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# Effects of Vesicular-Arbuscular Mycorrhizal (VAM) Fungi on the Leaf Nutritional Status of Four Grapevine Rootstocks

S. Caglar[1)] and A. Bayram[2)]
([1)]Department of Horticulture, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey and [2)]Cukurova Agricultural Research Institute, Adana, Turkey)

**Summary**

The effects of five VAM fungi (*Glomus etunicatum*, *Glomus caledonium, Glomus clarum, Glomus mosseae*, and mixed inoculum) on the nutritional status of four grapevine rootstocks (420 A, 41 B, 1103 P, and 'Rupestris du Lot') were investigated. One-year-old grapevine rootstock cuttings were rooted in perlite and transferred to black polyethylene bags filled with fumigated growing medium. A 40 g mycorrhizal soil band was used for each inoculation. The percentage of VAM inoculation, leaf area, N, P, K, and total sucrose contents of the leaves were determined.

Ten months after inoculation, grapevine rootstocks were colonized by the VAM fungi at frequencies ranging from 47.0 to 64.1 %. Inoculations with *G. etunicatum* and *G. clarum* significantly increased the leaf areas of 41 B, 420 A, and 1103 P rootstocks. The VAM fungi increased leaf P, but not N and K concentrations. Leaf total sucrose concentrations of the grapevine rootstocks were increased two- to four -fold with certain inoculums compared with the control.

**Key words.** *Vitis* – Mycorrhiza – macronutrients – sucrose – soil fumigation

## Introduction

Grapevines have been shown to be dependent on mycorrhizas (Possingham and Groot Obbing 1971) and, in vineyards, they form symbioses with naturally occurring vesicular-arbuscular mycorrhizal (VAM) fungi (Bonfante-Fasolo 1978; Menge et al. 1983). Mycorrhizas are widespread associations characterized by a bi-directional transfer of nutrients between plants and associated fungi, where plants provide fungi with sugar and the fungi help plants in the acquisition of mineral nutrients from the soil (Smith and Barker 2002). VAM fungal hyphae extend from the root into the soil, where they interface with soil particles. Because hyphae can extend from the root into the soil for several centimetres, they can absorb immobile nutrients from the soil. VAM fungi benefit grapevines by improving growth and nutrition (Menge et al. 1983; Schubert et al. 1988; Bavaresco and Fogher 1992; Mattheou et al. 1994; Karagiannidis et al. 1995).

Soil fumigation is a common practice when replanting grapevines to control phylloxera and other root disease problems. Unfortunately, this practice often eliminates beneficial organisms including VAM fungi from vineyards because native fungi are mostly found in topsoil (Nappi et al. 1985). Similarly, fumigated growing medium used for potted nursery plants generally lack VAM fungi. Previous work showed that grapevines pre-inoculated with VAM fungi grew better than uninoculated plants on fumigated or sterilized soils (Menge et al. 1983; Mazzitelli and Schubert 1989). Pot experiments (Karagiannidis et al. 1995) and field studies (Karagiannidis et al. 1997) confirmed that VAM fungi were responsible for improved P uptake by grapevines.

Grapevine nursery plant production is undertaken by grafting desired cultivars onto rootstocks. During the initial stages of nursery production, rootstocks are often grown in fumigated medium to avoid destructive soil-born pathogens. Beneficial effects of incorporating VAM fungi into the sterile growing medium used for nursery plant production has been reported for various horticultural plants such as peach (Larue et al. 1975), avocado (Menge et al. 1980), citrus (Camprubí and Calvet 1996), strawberry (De Silva et al. 1996), and woody ornamentals (Hall and Bowes 1984). This study was conducted to investigate the effect of VAM fungi inoculation on the nutritional status of four grapevine rootstocks at the nursery level.

## Materials and Methods

The experiment was carried out in a greenhouse at the Kahramanmaras Sutcu Imam University, Turkey, using four grapevine rootstocks (420 A, 41 B, 1103 P, and 'Rupestris du Lot') and five mycorrhizal genotypes (*Glomus etunicatum*, *Glomus caledonium, Glomus clarum, Glomus mosseae*, and a mixed inoculum, which consisted of an equal spore mixture of the mycorrhizas mentioned above). The experiment also included a non-mycorrhizal treatment for all grape rootstocks tested.

