Symbiosis of mycorrhizal fungi with Rubus spp., in four farms of the municipality of Pamplona, Norte de Santander

Simbiosis de hongos micorrícicos con Rubus spp., en cuatro fincas del municipio de Pamplona, Norte de Santander

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Francisco Rodríguez Rincón
Universidad de Pamplona. Pamplona (Colombia)
francrincon@unipamplona.edu.co

Leonides Castellanos González
Universidad de Pamplona. Pamplona (Colombia)
lclcastell.@gmail.com

Ana Adelaida Becerra Caicedo
Universidad de Pamplona. Pamplona (Colombia)
yaya.3456@hotmail.com

Leidy Yohana Leal Montes
Universidad de Pamplona. Pamplona (Colombia)
yohanita07@hotmail.es

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Abstract

Introduction— The blackberry crop is part of the prioritized productive chains in Norte of Santander, however, there are no research results on the benefits of Arbuscular Mycorrhizal Fungi (AMF) and the level of natural colonization of these with Rubus sp.

Objective— To determine the levels of colonization and mycorrhization of AMF in commercial varieties of blackberry and wild species of Rubus sp. in areas of the municipality of Pamplona Norte de Santander

Methodology— This work was carried out in the Sabaneta Baja, Sabaneta Alta and San Francisco veredas of the municipality of Pamplona, sampling four farms. Root samples were collected from commercial blackberry crops and neighboring wild species to evaluate the percentage of colonization and mycorrhization by AMF. ANOVAS were carried out using the SPSS statistical package.

Results— High levels of AMF symbiosis were found in the three commercial varieties of blackberry (colonization between 92% to 98%) and in the three species of Rubus sp. (colonization between 71% to 84%), as well as the presence of mycorrhizal structures of AMF in blackberry varieties and native species.

Conclusions— The biological variables of the AMF did not vary statistically between the commercial varieties, or wild species, but did between the different farms and in the variety × farm interaction for the commercial varieties. The biological variables of the AMF in the commercial varieties differed from the wild species located on the same farm.

Keywords— Blackberry crop; fungi; symbiosis; wild species; commercial varieties

Resumen

Introducción— El cultivo de mora forma parte de una de las cadenas productivas priorizadas en Norte de Santander, sin embargo, no existen resultados de investigación sobre los beneficios de los Hongos Micorrizógenos Arbusculares (HMA) y el nivel de colonización natural de estos con Rubus spp.

Objetivo— Determinar los niveles de colonización y micorrización de los HMA en variedades comerciales de mora y en especies silvestres de Rubus en zonas del municipio de Pamplona Norte de Santander.

Metodología— Este trabajo se realizó en las veredas Sabaneta Baja, Sabaneta Alta y San Francisco del municipio de Pamplona, muestreando cuatro fincas. Se recolectaron muestras de raíces en los cultivos de mora comercial y especies silvestres aledañas para evaluar el porcentaje de colonización y micorrización por HMA en el laboratorio. Se realizaron ANOVAS, por medio del paquete estadístico SPSS.

Resultados— Se constató altos niveles de simbiosis de los HMA en las tres variedades comerciales de mora (colonización entre 92% a 98%) y en las tres especies de Rubus (colonización entre 71% a 84 %), así como la presencia de estructuras de micorrización de los HMA en las variedades de mora y las especies nativas.

Conclusiones— Las variables biológicas de los HMA no variaron estadísticamente entre las variedades comerciales ni especies silvestres, pero si entre las diferentes fincas y en la interacción variedad × finca para las variedades comerciales. Las variables biológicas de los HMA en las variedades comerciales se diferenciaron de las especies silvestres ubicadas en la misma finca.

Palabras clave— Cultivo de mora; hongos; simbiosis; especies silvestres; variedades comerciales

I. Introduction

Several researchers recognize that Arbuscular Mycorrhizal Fungi (AMF) stimulate the growth, development and nutrition of plants, especially in soils of moderate and low fertility, mainly because the external hyphae increase the adsorption surface, beyond the zone of exhaustion surrounding the root [1].

The symbiotic associations between plant roots and fungi were named in 1885 by the German forest pathologist AB Fran as mycorrhiza, derived from the Greek that translates fungal root. Mycorrhizae, for many years they have been common in forest trees, today they are considered as the roots for the normal nutrition of most plants including cereals, vegetables, ornamental plants and tree [2].

