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Research Note

Effects of Mycorrhizal Inoculation on Root Morphology and Nursery Production of Three Grapevine Rootstocks

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Abstract: Grapevines form mutualistic symbioses with arbuscular mycorrhizal (AM) fungi that have been shown to enhance plant growth and nutrition. In the field, AM fungal populations may be low or nonexistent (in fumigated soils), suggesting the need for AM inoculation of grapevine plants at the nursery. Addition of AM fungal inoculum to rooting substrate could be an effective strategy for the nursery production of mycorrhizal plants. The effects of inoculation of three grapevine rootstocks on root morphology and growth were tested. Results indicated that inoculation with the AM fungus *Glomus aggregatum* in rooting beds of grapevine cuttings changed root morphology, increasing branching of first-order lateral roots. When rooted cuttings were transplanted to pots, with soil sufficient in P and including indigenous AM fungi, and grown for nine months, a significant growth enhancement was found in two of the inoculated rootstocks. *Glomus aggregatum*, alone or in synergy with the indigenous AM fungi, seemed to have a higher affinity for 161-49 Couderc, the roots of which were more extensively colonized and exhibited a greater positive growth response.

Key words: arbuscular mycorrhiza, grape, growth enhancement, root development

Grapevines are known to form arbuscular mycorrhizae (AM), a naturally occurring symbiosis between their roots and certain fungi belonging to the *Glomeromycota* (Schüssler et al. 2001). Although commonly present in vineyard soils (Menge et al. 1983, Schubert and Cravero 1985), AM fungi may not always be infective and/or effective. The number of AM fungal propagules in cultivated soils is a key factor for rapid and effective symbiosis (Habte and Fox 1993). Some soil characteristics (top-soil removal, construction of contour banks or bunds) and cultural practices (soil fumigation, tillage) may decrease or even eliminate indigenous AM fungal populations (Thompson 1994), thus advising AM inoculation of grapevines at transplant or in the nursery before transplant in the field (Linderman and Davis 2001).

The inoculation of different rootstocks and cultivars with selected AM fungi has been shown to increase grapevine growth and mineral nutrition in greenhouse experiments, particularly when the potting substrate is sterilized and P deficient (Schubert et al. 1988, Karagiannidis et al. 1995, Linderman and Davis 2001). In these inoculation experiments, the inoculum was usually applied when plants, previously rooted in non-mycorrhizal substrates, were transplanted in soil-containing pots, in an attempt to reproduce field conditions in which mycorrhizal inoculum is applied at transplant. In the field, the inoculated AM fungi must rapidly colonize the roots and enhance host-plant growth, which will be affected by the native AM fungal population (if any), fertility levels, and edaphic factors (Sylvia and Jarstfer 1994).

The production of mycorrhizal plants at the nursery may be a better alternative than inoculation when planting in the field. On one hand, the inoculated AM fungi will already occupy the root tissues, and on the other, the amount of inoculum needed could be lower. In this work, we investigated the effects of applying an AM fungal inoculum to rooting substrate in the nursery during propagation of three grapevine rootstocks.

Materials and Methods

Uniform two-bud semihardwood cuttings (10 cm long) were collected from five-year-old healthy plants of the following *Vitis* rootstocks: 196-17 Castel (196-17) ([*Vitis vinifera* L. cv. Murviedro X *Vitis rupestris* Scheele] X *Vitis riparia* Michx.); 110 Richter (110R) (*Vitis berlandieri* Planch. X *V. rupestris*); and 161-49 Couderc (161-49C) (*V. riparia* X *V. berlandieri*). These rootstocks are among those suitable for cultivation in vineyards of Rías Baixas, a denomination of origin that produces high-quality white wines in northwest Spain.

