Vitis 36 (2), 57–60 (1997)

Root colonization and spore population by VA-mycorrhizal fungi in four grapevine rootstocks

by

N. KARAGIANNIDIS[1]), D. VELEMIS[1]) and N. STAVROPOULOS[2])

[1]) National Agricultural Research Foundation, Soil Science Institute of Thessaloniki, Greece
[2]) National Agricultural Research Foundation, Greek Gene Bank, Greece

S u m m a r y : The occurrence of vesicular arbuscular (VA) mycorrhizae was investigated in an experimental vineyard at the Thessaloniki prefecture, in a soil which was poor in available P. The soil had not received any phosphorus fertilization for several years, nevertheless the leaves had an adequate P content (0.17 - 0.31 % of d.w.). The vineyard was planted with 4 introduced (Cinsaut, Syrah, Grenache, Carignan) and 4 local cultivars (Athiri, Roditis, Assyrtiko, Malagouzia), grafted to the rootstocks 110 R, 41 B, 140 Ru and 1103 P. Microscopic examination showed that all 4 rootstocks were colonized by VA-mycorrhizal fungi at frequencies ranging from 45 to 75 %. The number of vesicles varied between 16 and 47 and the number of arbuscules between 5 and 26 per cm of infected root. Spore number produced by the mycorrhizal fungi in the rhizosphere ranged from 196 to 280 per 100 g of soil. Spores of the genus *Glomus* were more commonly encountered, in particular those of *G. mosseae* and *G. macrocarpum*. The roots of 1103 P, followed by 41 B, 140 Ru and 110 R were most heavily colonized. The same pattern was observed with regard to the number of spores in the rhizosphere of these rootstocks. Grafted cultivars were found to have some influence on the degree of colonization of these rootstocks and on the population of spores, but had no effect on the formation of vesicles and arbuscules. The degree of colonization of roots by endomycorrhizal fungi was inversely related to the available P content of the soil. At the time of root sampling the P content of leaves was not directly related neither to the degree of root colonization nor to the P content of the soil. However, leaves were found to be adequately supplied with P in all cases. Therefore, the differences observed among the 8 cultivars can be attributed primarily to a variety-specific demand for P.

K e y w o r d s : grapevine rootstock, grapevine varieties, VA mycorrhiza, infection, P uptake.

Introduction

A symbiosis of grapevine with vesicular arbuscular (VA) fungi has been demonstrated by POSSINGHAM and GROOT OBBING (1971), GEBBING *et al.* (1977) and NAPPI *et al.* (1985). Different grapevine rootstocks, like 110 R, 41 B and 5BB, were shown to differ in their growth response when affected by an artificial inoculation with the mycorrhizal fungus *Glomus mosseae* (KARAGIANNIDIS *et al.* 1995). Under greenhouse conditions with sterilized soil various species of mycorrhizal fungi affected growth and development of young grapevines in different ways (MENGE *et al.* 1983).

The VA-mycorrhizal fungi are obligatory symbiotic organisms which supply the host plant with a number of nutrients (MANJUNAT and HABTE 1988), mainly P, from soils in which this element is not avaible to the plants in an easily soluble form, e.g. phosphorus salts, like apatites and certain phosphoric salts of Fe and Al (DIEDERICHS 1991).

According to the local Directorate for Agriculture, the vine-growing region of Thessaloniki is mainly planted with the cultivars Roditis (85 %) and Savatiano (5 %). 110 R and 41 B are used almost exclusively as rootstocks. To investigate the performance of other varieties and rootstocks, the Directorate for Agriculture established a pilot vineyard with 140 Ru, 1103 P, 110 R and 41 B, 8 years ago. Each rootstock was grafted to 8 different vigorous, moderate to high yielding wine varieties.

This study was initiated to examine the response of rootstocks to inoculation with native mycorrhizal fungi, regarding colonization percentage of the fungi on their roots, formation of vesicles and arbuscules and spore populations in the rhizosphere, the soil being poor in available P (no phosphorus fertilization for 8 years). In addition it should be clarified whether the factor "variety" had some effect on the parameters of plant infection and colonization by the endomycorrhizal fungi.

