



# Temporal dynamics of the mycorrhizal inoculum potential of fungal communities in reference ecosystems for strawberry cultivation in southern Brazil

Dinâmica temporal do potencial de inóculo micorrízico de comunidades fúngicas em ecossistemas de referência no cultivo do morangueiro no sul do Brasil

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One of the alternatives for making the strawberry production system more sustainable is arbuscular mycorrhizal fungi (AMF). However, there are few commercial inoculants based on AMF. This shortage can be attributed to the information scarcity on the temporal dynamics of mycorrhizal inoculum potential (MIP). Here, we investigated whether there is temporal variability in the MIP of AMF communities in cultivated and native forest soils in reference ecosystems for strawberry cultivation. The treatments were nine inoculants (eight soils containing AMF communities and *Rhizophagus clarus* as a control) not subjected (year 2016) or subjected (year 2020) to storage. The experimental design was entirely randomized, with three replicates. The inoculants used were grouped into three strata in terms of mycorrhizal colonization. *R. clarus* showed the greatest infective capacity on sorghum roots, followed by inoculants from native forest soils, and finally, inoculants from soils cultivated with strawberries. The highest MIP was observed in non-stored inoculants in the experiment set up in 2016. In conclusion, there is temporal variability in the MIP of AMF communities in cultivated and native forest soils in reference ecosystems for strawberry cultivation. Natural soils and those not stored have a greater capacity to colonize the roots of the plant host.

Keywords: *Fragaria X ananassa* Duch., *Sorghum bicolor* (L.) Moench, arbuscular mycorrhiza.

Uma das alternativas para tornar o sistema de produção do morangueiro mais sustentável são os fungos micorrízicos arbusculares (FMA). No entanto, existem poucos inoculantes comerciais à base de FMA. Essa carência pode ser atribuída à escassez de informações sobre a dinâmica temporal do potencial de inóculo micorrízico (PIM). Aqui, investigamos se há variabilidade temporal quanto ao PIM de comunidades de FMA em solos cultivados e de mata nativa de ecossistemas de referência no cultivo do morangueiro. Os tratamentos foram nove inoculantes (oito solos contendo comunidades de FMA e *Rhizophagus clarus* como testemunha) não submetidos (ano de 2016) ou submetidos (ano de 2020) ao armazenamento. O delineamento experimental foi inteiramente casualizado, com três repetições. Os inoculantes usados agruparam-se em três estratos quanto à colonização micorrízica. *R. clarus* apresentou maior capacidade infectiva nas raízes de sorgo, seguido pelos inoculantes oriundos de solos de mata nativa e após, como último estrato, os inoculantes oriundos de solos cultivados com morangueiro. O maior PIM foi observado em inoculantes não armazenados, no experimento estabelecido em 2016. Em conclusão, há variabilidade temporal quanto ao PIM de comunidades de FMA em solos cultivados e de mata nativa de ecossistemas de referência no cultivo do morangueiro. Solos naturais e aqueles não armazenados têm maior capacidade de colonizar as raízes do hospedeiro vegetal.

Palavras-chave: *Fragaria X ananassa* Duch., *Sorghum bicolor* (L.) Moench, micorriza arbuscular.

## 1. INTRODUCTION

Migrating from soil to substrate cultivation of strawberries (*Fragaria X ananassa* Duch.) is one of the initiatives to increase crop yields. However, due to the low availability of nutrients in substrates, hydroponic cultivation requires excessive use of chemical fertilizers [1]. Producers wishing to reduce the use of these inputs are therefore faced with a lack of biotechnological tools.

One alternative is arbuscular mycorrhizal fungi (AMF). However, there are few commercial inoculants based on AMF. This lack is due to the limited knowledge about the temporal dynamics of the mycorrhizal inoculum potential (MIP) of soils in reference ecosystems for strawberry cultivation.