Two rooting beds of sand:vermiculite (1:1, v:v) were established at the nursery. One was amended with inoculum of the AM fungus *Glomus aggregatum* Schenck & Smith

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emend. Koske, obtained from sand:vermiculite (1:1, v:v) pot cultures of *Tagetes erecta* L. The mycorrhizal inoculum consisted of rhizosphere soil-containing spores, external mycelium, and colonized root fragments. The most probable number of AM propagules per 100 mL/dry soil was 140 ± 35, using the method of Porter (1979). In the inoculated rooting bed, inoculum was mixed with the sand-vermiculite mix at 10% of the total volume. The uninoculated control bed contained the same amount of the sand-vermiculite mix. To ensure that both beds had common microflora (excluding AM fungi), we added to the uninoculated control bed a filtrate free of AM propagules obtained from a suspension of the mycorrhizal inoculum in water.

Before planting in the rooting beds, all cuttings received a rapid basal coating with a talc-based auxin preparation providing 8000 μg IBA.g^{-1}. There were 50 cuttings of 196-17 and 110R and 30 of 161-49C in each rooting bed, which were maintained under natural day length in a greenhouse from December to September, the day and night temperatures being 8 to 14°C and 3 to 7°C (winter) and 19 to 25°C and 9 to 12°C (spring and summer), respectively. Plants were watered as needed.

After nine months, rooted cuttings were removed and the roots carefully washed free of substrate. Rooting percentages were recorded. Cuttings were considered rooted if the root systems were large enough to withstand hand potting. The number of main root axes and first-, second- and third-order lateral roots, when present, were recorded in all rooted cuttings before potting.

For each rootstock, we selected five rooted cuttings of similar size from inoculated and uninoculated rooting beds for transfer to 1.5-L pots (one cutting per pot) containing unsterilized field soil with an uncharacterized AM fungal population. The soil was collected from the arable layer of an apple orchard at the Do Areeiro Phytopathological Station. The main soil characteristics were pH (H_2O) 5.6, organic matter 4.8%, HCO_3Na-extractable P 34 mg/kg, Ca^{2+} 7.00 cmol(+)/kg, Mg^{2+} 0.58 cmol(+)/kg, Na^+ 0.38 cmol(+)/kg, K^+ 0.28 cmol(+)/kg and Al^{3+} 0.20 cmol(+)/kg. All pots were randomly arranged in a growth chamber at 25/22°C day/night temperature, 80 to 90% relative humidity, and 16 hr photoperiod at 450 μmol/m^2/s, and watered as needed.

Grapevines were harvested nine months after transplant. The number of shoots and leaves per plant were recorded. Shoot dry weights were determined after drying at 70°C for 48 hr. Roots were washed clean of soil and fresh weights were determined. Each root system was longitudinally divided in two: one half was used to obtain dry weights and the other half was cleared and stained in trypan blue (Phillips and Hayman 1970) for estimating the percentage of root length colonized by AM fungi using the gridline intersect method (Ambler and Young 1977, Giovannetti and Mosse 1980).

The Kolmogorov–Smirnov test was applied to check that data were normally distributed. Statistical analysis of the data was then performed using a one-way analysis of variance. The significance of the F ratio was used to indicate statistical significance for $p \leq 0.05$.

Results and Discussion

After nine months, all 196-17 cuttings were well rooted both in inoculated and uninoculated rooting beds. However, the inoculation of *G. aggregatum* resulted in a slightly higher number of rooted cuttings of 110R and 161-49C (respectively 98% and 87% versus 86% and 73% of the uninoculated cuttings). Cuttings of 196-17 formed a vigorous root system, with numerous main root axes that showed almost no branching (Table 1). The rooting pattern of 110R and 161-49C was exactly the opposite: very few main roots, but numerous first-order lateral roots. Inoculation of *Glomus aggregatum* in the rooting bed significantly increased the number of first-order lateral roots in all three rootstocks (especially in 110R and 161-49C) and second-order roots in 110R. Effects of AM fungal inoculation on root morphology has also been observed in micropropagated SO4 (*V. berlandieri* Planch. X *V. riparia* Michx.), only two weeks after transplanting into pots, by Schellenbaum et al. (1991), who found no effect on the formation of main root axes but an increased number of first- and second-order lateral roots. This rapid change in root morphology might explain the benefits attributed by Lovato et al. (1992) to AM inoculation in the acclimation and growth of micropropagated grapevines and might have been related to the increased number of rooted cuttings obtained for the inoculated rootstocks 110R and 161-49C.

