Arbuscular Mycorrhizal Fungi (AMF) and their possibilities for cleaner blackberry production

Los Hongos Micorrícicos Arbusculares (HMA) y sus posibilidades para una producción más limpia de zarzamora

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Yerson Aranguren Ariza
Universidad de Pamplona. Pamplona (Colombia)
yersonarangure@gmail.com

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

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

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Abstract

Introduction— Blackberry (Rubus glaucus Benth) is among the promising fruit trees of great commercial importance in Colombia, and although it is proposed that the use of mycorrhizae in blackberry plays an important role in sustainable agroecosystems, there is no updated information on the subject.

Objective— To provide comprehensive and updated information on arbuscular mycorrhizal fungi in interaction with fruit species with emphasis on the cultivation of blackberry (*Rubus* sp.).

Methodology— A documentary investigation of articles was carried out with updated information that indicated the approach to Arbuscular Mycorrhizal Fungi (AMF) in blackberry, using as units of analysis the articles published by national and international journals and the databases selected during the period. from 2010 to 2020.

Results— A total of 20 original articles published were found, distributed like this: national magazines, 6 articles; international magazines, 7 articles; and in databases, 7 articles.

Conclusions— The use of AMF in fruit trees is encouraging in Colombia and to a certain extent also in the cultivation of blackberry, however, it is necessary to deepen on aspects related to the response capacity of each crop to mycorrhizal symbiosis, turning this into an obstacle to the larger-scale application of this biotechnology, as well as increasing scientific research on native AMF species and their mycorrhizal potential for achieving cleaner and more sustainable agriculture

Keywords— Fruit trees; nutrition; Rubus glaucus; symbiosis

Resumen

Introducción— La mora (Rubus glaucus Benth) se encuentra entre los frutales promisorios de gran importancia comercial en Colombia, y aunque se plantea que el uso de micorrizas en mora juega un papel importante en agroecosistemas sostenibles, no existe información actualizada sobre el tema.

Objetivo— Proporcionar información completa y actualizada sobre hongos micorrízicos arbusculares en interacción con especies frutales con énfasis en el cultivo de mora (*Rubus* sp.).

Metodología— Se realizó una investigación documental de artículos con información actualizada que indicaran el abordaje de los Hongos Micorrízicos Arbusculares (HMA) en zarzamora, utilizando como unidades de análisis los artículos publicados por revistas nacionales e internacionales y las bases de datos seleccionadas durante el periodo. de 2010 a 2020.

Resultados— Se encontraron un total de 20 artículos originales publicados, distribuidos así: revistas nacionales, 6 artículos; revistas internacionales, 7 artículos; y en bases de datos, 7 artículos.

Conclusiones— El uso de HMA en frutales es alentador en Colombia y en cierta medida también en el cultivo de mora, sin embargo, es necesario profundizar en aspectos relacionados con la capacidad de respuesta de cada cultivo a la simbiosis micorrízica, convirtiéndose esto en un obstáculo para la aplicación a mayor escala de esta biotecnología, así como incrementar la investigación científica sobre las especies nativas de HMA y su potencial micorrízico para lograr una agricultura más limpia y sostenible.

Palabras clave— Frutales; nutrición; Rubus glaucus; simbiosis

I. Introduction

The blackberry (*Rubus glaucus* Benth) is among the promising fruit trees of great commercial importance in Colombia [1]. It is a traditional crop product and its production volume has grown considerably in recent years, in addition to the fact that the Colombian blackberry variety is highly valued for its characteristics from the point of view of its contribution to human health [2].

The main blackberry-producing department in Colombia is Cundinamarca, which represents 26% of the annual production volume, followed by Santander and Antioquia, which contribute 22.8% and 6.2%, respectively. Blackberry production at the country level has increased significantly, so much so that between 2015 and 2018 the area planted in blackberry crops increased by 1.3% per year on average, reaching 15 696 hectares for the last year, with a production which amounted in the last year (2018) to 130,000 tons nationwide [3].

During the production of the blackberry crop, some of the main limitations are related to phytosanitary problems that originate in the seed, due to the fact that most of the established crops use asexual propagation through layers or stakes, which transmit fungal, bacterial and viral diseases. among the plants that cause great losses to the farmer [4]-[6].

