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Can we predict arbuscular mycorrhizal inoculation effects on vine plants? †

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Abstract: Scientific literature has demonstrated for more than 50 years the positive effect of arbuscular mycorrhiza fungi (AMF) on plant growth and stress tolereance. But it has been in the last 10 years that its application was implemented in agricultural systems. Recent reviews point to AMF as key to viticulture. In order to identify the most effective mycorrhizal species and detect the most dependent rootstocks on inoculation we reanalyzed published experiments were AMF were inoculated in vine plants. We created a database where we included all the results comparing the development of vine plants that have been inoculated with AMF against a control. We calculated inoculation dependence ID = ((Mean of inoculate treatment - Mean of control)/Mean of inoculate treatment) *100) to compare the effect of AM inoculation on the vine between very different experiments. Only two species of mycorrhiza (*Rhizophagus irregularis* and *Funneliformis mosseae*) have been studied on the same rootstock measuring the same variables in more than one study. No differences were found in ID of the different rootstocks, for the majority of measured response variables, when all the AMF species were analyzed together. The results obtained showed that plants cannot always benefit from AMF inoculation, and the effect of mycorrhizae can be positive or negative. The effect of mycorrhizal inoculation in vineyards is context dependent. This study has shown the need for prior pilot testing to determine the effect of a specific mycorrhizal species on certain rootstocks under specific growing conditions before their use can be recommended.

Keywords: rootstock; mycorrhiza; viticulture

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1. Introduction

Roots of most plants show mutualistic symbiosis with certain soil fungi name mycorrhizas. More than 200.000 cultivated and uncultivated plants are living in symbiosis with these fungi [1]. Scientists described seven types of mycorrhizae, but the most common are arbuscular mycorrhizae fungi (AMF) [2]. AMF belongs to the Glomales order of Zygomycetes, these colonize the root biotrophic and extend a mycelium outside the root system forming a complex net [3]. AMF has always been a key link in moving water and mineral nutrients from the soil to the plants in exchange for photosynthetic products [1,4]. Fungal hyphae are much thinner than roots, therefore they can penetrate small pores and acquire nutrients inaccessible to the plant's roots [5,6]. In addition to improved nutritional supply, AMF has many other positive effects on the plant in abiotic stresses, such as water stress [7], soil salinity [8] and biotic stresses, such as root diseases caused by necrotrophic pathogens, herbivorous arthropods or nematodes [9].

In order to maintain a high level of AMF in soils, Berruti et al (2016) suggested introducing AMF into the target soils to achieve this goal. The use of (AMF) inoculum recorded a good development and performance in several plants and soil conditions [10]. The mycorrhizal biotechnology has been widely used in horticulture and agriculture plant production [11,12]. AMF inoculum is mainly obtained from *in situ* systems that could be from *in vivo* cultivation on the roots of plants, whether in pots [13] or in the field of nurseries or farms [12,14].

Assembling the right consortia of plant phenotype and rhizosphere microbiome has also been postulated as one of the means for a new underground revolution that aims at an ecological intensification in agriculture [15]. This approach is promising but holds intrinsic associated risks [16]. The benefits of mycorrhizal inoculum can be highly context dependent [17] resulting in a high variability in plant response to AMF inoculation and a lack of trust in its general efficiency by the agricultural community.

In the case of viticulture, many studies have demonstrated the value of AMF maintaining healthy vines and grape quality [7,18]. These studies argue that strengthening the mycorrhizal community in the vineyard can provide significant benefits in a context of climate change [7,18]. However, specific information on the impact of the use of mycorrhizal inoculum on vineyard plants is not available.

The objective of this work is to analyze the results obtained in published research comparing the development of vine plants inoculated with AMF against a control. The aim is to determine whether the existing information allows us to identify the most effective species of mycorrhizal fungi for improving grapevine performance, as well as to detect those rootstocks most dependent on inoculation.

2. Materials and Methods

To build a database, searches were conducted for articles published from 1980 to 2019 in the Google Scholar. Keywords used were the following: mycorrhiza*, inocul*, vineyard*, rootstock*. The use of Boolean truncation '*' character ensured that we have variations of the word. For example, mycorrhizae, mycorrhizas, mycorrhizal, were included in our search.