The process of propagating and formulating inoculants involves a series of important steps, which are crucial for ensuring the final product's quality [2]. One of these steps considers the MIP of the material used as inoculant. MIP is defined as the number of viable fungal propagules responsible for the initial root infection of the host plant [3]. Extraradicular mycorrhizal mycelium, spores, and colonized root fragments represent the propagules that determine MIP, measured by mycorrhizal colonization in a host plant bioassay [4]. MIP is a quantitative attribute that indicates whether an AMF community is efficient in establishing symbiosis [5].

In addition, it is essential to know the richness of AMF in relation to the cultivation site, since the beneficial effects on plant growth and development do not depend only on the fungus, but also on the characteristics of the host and edaphoclimatic conditions [5]. To understand the structuring of AMF communities is one of the goals of ecology and the easiest way to comprehend the bionomics of these symbionts is by studying their diversity in ecosystems and relating their occurrence to biogeographic edaphoclimatic characteristics. Furthermore, exploring the MIP of these communities contributes to the development of inoculants and the strengthening of sustainable agriculture. The use of inoculants based on AMF can reduce the application of chemical inputs in agroecosystems and increase the productivity and/or crop quality [2].

Therefore, based on the hypothesis that native forest soils and non-stocked soils have higher MIP, we investigated whether there is temporal variability in the MIP of AMF communities in cultivated and native forest soils in reference ecosystems for strawberry cultivation.

## **2. MATERIAL AND METHODS**

### **2.1 Soil identification and sampling**

Soil samples were collected from four different sites with agricultural suitability for strawberry cultivation in Rio Grande do Sul (RS), Brazil [6], in 2016. The sites selected were in the Vale do Caí region [Bom Princípio (BP) and São José do Hortêncio (SH)] and the Serra region [Flores da Cunha (FC) and Ipê (IP)]. At each site, three samples of rhizospheric soil under strawberry cultivation (CS) and three samples of native forest soil (NF) were randomly collected, totaling 24 samples. All sites under CS were conducted in the conventional system, using chemical fertilizers and pesticides registered for the crop to control pests and diseases. Each sample was made up of four subsamples. Samples were collected with a spade from a depth of 0 to 10 cm. All the samples were packed in plastic containers (Figure 1).

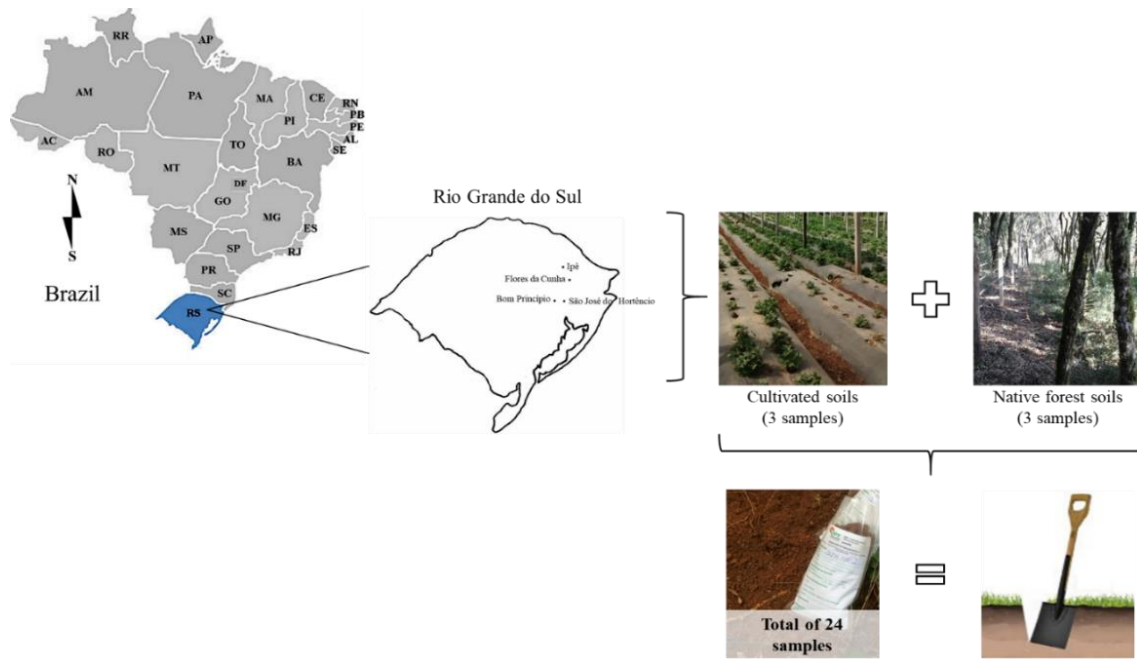


Figure 1: Scheme of identification and sampling of soils used in the study in Rio Grande do Sul, Brazil.