The main function of the mycorrhiza is to facilitate the plant acquisition and absorption of water, phosphorus and nitrogen, mainly; However, this association provides other benefits to plants, among which are: protection against attack by parasites, pathogenic fungi and nematodes, increasing their resistance to herbivores, influencing the production of defensive substances by the plant itself, limiting the absorption of toxic heavy metals such as zinc and cadmium that are housed in its hyphae, increasing the area of exploration of the root, which increases the flow of water from the soil to the plant, in addition to improving the physical and chemical properties of the soil through the enrichment of organic matter and the formation of aggregates through the adhesion of particles [1].

Agree with the 2013 Statistical Yearbook of the Ministry of Agriculture and Rural Development of Colombia, in the country there are 14 458 hectares planted with blackberry, with average yields of 7.22 tons per hectare. The departments of Cundinamarca, Santander and Antioquia stood out, together with The Eje Cafetero and Norte de Santander in production and yield of blackberry during the year 2012 [3].

In blackberry cultivation, the benefits of mycorrhizae on the nutrition and growth of blackberry plants are pointed out *Rubus* sp, micropropagated under ex vitro, greenhouse or field conditions, as well as tolerance to changes in the environment, achieving its physiological development with greater efficiency [4]. These effects are reflected in a stronger root system allowing the plant to absorb nutrients and tolerate eco-physiological stress [5].

Arbuscular mycorrhizae in blackberry plants could provide a better development of adaptability to different environments, acquisition and absorption of water, as well as protection against parasites, pathogenic fungi and nematodes attack [4].

The Departmental Strategic Plan for Science, Technology and Innovation of Norte de Santander 2014-2024 identified as a prioritized productive chain that of fruits and vegetables where the cultivation of blackberries is located [6]. In the case of the municipality of Pamplona (Colombia), the commercial varieties of *Rubus glaucus* Bent are cultivated: Mora de Castilla with thorns, Mora de Castilla without thorns and the new variety commonly called "Mora de uva", but mycorrhizae have not been used as a microbial inoculant to blackberry crops, due to lack of knowledge of the producer and the absence of scientific information on native mycorrhizae and their diversity.

On the other hand, different species of *Rubus* have been described as natural producers of wild blackberry foundin areas surrounding commercial crops and forested areas in the municipalities of Chitagá and Pamplona (Colombia). The species described were: *Rubus alpinus* Macfad, *R. floribundus* Kunth, *R. bogotensis* Kunth, *R. rosifolius* Smith, *R. adenotrichos* Schlechtendal and *R. urticifolius* Poir [7].

The knowledge of the symbiosis of arbuscular mycorrhizae in cultivated soils and in soils with wild species of *Rubus* sp., will allow to give a scientific contribution of its diversity and potential application in the medium and long term in the province of Pamplona, allowing to improve production in the cultivable varieties of blackberry. For this reason, the objective of this research was to determine the levels of colonization and mycorrhization of AMF in commercial varieties and in wild species of *Rubus* sp. in areas of the municipality of Pamplona Norte de Santander (Colombia).

II. Methodology

A. *Research location and root sampling*

This work was developed in the laboratory of the Microbiology and Biotechnology Research Group (GIMBIO) of the Department of Microbiology of the University of Pamplona (Colombia) and under field conditions in four farms located in the Sabaneta Alta, Sabaneta Baja and San Francisco veredas (Table 1), located towards the North of the municipality of Pamplona (Colombia). In these farms, samples were carried out in commercial blackberry plantations and in wooded areas where there was a presence of wild *Rubus* species. The annual average temperature varies between 8° C to 17°C, and average annual rainfall of 1042 mm.

Table 1.
Geographical location of the sampling areas in the municipality of Pamplona.

Municipality	Veredas	Farm name	Coordinate Latitude (N)	Coordinate Longitude (W)	Altitude (masl)
Pamplona	Sabaneta Alta	El Pino	7°39'51 "	72°66'52 "	2374
	Sabaneta Alta	La Esmeralda	7°24'34.7 "	72°39`6.87 "	2360
	Sabaneta Baja	El Recuerdo	7°26'7.18 "	72°40'7.22 "	2268
	San Francisco	El Salado	7°28'42 "	72°39`56``	2293

Source: Authors.