From more than 3000 articles, we selected only ones that include the addition of a mycorrhizal inoculum comparing it with a control treatment in vine plants. We exclude the articles that analyze the natural mycorrhizal colonization, focusing on analyzing the effect of AMF on disease resistance, articles with no present control, articles with not growth measurements or articles that no specify important information like growth conditions. The bibliographic references of the articles were used to find new works focused on the subject. Languages accepted were: English, Spanish, Portuguese German and Chinese.

From each study, we collected data on plant performances, with and without mycorrhizal inoculation considering: country: where the work has taken place; type: whether it is a greenhouse, outdoor conditions or field; the rootstocks used in the experiment; the species of AMF used in the experiment and the response variable measure.

To compare plant performances with and without inoculum, we extract the mean value of biomass, plant size or other growth measures. When the publication provided the dry weight separately from roots and stems, these were added to calculate the total dry weight. In some cases, the control was plants growing in a sterile substrate, but in other control growth under natural condition so presented a natural colonization by AMF, both studies were included.

To compare between studies, we calculated the degree of plant change associated with the inoculation of arbuscular mycorrhizal fungi expressed as Inoculation Dependency (ID), following the same calculation method than the mycorrhizal dependency [19].

$$ID (\%) = 100 (Xi - Xn)/Xi,$$

where Xi is the mean value of the response variable of mycorrhizal inoculated plant and Xn is the mean value of the response variable of non-mycorrhizal inoculated plant.

Different plant response variables were used as total dry and fresh biomass, shoot dry and fresh weight, root dry and fresh weight, the total number of leaves and total leave area.

Most publications conducted several experiments. We included all those results that compared inoculated vines against non-inoculated vines under the same conditions. In the cases that some plants were subjected to stress conditions, for example Cu contamination, P starvation or salinity, only the stress-free treatment data were included.

We describe the resulted data graphically using box plot diagrams to show data distribution among different studies in relation with: the genus of the AMF inoculated, the rootstocks. The diagrams that do not show dispersion and are shown as a horizontal line are due to the presence of only one data from one study.

3. Results

We end up having 23 publications, including one master thesis, with 106 experiments where more than one response variable was measured. Articles included were published in different journals such as the European Journal of Horticulture Science, Asian Journal of Crop Science, Vitis, South African Journal for Enology and Viticulture, Acta Horticulture, or Frontiers in Plant Science. Only the two more common species of mycorrhiza in inoculum (*Rhizophagus irregularis* and *Funneliformis mosseae*) have been studied on the same rootstock measuring the same variables in different experiments. This makes it difficult to perform statistical analysis and therefore, graphical comparisons considering a heterogeneous group of assays were made.

Among the articles used for this review, 15.38% were complete in field conditions and 76.92% in the greenhouse. Pots under no controlled ambient, were less common, 11.53% of the articles. In this review, different responses of vines to AMF inoculation, with 15.88% of negative responses and heterogeneous positive responses (ID from 0.76 to 88.52) were found.

Most of the experiments measured different response variables, which showed different IDs (Fig. 1). Comparing the more common ones, most of them showed clear positive effects, but with different dispersion (Fig. 1). Although all median values are positive, only the number of leaves showed no negative values.