*Plant material*

One-year-old grapevine rootstock cuttings (about 30 cm long) with one intact dormant bud were rooted in perlite under a mist propagation-bottom heat system in the greenhouse during early spring. The rooted grapevine cuttings were then transferred to black polyethylene bags (20×30 cm) filled with fumigated growing medium.

*Inoculation*

The mycorrhiza species were propagated on maize roots in a medium consisting of a 1:3:6 ratio of an organic manure:soil:sand mixture (ORTAŞ et al. 1999) at the Laboratory of Soil Science Department of Cukurova University. The potting medium consisted of a mixture of soil:sand:peat (3:1:1 v/v/v) containing 10 ppm P (Olsen's), and was fumigated with methyl bromide (200 g $m^{-2}$) under a plastic sheet for 48 h. For the inoculations, one-third of the polyethylene bags were filled with fumigated growing medium and, after placing 40 g of inoculum and rooted grape rootstock cuttings inside, the remaining bags were filled with the same fumigated growing medium. The inoculated and non-inoculated grapevine rootstocks were then grown in the greenhouse without any fertilizer application.

*Determination of infection*

At the end of the growing season, the plants were harvested and 0.2 g root samples were stained with trypan blue in lactic acid (KOSKE and GEMMA 1989). The percentage of root colonization was determined with a grid line intersect method (GIOVANETTI and MOSSE 1980) under a microscope (40×). The percentage of root colonization was calculated as follows: 100× colonized root number/total root number.

*Leaf area measurements*

Leaf area measurements were conducted on nine leaf samples from each treatment in mid-August with a planimeter (Sokkisha KP-90N, Japan).

*Determination of leaf N, P, K, and total sucrose contents*

Nine leaf samples for each treatment were collected from the sixth nodes of the shoots in mid-August and oven dried at 65 °C for 48 h. Total leaf N content was determined using the Kjeldahl method, P with the vanata-de-molybdate yellow colorimetric method using a spectrophotometer, and K by atomic absorption (CHAPMAN and PRATT 1961). Leaf total sucrose content was determined according to a modified anthron method (KAPLANKIRAN 1992).

*Statistical analyses*

The experiment had a completely randomised design with three replications of three rootstocks per replication. An analysis of variance (ANOVA) was conducted from the data, and a Duncan Multiple Range Test was performed ($P \leq 0.05$) to illustrate mean differences.

## Results and Discussion

Ten months after inoculation, the grapevine rootstocks were colonized by VAM fungi at frequencies ranging from 47.0 to 64.1 % (Table 1). This range was in accordance with a previous study conducted on grapevine rootstocks with native VAM fungi (KARAGIANNIDIS et al. 1997). In the present study, inoculation percentages of the roots with *G. etunicatum*, *G. mosseae,* and *G. caledonium* were higher than those with *G. clarum* or the mixed inoculum.

VAM fungi influenced the leaf area of grapevine rootstocks. The leaf areas of the four grapevine rootstocks differed greatly with the fungal genotype, and significant interactions ($P \leq 0.05$) between rootstocks and inoculations were found (Table 2). In this study, *G. etunicatum* and *G. clarum* increased the leaf areas in 41 B, 420 A and 1103 P rootstocks. However, in 'Rupestris du Lot', differences in leaf areas from the inoculums were not significant. There was a slight decrease in leaf areas in some instances, as can be seen in 420 A with *G. mosseae*, *G. clarum*, and *G.*

Table 1. Inoculation percentage of grapevine rootstocks with VAM fungi.

| Inoculation treatments | Percentage of inoculated roots |
|---|---|
| *G. etunicatum* | 64.1 (52.9) a [z] |
| *G. clarum* | 47.0 (41.9) b |
| *G. mosseae* | 62.6 (52.5) a |
| *G. caledonium* | 60.5 (51.4) a |
| Mixed | 52.0 (46.2) b |

