Original Research Article

Spore density and arbuscularmycorrhizal colonization in sunflower

ABSTRACT

The objective of this study was to evaluate the number of spores and mycorrhizal root colonization in Cerrado soil, under sunflower cultivation. Sampling of rhizospheric soil occurred in three periods: sowing, flowering and sunflower harvest. The experimental design was a randomized complete block design with four replications. The evaluated parameters were number of total spores in 50 g of soil and arbuscularmycorrhizal colonization. The mean number of spores was 247 and 232 in 2009 and 2010, respectively. For root colonization there was a difference between years. The maximum spore production occurred during the flowering period and mycorrhizal colonization was not influenced by the genotype. To accomplish, mycorrhiza favors the development of sunflower in lower levels of phosphorus in the soil.

Keywords: Helianthus annuus L.; soil; arbuscularmycorrhizal fungi.

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 arbuscularmycorrhizal fungi(AMF) is called mycorrhiza. Root colonization by arbuscularmycorrhizal fungi 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, AMs can be used as an alternative to reduce the use of agricultural inputs, mainly fertilizers of chemical synthesis.

The influence of arbuscularmycorrhizal 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.

The sunflower cultivation (*Helianthus annuus* L.) has aroused interest, especially in Brazilian Midwest, due to the broad adaptability to edaphoclimatic conditions, suitability for crop rotation and uses in the production of edible oil, biodiesel, ornamentation, animal food, among others [3,4].

Comment [V1]: What type of soil is it?

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Comment [V3]: t would be more convenient to put the number of spores either per gram of soil or per kilogram of soil. This gives more idea of the density of AMF present in the rhizosphere of the studied plant

Comment [V4]: But was it similar in the 3 stages of plant growth sampled?

Comment [V5]: I suggest to put percentages in which the colonization oscillated every year

Comment [V6]: t is important to mention from the objective of the work that the variations in the number of spores and radical colonization in different genotypes and under different level of phosphorus fertilization were evaluated.

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Considering that in the soils of the Cerrado Biome, for the optimization of the agricultural production, is necessary the use of a high amount of inputs, and that the agronomic efficiency is tied to the good indexes of soil quality, the present work aimed to evaluate the number of spores in different times and mycorrhizal colonization in Cerrado Biome soil, under cultivation of three sunflower genotypes.

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. The farm soil has clayed texture, with acid pH, average bases saturation of 50%, absence of aluminum and high content of organic matter (Table 1).

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

Year	pH $CaCl_2$	P	K	Ca	Mg	Al	H	MO	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

The experimental design applied in the field was randomized blocks, with four replications. The plots were formed by four rows of 6.0 meters, spaced in 0.8 meters, between rows, and 0.3 meters, between plants, considering as useful area the two central rows. Fertilization was carried out with 60-80-80 $kgha^{-1}$ of N-P-K and 2.0 kg ha^{-1} of boron.

The genotypes were M 734, Agrobel 960 and Helio 358, sown in 2009, and M 734, Embrapa 122 and HLA 860 H.O. in 2010. Rhizospheric soil sampling was obtained at 0-20 cm depth, in three periods: sowing (first half of March), flowering (60 days after sowing) and harvesting (after maturation). The rainfall distribution in the region, during the experimente, is shown in Table 2.

Table 2. Rainfall (mm $month^{-1}$) in Campo Verde - 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.

The evaluated parameters were total number of spores in soil, and arbuscularmycorrhizal colonization, whose root sampling occurred during crop harvest. The spore extraction was carried out by the wet sift methodology [5], 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 minutes. Subsequently, the samples were resuspended in 50% sucrose solution, centrifuged and washed. The spores were counted in a stereomicroscope in a petri dishes with vessels.

Comment [V12]: Is this a low dose of phosphorus? What is the dose that is used in a conventional way for the fertilization of this plant?

Comment [V13]: What was the reason why the genotypes used were selected? Why compare different genotypes in each year?

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For mycorrhizal colonization, the roots were washed, clarified with KOH (10%), acidified with diluted HCl and stained with trypan blue [7]. Ten segments of 1-2 cm in length were selected for slide assembly and quantification of colonization percentage under optical microscope.

Analysis of variance were preceded and the significant means were compared by Tukey test with 5% of significance.

3. RESULTS AND DISCUSSION

For the factor year, there was no difference in the number of spores of AMF (Table 3). This may occurred since the studied area adopted the minimum cropping system for more than 10 years. According to the authors [8], the association and mycorrhizal propagules dissemination is more affected in the initial phases of the occupation and use of the soil, with later stabilization.

Table 3. Quantification of spores of arbuscularmycorrhizal fungi in CerradoBiome soil, 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 b B	296 a A	267 a A	247 a
	Agrobel 960	185 ab B	342 a A	233 ab B	
	Helio 358	262 ab AB	311 a A	174 a B	
	Média	200 a B	317 a A	225 a B	
2010	M 734	234 ab AB	270 a A	147 b B	232 a
	Embrapa 122	191 ab A	254 a A	216 ab A	
	HLA 860 H.O.	271 a AB	320 a A	184 ab B	
	Average	232 AB	281 A	182 B	
	CV (%)		11,60		

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

For the periods, spore density in flowering was higher in the two years of study, with a general average of 317 in 2009 and 281 in 2010 (Table 3). The authors cited in the reference [9] confirm that maximum spore production can occur in the flowering period and in the final growth stage of the host.

According to the authors cited in the reference [2],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.