Arbuscular mycorrhizae enhance plant growth normally by increasing nutrient uptake (Marschner and Dell 1994). Thus, it could be argued that altered root morphology is a consequence of better nutrition of inoculated cuttings. However, after nine months in the rooting bed, there were no significant differences in fresh weight or size noted between inoculated and uninoculated grapevines of any rootstock (data not shown). Similar conclusions were reported

Table 1 Number of main root axes and first-, second-, and third-order lateral roots, of three grapevine rootstocks inoculated with *Glomus aggregatum*.

Rootstock	Treatment	Main root axes	Lateral roots		
			1st order	2d order	3d order
196-17	Uninoculated	27.8b[a]	0.3a	nd[b]	nd
	Inoculated	23.3a	6.2b	nd	nd
110R	Uninoculated	9.0a	26.5a	0.0a	0.0a
	Inoculated	8.5a	62.2b	17.1b	2.4a
161-49C	Uninoculated	7.1a	25.2a	0.9a	nd
	Inoculated	8.4a	38.6b	0.4a	nd

[a]For each rootstock and within each column, values followed by the same letter are not significantly different at $p \leq 0.05$.

[b]nd: not detected.

Table 2 Number of shoots, number of leaves, dry weight of shoots and roots, and mycorrhizal colonization in three grapevine rootstocks inoculated with *Glomus aggregatum*.

Rootstock	Treatment	Shoots	Leaves/ plant	Shoot dry wt (g/plant)	Root dry wt (g/plant)	Mycorrhizal colonization (% root length)
196-17	Uninoculated	5.8 ± 0.8a[a]	41.3 ± 6.7a	5.5 ± 0.6a	3.5 ± 1.3a	19.0 ± 1.3a
	Inoculated	5.3 ± 0.6a	43.3 ± 2.9a	7.9 ± 0.8b	4.7 ± 1.0a	23.4 ± 3.8a
110R	Uninoculated	12.8 ± 0.6a	73.5 ± 4.9a	3.3 ± 0.2a	0.7 ± 0.1a	30.2 ± 10.1a
	Inoculated	15.0 ± 2.3a	84.3 ± 9.5a	2.5 ± 0.4a	0.8 ± 0.1a	29.2 ± 3.4a
161-49C	Uninoculated	12.2 ± 0.8a	69.3 ± 2.8a	4.1 ± 0.5a	1.3 ± 0.2a	27.5 ± 3.4a
	Inoculated	16.4 ± 0.5b	96.8 ± 5.4b	7.9 ± 0.8b	2.4 ± 0.5a	49.5 ± 3.7b

[a]For each rootstock and within each column, values followed by the same letter are not significantly different at $p \leq 0.05$.

by Fortuna et al. (1998) when studying the effect of inoculation with *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe on rooting of micropropagated cherry plants. Altered root morphology may, instead, be due to changes in the hormonal balance induced by the AM symbiosis, which may affect the development of the rooting system, favoring the formation of lateral roots (Linderman 1992).

Uninoculated plants of all three rootstocks were colonized by indigenous AM fungi, showing AM root percentages of 20 to 30% (Table 2). Similar AM colonization percentages were found in inoculated rooted cuttings of 196-17 and 110R. However, inoculated rooted cuttings of 161-49C had significantly higher colonization compared to uninoculated rooted cuttings, which was associated with a highly significant increase in the number of shoots and leaves and shoot and root dry weights. Increased growth was also observed in inoculated 196-17 rooted cuttings, but there were no differences in mycorrhizal colonization between inoculated and uninoculated rooted cuttings. Inoculated 161-49C and 196-17 showed higher shoot dry weights compared to uninoculated rooted cuttings, but inoculation had no effect on the growth of 110R.