Materials and methods

The 8-year-old experimental vineyard was situated near Messimbria, 30 km west of Thessaloniki, at the core of the grapevine zone of the prefecture. Some physical and chemical caracteristics of the soil are shown in Tab. 1. In this experimental vineyard the rootstocks 110 R, 41 B, 140 Ru and 1103 P were used. The coloured, introduced varieties, Cinsaut, Syrah, Carignan, Grenache and the local white ones, Athiri, Roditis, Assyrtiko and Malagouzia were grafted to each rootstock. Each of the 32 rootstock-variety combinations included 10-12 vines as a block and was replicated 4 times. Leaf, root and soil samples from the rhizosphere of the plants were taken during the first week of July in 1996 from 3 randomly selected plants of each of the 32 combinations. The vineyard had not received any P fertilizer for 8 years.

Leaf samples were washed, dried at 72 °C, finely ground, ashed at 540 °C, dissolved in 6N HCl; their P contents were determined chromatographically (ALEXIADIS 1972). Soil samples were stored for ca. 15 d at low tem-

Correspondence to: Dr. NIKITAS KARAGIANNIDIS, NAGREF, Soil Science Institute of Thessaloniki P.O.Box 14569, GR-54110 Thessaloniki, Greece. Fax: (31) 471 280.

Table 1

Chemical characteristics of the experimental vineyard soil (sandy loam)

pH	$CaCO_3$ %	Electr. cond. $\mu S \cdot cm^{-1}$	Organic matter %	P ppm (Olsen)	K exch. ppm (am. ac.)	B ppm	Mn ppm	Zn ppm	Fe ppm	Cu ppm
7.60	1.32	0.49	1.28	6.60	290	0.67	18.3	1.12	2.12	0.60

perature until the number of spores of mycorrhizal fungi was determined following the method of wet sieving (GERDEMANN and NICOLSON 1963). Keys were used to identify certain fungal spores (TRAPPE 1982). The P content of the soil was determined using the Olsen method (OLSEN *et al.* 1954). After washing, the roots were stained with Congo blue in lactophenol (PHILLIPS and HAYMAN 1970). Then the colonization percentage by endomycorrhizal fungi and the number of vesicles and arbuscules were determined (KARAGIANNIDIS 1980; EZAWA and YOSHIDA 1994).

Statistical analysis of the data was carried out using 1-way and 2-way analysis of variance. Then, means were ranked using the Duncan's Multiple Range Test at 0.05 significance level. To assess the relation between the variables the package Statgraphics 3 was implemented, module Regression Analysis - Multiplicative Model $y = ax^{-b}$.

Results and Discussion

The degree of root infection of the 4 rootstocks by native mycorrhizal fungi was significantly different (Tab. 2). The roots of 1103 P with 75.8 % colonization were most heavily infected, followed by 41 B, 140 Ru and 110 R with root colonization percentages of 66, 56 and 46 %, respectively (averaged over all varieties). These results show the differential preference of the mycorrhizal fungi towards various species of *Vitis* and strengthen similar results of other authors reporting that root colonization by these fungi is genetically controlled (KESAVA RAO *et al.* 1990; MERCY *et al.* 1990; RAJU *et al.* 1990).

A parallel pattern to that of colonization was observed for the number of vesicles and arbuscules in root segments infected by these fungi. Examination with a light microscope showed that the number of vesicles ranged from 47.7 (1103 P) to 16.7 (110 R) per cm of infected root and the number of arbuscules from 26.3 to 4.8 (Tab. 2). The intensity of formation of the two fungal organs follows a similar pattern to the overall infection, being highest in 1103 P, intermediate in 41 B and 140 Ru and lowest in 110 R. The same trend was observed for the number of spores found in the rhizosphere of the grapevines which, compared to other perennial crops, was rather high and ranged from 279.2 (1103 P) to 195.7 (110 R) (Tab. 2) (ATAYESE *et al.* 1993; RAMAN *et al.* 1993). Apart from other factors the relative high spore number could be attributed to the season of sampling, since at the end of spring or the beginning of summer the number of spores reaches a maximum (AN *et al.* 1993; RABATIN 1979). All parameters estimated in this research are shown in Tab. 3.

It appears that the scion variety has a significant effect on the colonization of the root by the mycorrhizal fungi and the production of spores in the rhizosphere (Figure A, B). Stronger effects on spore production was initiated by the varieties Athiri (258.5 spores·100 g^{-1} soil) and Carignan (256.4), weaker effects by the varieties Roditis (228.4) and Assyrtiko (222.3). With regard to the colonization, Carignan (67 %) was found ranking first and Roditis (57 %) was the least effective. The formation of vesicles and arbuscules was not significantly affected by the grafted variety.