In each of the selected farms, root samples were taken from five plants with ages between 2 and 3 years from a blackberry field planted with the three commercial varieties that are grown in the area: Mora Uva, Mora de Castilla (MC) without thorns and Mora de Castilla with thorns.

Additionally, adult plant roots corresponding to native species naturally present in the forested areas of the El Pino and La Esmeralda farms were sampled (Table 2), which were previously identified and their taxonomic classification confirmed with the help of the specialist and the Herbarium of the University of Pamplona.

Table 2.
Native species present in the farms under study.

Native species	Farms
R. alpinus	El Pino,
R. urticifolius	El Pino, La Esmeralda
R. floribundus	El Pino, La Esmeralda

Source: Authors.

The plant selection in the field of each commercial variety per farm, was carried out following a directed systematic random sampling, which consisted of walking in a V-shape through the entire selected area. The same procedure was followed for taking samples of roots of the wild species in the wooded areas of each one of the farms.

Four mini pits ($10 \times 10 \times 20$ cm^3, width, length and depth respectively) were excavated around the plants, and at each point, at least 10 adventitious roots were taken at a depth of 20 cm. The four subsamples made up a composite sample that was placed in small transparent plastic bags and taken to the Microbiology Laboratory of the University of Pamplona in order to develop the process of quantifying the fungal colonization frequency and visual mycorrhization per plant.

B. *Root lightening and staining*

In order to facilitate quantification of roots colonized by AMF, the lightening and staining of roots was carried out using the methodology proposed by researchers in the United Kingdom [8], with some modifications. The procedure was as follows: initial washing of the adventitious roots with abundant running water to remove soil debris, then these were immersed in a 10% KOH solution, and left in a water bath (90°C) for 15 minutes. Then they were washed again and 10% KOH and 10% H_2O_2 were added, mixed in a 1:1 ratio (V/V), and placed again in a water bath at 90°C for 10 minutes. Next, the roots were washed with running water, a 1N HCl solution was added to them, they were left in a water bath for 10 minutes and they were washed again with water. Subsequently, the staining was carried out with a 0.05% Trypan Blue solution in acetic acid-glycerol-water (1:1:1), placing the roots in a water bath for 10 minutes. Afterward, the dye was removed, a lactic acid-glycerol-water solution was added, leaving the roots to rest to remove excess dye, and finally they were mounted on a slide and coverslips to observe them under the microscope with the same solution.

After staining the roots, the plate field intersection methodology recommended by English scientists was followed with some modifications [9]. This procedure consisted of placing five root fragments of each plant, each 1 cm long on slides, in such a way that the segments were parallel, lactoglycerol was added, the coverslip was placed and it was observed in the objective at 10X with a Zeiss Primo Star brand microscope. The observation began at the end of the first root, in this visual field the presence or not of colonization by AMF was indicated. It was passed to the next field vertically and again it was indicated whether or not there was colonization by the fungus. It continued until 10 fields per root and 50 fields per plant were covered, repeating the process in a total of the five plants per variety, or wild species.

To calculate the percentage of colonization of AMF by microscopic observation fields, was used (1):

$$\%\ \textbf{Total colonization by AMF} = \left(\frac{C}{T}\right)\textbf{100} \qquad (1)$$

C: Number of fields colonized by any AMF structure.
T: Total number of observed fields.

In order to specify the percentage of mycorrhization by hyphae, arbuscules and/or vesicles, the percentage of these structures in the microscopic field of observation was calculated visually, taking as 100% the tissue observed in 10 × 10. The percentage of each of these structures resulted from obtaining the average of 50 fields observed in the roots of each plant. For the calculation of the percentage of total visual mycorrhization of the three structures of the AMF, was used (1):

$$\%\ \textbf{visual micorrhization by AMF} = \%\ \textbf{HYPHAE} + \%\ \textbf{VESÍCLES} + \%\ \textbf{ARBUSCULES} \qquad (2)$$

With the information obtained from the five biological variables of the mycorrhizae (percentage of colonization, mycorrhization by hyphae, vesicles, arbuscules, and total mycorrhization of the three structures), several analyzes of variance were performed.