Results suggest that *G. aggregatum*, alone or in synergy with indigenous AM fungi, has a higher affinity for rootstock 161-49C, which resulted in a more extensive colonization of the roots and a greater enhancement of plant growth. Similarly, Carretero et al. (1999) found that *Glomus deserticola* Trappe, Bloss & Menge colonized the roots of micropropagated 110R to a greater extent than 140 Ruggeri; 110R had increased survival and growth. Linderman and Davis (2001) reported high mycorrhizal colonization in several *Vitis* rootstocks and cultivars for each AM fungal inoculum tested after four months of growth in P-deficient soil. In all combinations of inoculum and rootstock/cultivar, inoculation resulted in increased plant growth when compared to uninoculated controls.

It was once thought that AM fungi did not show host-specifity (Harley 1989), in contrast with other known plant symbionts such as *Rhizobium* and *Bradyrhizobium*. However, cases of host-preference have been described (Boyetchko and Tewari 1990) in terms of an increased root colonization and sporulation by AM fungi on certain plant species (Bever et al. 1996, Camprubí and Calvet 1996). These differences in the preference of an AM fungus for a specific plant host are not only interspecific (Schenck and Kinloch 1980, Zhu et al. 2000) but also intraspecific (Kurle and Pfleger 1994). In grapevine, Schubert et al. (1988) observed different affinities among several AM fungi and the rootstock 420A, finding that, after three months of growth, *Glomus monosporum* Gerdemann & Trappe and *G. occultum* Walker more successfully colonized roots and more effectively increased plant growth than *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. and *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske. This observation supports the hypothesis that host plants prefer certain AM fungi.

As shown by Linderman and Davis (2001), the beneficial effects of the mycorrhizal symbiosis are generally shown by plants in P-deficient soil. Karagiannidis et al. (1997) found that roots of 110R, 41B, 140 Ruggeri, and 1103 Paulsen were heavily colonized by indigenous AM fungi in vineyard soil deficient in P (the degree of mycorrhizal colonization being inversely related to soil P content), while leaves showed adequate P levels. In potted vineyard soil deficient in P, Bavaresco and Fogher (1996) reported low mycorrhizal colonization of 3309 C, SO4, and 41B by indigenous AM fungi (less than 20%), but found enhanced plant growth and mycorrhizal colonization when inoculated with *Glomus mosseae*. Our work shows that grapevine growth can be enhanced by inoculation of rooting beds in P-sufficient soil, a situation usually found in vineyards of the Rías Baixas in northwest Spain.

Conclusions

AM fungal inoculation has been shown to significantly enhance growth of grapevine rootstocks and cultivars when compared to uninoculated controls. In the present work, three grapevine rootstocks responded differently to *G. aggregatum* when inoculated in rooting beds before transplant to soil containing a native AM fungal population. These results suggest that attention must be paid to the interaction between introduced and native AM fungal populations to achieve optimal benefits from the mycorrhizal symbiosis for specific grapevine rootstocks or cultivars under field conditions.

Literature Cited

Ambler, J.R., and J.L. Young. 1977. Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Sci. Soc. Am. J. 41:551-556.

Bavaresco, L., and C. Fogher. 1996. Lime-induced chlorosis of grapevine as affected by rootstock and root infection with arbuscular mycorrhiza and *Pseudomonas fluorescens*. Vitis 35:119-123.

Bever, J.D., J.B. Morton, J. Antonovics, and P.A. Schultz. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. Ecol. 84:71-82.

Boyetchko, S.M., and J.P. Tewari. 1990. Root colonization of different hosts by the vesicular-arbuscular mycorrhizal fungus *Glomus dimorphicum*. Plant Soil 129:131-136.

Camprubí, A., and C. Calvet. 1996. Isolation and screening of mycorrhizal fungi from citrus nurseries and orchards and inoculation studies. HortScience 31:366-369.

Carretero, C.L., M. Cantos, and A. Troncoso de Arce. 1999. Efecto de la micorriza vesículo arbuscular en la aclimatación de patrones de vid micropropagados. Vitic. Enol. Profesional 60:28-36.

