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MYCORRHIZAL FUNGI *GLOMUS* SPP. AND *TRICHODERMA* SPP. IN VITICULTURE (REVIEW)

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Abstract

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Mycorrhizal fungi's *Glomus* spp. and *Trichoderma* spp. are natural components of the healthy vineyard soils. They have the ability to improve nutrition and root resistance to attack by pathogens, and contribute positively to soil structure and stability. Implementation of practices that favors their propagation should be important aspects of viticulture management. Mycorrhizal inoculations of grapevines are proposed as a biotechnological alternative of conventional systems to manage soil sickness problem and to maintain improved grapevine plant growth and health. The conducted studies in this review showed the beneficial effects of mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp. on grapevine growth/health status, plant nutrient uptake and soil fertility, as well on vineyard ecology in general.

Key words: *Glomus* spp., *Trichoderma* spp., viticulture, plant nutrient uptake, biocontrol

Introduction

Currently, in grape nursery and wine production sectors there is a widespread usage of chemical pesticides to repress plant pathogens, and of synthetic fertilizers to stimulate plant growth. Such treatments lead to a reduction of microflora complexity in the soil and to declining of the overall plant quality. While tolerance of various pathogens to pesticides increases, the soil impoverishment determines a reduction of yield and quality. The negative effects of pesticides and synthetic fertilizers is the soil sickness phenomena, whose symptoms usually follow the replant of fruit tree crops such as apple, peach, cherry, apricot, grapevine, etc. One of the main reasons is related to the disappearance of mycorrhizal fungi from the soil, which, in association with other antagonist microorganisms, are known to increase the absorbing surface of the root system, causing the improvement of nutrient uptake and plant growth in general, and an increasing plant resistance to pathogens (Schreiner and Bethlenfalvay, 1995). In order to reduce negative environmental effects and to increase soil fertility, the policies at European and Worldwide level tend to limit the use of chemical substances for both fertilization and plant defense. There is a necessity of innovating plant production processes to produce plant more tolerant to common pathogens and more efficient in using the natural soil resources (mineral elements, water, etc) in all the phases of cultivation, from nursery to vineyard.

Last decade numerous studies regarding the impact and effects of organic *versus* conventional systems on soil microbial dynamic have been conducted (Mäder et al., 2000a; Ryan et al., 2000; Ryan and Graham, 2002; Purin et al. 2006; Freitas et al., 2011). The results show that mycorrhizal fungi are important for the good nutrient status of young host plants in the nursery phase and during acclimatization period. The other significant result is the bioprotective effect of mycorrhization against soilborne fungal pathogens (Vierheiling et al., 2008). Moreover, several fungal pathogens are considered as a principal cause of soil fatigue in vineyards (Nogales et al., 2008), thus the mycorrhizal inoculation of grapevines is proposed as a biotechnological alternative of conventional systems to manage soil sickness problem and to maintain improved grapevine plant growth and health.

Mycorrhizal Fungi – *Glomus* spp.

Glomus spp. is the largest genus of arbuscular mycorrhizal (AM) fungi. All species form symbiotic relationships with plant roots. The AM symbiosis makes a significant con-

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tribution to the plant growth and nutrition (Smith and Read, 1997; Schüssler et al., 2001; Borde et al., 2009). The major effect on nutrition is result from the hyphal transport of immobile mineral ions. It is very important especially for the slowly diffusing mineral ions such as phosphorus (Smith and Gianinazzi- Pearson, 1988). Most of the mycorrhizal fungi used in viticulture are AM (Menge et al., 1983; Nappi et al., 1985; Schubert et al., 1988). The remarkable increasing in morphological parameters, nutrient uptake, phosphorus and chlorophyll content of grapevine plants after 60 and 120 days inoculations with AM *Glomus fasciculatum* was reported by Borde et al. (2009). The AM inoculated grape plant showed higher leaf number (50, 84% and 75, 29%), leaf area (254.43% and 209.96%), shoot length (22.91% and 34.88%) and root length (10.99% and 19.67%), over the non-inoculated control after 60 days and 120 days of AM inoculation, respectively. Meanwhile, plant phosphate content (430.79% and 525.13%) and total chlorophyll content (45.66% and 18.88%) also are accumulated substantially. AM fungal hyphae take up and transport N to hosts from inorganic N sources (Johansen et al., 1992; Hodge et al., 1998; Mäder et al., 2000b) and organic N sources (Hawkins et al., 2000; Hodge et al., 2001; Aristizabal et al., 2004). Indeed, AM fungi have been shown to enhance to a large degree N uptake of grapevines (Cheng and Baumgartner, 2004; Patrick et al., 2004).