For commercial varieties, a two-factor analysis of variance (4 farms × 3 varieties) was carried out considering a completely randomized design where the repetitions were the five plants evaluated in each field of each variety per farm. A two-factor analysis of variance (2 farms × 2 species) was also carried out considering a completely randomized design where the repetitions were the five plants evaluated in each species (*R. floribundus*, *R. urticifolius*) in the El Pino and La Esmeralda farms.

Secondly, the three commercial varieties of *R. glaucus* (Mora de Castilla without and with thorns and Mora Uva) were compared in a simple ANOVA, together with the native species of *R. floribundus*, *R. urticifolius*, present on the farms El Pino and La Esmeralda considering a completely randomized design of five treatments and as repetitions the 10 plants evaluated in the two farms.

Finally, a simple ANOVA was performed to compare the five biological variables of the mycorrhizae among the three wild species of *R. floribundus, R. urticifolius*, *R. alpinus* present in the El Pino farm. A completely randomized design of three treatments was considered and the five evaluated plants of the El Pino farm were considered as repetitions.

Before carrying out the analyzes, the assumption of normality was verified by the Kolmodorov Smirnov test. The means were compared by the Tukey test for $p < 0.05$. The SPSS version 21 statistical package was used.

III. Results and Analysis

The microscopic observation of root mycorrhization in commercial blackberry plants and wild *Rubus* plants in the different farms showed the presence of vesicles, hyphae and arbuscules, making strong symbiosis within the roots of the evaluated plants (Fig. 1).