*G. = Glomus*

[z]: Mean separation within columns by Duncan MRT ($P \leq 0.05$). Data in brackets were arcsine transformed

Table 2. The leaf areas of the grapevine rootstocks inoculated with different VAM fungi.

| Grapevine rootstocks | *G. etunicatum* | *G. clarum* | *G. mosseae* | *G. caledonium* | Mixed | Control |
|---|---|---|---|---|---|---|
| 41 B | 89.4 a [z] | 75.4 bc | 61.0 def | 65.6 cde | 68.0 cd | 41.6 gh |
| 420 A | 81.1 ab | 57.2 def | 52.0 fg | 50.4 fg | 58.6 def | 60.7 def |
| 1103 P | 53.4 efg | 62.0 def | 44.2 gh | 32.7 hij | 51.3 fg | 35.1 hi |
| 'Rupestris du Lot' | 23.9 ij | 22.1 j | 27.2 ij | 26.4 ij | 29.0 ij | 23.1 ij |

*G. = Glomus*

[z]: Mean separation within columns by Duncan MRT ($P \leq 0.05$)

*caledonium*, and in 1103 P with *G. caledonium*. A decrease in vegetative development by mycorrhizal activity has been rarely found in previous work, although a negative effect of some mycorrhiza species on plant development was reported for avocado seedlings (Da Silveira et al. 2002). Huat et al. (2002) demonstrated that mycorrhizal *Azadirachta excelsa* seedlings grew slower than uninoculated seedlings, despite the higher percentage of inoculum. The decreased leaf area in the present study may have been due to insufficient N uptake during leaf expansion and/or a greater drain on photosynthesis by these inoculums early in the season. This is because these rootstock × inoculum combinations had a significantly high number of spores per unit of rhizosphere soil (unpublished data). Waschkies et al. (1994) reported in grapevines that mycorrhizae have an influence in increasing the leaf area when compared with non-mycorrhizal plants. Van Staden and Davey (1979) suggested that cytokinins might have a specific role in promoting leaf growth by increasing the rate of cell division and cell expansion in mycorrhizae associated plants.

The leaf N, P, and K contents of the grapevine rootstocks are given in Table 3. Although not statistically significant, the leaf N contents varied depending on the genotype of the rootstocks and mycorrhizal species used. Inoculations with *G. clarum* appeared to have high leaf N concentrations in 41 B, 1103 P and 'Rupestris du Lot' rootstocks, and mixed inoculation gave a higher value than the other inoculations in 420 A. However, in general, great variation in leaf N content was observed. The leaf sampling time (mid-August) for N analysis may have been partly responsible for this variance because, at this time, leaves were mature and plants were aging because of high ambient temperatures. This might have affected the N content in the leaves. Sonawane et al. (1998) reported that N uptake was higher in mycorrhizal grape cultivars grown in P deficient soils than in control plants. In the present study, the soil:sand:peat mixture had a medium level of P (10 ppm, Olsen's), and thus the inoculums might not have been as effective in N uptake as in the previous study. Enhanced N uptake in mycorrhizal plants was explained by an increased N demand because of high P nutrition (George et al. 1995). However, in the present study, given the variation in the leaf N content of the grapevine rootstocks, we cannot reach any conclusion in this respect.

The plant response to VAM fungi has often been thought of exclusively as a P response. Nutrient analysis data clearly show that leaf P concentrations were apparently greater in the inoculated plants than in the control plants. This finding was in accordance with a previous study (Karagiannidis et al. 1995). Such an increase in leaf P concentration was commonly observed in mycorrhizal plants when the P supply was limited (Gebbing et al.