On the other hand, many practices of conventional modern agriculture, characterized by the high use of inputs, have brought instability to agricultural production systems, becoming contaminants for biotic systems (mainly plants) as well as environmental pollution (soil, air and water), threatening its stability [7].

The use of beneficial microorganisms such as mycorrhizae play a fundamental role in the purpose of obtaining a cleaner agriculture; among them, the fungi that form Arbuscular Mycorrhizae (AMF) stand out. Mycorrhizal "fungal roots" are symbiotic associations between specialized soil fungi and plants. So far, seven types of mycorrhizae are recognized, but several are very similar and interact symbiotically with about 80% of terrestrial plants, with which they are capable of forming vesicular-arbuscular mycorrhizae, found in almost all terrestrial ecosystems [7], [8].

The fundamental bases on which the arbuscular mycorrhizal symbiosis is established are nutritional. The plant supplies to the fungus carbonaceous compounds from photosynthesis, while the latter provides the plant with mineral nutrients, especially those that are less available, by virtue of the greater accessibility of the external mycelium of the fungus to more distant soil resources in the soil. Therefore, the economic importance of the mycorrhizal association lies in the harmonic relationship of nutritional support that is established between both organisms, with the bidirectional flow of nutrients [9]. Various studies have shown the beneficial effects of the symbiotic association between AMF and plants [10]-[13]. Some authors point out that plant nutrition is improved and plant tolerance to nematodes is increased [14].

In relation to this, some authors mention that AMF intervene in the growth and successful establishment of cultivable species, helping in the biological control of pathogenic fungi and nematodes, providing resistance to different stress conditions together with other edaphic microorganisms [15].

In nutrition, mycorrhizal fungi provide the plant with nitrogen (N), as well as poorly soluble substances such as potassium (K), copper (Cu), zinc (Zn), magnesium (Mg) and iron (Fe). In addition, different studies have shown that the AMF-plant symbiosis can reduce the translocation of Heavy Metals (MP) in different plant species, which means that the use of these fungi could be a biotechnological tool for managing agricultural systems with contamination by PM [16]-[19].

The use of mycorrhizae in blackberry plays a favorable role in sustainable production, since it can facilitate the acclimatization of plants growing in greenhouse conditions, which has been reported to improve the yields of plants that are subsequently subjected to stress. environmental [20], [21].

The adequate inoculation of these beneficial microorganisms represents a viable strategy for the micropropagation processes of blackberry plants in the acclimatization phase [22]. It is suggested that this has an positive impact on obtaining clean blackberry seed in the hardening phase, from tissue culture seedling inoculated with arbuscular mycorrhizal fungi, to improve plant survival and reduce the use of fertilizers [1].

That is why the implementation of arbuscular mycorrhizal fungi for agriculture in recent years is increasing, as a sustainable biological alternative for the improvement of agricultural production. Taking this background into consideration, the objective of this study was to provide comprehensive and up-to-date information on the fungi that form arbuscular mycorrhizae in interaction with fruit species, with an emphasis on blackberry (*Rubus* sp.) cultivation.

II. Methodology

The present investigation was carried out in the period between July 2020 and September 2020. A descriptive study was carried out [23], where the units of analysis were the articles published by the selected journals during the period from 2010 to 2020. divided into three stages:

A. *Compilation of updated information on arbuscular mycorrhizal fungi in interaction with blackberry species (Rubus sp.)*

A compilation of articles was made in Agrosavia magazine, Colombian Horticultural Science magazine, Mesoamerican Agronomy, Universitas Scientiarum, Agronomic Act, Colombian Agronomy, Colombian Biological Act, Bioagro, UDCA magazine and those of foreign magazines, Tropical Crops, Pastures and Forages, Agricultural Center, Remexca, Pastos Tropicales and Terra Latinoamericana, searching with two groups of keywords: mycorrhizal-arbuscular fungi and mycorrhizal-blackberry. Subsequently, the same keywords in Spanish and English were used in the databases Scopus, Springerlink, Science Direct and Francis and Taylor, from the facilities offered by the library of the University of Pamplona (Colombia).

In this descriptive study, through document analysis, we proceeded to search for and extract all those articles of a theoretical or empirical nature whose title will indicate the approach to AMF in blackberry.