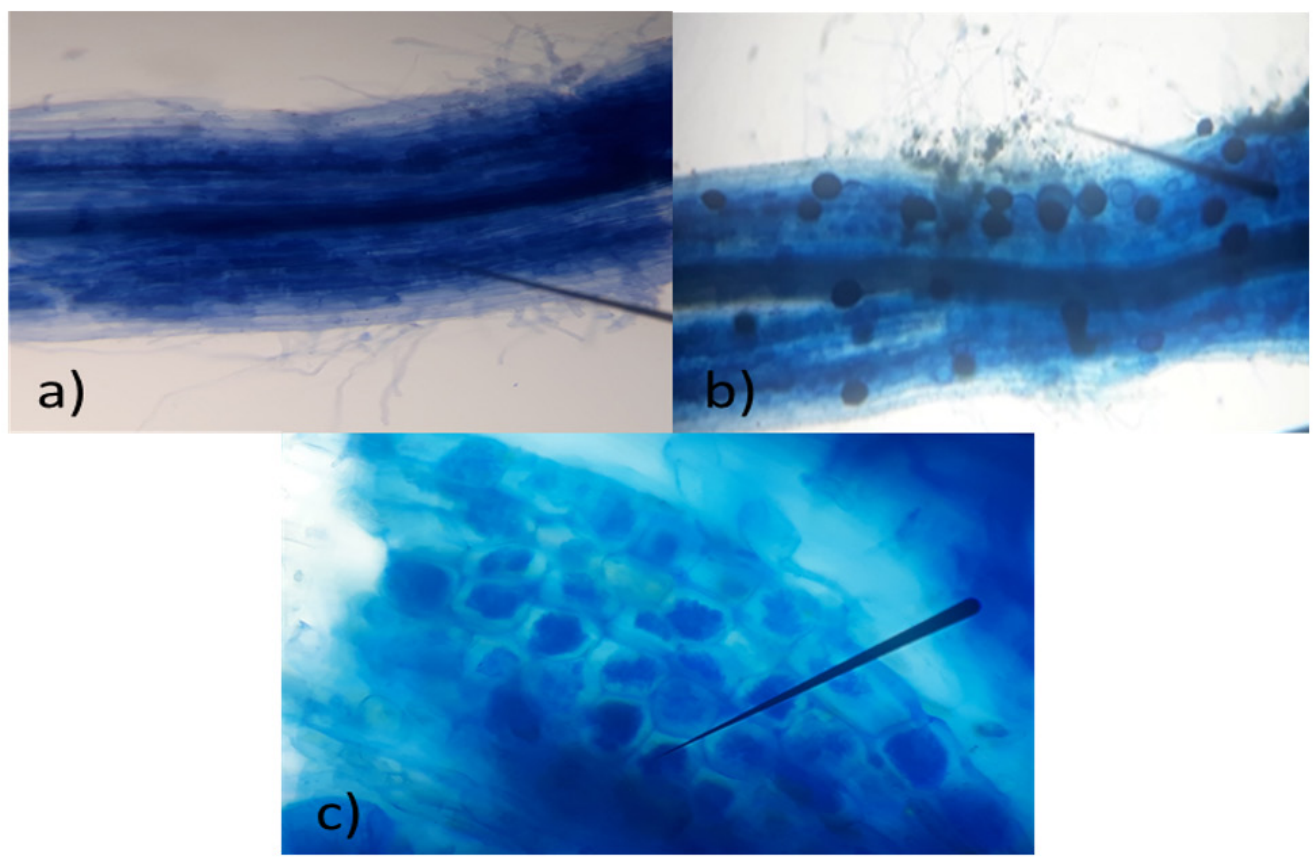

Fig. 1. AMF Structures of commercial varieties of blackberry plants evaluated
a) hyphae, b) vesicles, c) arbuscules, observed in a 10X microscope.
Source: Authors.

The hyphae found in the colonization of the roots are not septate and continuous thin filaments, some of them with intracellular and intercellular growth in the root. The vesicles presented varied shapes from round, oval, grouped or dispersed in the root, some contain hyphae; and the arbuscules were found within the cortical cells of the colonized roots. These results are similar to some images and studies carried out by other authors [4].

The result of the bifactorial ANOVA farms x commercial varieties for the five biological variables evaluated did not show a statistical difference between the three commercial varieties; however, a statistical difference was found between the three farms and the interaction between farms x varieties for a group of variables, both for commercial varieties and for native varieties (Table 3).

It was verified that there was an interaction between the variety and farm factor for the variables visual mycorrhization of vesicles and arbuscules and not for the rest of the variables, since the colonization percentage was very high, between 92 and 98% for commercial varieties (Table 3). Mycorrhization by vesicles was higher for Mora de Castilla with thorn in the El Pino farm (22.28%) that did not differ statistically from the Mora Uva on the El Pino farm and the MC without and with thorn and the Mora Uva on the La Esmeralda farm and Mora de Uva on the El Salado farm. The mycorrhization by arbuscules also showed a statistical difference, being greater for MC with spines in the El Salado farm but that did not differ from the MC without spines in that same farm, from Mora Uva and MC without spine at La Esmeralda farm and MC without thorns in the El Pino farm. It is noteworthy that for total mycorrhization (by hyphae, vesicles and arbuscules) there was no statistical difference for the variety × farm interaction.

Table 3.
Colonization and mycorrhization comparison for interaction between commercial varieties x farms.

Varieties	Farms	% colonization	Mycorrhization Structures (%)			
			Hyphae	Vesicles	Arbuscules	Total
Mora uva	El Pino	99.33a	16.05a	11.02ab	0.425d	27.51a
MC without thorn	El Pino	100.00a	6.52a	6.85b	9.07abc	22.45a
MC with thorn	El Pino	96.00a	7.35a	22.28a	0.699d	30.34a
Mora uva	La Esmeralda	92.00a	3.88a	11.13ab	4.95bcd	19.96a
MC without thorn	La Esmeralda	96.00a	5.14a	13.11ab	10.28ab	28.53a
MC with thorn	La Esmeralda	97.33a	2.33a	9.33ab	10.89ab	22.56a
Mora uva	El Recuerdo	98.00a	3.72a	1.22b	5.88abcd	10.83a
MC without thorn	El Recuerdo	96.66a	3.34a	8.44b	2.34bd	14,13a
MC with thorn	El Recuerdo	98.00a	1.54a	2.11b	5.79abcd	9.44a
Mora uva	El Salado	100.00a	8.45a	14.06ab	6.51abcd	29.02a
MC without thorn	El Salado	100.00a	6.04a	6.26b	12.72a	25.02a
MC with thorn	El Salado	100.00a	38.59a	11.22ab	9.65abc	16.99a
CV%		3.09	182.15	46.63	39.48	40.14
TE*		1.74	9.03	2.62	1.50	4.96

MC: Mora de Castilla variety; Unequal letters in the columns differ for $p \leq 0.05$;
CV: coefficient of variation; TE: typical error. Source: authors

The previous results show that the biological variables of the mycorrhizae were not influenced by the commercial varieties within each farm; but among the farms that were located on different soils and altitudes, the plants of the El Recuerdo farm were less prominent in mycorrhization by vesicles and arbuscules and curiously it is where the plants had less height.