Table 3. The Leaf N, P, K contents of the grapevine rootstocks inoculated with different VAM fungi.

| Inoculation treatments | 41 B | 420 A | 1103 P | 'Rupestris du Lot' |
|---|---|---|---|---|
| | | N ( %) | | |
| *G. etunicatum* | 1.8 | 2.1 | 1.8 | 2.1 |
| *G. clarum* | 2.5 | 1.8 | 2.6 | 2.6 |
| *G. mosseae* | 1.8 | 2.1 | 2.3 | 2.0 |
| *G. caledonium* | 1.9 | 2.3 | 2.1 | 2.1 |
| Mixed | 2.1 | 2.5 | 2.2 | 2.4 |
| Control | 2.6 | 2.0 | 2.0 | 2.1 |
| | n.s. | n.s. | n.s. | n.s. |
| | | P ( %) | | |
| *G. etunicatum* | 0.17 b [z] | 0.23 a | 0.23 a | 0.23 a |
| *G. clarum* | 0.22 a | 0.19 b | 0.22 a | 0.22 a |
| *G. mosseae* | 0.17 b | 0.23 a | 0.27 a | 0.20 a |
| *G. caledonium* | 0.16 b | 0.23 a | 0.27 a | 0.24 a |
| Mixed | 0.17 b | 0.19 b | 0.19 b | 0.25 a |
| Control | 0.14 b | 0.15 b | 0.19 b | 0.12 b |
| | | K ( %) | | |
| *G. etunicatum* | 1.11 | 1.19 | 1.26 | 1.15 |
| *G. clarum* | 1.11 | 1.22 | 1.24 | 1.11 |
| *G. mosseae* | 1.07 | 1.09 | 1.27 | 1.19 |
| *G. caledonium* | 0.98 | 0.96 | 1.11 | 1.11 |
| Mixed | 1.05 | 1.05 | 1.05 | 1.96 |
| Control | 1.10 | 0.88 | 0.92 | 1.05 |
| | n.s. | n.s. | n.s. | n.s. |

*G.* = *Glomus*
[z]: Mean separation within columns by Duncan MRT (P≤0.05)
n.s.: Non significant

Table 4. The effects of VAM fungi on the leaf total sucrose contents in grapevine rootstocks.

| Inoculation treatments | Leaf total sucrose content (g $100g^{-1}$) |
|---|---|
| *G. etunicatum* | 2.1 ab [z] |
| *G. clarum* | 0.9 b |
| *G. mosseae* | 3.2 a |
| *G. caledonium* | 1.6 ab |
| Mixed | 1.3 ab |
| Control | 0.8 b |

*G.* = *Glomus*
[z]: Mean separation within the column by Duncan MRT ($P \leq 0.05$)

1977; SONAWANE et al. 1998). However, MORIN et al. (1994) demonstrated that, in steam-pasteurized soils with high P contents, the addition of mycorrhizal fungi isolates increased leaf P concentrations in apple rootstocks. The effect of mycorrhiza on P uptake seems to be pronounced in fumigated or sterilized soils, as can be seen in our experiment. Increased N and P contents in the plants from mycorrhizal inoculation can contribute to plant growth. SCHUBERT et al. (1997) reported that VAM fungi stimulated grapevine plant growth in unsterilized soils. LINDERMAN and DAVIS (2001) demonstrated that, in low P fumigated soils, the growth response of inoculated grapevines by VAM fungi was pronounced, and depended on the plant genotype and fungal species used. The improved P uptake by the mycorrhizal inoculations could be important in transplanting grapevine plants into soils with low fertility. Differences in leaf K concentrations from the inoculums were not significant.

In grape rootstocks, inoculations with VAM fungi significantly increased the leaf total sucrose concentration compared with the control (Table 4). The highest leaf total sucrose content was obtained with *G. mosseae*. This value was four times greater than that of the control. Rootstocks inoculated with *G. etunicatum*, *G. caledonium*, and the mixed inoculum also had statistically higher leaf total sugar contents. Our finding confirms the result of SHIUCHIEN et al. (1988), who reported that mycorrhizal inoculations improved the sugar content of *in vitro* produced grape plantlets. This positive effect of mycorrhizal inoculations on the carbohydrate status was also reported in other horticultural plants such as citrus (NEMEC and GUY 1982) and avocado (DA SILVEIRA et al. 2002) plants. This may indicate the increased photosynthesis capacity of mycorrhizal plants. This, in turn, probably contributes to the nutrition of mycorrhizal colonies in the roots.

