Original Research Article

Spore density and arbuscular mycorrhizal colonization in sunflower grown in Campo Verde (Brazil)

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ABSTRACT

The objective of this study was to evaluate the number of spores and mycorrhizal root colonization in Cerrado soil (Red-Yellow Latosol) cultivated with different sunflower genotypes ~~of sunflower~~. Sampling of rhizospheric soil was performed at ~~occurred in~~ three growth stages~~periods~~: sowing, flowering and ~~sunflower~~ harvest. The experimental design was a randomized complete block ~~design~~ with four replications. ~~To proce~~Three ~~d the evaluations~~ different sunflower hybrids were tested in 2009 and 2010 cropping seasons~~selected: M 734, Agrobel 960 and Helio 358, in 2009, and M 734, Embrapa 122 and HLA 860 H.O. in 2010~~. Data collected comprised~~The measured parameters were~~ total number of ~~total~~ spores per ~~in~~ 50 g of soil at the three growth stages ~~in three periods: sowing, flowering and harvesting,~~ and the arbuscular mycorrhizal (AMF) colonization ~~was evaluated only in the final of the experiment~~. It came out that mycorrhizal colonization was not influenced by sunflower genotype, and the~~The~~ average spore densities ~~mean number of spores~~ measured gave ~~was~~ 4,94 ~~g soil~~$^{-1}$ 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 ~~occurred~~ at ~~during the~~ 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 ~~period and mycorrhizal colonization was not influenced by the genotype. The mycorrhizal colonization rate ranged from 21 to 28% in 2009 and from 28 to 48% in 2010. The number of spores varies from 153 to 342 in 2009 and 147 to 320 in 2010, and the maximum production occurs, on average, in the flowering period. Lower soil phosphorus levels favors arbuscular mycorrhizal colonization~~.

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Keywords: Helianthus annuus L.; soil phosphorus; plant nutrition, symbiosis,~~arbuscular mycorrhizal fungi,~~ 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 the use of soil microbial population has shown the way to link sustainability to 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 their ability to withstand adverse conditions.

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

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

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

2. MATERIAL AND METHODS

The experiment was carried out at Santa Luzia Farm, in Campo Verde (MT-Brazil), latitude 15°45'12"S and longitude 55°22'44"W. Soil in the experiment was 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 most crops grown under minimum soil tillage practiced over more than ten years. Over both cropping seasons considered (2009 and 2010), the preceding crop of sunflower was soybean.

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

Year	pH $CaCl_2$	P	K	Ca	Mg	Al	H	OM	CTC
		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 (V%)	Sand	Silt	Clay	Saturation (%)			
		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: aluminium; OM: organic matter; CTC: cation exchange capacity; H: hydrogen.

The experimental design used was a randomized complete block with four replications. Every plot was composed of four rows of 6.0 meters, with 0.8 m of inter-row spacing and 0.3 m spacing within rows (19.2 m^2). Two central rows (9.6 m^2) were weighted at harvest to determine crop yield. NPK and boron fertilizers were applied 30 days after sowing respectively at the following rates: 30-80-80 and 2.0 kg ha^{-1}. The 2010 cropping season was rainy compared to 2009, with respectively 974 and 442 mm of total precipitation (Table 2).

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

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.

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

Both parameters evaluated were total number of spores in soil, and arbuscular mycorrhizal colonization. The extraction of spores was carried out by the wet sift methodology [6], in which the soil was processed in a sieving systems (0.42 and 0.053 mm mesh) and centrifuged with water at 2800 rpm for 4 min. Subsequently, samples were re-suspended in 50% sucrose solution, centrifuged and washed. Spores were counted using a stereomicroscope in petri dishes.

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 of 1-2 cm in length were selected for slide assembly. Determination of root colonization percentage was made using an optical microscope (40x).

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

3. RESULTS AND DISCUSSION

Regarding the factor year, there was no significant difference in the number of spores of 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 dissemination of mycorrhizal propagules is much more affected during initial phases of land use.

Table 3. Spore densities Quantification of spores of arbuscular mycorrhizal fungi in Cerrado Biome soil over three growth stages of, under sunflower cultivation, in two years and three periods, in Campo Verde (– MT, Brazil).