The result of the ANOVA for the variables percentage of colonization, mycorrhization by hyphae and total mycorrhization did not show differences in the interaction, but did show a statistical difference between the farms (Table 4). The highest percentage of root colonization was observed in the El Salado farm (99.55%), the lowest for the La Esperanza farm (95.11%), and both El Pino and El Recuerdo farms were statistically intermediate. The El Salado farm showed greater mycorrhization by hyphae (17.69 %) without statistical difference with El Pino and La Esmeralda and the lowest in El Recuerdo with 2.87%. The total of structures (hyphae-vesicles-arbuscules) was also lower for the El Recuerdo farm with respect to the rest of the farms.

Table 4.
Colonization and mycorrhization between farms for commercial varieties.

	Colonization	Mycorrhization Structures (%)	
Farms	%	Hyphae	Total
El Pino	98.44ab	9.98a	26.76a
La Esmeralda	95.11b	3.78ab	23.69a
El Recuerdo	97.55ab	2.87b	11.47b
El Salado	99.55a	17.69a	23.68a
CV%	3.13	84.55	39.12
TE*	1.01	3.27	2.79

Unequal letters in the columns differ for $p \leq 0.05$; CV: coefficient of variation; TE: typical error. Source: authors

The result of the bifactorial ANOVA farms x native and commercial species did not show statistical difference between the La Esmeralda and El Pino farms, but showed difference between the species and varieties, and there was also a difference in the interaction of these two factors for the five variables under study. In the interaction, the colonization percentage was higher for MC without thorn, MC with thorn and Mora Uva in the El Pino farm and Mora Uva, MC without thorn and MC with thorn, *R. urticifolius* and *R. floribundus*, in La Esmeralda farm, and lower for *R. urticifolius* and *R. floribundus* in the El Pino farm with 76% and 71.33% colonization of AMF, respectively (Table 5).

Table 5.
Colonization and mycorrhization comparison for the interaction between commercial and wild varieties x El Pino and Esmeralda farms.

Varieties	Farms	% Colonization	Mycorrhization Structures (%)			
			Hyphae	Vesicles	Arbuscules	Total
Mora uva	El Pino	99.33a	16.10a	11.00bc	0.40e	27.50ab
MC without thorn	El Pino	96.00a	6.53b	6.86cde	9.06bc	22.43ab
MC with thorn	El Pino	96.00a	7.36b	22.30a	0.70e	30.36a
R. urticifolius	El Pino	76.00bc	2.00d	4.46f	5.73cd	12.23c
R. floribundus	El Pino	71.33c	5.20bcd	3.10e	2.83de	11.13c
Mora uva	La Esmeralda	92.00ab	3.90bcd	11.16bc	4.93d	19.96bc
MC without thorn	La Esmeralda	97.33 a	5.13bcd	13.10b	10.26b	28.56ab
MC with thorn	La Esmeralda	100.00a	2.33cd	9.33bcd	10.90ab	22.56ab
R. urticifolius	La Esmeralda	100.00a	5.06bcd	4.06f	14.56a	23.70ab
R. floribundus	La Esmeralda	100.00a	5.93bc	6.76cde	14.56a	26.93ab
CV%		6.53	21.39	23.26	17.50	14.82
TE*		3.50	0.73	1.23	0.74	1.93

MC: Mora de Castilla variety; Unequal letters in the columns differ for $p \leq 0.05$;
CV: coefficient of variation; TE: typical error.

Source: Authors.

Mycorrhization by hyphae also showed a statistical difference, being higher for the Mora Uva variety in the El Pino farm, respect to the other varieties and species in the El Pino farm. The lowest mycorrhization by hyphae was observed for *R. urticifolius* (2.00%) in the El Pino farm, but it did not differ statistically from the values exhibited by *R. floribundus* in that same farm and MC without spine, MC with spine and Mora Uva and *R. urticifolius* in the La Esmeralda farm.

The percentage of mycorrhization by vesicles showed the highest value for MC with spines in the El Pino farm, which was statistically different from the other varieties in the El Pino farm and in the La Esmeralda farm. The lowest value of mycorrhization by hyphae was observed for *R. floribundus* (3.10%) which did not differ statistically from the values shown by MC with spines, *R. urticifolius* and *R. floribundus* in the same farm and *R. urticifolius* and *R floribundus* at La Esmeralda farm.