### *Conclusion*

Because of fumigation, grapevine nursery plants often lack VAM fungi at the time of transplanting. In the present study, it was clearly shown that grapevine rootstocks at the nursery level benefited from VAM fungi in terms of increased leaf P and sucrose content. This, in turn, could improve plant development in the field. Furthermore, the leaf areas in certain mycorrhizal grapevine rootstocks were greater than in control plants at the end of the study. This indicates that VAM fungi can contribute to better plant development during nursery production. MOSSE (1972) stated that, because of the wide host range of VAM fungi, their specificity might be determined more by interactions between the fungal strain and the soil than between the fungus and its host. Therefore, an effort also needs to be made to identify the most efficient VAM fungi for each grapevine rootstock grown in the specific soil of any major growing area. Our results indicate the possible benefit of planting grapevine nursery plants pre-inoculated with VAM fungi.

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## References

BAVARESCO, L. and C. FOGHER 1992: Effect of root infection with *Pseudomonas fluorescens* and *Glomus mossea* in improving Fe efficiency of grapevine ungrafted rootstocks. Vitis **31**, 163–168.

BONFANTE-FASOLO, P. 1978: Some ultrastructural features of the vesicular-arbuscular mycorrhiza in the grapevine. Vitis **17**, 386–395.

CAMPRUBÍ, A. and C. CALVET 1996: Isolation and screening of mycorrhizal fungi from citrus nurseries and orchards and inoculation studies. HortSci. **31**, 366–369.

CHAPMAN, H.D. and P.F. PRATT 1961: Methods of analysis for soils, plants and waters. University of California, Div. Agric. Sci. Berkeley.

DA SILVEIRA, S.V., P.V.D. DE SOUZA and O.C. KOLLER 2002: Influence of arbuscular mycorrhizal fungi on vegetative growth of avocado rootstocks. Pesquisa Agropecuaria Brasileira **37**, 303–309.

DE SILVA, A., K. PATTERSON and J. MITCHELL 1996: Endomycorrhizae and growth of 'Sweetheart' strawberry seedlings. HortSci. **31**, 951–954.

GEBBING, H., A. SCHWAB and G. ALLEWELDT 1977: Mycorrhiza of vines. Vitis **16**, 279–285.

GEORGE, E., H. MARSCHNER and I. JACOBSEN 1995: Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. Bitotech. **15**, 257–270.

GIOVANETTI, M. and B. MOSSE 1980: An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytol. **84**, 489–500.

HALL, T.J. and S.A. BOWES 1984: Mycorrhizas and container-grown hardy ornamental stock (HONS) production. Glasshouse Crops Res. Ins. Ann. Rep. (1982), 113–114.

HUAT, K.H., K. AWANG, A. HASHIM and N.M. MAJID 2002: Effects of fertilizers and vesicular-arbuscular mycorrhizas on the growth and photosynthesis of *Azadirachta excelsa* (Jack) Jacobs seedlings. Forest Eco. and Man. **158**, 51–58.

KAPLANKIRAN, M. 1992: Methodes spectrophotometrique pour les analysys en hydrates de carbone dans les tissus de plante. J. Agric. Fac. of Ç. Ü. **7(3)**, 167–176.

KARAGIANNIDIS, N., N. NIKOLAOU and A. MATTHEAU 1995: Influence of three VA mycorrhiza species on the growth and nutrient uptake of three grape rootstocks and one table grape cultivar. Vitis **34**, 85–89.

KARAGIANNIDIS, N., D. VELEMIS and N. STRAVROPOULOS 1997: Root colonization and spore population by VA mycorrhizal fungi in four grape rootstocks. Vitis **36**, 57–60.

KOSKE, R.E. and J.N. GEMMA 1989: A modified procedure for staining roots to detect VAM. Mycol. Res. **98**, 486–505.

LARUE, J.H., W.D. MCCLELLAN and W.L. PEACOCK 1975: Mycorrhizal fungi and peach nursery nutrition. California Agriculture **29(5)**, 6–7.