The biogeographical and edaphoclimatic characterization of the four municipalities studied can be found in Table 1.

Table 1: Biogeographic and edaphoclimatic characteristics of the four sites selected for soil collection in Rio Grande do Sul, Brazil.

Characterization	Sites <sup>1</sup>			
	BP	FC	IP	SH
Geographic coordinates	29° 29' 22" S 51° 21' 12" W	29° 01' 50" S 51° 11' 30" W	28° 49' 20" S 51° 16' 32" W	29° 29' 33" S 51° 12' 24" W
Altitude (m)	37.00	100.00	750.00	756.00
Biome	Atlantic forest	Atlantic forest	Atlantic forest	Atlantic forest
Climate <sup>2</sup>	Humid subtropical type Cfa	Oceanic type Cfb	Oceanic type Cfb	Humid subtropical type Cfa
Soil type <sup>3</sup>	Haplic Chernosol	Humic Cambisol	Regolithic Neosol	Red-Yellow Argisol
Precipitation (mm) <sup>4</sup>	223.50	247.60	226.50	221.50
Temperature (°C) <sup>4</sup>	22.10	20.00	20.00	22.50
Humidity (%) <sup>4</sup>	62.00	61.20	60.90	62.00

<sup>1</sup>BP = Bom Princípio; FC = Flores da Cunha; IP = Ipê; SH = São José do Hortêncio. <sup>2</sup>Climate classification of Köppen-Geiger. <sup>3</sup>Classification according to Santos et al. (2018) [7] proposal. <sup>4</sup>Annual average for the year of collection of soils (2016).

AMF spores were extracted from 50 g of soil by the wet sieving method [8] and centrifugation in sucrose [9]. After the extraction, the spores were grouped by morphotypes (color, shape, and size), under a stereoscope microscope, according to Morton (1988) [10]. The identification of the species was performed under optical microscope, based on the morphological characteristics of the spores [11, 12].

The characterization of the AMF communities identified in the soils collected is shown in Table 2. The experiment was carried out in the municipality of Passo Fundo (28°15'46" S; 52°24'24" W), RS, Brazil, from September (spring) to November (spring) 2016 and repeated during the same period in 2020.

AMF used in this work are regulated by Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) of the Ministry of the Environment, Brazil, according to the registration number A198F50.

Table 2: Arbuscular mycorrhizal fungi (AMF) communities identified in the sampled soils in Rio Grande do Sul, Brazil.