The mycorrhization by arbuscules was higher for *R. urticifolius* and *R. floribundus* in the La Esmeralda farm, which did not differ with MC with spines on that farm, but with the rest of the native varieties and species. For the total mycorrhization, the lowest values were for *R. urticifolius* and *R. floribundus* in the El Pino farm, but they did not differ from Mora Uva variety in the La Esmeralda farm. The rest of the farms reached values higher than 20% of total visual mycorrhization.

These results show that farm factors combined with varieties or species can influence the levels of natural symbiosis, and that in general there was a tendency to be less colonization and mycorrhization structures for the native species *R. urticifolius* and *R. floribundus* in the El Pino farm in relation to the La Esmeralda, which did not occur in the commercial varieties.

The comparative analysis of the five varieties and species existing in the El Pino and La Esmeralda farms did not show significant statistical difference for the colonization percentage, nor for the percentage of mycorrhization by arbuscules and the total mycorrhization, but for the variables of mycorrhization by hyphae and vesicles. The mycorrhization by hyphae was higher for the Mora Uva variety without statistical difference with the MC variety without and with thorn and the *R. urticifolius* species. Regarding the mycorrhization by vesicles, it was higher for the MC variety with spines without statistical difference with MC without spines and Mora Uva (Table 6).

Table 6.
Colonization and mycorrhization comparison for commercial varieties and native species in the El Pino farm.

Varieties	% colonization	Mycorrhization structures (%)			
		Hyphae	Vesicles	Arbuscules	Total
Mora uva	95.66a	10.00a	11.08ab	2.66a	23.73a
MC without thorn	98.00a	5.83ab	9.98ab	9.66a	25.50a
MC with thorn	96.66a	4.85ab	15.81a	5.80a	26.46a
R. urticifolius	88.00a	5.56ab	4.93b	8.51a	19.03a
R. floribundus	85.66a	3.53b	4.26b	10.15a	17.96a
CV%	11.68	59.32	44.64	62.73	28.44
TE*	3.13	1.02	1.18	1.34	1.85

MC: Mora de Castilla variety; Unequal letters in the columns differ for p≤ 0.05; CV: coefficient of variation; TE: typical error.
Source: Authors.

The ANOVA result for the biological variables in the three wild species that were found in the El Pino farm, did not show statistical difference for the total colonization (between 71.33% and 84%), nor for the mycorrhization by hyphae (between 2.0% and 5.20%), by vesicles (between 3.10 and 4.46%), nor total mycorrhization (between 7.20% and 12.23%), only for mycorrhization by arbuscules showed a difference, with the highest value being 5.73% for *R. urticifolius* (Table 7).

Table 7.
Biological varieties in wild species of the El Pino farm.

Varieties	% colonization	Mycorrhization Structures (%)			
		Hyphae	Vesicles	Arbuscules	Total
R. alpinus	84.00a	3.73a	3.20a	0.30c	7.20a
R. urticifolius	71.33a	2.00a	3.10a	5.73a	12.23a
R. floribundus	76.00a	5.20a	4.46a	2.83b	11.13a
CV%	14.73	45.55	39.20	18.30	29.73
TE*	0.30	0.47	0.40	0.21	2.64

Unequal letters in the columns differ for p ≤ 0.05; CV: coefficient of variation; TE: typical error.
Source: Authors.

The higher percentage of mycorrhization in the El Pino farm, compared to the other farms, maybe due to the low agronomic practices that are used there, since soil is generally not tilled, but on the contrary, the plants are sown directly, they are hilled and the weeds are cleaned, leaving a lawn, which is manifested in the conservation of the soil structure, and therefore, of the associated microbiota.

The results showed that the colonization by mycorrhizae and the mycorrhizal structures do not present differences between native species, a result that is similar to that of the commercial varieties, despite the fact that these showed to have relatively higher values of colonization and mycorrhization structures.

Anthropic activity can affect both the dynamics and diversity of AMF [10]. In general, in these places where the native and commercial species and farms were evaluated, the anthropic activity is not high, which constitutes a positive factor in terms of the conservation of the mycorrhizal populations in the area.