Year	Genotype	Sowing	Flowering	Harvest	Average
		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	
	Average	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	

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Average	232 AB	281 A	182 B
CV (%)		11,60	

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

Spore density at the flowering stage was higher over both cropping ~~years seasons~~ investigated~~of study~~, with on average 317 and 281 in 2009 and 2010, respectively (Table 3). Maximum spore production may occur at flowering and final growth stages of host crop as reported by Smith and Read [10]. They also reported that increase in the amount of spores could be related to higher production of internal crop resistance structures in response to drought.

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

~~The spore density of AMFs is generally higher in agricultural systems, and variations may occur due to edaphoclimatic factors, growing time, agricultural practices as well as the implanted crop [2].~~

Smith and Read [11] reported ~~, studying the Cerrado biome, verified~~ that ~~the~~ arbuscular mycorrhizal fungi enhanced growth of annual crops and pasture ~~contribute to the growth of cultivated plants in annual cropping and pasture~~ systems, and its number of spores varied with~~also that the number of spores of the native fungi varies, with the~~ both crop species and farming ~~the cultivation~~ system ~~being determinant for the enrichment of the mycorrhizal fauna~~.

The ~~The i~~nteraction between ~~the~~ genetic factors and growth stages ~~the period~~ was significant, showing~~demonstrating~~ that the sporulation process was influenced by the~~sunflower genotypes genetic material influences the sporulation process~~. ~~However, the variations were low, indicating a stabilization of the mycorrhizal fungi sporulation.~~

Similar to our observations made in sunflower, it was reported in maize that ~~In a study performed with a maize crop, it was verified that~~ spore densities varied from 301 to 608 compared with ~~, whereas in a soybean cultivated soil the values ranged between~~ 239 to~~and~~ 287 in soybean [12]~~], similar to those obtained in the present work with sunflower~~. Mycorrhizal dynamics involving root colonization and sporulation occur in different ways depending on crops~~in different crops~~, due to the compatibility between AMF and ~~the~~ plant genetic traits ~~characteristics of plants~~ [13]. In addition to the symbiotic process, environmental, climatic and edaphic factors may generate changes in the ~~symbiotic process~~ [14].

In sugarcane, ~~the occurrence of~~ AMF colonization increased when sunflower was used as preceding ~~the~~ crop ~~was preceded by sunflower~~ [15]. Likewise, sunflower ~~favored~~ enhanced the inoculum potential of AMF in ~~the~~ soil with improvement in corn growth as a~~and the~~ subsequent crop~~corn growth~~ [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, ~~the~~ sunflower is an option to benefit from ~~the~~ soil mycorrhizal population in crop rotation~~/succession~~ systems.

As far as ~~For~~ the mycorrhizal colonization rate was concerned, no significant difference was observed within sunflower genotypes during both cropping years 2009 and 2010 considered~~a variation ranging from 21 to 28% was observed in 2009, and from 28 to 48% in 2010~~ (Table 4~~,) (~~ Figure 1)~~, with no difference between genotypes~~. ~~According to Janos [18],

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Mmycorrhizal dependence iscan be defined as the plant responseiveness to mycorrhization through increased growth, which may be influenced byrelated to soil the fertility and the availability of soilamount of phosphorus [18]. present in the soil.

Table 4. Average percentage of AMF colonization in soil under sunflower cultivation, in Campo Verde (– MT, Brazil), in 2009 and 2010.

Year	Genotype	Mycorrhizal colonization (%)	Average
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 the Tukey test (P =.05). CV: coefficient of variation.

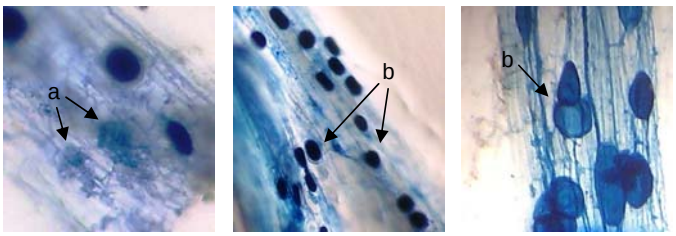

Figure 1. Sunflower root colonization by arbuscular mycorrhizal fungi (AMF). Fungal structures: arbuscules (a) and vesicles (b).