Phosphorus concentration in leaves varied significantly among varieties (Figure, C). The highest concentration was found in Assyrtiko (0.28 %) and the lowest in Grenache, Carignan and Roditis (0.21%). These differences could not be attributed to the effect of the endomycorrhiza or to that of the rootstock alone, but mainly to the variety-specific demand for P. Thus, Assyrtiko appears to have the highest demand for phosphorus. The P content of the soil was not significantly different at the various sampling sites; it was generally low, ranging from 5 to 7 ppm (Olsen). Consequently, it cannot be expected that at these very low concentrations soil P would have a substantial effect on mycorrhizal fungus. It is known from literature that high P concentrations in the soil drastically reduce mycorrhizal formation and spreading, since the plant will absorb soil P

Table 2

Number of spores per 100 g soil, mycorrhizal infection in roots (%), vesicles and arbuscules per cm infected root and soil P (ppm) of four grapevine rootstocks. Means followed by a different letter within a column are significantly different at the 0.05 level

Rootstock	Spores	Infection	Vesicles	Arbuscules	Soil P
110 R	195.65 d	46.08 d	16.63 d	4.75 d	7.77 a
41 B	255.25 b	66.08 b	33.92 b	18.13 b	5.60 b
140 Ru	228.96 c	56.04 c	23.54 c	10.33 c	5.88 b
1103 P	279.17 a	75.79 a	47.63 a	26.33 a	5.40 b

Table 3

Mycorrhizal infection in roots (%), number of spores per 100 g soil, vesicles and arbuscules per cm infected root, soil P (ppm) and P concentration in leaves (%) of 8 varieties grafted to 4 rootstocks. Means followed by a different letter within a column are significantly different at the 0.05 level

Rootstock	Variety	Spores	Infection	Vesicles	Arbuscules	Soil P	P in d.w.
110 R	Athiri	194 abc	50	16	5	7.7	0.18
	Grenache	154 c	36	14	3	10.6	0.20
	Assyrtiko	174 bc	47	16	5	8.2	0.21
	Carignan	223 ab	55	17	6	6.4	0.17
	Syrah	227 a	52	22	6	6.4	0.21
	Malagouzia	198 abc	46	17	5	6.6	0.26
	Cinsaut	217 ab	46	17	5	6.7	0.19
	Roditis	178 abc	37	15	3	9.6	0.20
	LSD 5 %	47	17.8	13.7	4.3	5.9	0.09
41 B	Athiri	265 ab	65	30	16	5.5	0.20
	Grenache	225 b	57	40	23	6.0	0.20
	Assyrtiko	268 ab	74	31	17	5.3	0.27
	Carignan	288 a	73	31	15	5.0	0.19
	Syrah	243 ab	65	36	20	5.8	0.21
	Malagouzia	267 ab	65	28	17	5.6	0.23
	Cinsaut	262 ab	70	36	17	5.6	0.21
	Roditis	224 b	61	39	21	6.0	0.19
	LSD 5 %	40	16.6	12.6	11.3	2.8	0.11
140 Ru	Athiri	272 a	41	30	12	437.0 b	0.24
	Grenache	261 ab	57	27	12	5.3 ab	0.23
	Assyrtiko	199 ab	60	21	10	6.2 ab	0.30
	Carignan	229 ab	56	24	8	5.7 ab	0.26
	Syrah	216 ab	57	25	9	5.8 ab	0.25
	Malagouzia	236 ab	59	26	12	5.8 ab	0.27
	Cinsaut	188 a	62	18	8	8.0 a	0.30
	Roditis	231 ab	56	21	11	5.8 ab	0.22
	LSD 5 %	71	18.6	13	8.8	2.8	0.09
1103 P	Athiri	304 a	87 a	60 a	31	3.3 b	0.28
	Grenache	288 ab	86 a	55 ab	31	4.6 b	0.17
	Assyrtiko	248 b	66 c	39 b	23	7.6 a	0.30
	Carignan	283 ab	84 ab	51 ab	29	5.0 ab	0.24
	Syrah	274 ab	70 abc	40 b	24	5.9 ab	0.31
	Malagouzia	275 ab	68 bc	39 b	22	6.2 ab	0.24
	Cinsaut	282 ab	74 abc	52 ab	26	5.2 ab	0.27
	Roditis	280 ab	72 abc	44 ab	25	5.4 ab	0.21
	LSD 5 %	39	14.9	15.4	9.5	2.7	0.18
all trials	LSD 5 %	47	15.4	12.5	7.9	3.8	0.11

directly and not through the mycorrhizae (DIEDERICHS 1991).

