EFFECTS OF DIFFERENT P SOURCES IN SOIL ON INCREASING GROWTH AND MINERAL UPTAKE OF MYCORRHIZAL *VITIS VINIFERA* L. (cv VICTORIA) VINES

EFFET DES DIFFÉRENTES SOURCES DE P DU SOL SUR LA CROISSANCE DES RAMEAUX ET L'ABSORPTION MINÉRALE DU CÉPAGE DE VIGNE VICTORIA (*VITIS VINIFERA* L.) MYCORHIZÉ

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Abstract: The effect of different P-sources on growth, leaf chemical composition, and fruit soluble solids and acid content was evaluated in mycorrhizal Victoria grapevine variety grafted onto the rootstocks 3309C or 110R. Mycorrhizal and non mycorrhizal plants were grown in 20 L pots containing 20 kg soil supplemented with different P-forms: (Calcium bis-dihydrogen-phosphate, tri-calcium phosphate, aluminium phosphate, iron (III) phosphate) with different solubility, equivalent to 90 kg $P.ha^{-1}$. The percent of mycorrhizal root colonization was higher in insoluble P-form treatments compared to control or to soluble P-form treatment (CaDP), ranging from 66 to 84 % in treatments receiving insoluble P, from 36.67 to 38.33 % in control and from 25.33 to 27.33 % in soluble P-form treatments. The roots of 110R rootstock showed higher colonization rate compared to the 3309C. Mycorrhizal colonization increased both the pruning weight and number of nodes of the vines, up to 9 and 1.9 times respectively, according to the root-stock-P form combination. Mycorrhizal vines showed increased leaf concentrations in N, P, K, Ca. Fruit total soluble solids of mycorrhizal vines were about 30 % lower compared with those of the non mycorrhizal vines. Both factors, mycorrhizal colonization and P-forms had no significant effect in fruit titratable acidity.

Résumé : Les effets des différentes sources de P sur la croissance, la composition minérale des feuilles et la teneur des raisins en solides solubles totaux et en acides ont été évaluées chez le cépage de vigne Victoria greffé sur les porte-greffes 3309C et 110R. Les plants de vignes mycorhizés et non-mycorhizés ont été cultivés dans des pots contenant 20 Kg de sol ayant reçu des quantités de phosphore (phosphate de calcium bis-di-hydrogène, phosphate tri-calcique, phosphate d'aluminium et phosphate de fer) de solubilité différente, équivalent à 90 kg P ha^{-1}. Le taux de colonisation des racines par les mycorhizes était plus élevé dans les traitements avec les formes insolubles de phosphore (de 66 à 84 %) que dans les cas de traitements avec des formes solubles (de 36,67 à 38,33 %) ou des plants témoins (de 25,33 à 27,33 %). Les racines du porte-greffe 110R ont présenté une colonisation mycorhizienne plus élevée par rapport au 3309C. La colonisation mycorhizienne a augmenté le poids de bois de taille et le nombre de nœuds des rameaux : 9 et 1,9 fois respectivement, selon la combinaison porte-greffe forme de P. Les vignes mycorrhizées ont présenté des concentrations plus élevées en N, P, K, Ca, Mg et B par rapport aux vignes non-mycorhizées. Le taux de solides solubles totaux des raisins de vignes mycorrhizées a été inférieur de 30 % par rapport aux vignes non-mycorhizées. L'acidité totale n'a pas été influencée ni par la mycorhization, ni par les formes de phosphore.