In other studies it has been found that the use of mycorrhizae can help blackberry seedlings to tolerate medium concentrations of salinity in the soil, by allowing a greater absorption of nutrients such as phosphorus and a lower intake of sodium [11], very important aspects because most of the soils of Pamplona (Colombia) are low in phosphorus content [12], [13], although the situation with salinity is unknown.

On the other hand, it is suggested that environmental differences are also important factors that determine AMF spore density in ecosystems; It is known that the higher the temperature and the luminosity on the soils, the production of AMF spores increases [1], which could explain why commercial varieties present greater mycorrhization than native species located mostly in forested areas, and because in other studies carried out in coffee plantations, it was found that the abundance of AMF was independent of the degree of disturbance or type of land use under those specific conditions of low anthropic activity [14].

According to different American authors [15], [16], permanent changes in the edaphic environment reflect the existing dynamism which are observed in parameters such as humidity, temperature, nutrient availability, due to natural conditions or the effect of cultural practices to improve crop productivity. Additionally, the soil can suffer degradation and contamination processes with toxic chemicals for plants and microorganisms, so it is advisable not to use agrochemicals in the studied areas.

Under these conditions the development of AMF may be affected, finding that the soil environment can favor it at one time and reduce it at another, either by direct effects on AMF communities or indirectly by its effects on the host plant.

The present results indicate that studies should be continued to identify the AMF species present in the soil samples taken together with the roots, confirming the families, genera and species in each farm and variety or native species by morphological and molecular techniques. In addition, studies should be carried out in other places of the Pamplona municipality, both in commercial varieties and in native *Rubus* species to verify if the levels of colonization and mycorrhization structures are similar to those observed in the present study and to conserve the native species of mycorrhizae with potential use instead of chemical fertilizers and reduce environmental pollution.

It is necessary to verify the effects of native AMF species on commercial blackberry crops, since it is not enough that the colonization percentage is high, but rather the contributions that it makes to the plant in symbiosis, since in research carried out in greenhouse with blackberry seedlings it has been proven that the adequate inoculation of these beneficial microorganisms represents a viable strategy for the micropropagation processes of blackberry plants in the acclimatization phase [17].

The present results encourage to continue studies to obtain native strains of this area, multiply them and use them in obtaining blackberry propagation material in greenhouses or nurseries, especially in hardening centers, since some researchers [5], verified that the seedlings inoculated with AMF strains presented beneficial effects on growth and development such as accumulation of biomass, both in the aerial and root part, and positive effects on the foliar area and the bearing of the plants, as well as beneficial effects related to plant nutrition, expressed in the absorption of essential elements such as phosphorus, nitrogen, calcium and magnesium.

On the other hand, it is proposed to continue the research including comparisons with other farms, where a conventional blackberry production system is used, since verified that the farms with agroecological management provided adequate characteristics to support a higher density of vesicle arbuscular mycorrhizal spores than in the soils of farms with intensive agricultural management, being an important property for the structural stability of AMF populations [18].

IV. Conclusions

High levels of symbiosis of vesicle arbuscular fungi were found in the three commercial varieties of blackberry (colonization between 92% to 98%) and in the three wild species of *Rubus* sp. (colonization between 71% to 84%), as well as the presence of mycorrhization structures in blackberry varieties and native species.

The biological variables of the AMF (colonization and mycorrhization structures) did not vary statistically between the commercial varieties, but did between the different farms, presenting a statistical difference in the variety x farm interaction, with a tendency to be lower in the varieties located on the La Esmeralda farm, which presented lower height.

The biological variables of AMF in commercial varieties differed from wild species located on the same farm. There was also statistical difference between the farms and in the interaction variety-species × farm; however, the native species within a farm did not present statistical difference for these variables.

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Francisco Rodríguez Rincón. Universidad de Pamplona. Pamplona (Colombia). https://orcid.org/0000-0002-7955-7850

Leonides Castellanos González. Universidad de Pamplona. Pamplona (Colombia). https://orcid.org/0000-0001-9285-4879

Ana Adelaida Becerra Caicedo. Universidad de Pamplona. Pamplona (Colombia). https://orcid.org/0000-0001-9545-5953

Leidy Yohana Leal Montes. Universidad de Pamplona. Pamplona (Colombia). https://orcid.org/0000-0001-5517-8947