A number of findingsSilva et al [19]showed different patterns in found an AMF colonization depending on plant or crop species like arboreal (between 11 and 54%) for arboreal species, crop rotation systems (; from 33 to 49%), cassava for different crop rotation/succession systems [20]; from in different locations (31 to 71%), and banana (when associated to the cassava crop in different localities [21]; and from 40 to 75%) [19-22] in banana plants [22].

Regarding the factor year, A greater mycorrhizal colonization was observed in 2010 there was a greater mycorrhizal colonization, which can be exp due to lained by the a lower soil phosphorus content in the soil (Table 1). This is in line of findings reported in the literature stating thatThe effect of the an increase in phosphorus availability is associated to aand decrease in plant-mycorrhiza symbiosis is negative [23-24] and emphasized in the literature [24]. These findings were corroborated by observations made in sunflower where a significant reduction in AMF colonization resulted from phosphorus rates higher than 30 mg/kg of soil.Therefore, the reduction in the P content may lead to an increase in plant colonization. Alson in sunflower, Oliveira et al [22] reported that When evaluating diffcrent doses of P_2O_5 in the mycorrhiza colonization, it was verified that doses higher than 30 mg P kg soil^{-1} decreased AMF colonization in sunflower [25].

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By studying sunflower hybrids, it was verified that higher P rates doses of P decreased sporulation and AMF colonization [22]. According to Sarah and Ibrar [26], AMFIn the same crop, another study reported a colonization percentage in sunflower ranging from within 66 to 71% with a , and spore density about 155 to 294 were associated to ; the soil, however, had a lower soil phosphorous content if compared with the present work [26]. In contrast, different authors [27-28] reported that a lower colonization in sunflower could produce similar or higher spore densitiesContrasting the results by Balota et al. [26] with those of this research, a lower colonization was capable of producing similar or higher quantities of spores.

In the same way, when comparing the results by Alves et al. [27] with the present work, it was observed that a lower colonization was able to produce similar or higher quantities of spores; therefore, the efficiency in mycorrhiza species perpetuation was superior.

In general, the AMF-plant relationship can be mediated by soil nutrient levels, present in the soil, since these fungi increase the root exploration area, with therefore contributing to a greater absorption of nutrients by the plantimprovement in plant nutrition. , As thAse increase in soil phosphorus increases, decreases the root mycorrhizal colonization and plant dependence to mycorrhization decreaseand the plant dependence to mycorrhization [28] in soils with low levels of phosphorus. This is similar to, typical of the Cerrado biome where sunflower cultivation is enhanced by , the AMF colonization favors sunflower cultivation [25].

TBesides, there is evidence that AMF colization in the mycorrhizal sunflower ratio enables greater plant resistance to heat, showing a positive n interesting impact in Cerrado production systems , which areis characterized by high temperatures [29].

Moreover, tThe potential of AMF colonizations as biofertilizer for oleaginous crops is reinforced, especially for low fertility soils with low fertility, assince this practice improves cropallows reaching adequate levels of production with less mineral use of synthetic fertilizer applicationss and therefore promotes a sustainable, making the p production system more sustainable [26].

Therefore, colonization and mycorrhizal sporulation vary according to the sunflower genotype and the evaluation period. There was intense AMF activity in the flowering period, a moment in which the plant requires a high nutritional supply for grain production.

4. CONCLUSION

The study showed that arbuscular mycorrhizal colonization was enhanced in lower soil phosphorus conditions, and it was not significantly influenced by sunflower genotypes. In contrast, it was significantly influenced by sunflower growth stages, 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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The number of spores varied from 153 to 342 in 2009 and 147 to 320 in 2010, and the maximum production occurs, in average, in the flowering period.

Mycorrhizal colonization in sunflower is not influenced by the genotype, and the average percentage was 24 (2009) and 38 (2010).

~~Lower soil phosphorus levels favor arbuscular mycorrhizal colonization.~~

COMPETING INTERESTS

We declare that no competing interest~~s~~ exist.

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