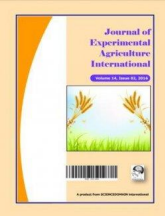

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Spore Density and Arbuscular Mycorrhizal Colonization in Sunflower Grown in Campo Verde (Brazil)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DSP, DAF, DTSC and ABBF designed and wrote the protocol. Authors DSP, DAF, DTSC and ABBF conducted the experiment and wrote the first draft of the manuscript. Authors DSP, DAF, JGA, ECC and MHS managed the analyses of the study. Authors DSP, DAF, DTSC, ABBF, JGA, ECC and MHS discussed the results and improved the writing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to evaluate the number of spores and the mycorrhizal root colonization in a Cerrado soil (Red-Yellow Latosol) cultivated with different sunflower genotypes. The sampling of the rhizospheric soil was performed at three growth stages: Sowing, flowering, and harvest. The experimental design was in completely randomized blocks with four replications. Three different sunflower hybrids were tested in the 2009 and 2010 cropping seasons. The collected data comprised the total number of spores per 50 g of soil at the three growth stages, along with

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arbuscular mycorrhizal fungi (AMF) colonization. It was verified that the mycorrhizal colonization was not influenced by the sunflower genotypes, and the mean spore densities were equivalent to 4.94 and 4.64 g soil-1 in 2009 and 2010, respectively. More importantly, AMF colonization was enhanced by lower soil phosphorus levels. The maximum spore production was obtained at flowering, with mycorrhizal colonization rates ranging from 21 to 28% and from 28 to 48% in 2009 and 2010, respectively. The number of spores also varied from 153 to 342 and from 147 to 320 in 2009 and 2010, respectively.

Keywords: Helianthus annuus L.; soil phosphorus; plant nutrition; symbiosis; root colonization.

1. INTRODUCTION

Soil quality and the viability of improvements through chemical, physical, and biological management are essential factors for success in agricultural production. In this context, the study and use of the soil microbial population have pointed the way to link sustainability with efficiency.

The symbiotic association between plant and fungi is called mycorrhiza. Root colonization by arbuscular mycorrhizal fungi (AMF) generates several improvements; the plant provides photosynthates to the fungus, and this, through the branching and extension of the mycelium, increases the area of nutrient absorption for the plant [1]. Thus, AMFs can be used as an alternative to reduce the use of agricultural inputs, mainly fertilizers of chemical synthesis.

The influence of arbuscular mycorrhizal fungi acts not only on soil particles aggregation but also on plant growth, providing essential nutrients [2] and improving the ability to withstand adverse conditions.

An increase was observed in the capitulum diameter, achenes weight, and achene yield when studying the AMF inoculation in sunflower. These traits were related to the better development of plants through their association with AMFs, due to the higher absorption of nutrients such as P, K, and Fe [3].

Sunflower (*Helianthus annuus* L.) cultivation has successfully aroused interest, especially in the Brazilian Midwest, due to its broad adaptability to edaphoclimatic conditions, suitability for crop rotation and use as edible oil, biodiesel, ornamental crop, animal feed, etc. [4,5].

The present work aimed to evaluate three sunflower genotypes based on their root mycorrhizal colonization at three different growth stages in soils of the Cerrado biome.

2. MATERIALS AND METHODS

The experiment was conducted at the Santa Luzia Farm, in Campo Verde (MT-Brazil), latitude 15°45'12" S and longitude 55°22'44" W. The soil used in the experiment was classified as Red-Yellow Latosol, with the following properties: clayed texture, acidic pH, 50% average base saturation, absence of aluminum and high organic matter content (Table 1). Soybean and corn were the most frequent crops, grown under minimum soil tillage, and practiced over more than ten years. Soybean preceded the sunflower crop for both considered cropping seasons (2009 and 2010).

Table 1. Chemical and physical properties of the soil under sunflower cultivation after harvest in 2009 and 2010 at the Santa Luzia farm, Campo Verde (Brazil)

Year	pH	P	K	Ca	Mg	Al	H	OM	CEC
	$CaCl_2$	mg dm^{-3}		$cmol_c dm^{-3}$				gdm^{-3}	$cmol_c dm^{-3}$
2009	5.1	21.8	76	3.2	0.9	0	4.4	37.8	8.7
2010	4.9	8.0	80	3.3	0.7	0	5.5	39.9	9.7
	Bases saturation	**Sand**	**Silt**	**Clay**		**Saturation (%)**			
	(V%)	**g kg^{-1}**				**Ca**	**Mg**	**K**	**H**
2009	49.3	196	133	671		36.7	10.5	2.3	50.7
2010	43.3	172	200	628		33.9	6.8	2.1	56.7