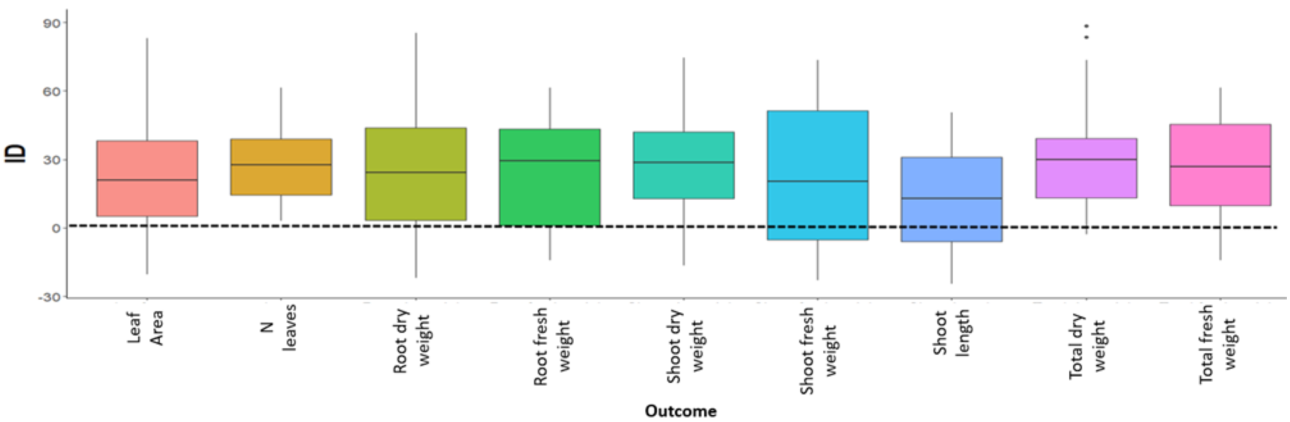

Figure 1. Degree of plant biomass change associated with the inoculation of arbuscular mycorrhiza **Figure 110.** Richter and 1103P present some negative effect (Fig. 2). The rootstocks showing the greatest positive effect on shoot dry weight were 3309C, SO4, Pusa Navrang and FPS93. If the shoot dry weight is analyzed in relation to the different genera of AMF inoculated, it is observed that several species combination (Mix) shows a slightly more positive effect with respect to the rest of the experiments where only species of the same genera were used (Fig. 3).

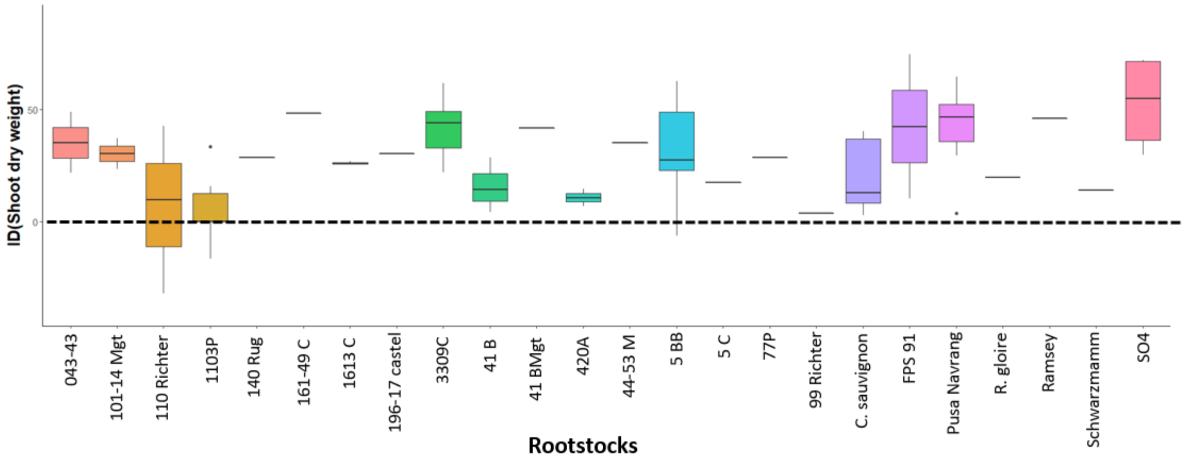

Figure 2. Different rootstocks shoot dry weight change associated with the inoculation of AMF expressed as Inoculation Dependency (ID). Lines in each boxplot represent the minimum (whisker), lower quartile, median, upper quartile, and maximum (whisker). The diagrams that do not show dispersion and are shown as a horizontal line are due to the presence of only one data from one study.