Vineyard cover crops have been shown to enhance indigenous populations of AM fungi in vineyard soils and grapevine roots (Baumgartner et al., 2005; Cheng and Baumgartner, 2005).

Overlap of grapevine and cover crop roots may encourage interactions among grapevines, cover crops, and AM fungi, such as the formation of common mycorrhizal networks via AM fungal links (Leake et al., 2004). It was found evidence of direct nutrient transfer from cover crops to grapevines (Cheng and Baumgartner, 2004). N transfer was significantly greater from the grass, *Bromus hordeaceus* L. ssp. *molliformis* (Lloyd) Maire & Weiller cv. Blando, to the grapevine than from the legume, *Medicago polymorpha* L. cv. Santiago, to the grapevine. Certain cover crop species may be better than others at enhancing AM fungi-mediated nutrient transfer from cover crops to grapevines, possibly by hosting different AM fungal species or by supporting a greater root biomass. Cover crop management practices, specifically mowing and tilling, may affect AM fungi-mediated nutrient transfer from vineyard cover crops to grapevines (Cheng and Baumgartner, 2006). A pilot experiment was conducted to determine the effects of soil salinity and inoculation with *G. fasciculatum* on growth (shoot length, leaf number, internode length, and total dry weight), spore count and root colonization of 5 grape rootstocks. All the inoculated rootstocks indicating the beneficial role of mycorrhizal inoculation for improving plant growth and salt tolerance (Belew et al., 2010).

AM fungi, by forming symbioses with plants, can increase the availability of certain essential plant nutrients such as phosphorus (P), zinc (Zn) iron (Fe) and copper (Cu), which are considered to have slow mobility in the soil (Bavaresco and Fogher, 1992; Ortas and Rowell, 2004; Ortas and Akpinar, 2006; Ortas and Varma, 2007; Bucher, 2007; Schnepf et al., 2008). Many studies have shown that grapevines pre-inoculated with *Glomus* spp. fungi grow considerably faster than non-inoculated plants on sterilized growing media. The aim of such studies has been mainly to investigate the effects of *Glomus* spp. inoculations on nutrient uptake and grape yield and quality. (Bavaresco and Fogher, 1996; Nikolaou et al., 2003; Schreiner, 2003; Aguin et al., 2004; Karagiannidis et al., 2007; Almaliotis et al., 2008). Considering the results, reported by Ozdemir et al. (2010), *G. mosseae* appears to have a greater effect on shoot growth parameters, and *G. intraradices* appears to have a greater effect on root growth parameters and leaf P and Zn concentrations. Therefore, *G. intraradices* would be anticipated to aid vines to overcome element deficiencies in especially areas where P and Zn deficiency predominate. Kara et al. (2011) reported positive effects of *Glomus* spp. applications on vegetative development of grape cuttings. The authors used two commercial AM preparations as Mycosym® (*G. intraradices*) and MycoApply® (*G. mossae*, *G. intraradices*, *G. aggregatum* and *G. etunicatum*) which have been tested on young plants of table grape varieties and rootstock 41 B in greenhouses. Stomatal conductance, transpiration rate and midday xylem water potential were higher in the *G. etunicatum* hosts grapevine young plants during the transplanted period (van Rooyen et al., 2004). These results indicate that AM inoculation can influence the water relations, thereby improving photosynthetic performance and potential survival during the initial growth stages of the grapes. The researches carried out put in evidence an infective capacity of *G. intraradices* and a higher effectiveness to those of many other mycorrhizal fungi species. It colonizes the plant roots and provides them with mineral elements and water, by extracting from soil through an external net of hyphas, whilst the plant supplies the micro-organism radical organic compounds. The variety *G. intraradices* is autochthon of the Mediterranean basin, therefore it well fits to the climate of this region (Garcia-Garrido et al., 2002).