In contrast, if we compare the infection rate and spore numbers in the root zone with the respective P concentration in the soil and we implement another statistical design (Regression Analysis - Multiplication Model $y = ax^{-b}$), we find an inverse correlation between the number of spores ($r = -0.944$, $r^2 = 0.891$) and root infection ($r = -0.928$, $r^2 = 0.862$) with a soil P concentration which is apparent even if the range of fluctuation of soil P is very narrow. Other authors stress the fact that mycorrhizal formation is particularly high in cases where soil P concentration tends towards zero (VELEMIS *et al.* 1995). In the current work an analogous positive correlation was found between spore population in the soil and percent colonization of the roots ($r = 0.998$, $r^2 = 0.997$). In literature results obtained with other species are contradictory indicating either a positive correlation between these two parameters or no correlation at all (BOYTECHKO and TEWARI 1990).

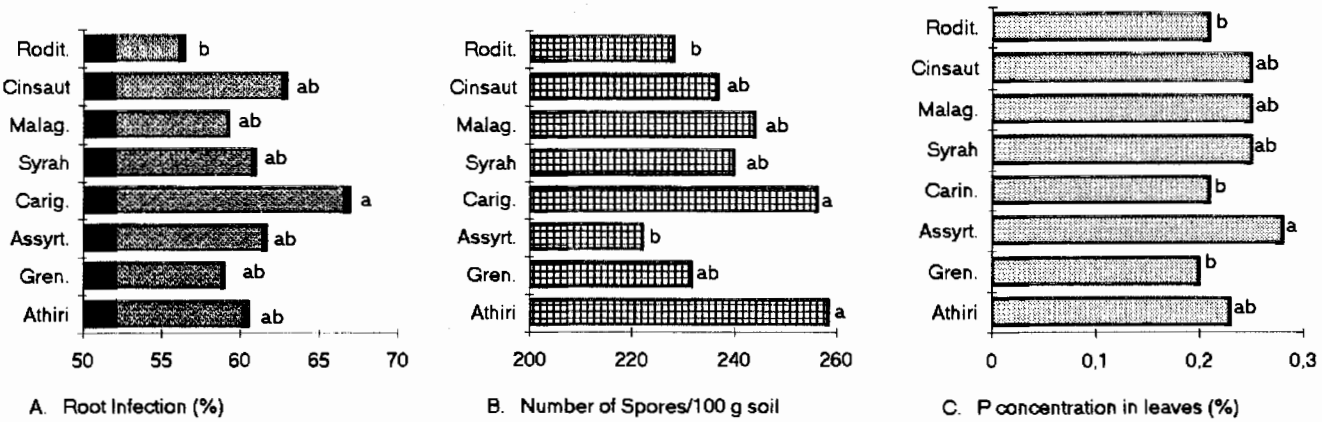

Figure: Mycorrhizal infection in roots, number of spores and P concentration in leaves of 8 different grapevine varieties. Bars marked by different letters are significantly different at the 0.05 level (Duncan's Multiple Range Test).

No correlation was found between P concentration in leaves and percent colonization in the roots.

Examining the values of P concentration in the leaves and applying the method of stepwise selection we find that for P nutrition of grapevines the main responsible factors are "soil P", "colonization" and "rootstock", from which, after selection, the factors "colonization" and "rootstock" are most effective (F-remove values 32.38 and 4.67 respectively, value F-to-remove: 4.00). Since the latter two factors are the most important, we recommend to provide optimal conditions for the growth and distribution of the locally adapted native mycorrhizal fungi, by use of appropriate cultural practices, and to select suitable rootstocks responding optimally to mycorrhizal symbiosis.

The main conclusion derived from this research is that the native vesicular arbuscular mycorrhizal fungi contribute to the grapevine's P nutrition so that regular P fertilization becomes unnecessary. Thus we can benefit both, directly from a reduction of production cost and indirectly by reducing a specific form of environmental pollution, the "eutrophism" of water, which is partly attributed to excessive P fertilization.

References

AN, Z. Q.; GUO, B. Z.; HENDRIX, J. W.; 1993: Population of spores and propagules of mycorrhizal fungi in relation to the life cycles of tall fescue and tobacco. Soil Biol. Biochem. **25**, 813-817.