P: phosphorous; K: potassium; Ca: calcium; Mg: magnesium; Al: aluminum; OM: organic matter; CEC: cation exchange capacity; H: hydrogen

A randomized complete block design with four replications was employed in the experiment. Each plot consisted of four 6.0 m rows, with 0.8 m of inter-row spacing and 0.3 m of within-row spacing (19.2 m²). Two middle rows (9.6 m²) were weighed at harvest in order to determine the crop yield. NPK and boron fertilizers were applied 30 days after sowing at the following respective rates: 30-80-80 and 2.0 kg ha^{-1}. The 2010 cropping season was rainy compared to 2009, with 974 and 442 mm of total precipitation, respectively (Table 2).

Three different sunflower hybrids were evaluated based on their response to fungal colonization. Hybrids M 734, Agrobel 960 and Helio 358 were used in 2009, whereas the M 734, Embrapa 122 and HLA 860 H.O., were used in 2010. The sampling of the rhizospheric soil was performed at harvest in the 0-20 cm depth layer and at three different growth stages, namely sowing (first half of March), flowering (60 days after sowing) and harvest (after maturation).

The two evaluated parameters were the total number of spores in soil and the arbuscular mycorrhizal colonization. The extraction of spores was performed by wet sifting [6], in which the soil was processed through a sieving system (0.42 and 0.053 mm mesh) and centrifuged in water at 2800 rpm for 4 min. Subsequently, the samples were re-suspended in a 50% sucrose solution, centrifuged, and washed. The spores were counted Petri dishes, using a stereomicroscope.

In order to check the mycorrhizal colonization, crop roots were washed, clarified with KOH (10%), acidified with diluted HCl [7] and stained with trypan blue [8]. Ten segments with 1-2 cm in length were selected for slide assembly. The determination of the root colonization percentage was performed using an optical microscope (40x).

The analysis of variance was calculated using the Sisvar 5.8 software package, and significant differences between the means were determined following Tukey's test at 5%.

3. RESULTS AND DISCUSSION

With regard to the factor year, there was no significant difference in the number of spores of the AMF (Table 3). This could be explained by a general improvement in soil fertility resulting from the practice of minimum soil tillage over more than 10 years. According to Carrenho et al. [9], the disseminationof mycorrhizal propagules is much more affected during the initial phases of land use.

Table 2. Rainfall distribution (in mm/month) over the 2009 and 2010 cropping seasons in Campo Verde (Brazil), from February to July

Year	February	March (S)	April	May (F)	June	July (H)	Total
2009	262	132	16	10	22	0.2	442.4
2010	385	206	325	55	3	2	974.0

S: sowing; F: flowering; H: harvest

Table 3. Spore densities of arbuscular mycorrhizal fungi in the Cerrado Biome soil over three sunflower growth stages, Campo Verde (Brazil)

Year	Genotype	Sowing	Flowering	Harvest	Mean
		n° spores 50 g $soil^{-1}$			
2009	M 734	153 bB	296 aA	267 aA	247 a
	Agrobel 960	185 abB	342 aA	233 abB	
	Helio 358	262 abAB	311 aA	174 aB	
	Mean	200 B	317 A	225 B	
2010	M 734	234 abAB	270 aA	147 bB	232 a
	Embrapa 122	191 abA	254 aA	216 abA	
	HLA 860 H.O.	271 aAB	320 aA	184 abB	
	Mean	232 AB	281 A	182 B	
	CV (%)	11,60			

Means followed by different letters, uppercase in the line and lowercase in the column, differ from each other by Tukey's test (P =0.05). CV: coefficient of variation

Spore density was higher at the flowering stage for both cropping seasons, with means equivalent to 317 and 281 in 2009 and 2010, respectively (Table 3). Maximum spore production may occur at the flowering and final growth stages of the host crop, as reported by Smith and Read [10]. These authors also reported that an increase in the number of spores could be related to the higher production of internal crop resistance structures in response to drought.

The higher AMF spore density is common in agricultural systems, and its variations are influenced by a number of factors such as soil, climate, growth stage, farming practices, and crop species.

Smith and Read [11] reported that arbuscular mycorrhizal fungi enhanced the growth of annual crops and pasture systems, and its number of spores varied with both crop species and farming system.

The interaction between genetic factors and growth stages was significant, showing that the sporulation process was influenced by the sunflower genotypes.

In a similar manner to our observations with sunflower, spore densities varying from 301 to 608 for a maize crop, compared with 239 to 287 in a soybean crop have been reported [12]. Mycorrhizal dynamics involving root colonization and sporulation occur in different ways depending on the crops, due to the compatibility between AMF and plant genetic traits [13]. In addition to the symbiotic process, environmental, climatic, and edaphic factors may also generate changes in the symbiotic process [14].

