in coffee plantations grown under different soil chemical and physical conditions? 2) Are mycorrhizal parameters in roots, rhizosphere, and CP influenced by soil depth?

Materials and methods

Soil sampling was conducted in a coffee production area of Caquetá, Colombia. The area is at an altitude between 882 and 1450 m.a.s.l., with a mean annual temperature of 17 ºC, annual rainfall of 3800 mm and air humidity of 80 %. The soil type in the area is Oxisol. The sampling was carried out in a total of 12 coffee farms, in two farms each located in Doncello (DO) (1º41'N, 75º20'W), Florencia (FL) (1º38'N, 76º01'W), Puerto Rico (PR) (1º51'N, 75º16'W), Paujil (PA) (1º35'N, 75º22'W), Montañita (MO) (1º46'N, 75º24'W) and San Vicente (SV) (2º16'N, 74º58'W), in 2013. Coffee varieties were "Caturra" and "Arabica".

* Corresponding author

Six samples were taken in the two farms in Florencia before CP application; these were used as controls. All farms had applied and incorporated one kg of fresh CP in a one m radius around the stem of the trees in coffee plantations, 6 - 10 months before sampling. Four soil samples were collected at a distance of 50 cm from the trunk of a plant, with three plants per farm. Samples were collected with a soil corer of 3.8 cm diameter to 10-cm depth. Each sample was divided for the upper soil layer (0-5 cm, top) and the lower (5-10 cm, bottom), placed in plastic bags, and brought to the laboratory. Samples were thoroughly homogenized and separated into decomposing CP, roots and soil. The proportion of CP, roots and soils were estimated as their fresh weight. To characterize the CP at each depth, the areas of 15 randomly selected fragments were measured using Sigma Scan Pro 5.0 (SPSS Inc.); the area was used as an indicative for decomposition stage of CP – smaller size indicating more advanced decomposition. Soil water content was measured using subsamples by drying at 80 ºC for 72 h.

Sub-samples of soils from the same farm were mixed and homogenized. This pooled sample (400 g) was sent to the Instituto Geográfico Agustín Codazzi (IGAC), Bogota, for physical and chemical characterization: texture was determined by Bouyoucos method (Bouyoucos, 1962), pH in H_2O (1:1) by potentiometer, cation exchange capacity (CEC) in KCl, saturation of bases in %, organic C according to Walkley-Black (Nelson and Sommers, 1982), and plant available P according to Bray II (Fixen and Grove, 1990). A dried pooled CP sample (2 g) of each farm was sent to IGAC for the determination of C and N content, by dry combustion and thermal conductivity using an elemental analyzer (LECO 1000).

External mycelia of AM fungi were measured according to Herrera et al. (1986) as follows: a 2 % H_2O_2 dilution was added to a defined air-dried sample, blended for 30 s, rinsed on a 45 µm-mesh-opening sieve, air dried for 48 h and weighed. A sub-sample of 0.02 g of this was mixed with 2 drops of glycerin (100 %) on a microscope slide. The number of coenocytic hyphae that intersected four squared transects (two horizontal and two vertical) on each slide were counted at 100 times magnification under the microscope. The mycelium length per gram of dry sample was calculated as: average number of intersections/transect x 1.57 mm/intersection x 20 transects/22 mm square cover-slip x weight of material from the 45 µm sieve/0.02 g sub-sample (modified from Herrera et al. (1986)). The results were expressed in meters of root external AM mycelium per gram of dry sample.

Sub-samples of CP fragments were cleared by the Koske and Gemma (1989) technique, as modification of Phillips and Hayman (1970). Decomposing CP was carefully cleaned and submerged in KOH, extremely lignified tissues were exposed to a mix (1:1 v/v) of 2 % H_2O_2-NH_4OH (Schenck, 1982). Cleared samples were acidified with 1 % HCl during 60-600 s and AMF mycelium was stained with acid trypan blue (0.5 g L^{-1} trypan blue) at 85 ºC for 1 h. To assess the AMF colonization of CP, small randomly selected CP fragments were placed adjacent on microscope slides to form lines 2 mm broad and 18 mm long, and mounted in polyvinyl-lacto glycerol under a cover slip. On each slide nine transects of each line were observed for AMF colonization. CP fragments were considered to be colonized if they contained either coenocytic mycelium with unilateral angular projections (Nicholson, 1959) or coenocytic hyphae with terminal vesicles or spores. CP colonization rate was estimated as the number of colonized intersections, divided by the total number of observed intersections multiplied by 100.