B. *Comparison of the level of existing Colombian information on the benefits of arbuscular mycorrhizal fungi in blackberry species*

Comparisons and assessments were made on the information collected in Colombian journals and on studies carried out in Colombia with respect to foreign journals and the results of other territories, looking for what studies have not been carried out in Colombia and could be recommended as new research topics.

C. *Suggested research topics on arbuscular mycorrhizal fungi in interaction with blackberry species*

The results of the articles were analyzed with a view to making suggestions based on the information consulted in national journals focused on topics that are not found in them and that is why it is necessary to address new research topics.

III. Results y Discusion

Directly related to the development of the activities carried out in the three phases of the methodology, the following conditions have been determined regarding Arbuscular Mycorrhizal Fungi (AMF) and their possibilities for cleaner blackberry production.

A. *Results of exploration at national and international level.*

The search for articles in journals and databases yielded a total of 20 original articles published in the period between 2010 and 2020 (all in the first 10 years), distributed as follows: through Google and Google Scholar, 7 articles were located in national magazines; 6 articles in international journals; and 7 articles in journals from the database of the digital library of the University of Pamplona, which were selected according to the approach of the HMA. The selected articles corresponded to topics where the benefits of AMF in fruit trees and blackberry cultivation are evident.

B. *Arbuscular Mycorrhizal Fungi (AMF)*

AMFs are associated with most plant species under natural conditions [24], It is estimated that more than 90% of plant communities on the planet have the characteristic of forming mycorrhizal symbiosis. This corroborated what had already been stated in relation to the ecosystems considered to be AMF as those with the greatest worldwide distribution, both due to the large number of possible hosts and their geographical distribution, since they have been reported from the Amazon, where they are predominant, up to the Arctic [25].

The use of mycorrhizae in agriculture is increasing, their multiple benefits in agroecosystems promote their application in agricultural systems, in addition, another important aspect derives from the economic part, since in relation to chemical fertilization, mycorrhizae provide an advantage being cheaper, which allows their access to low and medium-scale producers [24].

According to this, during the documentary review, various works related to AMF in agriculture were located, involved in different research topics in different crops [22], [26]-[40]. Many of these works were aimed at topics where positive results are highlighted with the application of AMF alone or in consortia, as well as its application with other microorganisms.

In the majority, the effects on crops are ratified when this symbiotic association is established in different aspects related to their nutrition and protection, since AMF colonize the intraradical tissue of the host plant, developing characteristic structures of the symbiosis (arbuscules and vesicles) (Fig. 1), as well as extraradical mycelium, which interacts with the rhizosphere ecosystem and is responsible for the absorption of nutrients from the soil [41].