Sites	Ecosystem	Mycorrhizal community <sup>1</sup>
Bom Príncipe	Cultivated soil	<i>Acaulospora koskei</i> , <i>A. rehmsii</i> , <i>Claroideoglossum</i> aff. <i>luteum</i> , <i>C. claroideum</i> , <i>C. etunicatum</i> , <i>Funneliformis</i> aff. <i>mosseae</i> , <i>Glomus</i> aff. <i>versiforme</i> , and <i>Glomus</i> sp. ( <i>caesaris</i> like)
	Native forest soil	<i>Acaulospora</i> aff. <i>scrobiculata</i> , <i>A. colossica</i> , <i>A. scrobiculata</i> , <i>Acaulospora</i> sp., <i>Acaulospora</i> sp1 ( <i>E. infrequens</i> like), <i>Acaulospora</i> sp2 ( <i>excavata</i> like), <i>Ambispora leptoticha</i> , <i>Claroideoglossum</i> aff. <i>luteum</i> , <i>C. claroideum</i> , <i>C. etunicatum</i> , <i>F. mosseae</i> , <i>Glomus microaggregatum</i> , <i>Glomus</i> sp1, and <i>Glomus</i> sp2
Flores da Cunha	Cultivated soil	<i>C. claroideum</i> , <i>C. etunicatum</i> , <i>Funneliformis</i> aff. <i>geosporum</i> , <i>Glomus</i> aff. <i>versiforme</i> , <i>Glomus</i> sp. ( <i>caesaris</i> like), and <i>Glomus</i> sp2
	Native forest soil	<i>A. colossica</i> , <i>A. koskei</i> , <i>A. lacunosa</i> , <i>A. mellea</i> , <i>A. tuberculata</i> , <i>C. claroideum</i> , <i>Dentiscutata savannicola</i> , <i>F. mosseae</i> , <i>Glomus</i> aff. <i>manihotis</i> , <i>Racocetra</i> sp., and <i>R. verrucosa</i>
Ipê	Cultivated soil	<i>A. colossica</i> , <i>Acaulospora</i> sp., <i>Cetraspora pellucida</i> , <i>C. etunicatum</i> , <i>D. erythropha</i> , <i>D. heterogama</i> , <i>D. rubra</i> , <i>Funneliformis</i> aff. <i>geosporum</i> , <i>F. mosseae</i> , <i>Gigaspora</i> sp., <i>Glomus</i> aff. <i>caledonium</i> , <i>Glomus</i> aff. <i>manihotis</i> , <i>Glomus</i> sp. ( <i>caesaris</i> like), and <i>Glomus</i> sp1
	Native forest soil	<i>Acaulospora</i> aff. <i>lacunosa</i> , <i>Acaulospora</i> aff. <i>scrobiculata</i> , <i>A. colossica</i> , <i>A. koskei</i> , <i>A. lacunosa</i> , <i>Acaulospora</i> sp. ( <i>colossica</i> like M+), <i>A. spinosa</i> , <i>Claroideoglossum</i> aff. <i>luteum</i> , <i>C. etunicatum</i> , <i>D. biornata</i> , <i>F. geosporum</i> , <i>F. mosseae</i> , <i>Glomus</i> sp1, <i>Glomus</i> sp2, and <i>Scutellospora calospora</i>
São José do Hortêncio	Cultivated soil	<i>A. foveata</i> , <i>Claroideoglossum</i> aff. <i>luteum</i> , <i>C. claroideum</i> , <i>C. etunicatum</i> , <i>Funneliformis</i> aff. <i>geosporum</i> , <i>Funneliformis</i> aff. <i>mosseae</i> , <i>F. mosseae</i> , <i>Glomus</i> aff. <i>versiforme</i> , <i>Glomus</i> sp. ( <i>caesaris</i> like), and <i>Glomus</i> sp2
	Native forest soil	<i>A. mellea</i> , <i>Acaulospora</i> sp2 ( <i>excavata</i> like), <i>Claroideoglossum</i> aff. <i>luteum</i> , <i>C. claroideum</i> , <i>C. etunicatum</i> , <i>F. geosporum</i> , <i>F. mosseae</i> , <i>Gigaspora</i> sp., <i>Glomus</i> aff. <i>heterosporum</i> , <i>Glomus</i> sp1, <i>Glomus</i> sp2, and <i>Scutellospora</i> sp1

<sup>1</sup>Classification of Glomeromycota by Redecker et al. (2013) [13].

## 2.2 Experimental design

The treatments, designed in a bifactorial scheme, were nine inoculants [eight soils containing AMF communities (Table 2) and a control composed of *Rhizophagus clarus* (Nicolson & Schenck) C. Walker & A. Schüß. Schüßler], not subjected (year 2016) or subjected (year 2020) to storage. The experimental design was completely randomized, with three replications. Each replication consisted of one sorghum plant (*Sorghum bicolor* (L.) Moench). *R. clarus*-based inoculant came from the International Culture Collection of Glomeromycota (CICG).

## 2.3 Procedures

After collection, the soil samples remained at room temperature for four days and were then stored under refrigeration (2 to 4°C) for four months in plastic containers until the first experiment was set up.