Roots from each sample were cleared according to the Koske and Gemma (1989) method, as a modification of Phillips and Hayman (1970). The cleared roots were acidified with 1 % HCl during 300-1200 s and the AM fungal structures were stained with acid trypan blue (0.5 g L^{-1} trypan blue) at 85 ºC for 1 h. The stained roots were mounted on microscope slides for assessment of percent AM colonization by the magnified intercept method (McGonigle et al., 1990).

For statistical analyses, AM root colonization and soil water content did not require normalization. Colonization of CP by AM mycelium was normalized by $x^{-1/2}$ transformation; the external mycelium was normalized by Sen x transformation and the remained variables were normalized by $-\text{Log}_{10}(x+1)$ transformation (Zar, 1999). In each location, no large numerical differences were observed in soil characteristics between the two farms, except for El Vergel in Puerto Rico, where P content was much higher. Thus, we decided to process the data for the AM parameters by location. Two-way ANOVA and the least significant difference (LSD) post-hoc test (Statistica ver. 6.0 programme of Statsoft, 2001) were used to evaluate statistical differences between locations and soil depths for CP and root colonization by AMF, soil water content, AM mycelium length, root and CP fractions. The Kruskal-Wallis test was employed to compare the area of CP fragments between locations and soil depths, separately. Spearman's rank correlation coefficients were computed for all pairwise combinations of measured variables (Zar, 1999).

Results

Soils in the study area were clay-sandy-loams with a pH between 3.8 and 4.7. The contents of organic C and for plant available P were low, except for El Vergel with an intermediate level of P (Tab. 1). CEC was very low in all locations. Highest C contents were found in Doncello and Florencia with 2.6 - 3.6 %; it varied in the other locations from 1.9 % to 2.7 %. Content of N in soils was between 0.08 - 0.22 %.

Significant differences were found among locations in soil water contents, CP fragment areas, AM root colonization and root external mycelium length (Tab. 2). Root colonization was lower in control plants than in CP-amended plants. Significantly more roots and CP fractions, as well as higher AM root colonization were observed in the top soil layer than in the bottom layer (Tab. 2). However, there were no significant interactions between locations and soil depths. Calculating the root external mycelium length in relation to percentage of root or CP fraction in soil, more mycelium per unit root was found in deeper soil layers, while there was no such clear indication of mycelium length with the CP fraction (Tab. 3). On the other side, soil depth did not influence the mycelium length per AM colonized-root, but mycelium length per AM colonized-CP was consistently lower in the bottom soil layer (Tab. 3).

The pH, CEC, organic C, base saturation, available P, and water contents did not indicate any influence on AM root colonization or on AM colonization of CP. C:N ratios in CP, organic C and soil water content were negatively correlated with AM mycelium in soils ($r = -0.8738$, $P = 0.023$; $r = -0.7096$, $P = 0.010$; $r = -0.6551$, $P = 0.02$, respectively).

More CP fragments, more roots, and higher AM root colonization were found in the top soil layer than in the bottom layer. Although there were no significant differences between soil depths in CP colonization with AM or external mycelium length (Tab. 2), in the majorities of the locations, more mycelium per AM colonized-CP was found in the upper soil layer (Tab. 3).

Discussion

The present study showed that CP amendments resulted in higher AM root colonization of field grown coffee (66 ± 19 % vs. 35 ± 6 % without amendment). Osorio et al. (2002) reported increases in height, leaf number, root, shoot and total dry weight but not in root colonization of coffee seedlings amended with CP under greenhouse conditions. Our results indicate and confirm that amendments

Tab. 1: Chemical properties of soils and decomposing coffee pulp (CP) in six locations of Caquetá, Colombia.

Location[a]	Farm	Soil					CP		
		pH	Cation Exchange Capacity meq/100 g	Base Saturation %	Organic Carbon %	Available Phosphorus mg / kg[b]	% C	% N	C:N
DO	Los Naranjos.	4.1	2.9	81.9	2.4	nd	3.5	0.13	26.9
	La Argelia.	3.9	3.3	82.8	1.8	nd	2.6	0.08	32.5
FL	La Primavera.	3.9	4.7	91.4	2.8	0.6	3.6	0.18	20.0
	Las Brisas.	3.9	3	89.6	1.9	nd	3.2	0.21	15.2
MO	Peñas Altas.	4.5	1	26.8	1.7	3	2.7	0.18	15.0
	La Esperanza.	4.3	1.6	42.7	1.4	nd	2.4	0.15	16.0
PA	La Florida.	3.8	3.2	89.6	1.7	0.6	2.9	0.22	13.2
	Sombra Palestina.	3.9	2.3	83.7	1.9	2.6	2.9	0.11	26.4
PR	La Floresta.	4.6	1.4	32.6	0.5	4.3	2.1	0.22	9.6
	El Vergel.	4.7	1.1	24.9	1.8	24.5	2.6	0.21	12.4
SV	La Lindosa.	4.5	0.7	32.7	1.2	nd	1.9	0.15	12.7
	El Cedral.	4.4	1.4	63.1	1.3	nd	2.2	0.21	10.5

[a] DO: Doncello; FL: Florencia; MO: Montañita; PA: Paujil; PR: Puerto Rico; SV: San Vicente.
[b] nd: not detected.