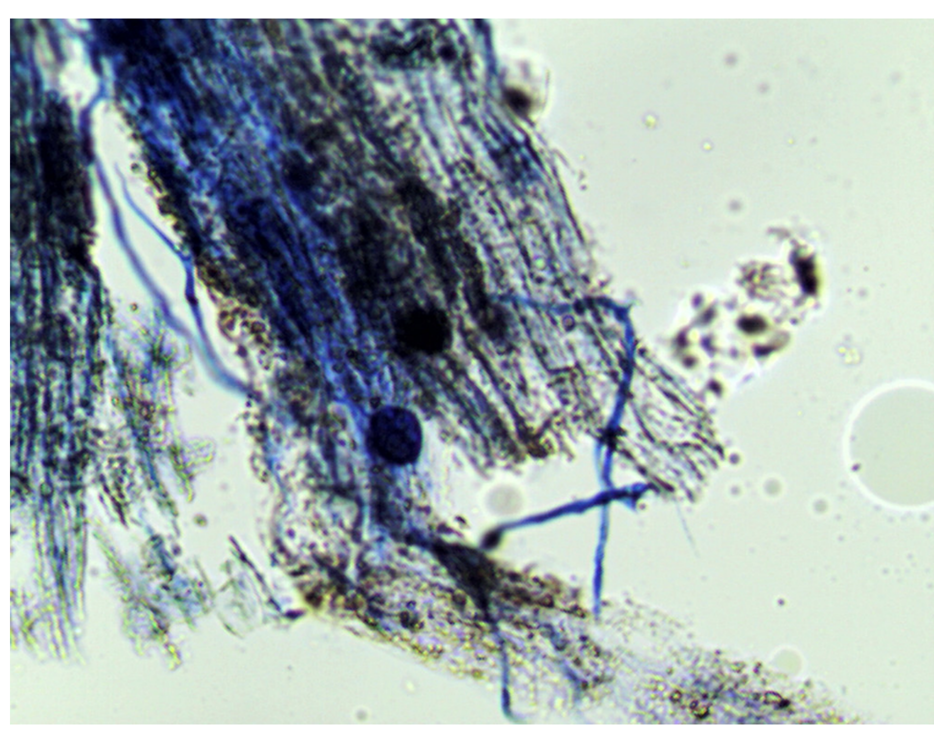

Fig. 1. Blue colored hyphae, vesicles and arbuscules of an AMF within a grass root.
Source: [42].

C. *AMF in fruit trees worldwide*

In general, the information collected on the application of AMF in fruit species worldwide is not extensive in the last decade, however, several studies related to this interaction in crops of interest such as avocado, guava and papaya were found. Although the information found is not abundant, the results of the investigations positively show the applicability of AMF in these fruit trees.

In avocado, mycorrhization had positive and significant effects on the development of inoculated plants compared to non-mycorrhized plants, showing an increase in growth variables such as a greater number of leaves and foliar area, greater fresh weight of the aerial part and greater height [36], in addition, greater development is provided by increasing plant height up to 54%, stem diameter (up to 36%), number and length of leaves (48% and 40% respectively), as well as root fresh weight (up to 85%) [43].

It was reported that for the stem diameter variable in this crop, the use of *Scutellospora pellucida* strains is recommended and for a better performance in height of *Acaulospora delicata* plants [37]. Likewise, it has been shown that the use of efficient AMF strains stimulates the development of avocado rootstocks under nursery conditions, which has repercussions on obtaining higher quality plants and, in turn, constitutes a nutritional alternative for this crop [38].

In results from previous years, it had been reported that AMF generated a significant increase in the absorption of mineral elements (N, P, K, Mg, Cu and Zn) induced by AMF and also increased the amounts of carbohydrates in the part aerial of plants [44].

Similarly, the species Scutellospora heterogama, *Acaulospora scrobiculata*, *Glomus etunicatum* and *Glomus clarum* are more effective in root colonization and in promoting better vegetative growth of plants in the rootstock phase, during the plant production period and after transplanting to the orchard [45]. In other studies, it had also been determined that the application of fungi of the genus *Trichoderma* for the control of phytopathogens of the avocado root favor the increase of AMF spores in the rhizosphere of this crop [39].

Regarding the benefits of AMF in guava [30], it is mentioned that a 25% reduction in mineral fertilization can be achieved. The use of AMF as growth promoters has shown a differential effect when they have been inoculated with native AMF, generating better development and quality of the guava plant, also finding that the growth of guava plants propagated by seed increased when they were applied some mycorrhizal consortium [35]. These results allow promoting the use of AMF as a practice that could be recommended for the sustainable production of guava plants under nursery conditions.

On the other hand, research in papaya cultivation shows that the results obtained in the advances in the application of AMF show their benefits. In several investigations it was verified that the plants inoculated with AMF showed increases in growth with respect to the plants without inoculation, taking better advantage of the available P of the soil and its adaptation in the field [31], [32], responding positively to the inoculation and its importance of mycorrhize in the nursery, before transplanting in the field, significantly favoring the growth of papaya plants without the addition of P through inoculation with Glomus sp. Zac-2 [33].

Early mycorrhization in papaya crops reduces the need for P fertilization during the nursery stage, obtaining benefits expressed in terms of plant development and nutritional status, which are significantly greater than those derived from the application of P, in general. Mycorrhizal papaya plants exhibit higher shoot biomass and macroelement contents than plants without mycorrhizae at any P level [40]. In economic terms, this can represent savings for nurserymen, by reducing or eliminating the application of phosphate fertilizers and reducing the time the plant stays in the nurseries.

In addition, the use of AMF consortiums promotes papaya growth and, therefore, could be used in the nursery or greenhouse phase [34], since papaya cultivation depends considerably on its association with arbuscular mycorrhizal fungi, especially in P-deficient substrates [32].

In peach nurseries, studies had also been carried out since the beginning of the century where positive results had been obtained with the arbuscular mycorrhizal symbiosis, as a way to improve the health and nutritional status of plants [46].