The nine non-stored inoculants were used to set up the experiment in September 2016. The eight AMF communities (Table 2) and the *R. clarus* fungal isolate remaining from the 2016 experiment were stored for four years in a refrigerator and used to finalize the study in September 2020.

## 2.4 Mycorrhizal species richness

After identifying the AMF species, we obtained the species richness of the communities of these microorganisms by counting the number of species found in the soil samples [5].

## 2.5 Mycorrhizal inoculum potential

The MIP analysis was carried out according to the medium colonization potential bioassay described by Moorman and Reeves (1979) [14], in a 1:1 ratio [50% native soil and 50% diluent (sand)], for both non-stored inoculants (2016) and those subjected to storage (2020). Sorghum was grown in polystyrene tubes with a volume of 250 cm<sup>3</sup>. In September 2016 and 2020, the tubes were filled with the collected soils and three seeds were sown.

The tubes were kept on metal benches, 1.2 m above the soil surface, in a greenhouse (90 m<sup>2</sup>) with a semicircular roof, installed in a northwest-southeast direction. The galvanized steel structure is covered with low-density polyethylene film with an anti-ultraviolet additive and a thickness of 150 microns, and the sides are covered with anti-aphid mesh.

The irrigation used was mechanized sprinklers with a flow rate of 2 L.min<sup>-1</sup> per unit. In both years, the evaluation was carried out 45 days after sowing, in October. The area of the plant and the soil + diluent were discarded and the roots were washed to remove excess inoculant.

To check MIP, after removing the plants from the tubes, the roots were prepared according to Phillips and Hayman (1970) [15] and their mycorrhizal colonization (MC, %) was determined according to Trouvelot et al. (1986) [16].

## 2.6 Data analysis

The data obtained was submitted to analysis of variance (Anova) and, when significant, the means of the treatments were compared using the Tukey test, at a 5% probability of error, with the aid of the Sisvar<sup>®</sup> program [17].

# 3. RESULTS AND DISCUSSION

## 3.1 Mycorrhizal species richness

There was variability in the richness of AMF species in the soils collected. The native forest soils and the soil cultivated in Ipê were superior in terms of the number of mycorrhizal species compared to the average and the other soils cultivated with strawberries (Figure 2).

The native forest soil in the municipality of Ipê had 60% more species richness than the cultivated soil in the municipality of Flores da Cunha. In the native forest soils, there was a 27% variation between the sites with the highest and lowest species richness. In cultivated soils, this variation was 57% (Figure 2).

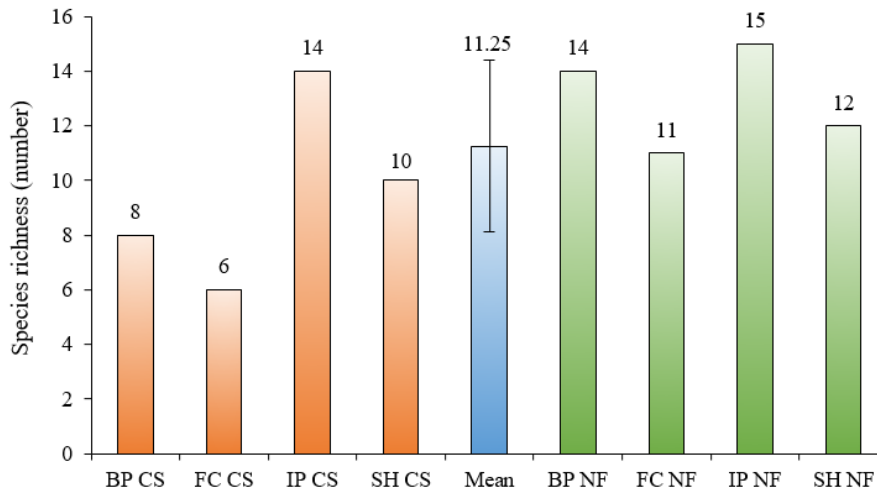


Figure 2: Species richness of Arbuscular mycorrhizal fungi communities in Rio Grande do Sul, Brazil. BP = Bom Princípio; FC = Flores da Cunha; IP = Ipê; SH = São José do Hortêncio; CS = cultivated soils; NF = native forest soils.