The authors cited in the reference [10] studyingCerrado biome verified that the arbuscularmycorrhizal fungi contribute to the growth of cultivated plants in annual cropping ans pasture systems and the number of spores of the native fungi varies, being the crop and the cultivation system determinant for the enrichment of mycorrhizal fauna.

The interaction between the genetic factors and the period was significant, demonstrating that the genetic material influence the sporulation process. However, thevariations were low indicating the stabilization of the mycorrhizal fungi sporulation.

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Comment [V20]: Why are there two columns where the results of the average comparison analysis are presented? there seems to be one more column in each sampled period in each evaluated variety

Comment [V21]: Why not perform the statistical analysis comparing between periods of culture in the same genotype and also among the 3? Interestingly, M734 presents for this year a number of spores opposite to that observed in 2009, to what do you attribute it?

Comment [V22]: Average?

Comment [V23]: Why use the coefficient of variation instead of the standard deviation to make the statistical analysis? Is there a difference of more than 100 spores between the first genotype and the last one in the sowing and harvest periods in 2009 and even then no statistically significant differences were found?

Comment [V24]: To what is attributed that the highest density of spores can be obtained at the time of flowering and crop harvest?

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Comment [V28]: What is the average number of spores that can be found in a soil similar to the one in your study area? and in a native area?

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In a carried study was verified that spore densities varies from 301 to 608 for maize crop, whereas in soybean cultivatedsoil the values were between 239 and 287 [11], similar to those obtained in the present work with sunflower. Mycorrhizal dynamics involving root colonization and sporulation occur in different ways in different crops due to the compatibility between AMF and the genetic characteristics of plants [12]. In addition, environmental, climatic and edaphic factors generate changes in the symbiotic process [13].

In sugarcane the occurrence of AMF increase when the crop was preceded by sunflower [14]. Likewise, sunflower favored the inoculum potential of AMF in the soil, and subsequent corn growth [15].

Annual crops, green manures and forage species have a high degree of mycorrhizal dependency, acting as a soil conditioning, multiplying the native mycorrhizal community [16,13]. In this sense, sunflower is an option to benefit the soil mycorrhizal population in crop rotation / succession systems.

For the mycorrhizal colonization rate, it was observed a variation from 21 to 28% in 2009 and 28 to 48% in 2010 (Table 4), with no difference between genotypes. According to the authors cited in the reference [17], mycorrhizal dependence can be defined as the plant's responsiveness to mycorrhization through increased growth, which may be related to the fertility and amount of phosphorus, present in the soil.

About the factor year, in 2010 there was a higher mycorrhizal colonization, which can be explained by the lower phosphorus content in the soil (Table 1). The correlation between the phosphorus content and mycorrhizal colonization is negative [18] so, the reduction in the P content may lead to an increase in plant colonization. Studing sunflower hybrids, it was verified that higher doses of P decreased sporulation and AMF colonization [19].

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.

In general, the relationship AMF-plant can be mediated by nutrient levels, present in the soil, since these fungi increase root exploration area, contributing to a greater absorption of nutrients for the plant. As the increase in soil phosphorus decreases the root mycorrhizal colonization and the plant dependence to mycorrhization [20], in soils with low levels of phosphorus, typical of the Cerrado biome, the AMF favors sunflower cultivation [21].

Studying, AMF inoculation in sunflower, it was observed an increase in chapter diameter, thousand achenes weight and achenes yield, parameters that were related to the better development of the plants through the association with AMFs, due the higher absorption of nutrients as P, K and Fe.

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Comment [V31]: This paragraph should be put once it has been demonstrated that the capacity of colonization of the splantas by the AMF was high, otherwise it would not have sustenance, because the fact of finding a good number of propagules does not necessarily guarantee that these will be able to interact with the cultivation of interest in the first instance and in the second to promote the benefits reported in the literature

Comment [V32]: The percentages of colonization observed in all genotypes in the 2 years of sampling are low. In the literature, good colonization arises when the colonization oscillates around 40 and 60%. It would be convenient to include percentages of radical colonization reported for sunflower in other works.

Comment [V33]: This explanation has no support because although the soil could have a low initial content, it was compensated by the fertilization that was applied to the crop. On the other hand, how does it explain the substantive decrease of P presented in the soil from 2009 to 2010? This could be the result of a high demand for the crop or a leaching due to the amount of rainfall that occurred before sowing?

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In addition, there is evidences that mycorrhizal-sunflower ratio enables greater plant resistance to heat, showing an interesting impact in Cerrado production systems, which is characterized by high temperatures [23].

Moreover, the potential of AMFs as biofertilizer for oleaginous crops is reforced, especially for soils with low fertility, since the practice allows to reach adequate levels of production, with less use of synthetic fertilizers making the productive system more sustainable [19].

Therefore, colonization and mycorrhizal sporulation vary according to the sunflower genotype and the evaluation period. On flowering period there were intense AMFs activity, moment that is required to the plant a high nutritional supply for grain production.

4. CONCLUSION

The number of spores has low variation, demonstrating that the system is stable. The maximum production occurs in flowering period.

Mycorrhizal colonization in sunflower is not influenced by the genotype.

Lower soil phosphorus levels favorsarbuscularmycorrhizal colonization.

COMPETING INTERESTS

We declare that no competing interests exist.

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Comment [V37]: How does this information relate to the results obtained in this study?

Comment [V38]: The conclusions are contradictory and only partial, it is necessary to abound more in the analysis of the results observed in number of propagules reported by genotype and year of sampling

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