D. *AMF in fruit trees in Colombia*

The compilation of documents related to AMF in fruit trees in the last 10 years at the country level is very scarce, only information was found in crops such as avocado and custard apple and banana. This indicates the lack of research on the benefits of AMF in other crops and applied to various topics. The scarcity of studies based on this interaction limits obtaining results that could be adapted to various crops of economic interest for the country, since fruit production is increasing rapidly in recent years, however, the documents found positively show the application of the HMAs.

However, the inoculation of arbuscular mycorrhizal fungi in fruit trees in Colombia show encouraging results in banana. The implementation of AMF combined with biofertilizers contributed to the growth and establishment of seedlings [47].

In the avocado, for example; prior to the current decade, greater resistance to transplant stress had already been reported, because AMF improves water absorption capacity [48], and also favors the accumulation of total biomass in the nursery phase [49]. It is pointed out that the most developed morphological type of colonization in avocado corresponds to the "Paris" type, characterized by the extensive development of intracellular hyphae, which extend directly from cell to cell, within the root cortex [28].

On the other hand, within the benefits of the use of AMF in cherimoya, it was determined that they contribute to improve the absorption of nutrients, mainly phosphorus, and increase dry matter, which is reflected in the development of the seedling. In addition, it is expressed in increased seedling survival; increased plant growth in less time, thus reducing the time spent in the nursery, savings in fertilizer costs; and increases in production and product quality [29]. This confirms what was stated in general for AMF by other authors [50].

The results in Colombia also correspond to what was pointed out by some researchers in the sense that most fruit tree species require a relatively long period of growth in the nursery before the definitive transplant. At this stage, the use of AMF shows great potential because they play an important role in the growth and nutrition of fruit species [51].

E. *HMAs in blackberry globally*

Studies on AMF related to fruit trees and in particular to blackberries at the international level have not been registered in the last 10 years, during the collection of information no articles or works related to this interaction were located. This indicates that countries where the blackberry is native, such as Ecuador, Mexico or Honduras [52], have not been interested in researching the subject, which, from this, it is not possible to have a clear idea about the benefits of arbuscular mycorrhizae in blackberry cultivation, therefore, limits the investigation to obtaining information only at the national level.

F. *HMA in blackberry in Colombia*

Regarding the country level, the published information on the relationship of AMF in blackberry is very limited, however, the results obtained by these investigations are very positive and viable to be applied in the productive processes of this crop. The limitation in documents on the subject indicates a lack of research related to arbuscular mycorrhizal fungi.

The studies carried out in the country positively show the applicability and viability of HMA in default. A system for in vitro mycorrhization in blackberry plants was evaluated, where the micropropagated plants were successfully associated, for the first time, with an arbuscular mycorrhizal forming fungus under in vitro conditions, allowing the development of the HMA *Glomus* sp symbiotic system, associated with the roots of micropropagated Castilla blackberry seedlings [26].

The inoculation of these fungi in micropropagated blackberry seedlings favors the establishment, development and vigor of the seedlings, and can also improve their survival (≥ 80%) in the hardening and acclimatization stages [22].

More than 10 years ago it had already been pointed out that during the stages of acclimatization and hardening of blackberry microseedlings, the inoculation of native AMF classified as *Glomus* sp. and *Acaulospora* sp. presented beneficial effects on growth and development and better adaptation to the environment, expressed in size, accumulation of foliar and radical biomass, greater leaf area and better nutritional status expressed in greater absorption of essential nutrients (P, N, Ca and Mg) [1].

However, more recently it has been found that the use of mycorrhizae can help blackberry seedlings to tolerate medium concentrations of salinity in the soil, by allowing greater absorption of nutrients such as phosphorus and less sodium intake [27].

It had also been pointed out that, among other benefits, AMFs promote the transport of key nutrients in the aerial growth of blackberries, such as nitrogen, allowing a greater accumulation of dry matter in aerial organs. It was also determined that mycorrhizae allowed blackberry seedlings to absorb potassium to a greater extent, instead of absorbing sodium (mycorrhizae allowed greater selectivity for this nutrient) [27].

In this way, it has been confirmed that the adequate inoculation of these beneficial microorganisms represents a viable alternative for the micropropagation processes of blackberry plants in the acclimatization phase, since these plants could later be transplanted in the field, which would contribute to an easy adaptation to different environmental conditions as referred by some authors [26].