There was a reduction in the richness of mycorrhizal species from natural soils to cultivated soils of 46%, 43%, 17%, and 7% in the municipalities of Flores da Cunha, Bom Princípio, São José do Hortêncio, and Ipê, respectively (Figure 2).

The greater richness of AMF in natural soils is related to the edaphic anthropization of cultivated soils. Intensive agricultural practices, such as the use of chemical inputs, have a negative impact on AMF communities and this reduces their richness [18].

The composition of mycorrhizal communities is structured by biogeographical and edaphoclimatic conditions [19]. Although edaphic factors are the main cause of the structuring of AMF communities [20], the literature indicates that AMF have an idiosyncratic response in relation to the plant community [21].

When comparing the ecosystems in terms of the presence of hosts (Figure 1), the native forest soils were heterogeneous in relation to the cultivated soils and this may explain the greater diversity of AMF species in natural soils, as plant plurality benefits the richness of mycorrhizal species [22]. Regardless of the phytophysiognomy, the floristic composition established in the native forest soils was similar and so there was less variation in AMF species in natural soils (27%) compared to cultivated soils, which had greater species variation (57%), probably due to edaphic filtering.

The vegetation cover of the soils may also have favored the greater diversity of AMF in natural ecosystems [5]. One noticeable difference between the natural and agricultural ecosystems was the vegetation cover. In all the natural ecosystems, the native forest soils were under vegetation cover, while the cultivated soils were mulched (Figure 1). This indicates that conventional agricultural practices and soil use/management cause a loss of AMF diversity and, through selection pressure, favor the sporulation of certain ecological groups of AMF, known as generalists or dominants.

### 3.2 Mycorrhizal inoculum potential

According to the Anova, there were significant differences between inoculants and storage in terms of the MC of the sorghum root system (Table 3).

Table 3: Summary of analysis of variance for mycorrhizal colonization of sorghum roots grown with on-farm Arbuscular mycorrhizal fungi (AMF) inoculants, whether or not subjected to storage.

Causes of variation	DF <sup>1</sup>	Mean square
		MC (%)
Storage	1	9868.51**
Inoculants	8	820.83**
Interaction	8	189.35 <sup>ns</sup>
Residue	36	94.44
Total	53	
Mean		71.66
CV (%) <sup>2</sup>		13.56

<sup>1</sup>DF = degrees of freedom; MC = mycorrhizal colonization. <sup>2</sup>CV = coefficient of variation. \*\*Significant at the 1% probability level ( $p < 0.01$ ). <sup>ns</sup>Not significant ( $p \geq 0.05$ ).

The inoculants used were grouped into three strata with regard to the MC attribute (Table 4). The *R. clarus* isolate (control) showed the greatest infective capacity on sorghum roots, followed by inoculants from native forest soils and then, as the last strata, inoculants from strawberry soils (Table 4). The AMF structures observed in the roots were hyphae, vesicles, and arbuscules (Figure 3).

Table 4: Effect of inoculants and storage on the mycorrhizal inoculum potential (MIP) of the root system of sorghum plants.

On-farm inoculants <sup>1</sup>	MC (%) <sup>2</sup>
BP CS	56.66±9.87 c
FC CS	65.00±9.97 c
IP CS	61.66±8.53 c
SH CS	63.33±7.94 c
BP NF	83.33±8.02 b
FC NF	70.00±7.41 b
IP NF	73.33±9.06 b
SH NF	78.33±8.94 b
RC	93.33±9.62 a
Storage	
Without (2016)	85.18±6.97 a
With (2020)	58.14±7.69 b
Mean	71.66
CV (%) <sup>3</sup>	13.56

Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ( $p \leq 0.05$ ). <sup>1</sup>BP = Bom Princípio; FC = Flores da Cunha; IP = Ipê; SH = São José do Hortêncio; CS = cultivated soils; NF = native forest soils; RC = *Rhizophagus clarus*. <sup>2</sup>MC = mycorrhizal colonization. <sup>3</sup>CV = coefficient of variation.

