

Received: 10 March 2023  
Published: 28 July 2023

## Efficiency in the Sporulation and Colonization of the Mycorrhizal-Forming Fungus Morphospecies *Glomus claroides* IN *Bothriochloa pertusa* (L) A. Camus

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### Abstract

*The objective of this study was to evaluate the sporulation and colonization capacity of Glomus claroides in situ on Bothriochloa pertusa. The results of the in situ trial show that the arbuscular mycorrhizal fungal morphospecies (AMF) G. claroides isolated from different localities (Tolú and Corozal) have different sporulation and colonization patterns, but in both treatments it was found that the arbuscular mycorrhizal fungal morphospecies has the ability to speculate and colonize roots of B. pertusa grass. G. claroides is a predominant AMF morphospecies in both livestock farms in the municipality of Tolú and Corozal and can simultaneously use B. pertusa as a host plant for the production of inoculums for use as biofertilizers.*

**Keywords:** *inoculum, fungi, soil, spores, colonization, pasture.*

### 1. INTRODUCTION

In recent years, plant-fungal interactions, especially with arbuscular mycorrhizal fungi (AMF), have attracted attention. AMF, represent symbiotic associations between plants and fungi based on the exchange of metabolites and nutrients. More than 95% of terrestrial plants are capable of symbiosis with mycorrhizae.

Arbuscular mycorrhizal fungi are present in the rhizosphere of many plant species and interact with various bacterial populations. This interaction is established during all stages of the mycorrhizal life cycle, from spore formation, germination, colonization within plant roots to the emission of external hyphae (Toljander et al., 2006).

Under natural agroecosystem conditions, arbuscular mycorrhizae are not simply interactions between a plant root and a particular fungal species, but constitute a complex community formed by different fungal species and root systems. There is evidence that arbuscular mycorrhizal fungi originate an extensive network of external mycelium that explores the soil in search of resources and interconnects the roots of plants of the same or different species (Simard and Durall, 2004). This association is defined as a "mutualism-parasitism" continuum, i.e. it is analyzed from a "cost-benefit" perspective, considering the development of both plants and fungi, environmental and edaphic conditions and mutual genetic recognition factors (Johnson et al., 1997). In different ecosystems on earth, selection seems to have favored certain attributes of mycorrhizae and their involved symbionts, leading to great structural and functional diversification.

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Pastures on the northern coast of Colombia are made up of high productive potential grasses such as guinea (*Panicum maxima*), angletón (*Dichanthium aristatum*), puntero (*Hyparrhenia rufa*) and pará (*Brachiaria mutica*), some naturalized species such as Colosuana or kikuyina (*Bothriochloa pertusa*), becoming an exclusive source of animal feed. Colosuana grass covers a total of 274,005 ha, distributed in 19 municipalities in the department of Sucre (Aguilera, 2005).

Based on the importance of this natural symbiosis and the value of this process for pasture crops in the region and the need to know the types of arbuscular mycorrhizal fungi associated with *Bothriochloa pertusa* (L) A. Camus, the present study was carried out with the purpose of evaluating the sporulation and colonization capacity of the morphospecies *Glomus Claroides* in situ in *B. pertusa* grass as an alternative for the massive production of inoculum of these morphospecies as an alternative biofertilizers.

## 2. MATERIALS AND METHODS

2.1 Sampling. Sampling of soil and colonized roots was carried out in cattle farms in the municipalities of Tolú and Corozal in the department of Sucre established with predominantly *Bothriochloa pertusa* (L) A. Camus pasture. Sampling was carried out as follows: A PVC tube of 3.8 cm diameter and 25 cm length was used to take the samples at a depth between 0-20 cm, introducing, rotating and extracting the cylinder with the sample (soil and roots). Between 15-20 samples were taken on each farm, and these were homogenized per farm to form a sample weighing 2000 grams. These samples were used for spore isolation, determination of the percentage of colonization in roots and identification of morphospecies present in situ in each locality (Pérez et al., 2012).

2.2 Field experiment. To carry out the field experiment, the most abundant morphospecies of arbuscular mycorrhiza-forming fungi in both locations were used as evaluation criteria. Once the predominant morphospecies had been selected, the field evaluation was set up. The experiment was carried out in situ on a livestock farm in the municipality of Sincelejo. For this test, 14 plots of 0.5 x 0.5 m by 0.20 m depth were constructed, with a separation of 1.5 m between plots and 2 m between replications. The treatments used were: T1: *Glomus Claroides* isolated from the municipality of Tolú; T2: *Glomus Claroides* isolated from soil of a cattle farm in the municipality of Corozal; T3: soil without inoculation.