Glomus spp. enhances plant resistance to abiotic or biotic stresses (Sylvia and Williams, 1992; Hooker and Black, 1995; Pozo et al., 2009). Most studies on protection against soil-borne diseases report reductions in incidence of damages symptoms caused by pathogenic fungi from genera *Rhizoctonia*, *Fusarium*, *Verticillium*, *Phytophthora*, *Pythium* and

Aphanomyces. Whipps (2004) and Pozo et al. (2009) compiled detailed reviews of these studies. Similarly, a reduction in deleterious effects caused by parasitic nematodes, such as *Pratylenchus* and *Meloidogyne*, is common in mycorrhizal plants (de la Peña et al., 2006). Inoculation of the grapevine roots (*Vitis amurensis* Rupr.) with the AM fungus *G. versiforme* significantly increased resistance against the rootknot nematode *Meloidogyne incognita* by transcriptional activation of the Class III chitinase gene *VCH3* (Li et al., 2010). It is therefore necessary to identify which chitinase gene(s) is induced following inoculation with AM fungus, and to confirm whether this increase in gene activity mediates subsequent resistance of the mycorrhizal grapevines to nematodes. Petit and Gubler (2006) examined the influence of *G. intraradices* (INVAM CA 501) on grape black foot disease caused by the fungus *Cylindrocarpon macrodidymum* on *Vitis rupestris* cv. St. George under controlled conditions. Eight months following inoculation with the pathogen mycorrhizal plants developed significantly less leaf and root symptoms than nonmycorrhizal plants. Results from this study suggest that preplant applications of *G. intraradices* may help prevent black foot disease in the nursery and in the vineyard.

Indeed, progresses in basic knowledge of grapevine - *Glomus* spp. interactions, identification of markers associated with induced resistance, as well as the generation of predictive models for the outcome of particular interactions will have important practical implications in the biological control and integrated management of pests and diseases in viticulture.

Mycorrhizal Fungi – *Trichoderma* spp.

Trichoderma species are green-spored ascomycetes present in nearly all types of temperate and tropical soils. They can often be found in decaying plant material and in the rhizosphere of plants (Schuster and Schmoll, 2010). Colonization of the root tissues are only limited at the root cortex due to the deposition of callose which restrict the penetration of hyphae. The callose barriers made *Trichoderma* spp. become harmless to the plants (Vinale et al., 2008). Elicitors produced by *Trichoderma* spp. during penetration stimulate the activation of plant defense system, causing an increase in the production of defense-related plant enzymes, such as chitinase, glucanase, and enzymes associated with the biosynthesis of phytoalexins.

Weindling (1934) first reported the use of the fungus *Trichoderma* spp. as a biocontrol agent for controlling plant disease. Subsequently, many studies have shown that *Trichoderma* spp. is the most effective biocontrol agents for managing plant disease. *Trichoderma* controls the pathogen via a mycoparasitism process in which it grows towards the pathogenic fungi, soils around them, and secretes cell wall degrading enzymes that limit their growth (Vinale et al., 2008; Naher et al., 2012). Members of the genus *Trichoderma* are filamentous fungi that can be isolated from many soil types, being part of a healthy soil environment, and that have species found worldwide.

Failure to comply with phytosanitary requirements grapevine pruning/reconstruction open wounds often lead to so-called "trunk diseases" caused by *Eutypa lata*, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, *Fomitiporia mediterranea*, *Botryosphaeria* spp. and *Phomopsis* spp. (Halleen et al., 2005). *T. harzianum* and *T. atroviride* -based treatments of grapevine wood gave up to 90% decrease in colonization of many of these patogenes (Hunt et al., 2001; Hunt and Harvey, 2006; John et al., 2008; Mutawila et al., 2011). At the moment the prevention of pruning wounds infection by following correct cultural practices remain the main way to manage the disease but the possibility of introducing microorganisms such as biological control seems to represent an alternative or a complementary strategy (Pellegrini and Pertot, 2013).

Most commercially important cultivars of the European grapevine (*Vitis vinifera*) are highly susceptible to downy mildew caused by the obligate biotrophic oomycete *Plasmopara viticola*. Therefore, adequate control by fungicides is crucial, particularly in rainy climates. Copper-based fungicides are commonly used to control the disease in organic vineyards. Dagostin et al. (2011) identify that mycorrhizal fungi are highly effective against grapevine downy mildew, less toxic than copper and compatible with the principles of organic farming, as outlined by the International Federation of Organic Agriculture Movements (IFOAM, www.ifoam.org). *T. harzianum* strain T39 treatment enhanced the expression of defence-related genes in the responsive cultivars, before and after *P. viticola* inoculation. A positive correlation between the efficacy of T39 and the expression level of defense-related genes was found in Primitivo and Pinot noir grapevine cultivars (Banani et al., 2013). When the *Trichoderma*- biotized plants were challenged with a pathogen, defense gene expression and protective enzyme activity were enhanced compared with inoculated control plants (Brotman et al., 2012). Palmieri et al. (2012) proved that proteins affected by *P. viticola* in T39- treated plants (CL3) are mainly associated with response to stress, photosynthesis, redox signaling, and energy metabolism. Although T39 appears to be a promising alternative for controlling downy mildew in the vineyard, the key components of the defense mechanism need to be identified in order to better understand how this method of biocontrol functions and how to maximize its efficacy.