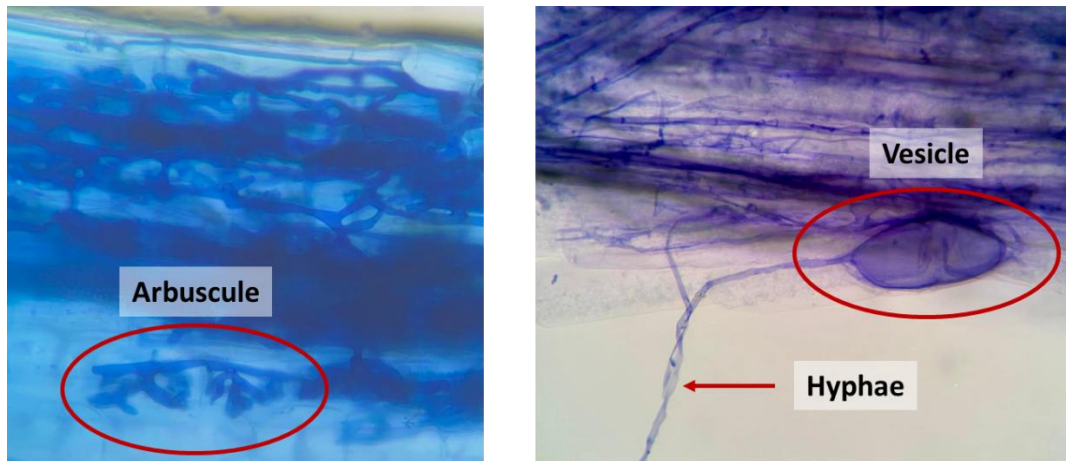


Figure 3: Hyphae, vesicle, and arbuscule visualized in sorghum roots. Optical microscope observation, with magnification of 400 x.

The highest MIP was observed in non-stored inoculants in the experiment set up in 2016 (Table 4). However, even when stored for four years (2020), the eight soils collected from reference sites for strawberry cultivation proved capable of maintaining viable mycorrhizal communities (Table 2), which were associated with the roots of the plant host (Table 4).

MIP provides information on the activity of AMF communities in the soil [23]. Thus, our results indicated that in native forest soils AMF propagules are more active and at appropriate levels ( $\geq 60\%$ ) to establish symbiosis, in a short period of time, compared to cultivated soils (Table 4).

The higher MIP observed in natural soils (Table 4) may be related to the greater diversity of AMF in these ecosystems (Figure 2). While agricultural soils had an average of 9.5 fungal species making up the mycorrhizal community, native forest soils had an average of 13 AMF species (Figure 2). The greater the diversity of species that make up a mycorrhizal community, the more effective the association between the plant host and the fungal symbionts [5]. It should be noted that the diversity of AMF contributes to the coexistence, productivity, and maintenance of flora in ecosystems [24].

Large-scale multiplication of AMF for the formulation of an inoculant can be done in aeroponic, hydroponic, or *in vitro* systems [25]. However, one option for increasing the use and acceptance of an AMF-based biofertilizer is to develop an inoculant produced by the on-farm method, obtained on site where it will later be used, in order to avoid the costs associated with buying a commercial product [26]. The on-farm inoculant can be developed under natural environmental conditions, multiplying mycorrhizal species indigenous to a given ecosystem, and/or with known efficiency [27]. Under these conditions, the fungal species and the plant host are physiologically more compatible, which can ensure greater benefits from the association.

AMF are of great value to the functioning and sustainability of ecosystems [28]. Thus, studies of the diversity of AMF, the MIP of these microorganisms and their functions in different edaphic aptitudes contribute to understanding the impact of changes in land use in the agroecosystem context [29].

#### 4. CONCLUSION

There is temporal variability in the MIP of AMF communities in cultivated and native forest soils in reference ecosystems for strawberry cultivation. Natural soils and those not stored have a greater capacity to colonize the roots of the plant host. Studies are recommended to boost the on-farm production of inoculants with these mycorrhizal communities in order to benefit strawberry growers in terms of yield and/or fruit quality.



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