Sterilization of the corresponding treatments was done by applying the fungicide Basamid G, at a dose of 10 grams/plot (0.25 m<sup>2</sup>), the soil was mixed with the product to a depth of 0.2 m and irrigation was applied in order to distribute the fungicide in the soil. The plots were covered with black plastic for 8 days. After this time the plots were uncovered, irrigation was applied and 10 g of sexual seeds of the grass species *B. pertusa* were sown. Ten days after germination and when the seedlings were 5 cm high, holes were made near the root system of the seedlings and inoculums (soil + spores + fragments of colonized roots) were applied, following the guidelines of Safir, (1996). The inoculum used corresponded to soil mycorrhizal with spores and roots colonized with *Glomus Claroides* morphospecies. The amount of spore/gram of soil was 800 spores.

2.3 Evaluation of the experiment.

Spore count and percentage of colonization in roots. After 120 days, a soil and root sample per treatment was taken and spore counts and percentage of infection in roots were carried out individually for each treatment and repetition using methodologies proposed by Pérez and Vertel, 2010; Peroza and Pérez, Pérez et al., 2012; Pérez et al., 2015.

### 3. RESULTS AND DISCUSIÓN

Figure 1 shows the observed characteristics of the species *Glomus Claroides* Shenck & Smith, 1982, isolated from cattle soils in the municipality of Tolú.

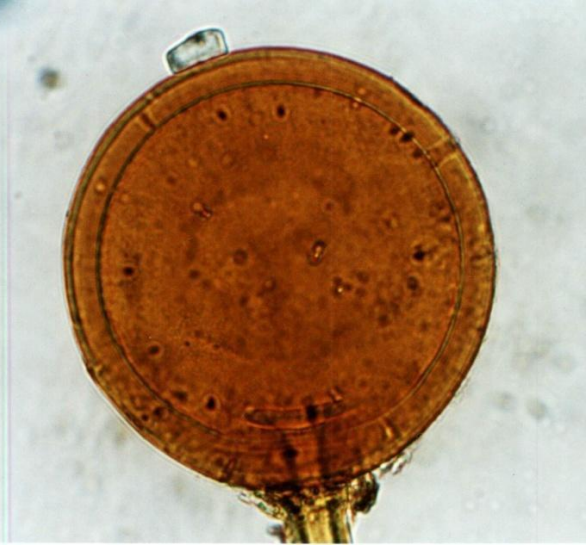
Locality: Tolú	OBJ 40 X OC 10X
DESCRIPTION OF THE SPORE:	
Shape: Globose Diameter ( $\mu\text{m}$ ): 114 Color: Water: Yellow L.V.P: Dark yellow Cytoplasmic content: Granular with pores Surface structure: Smooth Wall Composition and type of: Number of layers: 2 layers: Single Laminated Wall width ( $\mu\text{m}$ ): 7.8	
Hyphal junction width: Hyphal diameter ( $\mu\text{m}$ ): 8.6 Pore diameter ( $\mu\text{m}$ ): 3.9	Taxonomic determination: Genus: <i>Glomus</i> morphospecies: similar to <i>Glomus Claroides</i> Shenck & Smith, 1982
Additional observations: Open hyphae with open pores. Présense of Canals.	

Figure 1. Morphological and structural characteristics of the morphospecies *Glomus Claroides* Shenck & Smith, 1982, isolated from cattle farms in the municipality of Tolú, department of Sucre, Colombia.

Figure 2 describes the observed characteristics of the species *Glomus Claroides* Shenck & Smith, 1982, isolated from cattle soils in the municipality of Corozal.


Locality: Corozal	OBJ 40 X OC 10X
DESCRIPTION OF THE SPORE:	
Shape: Globose Diameter ( $\mu\text{m}$ ): 88 Color: Water: Yellow P.V.L: Dark Yellow Cytoplasmic content: Granular Surface structure: Ornamented Wall composition and type: Number of layers: 1 layer: Evanescent to lamellar Wall width ( $\mu\text{m}$ ): 7.2	
Hyphal junction width and type: Hyphal diameter ( $\mu\text{m}$ ): 7.8 Pore diameter ( $\mu\text{m}$ ): 3.2	Taxonomic determination: Genus: <i>Glomus</i> morphospecies: similar to <i>Glomus claroides</i> Schenck & Smith, 1982
Additional observations: Open hyphae with occluded pore. Presence of oily globules oily globules and folds.	