Tab. 2: Soil water content, fraction of roots and coffee pulps (CP) in soils, area of CP fragments, and mycorrhizal parameters investigated in top layer (0-5 cm) and bottom layer (5-10 cm) at different locations of Caquetá, Colombia.

Location[a]	Soil depth	Water content %	Roots fraction %	CP fraction %	CP fragments mm^2	Mycorrhizal root colonization %	AM external mycelium (m g^{-1} soil)	CP mycorrhizal colonization %
DO	Top	44±4 d	0.9±0.7	3.5±2.6	19.0±16.7 a	81.6±17.3 c	1.9±1.7 a	17.1±20.4
	Bottom	41±3	0.2±0.1	0.9±0.8	11.9±11.9	74.7±12.9	1.3±0.9	25.4±30.0
FL	Top	47±3 e	0.7±0.3	2.0±0.3	16.4±11.2 a	69.8±16.4 b	4.4±2.0 a	13.3±9.2
	Bottom	43±3	0.1±0.1	1.2±1.3	17.7±16.1	53.2±17.7	2.0±1.3	13.4±6.9
MO	Top	32±3 ab	1.5±1.1	2.7±2.8	9.4±8.7 a	62.2±17.4 b	4.2±2.6 ab	7.4±7.0
	Bottom	33±3	0.2±0.1	0.6±0.4	9.5±7.2	59.2±24.7	3.7±0.5	8.5±9.9
PA	Top	35±3 bcd	0.6±0.4	1.9±1.9	238.7±275.1 b	78.3±10.4 bc	6.2±4.7 abc	16.6±13.7
	Bottom	39±9	0.1±0.0	1.0±1.2	289.5±225.6	63.6±6.9	2.8±2.1	16.5±18.6
PR	Top	35±2 bc	0.6±0.3	0.5±0.3	9.5±12.0 a	62.7±24.6 bc	5.2±3.7 bcd	9.6±9.4
	Bottom	34±6	0.2±0.1	1.0±1.2	7.3±5.9	68.0±19.0	5.5±1.5	14.4±15.6
SV	Top	27±6 a	0.8±0.6	1.1±0.3	297.8±274.2 b	71.2±14.2 b	6.8±4.0 bcd	13.9±16.2
	Bottom	28±6	0.2±0.2	0.8±0.6	283.4±263.6	44.8±23.5	6.7±1.1	17.2±20.5
C[b]	Top	43±2 d	-	-	-	37.2±5.0 a	2.5±0.9 a	-
	Bottom	39±3	-	-	-	32.5±6.6	2.1±0.8	-
ANOVAs								
Location (L)		22.39**	1.75	1.61	5.94**	7.75**	6.74**	0.83
Depth (D)		0.87	41.24**	9.54**	1.36	6.60**	3.81	0.54
L x D		1.36	1.24	2	-	1.14	0.96	0.11

* Significant at $P < 0.025$; ** Significant at $P < 0.001$
[a]: DO: Doncello; FL: Florencia; MO: Montañita; PA: Paujil; PR: Puerto Rico; SV: San Vicente.
[b]: Control sample.
-: Not evaluated.
Means of column that share the same letter are localities that do not differ significantly.

Tab. 3: Relations of root external mycelium length (m g^{-1}) with root fraction (% w/w), CP fraction (%) in soil, AM colonized-root (%), and AM colonized-CP fragments (%).