El-Mohamedy et al. (2010) sets that *Trichoderma harzianum* cultured on sugar cane bagasse, *Trichoderma har-*

zianum (spore suspension 5×10^6 cfu/ml) and plant guard (Biocide®), successfully controlled *Fusarium solani*, *F. oxysporium* and *Macrophomina phaseolina*, the main pathogens of root rot disease on grapevines. *T. harzianum* caused a reduction of 80.0, 84.4 and 88.9% of linear growth of the same pathogens respectively. Damage of root-rotting pathogen fungi is performed through the release of digestive enzyme chitinase, which dissolves the chitin in the cell wall of the pathogen. Once damaged, the pathogen itself becomes a prey of other soil organisms. *Trichoderma* spp. can also attack and parasitize other fungi.

Only a few selected strains of *T. harzianum* showed to suppress plant diseases, due to their not adaptability to different plant species and pathogens. To overcome these limitations, researchers at Cornell University produced a hybrid strain with enhanced vigor and a larger adaptability. It was called strain T-22 and it is the active ingredient of different commercial biocontrols. It works as a deterrent, protecting the root system from the attack of pathogenic fungi like those from genera *Fusarium*, *Pythium*, *Rhizoctonia* and *Thielaviopsis*. Because it is a living organism, *T. harzianum* T-22 can grow along the entire length of the root system along which it establishes a barrier against pathogen attack. As long as the root system remains active in its growth, *T. harzianum* T-22 will continue to grow feeding the released plant waste products. In this way, it subtracts the nutrient that pathogens might use to feed up. Plant tissue that is under attack before the application of T-22 spores will not experience the full benefits of using the biocontrol agent. In addition, enlargement of the root system, is more likely to explore more efficiently soil and to use water and nutrients. The better "plumbing" in the roots means that more of the nutrients will make their way to the leaves, flowers or fruits. Therefore, in addition to disease control, the T-22 increases the overall health and development of a plant (Harman, 2006; Woo and Lorito, 2007; Vinale et al., 2008). Honey fungus (*Armillaria mellea*) is an important pathogen that can cause severe damage to infected orchards and vineyards. Percival et al. (2011) reported that application of *T. harzianum* strain T22 (Trianum®) reduced *A. mellea* severity by 12.5 – 65.7% over the two-year field study. Findings of this study strongly indicate that air-spading followed by inoculation with *T. harzianum* T22 appears to offer promise as a joint cultural/bio-control strategy for the management of *A. mellea*.

Relatively recently, *Trichoderma* isolates have been identified as being able to act as endophytic plant symbionts. The strains become endophytic in roots, but the greatest changes in gene expression occur in shoots. These changes alter plant physiology and may result in the improvement of abiotic stress resistance, nitrogen fertilizer uptake, resistance to pathogens and photosynthetic efficiency (Harman et al., 2012; Hermosa et al., 2012).

Conclusion

One of the main European viticulture problems is the decline of soil fertility due to a reduction of the natural soil harmony. This has led researchers towards the development of alternative solutions that are not based on the use of chemical pesticides and fertilizers to the soil for guaranteeing the resistance of plants to pathogen agents. The demonstration of the importance is establishing symbiosis between root system and mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp., which makes possible an enduring protection of cultivated plants and a better use of nutrients, improving plant tolerance to the diseases.

Recent studies have indicated the soil microflora management as the best solution for favoring the expansion of those microbes that are essential for a good crop growth. A growing number of research scientists confidently recommend the use of mycorrhizal fungi *Glomus* spp. and *Trichoderma* spp. associated to biocontrol microorganisms, as a sustainable solution for increasing plant tolerance to biotic and abiotic stress, for increasing plant productivity in degraded soils and for reducing the agricultural environmental impact.

However, more studies concerning the symbiotic effectiveness between different mycorrhiza fungal species and particular grape cultivars and rootstocks are needed in order to successfully implement it into the vineyard agronomical practices.

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