G. *Assessment of the level of information.*

According to the information, at the national level, 7 articles were found, of which 4 correspond to the cultivation of blackberries and 3 to fruit trees, respectively, while at the international level the 8 articles located are related only to fruit trees. This indicates that, in the country, research on AMF in blackberry cultivation has advanced due to the fact that its economic importance has grown vertiginously in recent years, generating a growing interest in the beneficial arbuscular mycorrhizal fungi, while, in the last 10 years, no research was found related to AMF in blackberry, which indicates that countries like Mexico, one of its main producers worldwide, have not generated any interest in studying this relationship. On the other hand, the information on AMF in fruit trees in the country is very limited, while at the international level more research has been done on the subject in various fruit trees, indicating a greater interest [53].

Regarding the studies carried out at the national level in fruit trees, only the relationship of AMF in avocado, cherimoya and banana crops has been investigated, where the results have been promising in terms of their effectiveness in the application, this somehow means an advance in research on the subject, however, much more could be investigated on the effectiveness and applicability of these fungi in other crops of economic interest for the country. At the international level, research has been carried out on other crops such as avocado, guava, peach and papaya.

H. *Suggestion on research topics.*

In this exploration, it was found that, in Colombia, although there are a number of works carried out on AMF in arrears, there is also a deficit due to the study of some topics. Likewise, there is also much to investigate in other crops of great interest. The foregoing and in light of the objective of this article, to highlight the advances of the Colombian scientific community that conducts research on AMF. It is evident that although it is not enough, at least there is a path traveled, but it is longer the path of what remains to be investigated, since, when comparing progress with international results, the list of pending issues is longer.

Therefore, research should be carried out on topics related to; the use of native strains of AMF in commercial blackberry for the elaboration of biofertilizers, which could present greater possibilities of effectiveness in the field, because they are adapted to the soil conditions of each region. Research on other native communities of mycorrhizal fungi in commercial blackberry, the mycorrhizal potential of the soil and its nutritional levels, in order to enhance its use in blackberry.

Research on the application of AMF in blackberry at the field level, on issues related to their nutrition and protection against pathogens most be increase. Thus, neither have AMF surveys been carried out in native blackberry species, which could be recommended for their application in commercial blackberry.

No research has been carried out on the morphological and molecular identification of native strains of *Glomus* sp. and *Acaulospora* sp., which have been used in blackberry. There are no investigations beyond the use of commercial strains in blackberries such as *Glomus* sp., *Entrophospora colombiana* and *Acaulospora mellea,* other commercial strains have not been used in blackberries such as *Scutellospora heterogama, Acaulospora mellea* and *Glomus* spp. Likewise, there is no bank of native strains of the AMF species collected in blackberry that allows for solid sustainability of the results obtained.

Similarly, no research has been found so far that has defined the native or commercial AMF species that are most effective in the case of blackberries, nor have research results been seen on which are the commercial bio-products of Colombia. more effective for blackberry.

In relation to this, it is suggested to carry out research to determine the effect of the different commercial species of mycorrhizae for blackberry.

Also, investigations are suggested to determine which are the species in commercial blackberry that best results are obtained before the inoculation of native or commercial AMF, which also leads to suggesting investigations of the native AMF species with the commercial species and carrying out a detailed prospecting. of native species in blackberry.

Finally, it is suggested to investigate the ecology of mycorrhizal species and how they adapt to the soil climate, since knowledge about the interactions between edaphic conditions and the ecology of native AMF is still limited.

IV. Conclusions

The use of AMF in blackberry in the country is encouraging, but aspects about the response capacity of each crop to mycorrhizal symbiosis remain to be defined, becoming an obstacle to the larger-scale application of this biotechnology for commercial purposes.

The inoculation of native spores of *Glomus* sp. and *Acaulospora* sp., has shown beneficial results for inoculation in commercial blackberry.

The lack of scientific research on the AMF species and their mycorrhizal potential limits further promoting their use in clean and sustainable blackberry agriculture, despite the existing history in other fruit crops and to a certain extent in blackberry.

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Yerson Aranguren Ariza. Universidad de Pamplona. Pamplona, Colombia). https://orcid.org/0000-0002-5245-1837

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

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