Location[a]	Soil depth	Mycelium per root fraction (m g^{-1} / %)	Mycelium per CP fraction (m g^{-1} / %)	Mycelium per AM-colonized-root (m g^{-1} / %)	Mycelium per AM colonized-CP fragment (m g^{-1} / %)
DO	Top	2.1	0.5	0.02	0.11
	Bottom	6.5	1.4	0.02	0.05
FL	Top	6.3	2.2	0.06	0.33
	Bottom	20	1.7	0.04	0.15
MO	Top	2.8	1.6	0.07	0.57
	Bottom	18.5	6.2	0.06	0.44
PA	Top	10.3	3.2	0.08	0.37
	Bottom	28	2.8	0.04	0.17
PR	Top	8.7	10.4	0.08	0.54
	Bottom	27.5	5.5	0.08	0.38
SV	Top	8.5	6.2	0.10	0.48
	Bottom	33.5	8.3	0.15	0.39
C[b]	Top	-	-	0.07	-
	Bottom	-	-	0.06	-
ANOVA					
Depth (F)		14.82*	0.02	0.02	2.05

* Significant at $P < 0.05$
[a] For abbreviations see Tab. 1
[b] Control sample.
-: Not evaluated.

with organic manure have positive effects on AM root colonization of adult plants under field conditions (MUTHUKUMAR and UDAIYAN, 2000; GOSLING et al., 2010; MONTALBA et al., 2010). Amendments of CP consistently resulted in more CP fragments in the upper soil layer, which is not surprising as CP was only superficially incorporated. Root distribution of coffee plants was highest in this layer, too, as well as AM root colonization (Tab. 2). We had expected more roots in deeper soil, as the upper layer was more affected by seasonal and daily changes in water contents and temperatures. Perhaps the superficial proliferation of nutrimental sources led to more roots and higher AM rates in the upper layer as was earlier reported by CUENCA et al. (1983) for coffee grown under shade trees.

In this study there was no relationship between C:N ratio of CP and the CP fragment areas, indicating that CP decomposition processes were similar at all locations. It can generally be assumed that the microbial activity and organic matter decomposition is high at soil temperatures of 15-25 ºC in the tropics, when water is not limiting the microbial activity. We found that the absolute AM mycelium length in soils was inversely related to C:N ratios of CP, together with soil water content and organic C. The decreased absolute presence of root external AM mycelium with higher water contents must be related to the likely higher release and availability of mineralized nutrients from CP, and potentially to poor soil aeration in water saturated soils. Both factors, lack of soil aeration (MUTHUKUMAR et al., 1997; BRAMLEY et al., 2007) and higher nutrient availability (SMITH and READ, 2008; TALBOT et al., 2008; FITTER, 2011) were reported to have negative impacts on AM.

Colonization of CP by mycelium of AM fungi was so far unknown. It is of interest to note that the colonization of CP was not significantly influenced by different soil physical and chemical characteristics at the different locations, nor by soil depth. Also, the colonization rate of CP itself was totally independent on CP fragment size, CP nutrient content or C:N relation in CP, indicating that AM mycelium can explore this source of organic manure at all stages of decomposition for nutrient extraction. These nutrients are theoretically then moved to the plant by the root external mycelium. Although the total external mycelium was decreased by more nutrient availability, the relative root external mycelium length was not impacted by CP fractions size and by the AM colonized CP fractions, at the two soil depths. This may indicate that the root external mycelium was not sensitive to nutrients potentially released by CP. Further on, relating root external mycelium length to root growth, it is clear that relatively more mycelium per unit root was produced in the lower soil layer. This may lead to the conclusion that root external mycelium is much more important for nutrient acquisition from the lower soil layer, since there the distance between roots and CP fractions was bigger than in the upper soil layer. This may be of great biological importance because the nutrient uptake by the external mycelium may compensate for the patchiness of CP and heterogeneity in nutrient availability at a micro-site level (DEGENS et al., 1996), and thus may lead to a more uniform nutrient uptake by plants (PEDERSEN and SYLVIA, 1996). Thus, in general, AMF mycelium may result in a greater nutrient acquisition form nutrient-rich patches like CP (VAIDYA et al., 2008). We conclude that the application of CP, and the direct uptake of nutrients from organic sources like CP via AM has great biological importance for a sustainable nutrition of coffee in soils with low nutrient availability of the tropics.

Acknowledgements
The authors are thankful to the Amazonia University, Caquetá, Colombia, and the Federación Nacional de Cafeteros de Colombia (FEDECAFE), for the economic support. The logistic support of FEDECAFE Regional Caquetá is highly acknowledged. Thanks are given to the Grupo de Investigaciones en Microorganismos

Simbiontes (GIMS), specially to Luis Antonio Franco, Faver Alvarez Carrillo and Juan Carlos Suarez for facilitating the sampling and laboratory activities.

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Address of the authors:

Dr. Raul Posada, Diagonal 151b No. 136A-75 Cs 93. Villas de Hato Chico II, Bogotá, Colombia

E-mail: raulposada@hotmail.com

Priv. Doz. Dr. habil. Ewald Sieverding, Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Garbenstr. 13, D-70593 Stuttgart-Hohenheim, Germany

E-mail: sieverdinge@aol.com