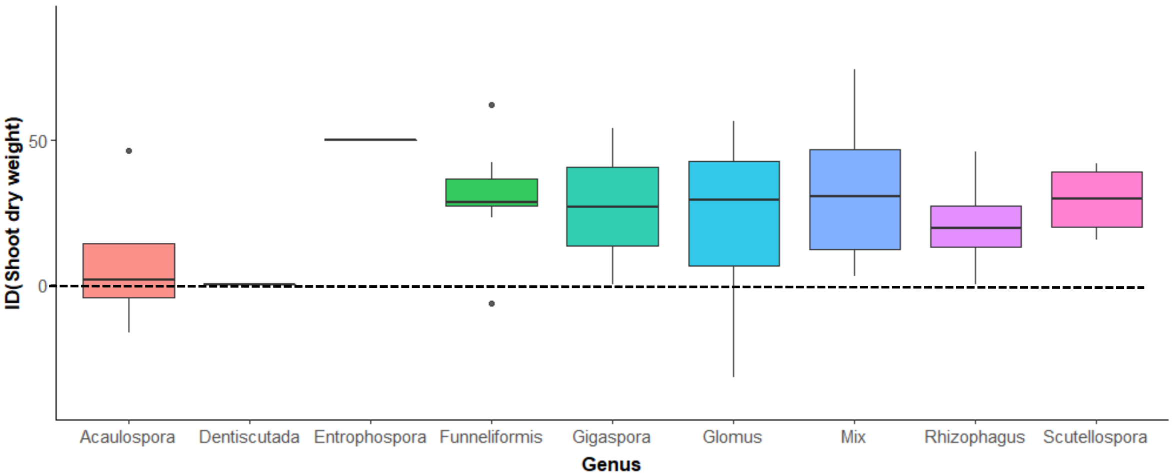

Figure 3. Shoot dry weight change associated with the inoculation of AMF expressed as Inoculation Dependency (ID) for the experiments made with fungus of different genera. Lines in each boxplot represent the minimum (whisker), lower quartile, median, upper quartile, and maximum (whisker). The diagrams that do not show dispersion and are shown as a horizontal line are due to the presence of only one data from one study.

4. Discussion

Mycorrhizal efficiency must be considered within a well-defined set of conditions: it depends on the fungal inoculate, rootstocks, as well as the measure variable. The results obtained show that plants can't always benefit from AMF, and the effect of mycorrhiza can be neutral or negative depending on specific experimental conditions. The measure parameters used as a response variable are crucial since not all the parameters showed the same response to inoculation. Other studies have shown that certain rootstock-AMF combinations can generate an increase, but also a decrease in certain specific parameters such as leaf area [20]. It has also been shown that certain inoculum have a greater effect on specific parameters than others [21].

Furthermore, fungal species preferences toward rootstocks can also affect mycorrhizal efficiency. *Glomus aggregatum*, for example, seemed to have a higher affinity for 161-49 Couderc than 196-17 castel [22]. Some studies have shown that the effect of mycorrhizae on grapevine depends on the combination between the inoculum and plant [23].

An important dispersion of results for inoculation experiments with the same genus or even with the same mycorrhiza species was observed. For example, Schreiner (2007), using the same grapevines, the same soil and the same AMF species found different effects depending on the origin of the fungi. Also, the same mycorrhizal type can have different levels of effectiveness depending on where it is used, under field or greenhouse conditions. In Camprubi's work (2008), *Glomus intraradices* had a more positive effect on grapevine under greenhouse conditions than under field conditions. In case of using an experimental inoculum, knowing what the origin of mycorrhiza (to what plants it was originally associated) used can make a difference. AMF that was originally associated with citrus has a more positive effect on vineyards than the same species was originally associated with grapevines [24].

Despite the use of arbuscular mycorrhizae is advocated as a potent solution to improve vineyard culture systems; our review shows that the effect of mycorrhizal inoculation in the vineyards is context-dependent. Several experiments showed neutral and even negative responses of certain combinations of rootstocks, mycorrhizae and environmental conditions are shown. The high heterogenetic among experiments make difficult to determine that inoculum presented more positive effects or which rootstocks are more positive affect by AMF. However, our data indicate that resistant rootstocks could be less favored by inoculation and the mixture of several AMF species could have more positive effects, while the species of the genus *Aculospora* more negative. This bibliographic review has demonstrated the need for previous pilot tests to determine the effect of specific mycorrhizal species on certain rootstocks in specific culture conditions before being able to advise its use a t a commercial scale.

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