Figure 2. Morphological and structural characteristics of the morphospecies *Glomus Claroides* Shenck & Smith, 1982, isolated from cattle farms in the municipality of Corozal, department of Sucre, Colombia.

Figure 3 shows the results of the sporulation test of the morphospecies *Glomus claroides* Schenck & Smith, 1982 isolated from soils of two cattle farms (Tolú and Corozal) and evaluated the in situ sporulation capacity in the *Bothriochloa pertusa* (L) A. Camus pasture. The results of the in vitro test show that the sporulation rates of *Glomus claroides* Schenck & Smith, 1982 isolated from soils of two cattle farms in the municipality of Tolú ranged between  $1568 \pm 1704$  spores/ g of soil, while the sporulation of the isolated morphospecies from the municipality of Corozal was  $1705 \pm 2799$  spore/g soil, showing a higher sporulation capacity of the isolated morphospecies from the municipality of Corozal on host plant with *Bothriochloa pertusa* (L) A. Camus.

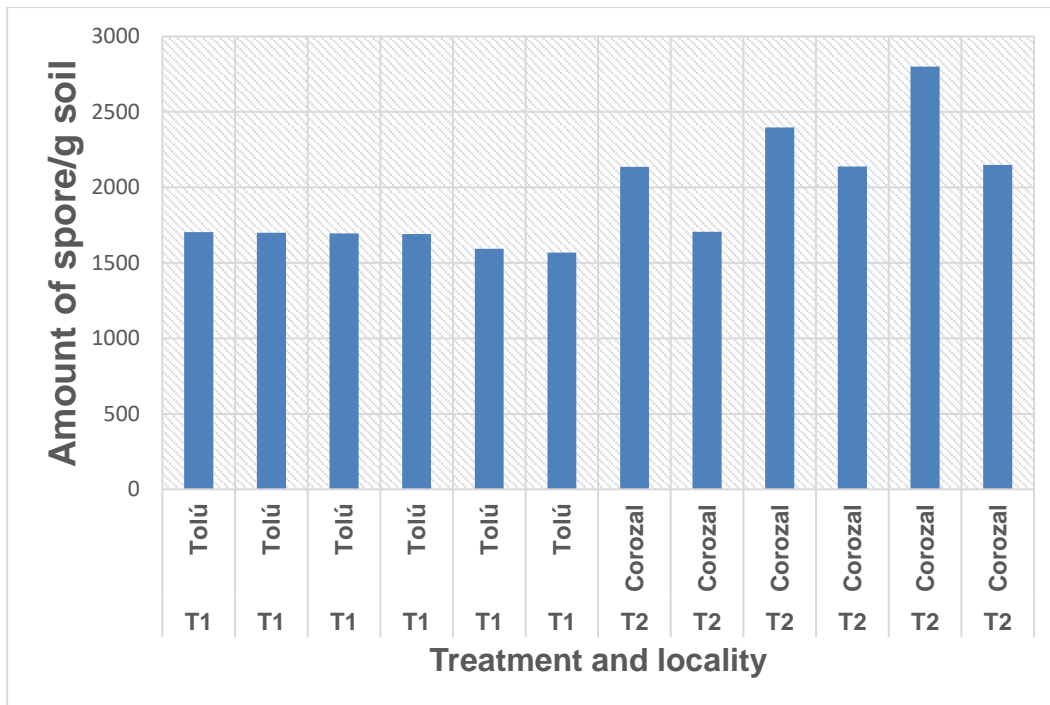


Figure 3. Evaluation of in situ sporulation on *Bothriochloa pertusa* (L) A. Camus of the morphospecies *Glomus claroides* Schenck & Smith, 1982 from two different localities (Tolú and Corozal).