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.

MATTHEOU, A., N. KARAGIANNIDIS and N. NIKOLAOU 1994: Seasonal changes of leaf nutrient levels of grapevine over two dry years. Agricoltura Mediterranea 124, 187–196.

MAZZITELLI, M. and A. SCHUBERT 1989: Effects of several VAM endophytes and artificial substrates on *in vitro* propagated *Vitis Berlandieri* x *rupestris* "1103 P". Agriculture, Ecosystems and Environment. Elsevier Science Publ. Amsterdam, 289–293.

MENGE, J. A., J. LARUE, C.K. LABANAUSKAS and E.L.V. JOHNSON 1980: The effect of two mycorrhizal fungi upon growth and nutrition of avocado seedlings grown with six fertilizer treatments. J. Amer. Soc. Hort. Sci. **105**, 400–404.

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.

MORIN, F., J.A. FORTIN, C. HAMEL, R.L. GRANGER and D.I. SMITH 1994: Apple rootstock response to vesicular-arbuscular mycorrhizal fungi in a high phosphorus soil. J. Amer. Soc. Hort. Sci. **119**, 578–583.

MOSSE, B. 1972: The influence of soil type and endogen strains on the growth of mycorrhizal plants in phosphate deficient soils. Rev. Ecol. Biol. Sol. **9**, 221–223.

NAPPI, P., R. JODICE, A. LUZZATI and L. CORINO 1985: Grapevine root system and VA mycorrhizae in some soils of Piedmont (Italy). Plant and Soil **85**, 205–210.

NEMEC, S. and G. GUY 1982: Carbohydrate status of mycorrhizal and nonmycorrhizal citrus rootstocks. J. Amer. Soc. Hort. Sci. **107**,177–180.

ORTAŞ, I., B. ERGÜN, D. ORTAKÇI, S. ERCAN and Ö. KÖSE 1999: The production technique of mycorrhizal spore for using in large arable land. Tr. J. of Agriculture and Forestry **23,** 959–968.

POSSINGHAM, J.V. and J. GROOT OBBING 1971: Endotrophic mycorrhiza and the nutrition of grape vines. Vitis **10**,120–130.

SCHUBERT, A., S. CAMMARATA and I. EYNARD 1988: Growth and root colonization of grapevines inoculated with different mycorrhizal endophytes. HortSci. **23**, 302–303.

SCHUBERT, A., S. CAMMARATA and O. ARIUSSO 1997: Inoculation of grapevine plants with VA mycorrhizal fungi in unsterile soil. In: BOURD, J. and R.J. POUGET (eds): Physiologie de la vigne. 3e symposium International sur la physiologie de la vigne, 303–308.

SHIUCHIEN, K., L. JIANGSHAN and B. KUOCHANG 1988: Effect of vesicular-arbuscular mycorrhizae on the growth of grape plantlets produced by tissue culture. Acta Hort. Sinica **15(2)**, 77–82.

SMITH, S.E. and S.J. BARKER 2002: Plant phosphate transporter genes help harness the nutritional benefits of arbuscular mycorrhizal symbiosis. Trends Plant Sci. **75**, 189–190.

SONAWANE, R.B., B.K. KONDE, D.V. INDI and P.V. WANI 1998: Symbiosis between grapevine varieties and VAM fungi for uptake of nitrogen and phosphorus. J. of Maharashtra Agric. Univ. **(22)2**, 184–186.

VAN STADEN AND J.V. DAVEY 1979: The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ. **2**, 93–106.

WASCHKIES, C., A. SCHROPP and H. MARSCHNER 1994: Relations between replant disease and root colonization of grapevine (*Vitis* sp.) by *Fluorescent pseudomonas* and endomychorrizal fungi. Plant and Soil **162**, 219–227.

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Addresses of authors: S. Caglar, Department of Horticulture, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, 46100 Kahramanmaras, Turkey and A. Bayram, Cukurova Agricultural Research Institute, Adana, Turkey, e-mail: sgcaglar@ksu.edu.tr.