ALEXIADIS, K.; 1972: Physical and Chemical Soil Analysis. Thessaloniki.

ATAYESE, M. O.; AWOTOYE, O. O.; OSONUBI, O.; MULONGOY, K.; 1993: Comparisons of the influence of vesicular-arbuscular mycorrhiza on the productivity of hedgerow woody legumes and cassava at the top and the base of a hillslope in alley cropping systems. Biol. Fertil. Soils **16**, 198-204.

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

DIEDERICHS, C.; 1991: Influence of different P sources on the efficiency of several tropical endomycorrhizal fungi in promoting the growth of *Zea mays* L. Fert. Res. **30**, 39-46.

EZAWA, T.; SAITO, M.; YOSHIDA, T.; 1995: Comparison of phosphatase localization in the intraradical hyphae of arbuscular mycorrhizal fungi *Glomus* spp. and *Gigaspora* spp. Plant and Soil **176**, 57-63.

GEBBING, H.; SCHWAB, A.; ALLEWELDT, G.; 1977: Mykorrhiza der Rebe. Vitis **16**, 279-285.

GERDEMANN, J. W.; NICOLSON, T. H.; 1963: Spores of mycorrhizal endogene species extracted from soil by wet sieving. Trans. Brit. Mycol. Soc. **46**, 234-235.

KARAGIANNIDIS, N.; 1980: Untersuchungen über die Effizienz der vesikulär-arbuskulären (VA)-Mykorrhiza verschiedener Herkunft bei unterschiedlichen tropischen und subtropischen Pflanzen, Phosphatformen, Boden-pH-Werten und Bodentemperaturen. Diss. Univ. Göttingen.

- -; NIKOLAOU, N.; MATTHEOU, A.; 1995: Wirkung dreier VA-Mykorrhizapilze auf Ertrag und Nährstoffaufnahme von drei Unterlagen und einer Tafeltraubensorte. Vitis **34**, 85-89.

KESAVA RAO, P. S.; TILAK, K. V. B. R.; ARUNACHALAM, V.; 1990: Genetic variation for mycorrhiza-dependent phosphate mobilization in groundnut (*Arachis hypogaea* L.). Plant and Soil **122**, 137-142.

MANJUNATH, A.; HABTE, M.; 1988: Development of vesicular-arbuscular mycorrhizal infection and the uptake of immobile nutrients in *Leucaena leucocephala*. Plant and Soil **106**, 97-103.

MENGE, J. A.; RASKI, D. J.; LIDER, L. A.; JOHNSON, E. L.; JONES, N. O.; KISSLER, J. J.; HEMSTREET, C. L.; 1983: Interactions between mycorrhizal fungi, soil fumigation and growth of grapes in California. Amer. J. Enol. Viticult. **34**, 117-121.

MERCY, M. A.; SHIVASHANKAR, G.; BAGYARAJ, D.J.; 1990: Mycorrhizal colonization in cowpea is dependent and heritable. Plant Soil **121**, 292-294.

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

OLSEN, S. R.; COLE, C. V.; WATANABE, F. S.; DEAN, L. A.; 1954: Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Dept of Agric. Circular **939**, 19.

PHILLIPS, J. M.; HAYMAN, D. S.; 1970: Improved procedures for cleaning root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. **55**, 158-161.

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

RABATIN, S. C.; 1979: Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by *Glomus tenuis*. New Phytol. **83**, 95-102.

RAJU, P. S.; CLARK, R. B.; ELLIS, J. R.; DUNCAN, R. R.; MARANVILLE, J. W.; 1990: Benifit and cost analysis and phosphorus efficiency of VA mycorrhizal fungi colonizations with sorghum (*Sorghum bicolor*) genotypes grown at varied phosphorus levels. Plant Soil **124**, 199-204.

RAMAN, N.; NAGARAJAN, N.; GOPINATHAN, S.; SAMBANDAN, K.; 1993: Mycorrhizal status of plant species colonizing a magnesite mine spoil in India. Biol. Fertil. Soils **16**, 76-78.

TRAPPE, J. M.; 1982: Synoptic keys to the genera and species of zygomycetous mycorrhizal fungi. Phytopathology **72**, 1102-1108.

VELEMIS, D.; KARAGIANNIDIS, N.; MANOLAKIS, E.; 1995. Einfluß von Mykorrhizapilzen auf den P-Gehalt der Blätter bei Kiwipflanzen. Erwerbsobstbau **3**, 88-91.

Received November 22, 1996