Figure 4 shows the results of the colonization test of the morphospecies *Glomus claroides* Schenck & Smith, 1982 isolated from soils of two cattle farms (Tolú and Corozal) and evaluated for its in situ colonization capacity on *Bothriochloa pertusa* (L) A. Camus pasture. The results of the in vitro test show that the sporulation rates of *Glomus claroides* Schenck & Smith, 1982 isolated from the soils of two cattle farms in the municipality of Tolú ranged between  $30 \pm 64$  % of colonization in the roots of the pasture analyzed, while the sporulation of the isolated morphospecies from the municipality of Corozal was  $43 \pm 68\%$ , showing a higher colonization capacity of the isolated morphospecies from the municipality of Corozal on the host plant with *Bothriochloa pertusa* (L) A. Camus.

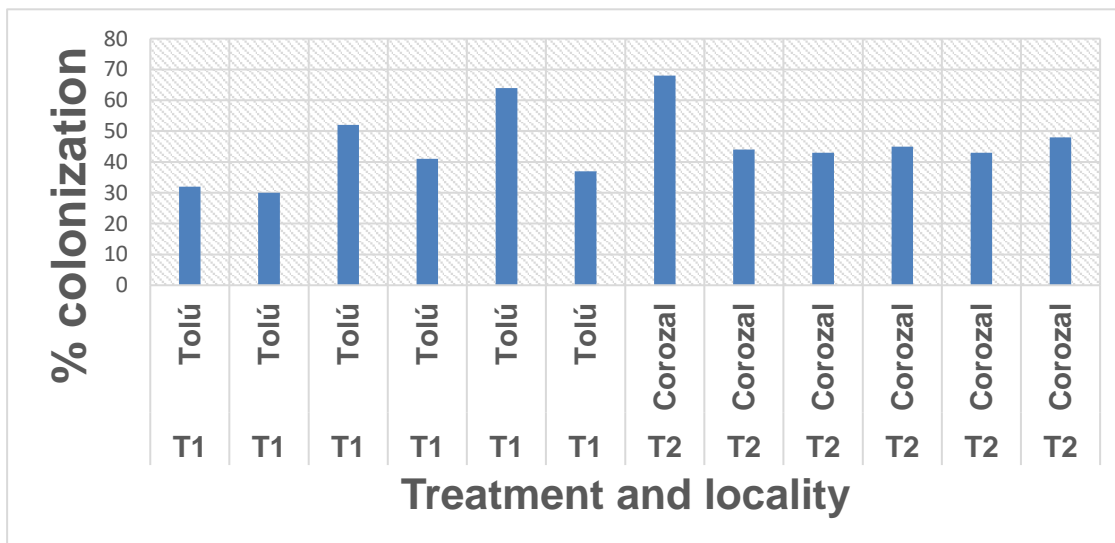


Figure 4. Assessment of in situ colonization on *Bothriochloa pertusa* (L) A. Camus of the morphospecies *Glomus claroides* Schenck & Smith, 1982 from two different localities (Tolú and Corozal).

The establishment of the symbiosis will depend on the interactions between the fungus, the plant and the environmental conditions. Their presence implies that processes of recognition between symbionts, compatibility and specificity occur, which condition their expression and lead to the morphological and functional integration of the associations (González, 1996). AMF sporulation under field conditions is determined by: soil physico-chemical conditions (pH, microbial respiration, phosphorus content, temperature, aeration, texture and organic matter content) (Pérez and Vertel, 2010), climatic conditions (light intensity and duration, humidity, rainy and dry seasons) and by agronomic practices (soil preparation, pesticide application and cultural practices) (Aguilera, 2005).

Although the importance of arbuscular mycorrhizal (AMF) plant-fungi symbiosis is recognized worldwide, there are some aspects about the structure and function of the communities in tropical agroecosystems that have not been studied. In Colombia and especially on the North Coast, most of the studies have been related to the benefits of the symbiosis in different hosts, especially in aspects of productivity, plant nutrition and fertilizer substitution, which has allowed determining the potential use of these microorganisms in conventional production systems or in clean production systems (Rey et al., 2005).

#### 4. CONCLUSION

*Glomus claroides* is a morphospecies with the ability to sporulation and colonization roots of *Bothriochloa pertusa*, with colonization rates of more than 50%, making this pasture an alternative host-trap plant for the production of inocula of arbuscular mycorrhizal fungi.

#### 5. ACKNOWLEDGEMENT

The authors would like to thank the Microbiological Research Laboratory of the University of Sucre for their support in carrying out this study.

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