Fortuna, P., S. Morini, and M. Giovannetti. 1998. Effects of arbuscular mycorrhizal fungi on *in vivo* root initiation and development of micropropagated plum shoots. J. Hortic. Sci. Biotechnol. 73:19-28.

Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Habte, M., and R.L. Fox. 1993. Effectiveness of VAM fungi in nonsterile soils before and after optimization of P in soil solution. Plant Soil 151:219-226.

Harley, J.L. 1989. The significance of mycorrhiza. Mycol. Res. 92: 129-139.

Karagiannidis, N., N. Nikolau, and A. Mattheou. 1995. Influence of three VA-mycorrhiza species on the growth and nutrient uptake of three grapevine rootstocks and one table grape cultivar. Vitis 34:85-89.

Karagiannidis, N., D. Velemis, and N. Stavropoulos. 1997. Root colonization and spore population by VA-mycorrhizal fungi in four grapevine rootstocks. Vitis 36:57-60.

Kurle, J.E., and F.L. Pfleger. 1994. The effects of cultural practices and pesticides on VAM fungi. *In* Mycorrhizae and Plant Health. F.L. Pfleger and R.G. Linderman (Eds.), pp. 101-131. APS Press, St. Paul, MN.

Linderman, R.G. 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. *In* Mycorrhizae in Sustainable Agriculture. G.J. Bethlenfalvay and R.G. Linderman (Eds.), pp. 45-70. ASA Special Publication, Madison, WI.

Linderman, R.G., and E.A. Davis. 2001. Comparative response of selected grapevine rootstocks and cultivars to inoculation with different mycorrhizal fungi. Am. J. Enol. Vitic. 52:8-11.

Lovato, P.E., J.P. Guillemin, and S. Gianinazzi. 1992. Application of commercial arbuscular endomycorrhizal fungal inoculants to the establishment of micropropagated grapevine rootstock and pineapple plants. Agronomie 12:873-881.

Marschner, H., and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. *In* Management of Mycorrhizas in Agriculture, Horticulture and Forestry. A.D. Robson et al. (Eds.), pp. 89-102. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Menge, J.A., D.J. Raski, L.A. Lider, E.L.V. Johnson, N.O. Jones, J.J. Kissler, and C.L. Hemstreet. 1983. Interactions between mycorrhizal fungi, soil fumigation, and growth of grapes in California. Am. J. Enol. Vitic. 34:117-121.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

Porter, W.M. 1979. The 'most probable number' method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res. 17:515-519.

Schellenbaum, L., G. Berta, F. Ravolanirina, B. Tisserant, S. Gianinazzi, and A.H. Fitter. 1991. Influence of endomycorrhizal infection on root morphology in a micropropagated woody plant species (*Vitis vinifera* L.). Ann. Bot. 68:135-141.

Schenck, N.C., and R.A. Kinloch. 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia 72:445-446.

Schubert, A., and M.C. Cravero. 1985. Occurrence and infectivity of vesicular-arbuscular fungi in north-western Italy vineyards. Vitis 24:129-138.

Schubert, A., S. Cammarata, and I. Eynard. 1988. Growth and root colonization of grapevines inoculated with different mycorrhizal endophytes. HortScience 23:302-303.

Schüssler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol. Res. 105:1413-1421.

Sylvia, D.M., and A.G. Jarstfer. 1994. Production of inoculum and inoculation with arbuscular mycorrhizal fungi. *In* Management of Mycorrhizas in Agriculture, Horticulture and Forestry. A.D. Robson et al. (Eds.), pp. 231-238. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Thompson, J.P. 1994. What is the potential for management of mycorrhizas in agriculture? *In* Management of Mycorrhizas in Agriculture, Horticulture and Forestry. A.D. Robson et al. (Eds.), pp. 191-200. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Zhu, Y.G., A.S. Laidlaw, P. Christie, and M.E.R. Hammond. 2000. The specificity of arbuscular mycorrhizal fungi in perennial ryegrass-white clover pasture. Agric. Ecosyst. Environ. 77:211-218.