In sugarcane, the AMF colonization increased when sunflower was used as a preceding crop [15]. Likewise, sunflower enhanced the inoculum potential of AMF in soil, with improvement in maize growth as a subsequent crop [16]. Annual crops, green manures, and forage species present a high degree of mycorrhizal dependency, acting as soil conditioners by multiplying the native mycorrhizal community [17,14]. In this sense, sunflower is an option to benefit from soil mycorrhizal population in crop rotation systems.

As far as the mycorrhizal colonization rate was concerned, no significant difference was observed within sunflower genotypes during the 2009 and 2010 crop years (Table 4, Fig. 1). Mycorrhizal dependency is defined as the plant response to mycorrhization through increased growth, which may be influenced by soil fertility and by the availability of soil phosphorus [18].

A number of findings showed different patterns in AMF colonization rates depending on the plant species or crop systems, such as arboreal species (11-54%), different crop rotation systems (33-49%), cassava in different locations (31-71%), and banana (40-75%) [19-22].

A greater mycorrhizal colonization was observed in 2010 due to lower soil phosphorus content (Table 1). This is in accordance with findings reported in the literature stating that an increase in phosphorus availability is associated with a decrease in plant-mycorrhiza symbiosis [23-24]. These findings were corroborated by observations performed with sunflower, in which a significant reduction in AMF colonization resulted from phosphorus rates higher than 30 mg/kg of soil [25].

Regarding sunflower, Oliveira et al. [22] also reported that higher P rates decreased sporulation and AMF colonization. According to Sarah and Ibrar [26], the AMF colonization percentage in sunflower ranging from 66 to 71%, along with a spore density from 155 to 294 were associated with lower soil phosphorous content. Conversely, different authors [27-28] reported that the lower colonization in sunflower could produce similar or higher spore densities.

The AMF-plant relationship can be mediated by soil nutrient levels, since these fungi increase the root exploration area, occurring, therefore, an improvement in plant nutrition. As soil phosphorus increases, root mycorrhizal colonization and plant dependence to mycorrhization decrease [28] in soils with low levels of phosphorus [25]. This is similar to the Cerrado biome, where sunflower cultivation is enhanced by AMF colonization.

There is evidence that AMF colonization in sunflower enables a higher plant resistance to heat, with a positive impact in Cerrado production systems, which are characterized by high temperatures [29]. The potential of AMF colonization as biofertilizer for oleaginous crops is reinforced, especially for low-fertility soils, as this practice improves crop production with less mineral fertilizer applications and, therefore, promotes a sustainable production [26].

Table 4. Mean percentage of AMF colonization in soil under sunflower cultivation, Campo Verde (Brazil)

Year	Genotype	Mycorrhizal colonization (%)	Mean
2009	M 734	28 a	24 b
	Agrobel 960	21 a	
	Helio 358	22 a	
2010	M 734	38 a	38 a
	Embrapa 122	48 a	
	HLA 860 H.O.	28 a	
CV (%)		16,24	

Means followed by different letters in the column differ from each other by Tukey's test (P =.05). CV: coefficient of variation

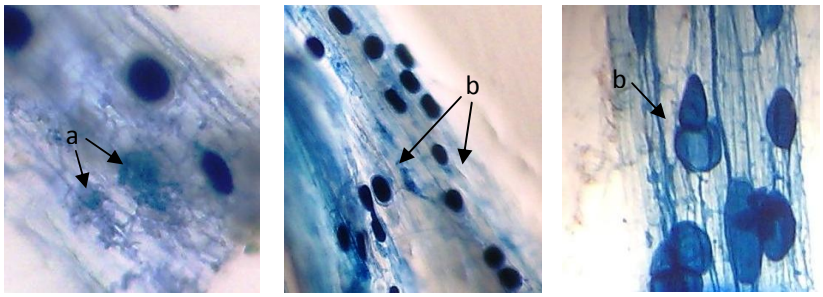

Fig. 1. Sunflower root colonization by arbuscular mycorrhizal fungi (AMF). Fungal structures: arbuscules (a) and vesicles (b)

4. CONCLUSION

The study showed that the arbuscular mycorrhizal colonization was enhanced in lower soil phosphorus conditions, and it was not significantly influenced by the sunflower genotypes. In contrast, it was significantly influenced by the sunflower growth stages, with the maximum number of spores being observed at flowering, with values ranging from 153 to 342 in 2009 and from 147 to 320 in 2010.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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