Key words: grapevine (*Vitis vinifera* L.), mycorrhizae, alternative P-sources, nutrient uptake

Mots-clés : vigne (*Vitis vinifera* L.), mycorhize, sources alternatives de P, absorption minérale

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are widespread in nature, living symbiotically with the root system of a great number of plant species. They play an important role in plant community and often the growth of mycorrhizal plant is increased in comparison to non-mycorrhizal plants, particularly at low phosphorus availability. Several investigations have examined the effect of the AM fungi on plant growth and nutrient uptake. The beneficial effect of these fungi on plant growth has largely been attributed to a higher uptake of P (NADIAN *et al.*, 1996; THOMPSON, 1994). Fungal development, both within and around the roots, provides addi-

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tional surface area in root system. The external hyphae are able to absorb and translocate phosphorus to the host from soil outside the root depletion zone (KARAGIANNIDIS and NIKOLAOU, 1999). Thereby, under conditions where P diffusion in soil is slow and root density not very high, more soil P is spatially available to mycorrhizal than to non-mycorrhizal plants (BOLAN, 1991). However, non-mycorrhizal plants are more responsive than mycorrhizal plants to fertilizer applications, and fungal development can be severely reduced in the cases of high P fertilization (GIANINAZZI-PEARSON, 1986). It is well known that if phosphate fertilizer is added to a normal soil, for the most part it is rapidly converted to insoluble forms not directly available for plants. It has been reported that plants take up a small fraction of phosphate P fertilizer and that it is rated between 1 and 10 %, at least 90 % of phosphate P being insoluble (CABALA-ROSAND and WILD, 1982), consisting an important but unavailable reserve in the soil. On the other hand mycorrhiza permit a better exploitation of phosphate reserves in soils and therefore more rational use of applied fertilizer. The primary sources of phosphate for fertilizers are certain mineral rock phosphates derived from the phosphate of organisms living in past and containing up to 80 % of apatite usually in form of fluorapatite. The rising cost of industrial fertilizers, the diminishing world reserves of phosphate as well as the impact that these can have on the environment, have all prompted increasing interest in the symbiotic associations as a possible mean of improving the efficiency of fertilizer use in agriculture. On the other hand several studies of AM symbiosis in grapevine have been published indicating that grapevine roots can be normally mycorrhizal and that mycorrhizal colonization improve grapevine nutritional status (GEBBING *et al.*, 1977; NAPPI *et al.*, 1985; KARAGIANNIDIS *et al.*, 1995; KARAGIANNIDIS and NIKOLAOU, 1999).

The purpose of the present research work was to examine the efficiency of the endomycorrhizal fungus *Glomus mosseae* on the growth and chemical composition of grape vine plants grown in soil supplemented with four P fertilizers of different solubility in order to assess the use of insoluble phosphate as P fertilizers in grapevine, after a single grinding of rock phosphate.

MATERIALS AND METHODS

I - MATERIALS

The experiment was conducted at the experimental farm of Aristotle University of Thessaloniki using potted plants grown outdoors. Treatments were a factorial (2 x 2 x 5) combination of two grapevine rootstocks (3309C, 110R), root mycorrhizal infection (presence or absence) and four phosphorus forms plus the untreated plants, replicated 4 times. The rootstocks used in this trial are of very different genetic parentage and agronomical properties. The rootstock 3309C is a *Riparia* x *Rupestris* hybrids, of moderate vigour, sensible to drought while 110R is a *Rupestris* x *Berlandieri* hybrids of increased vigour and drought resistance.

The first year of experimentation grafted vines were grown in 6L pots containing a sterilized pot medium. At the beginning of the next vegetative period, four uniform plants for each treatment were transplanted in pots containing 20 kg fine soil (< 2 mm), collected from 0-10 cm depth in field at Soil Science Institute of Thessaloniki. Soil physico-chemical properties are given in table I.

Mineral nutrients were mixed into the pot medium at rates corresponding to 200 kg ha^{-1} N (NH_4NO_3), 80 kg ha^{-1} K (K_2SO_4), 20 kg ha^{-1} Mg ($Mg.SO_4$ $6H_4O$). Four P- sources of different solubility were applied as P-fertilizers equivalent to 90 kg ha^{-1}. [Calcium bis-dihydrogen phosphate (CaDP), tri-Calcium phosphate (tCaP), aluminium phosphate (AlP), and iron (III) phosphate (FeP)]. In the same time a treatment without P-fertilizer was also included as a control. Among the fertilizers, nitrogen was applied every year whereas K, Mg and insoluble P-forms were applied once at the beginning of the experimentation. At drought period watering was done with 3 L of water every 2 or 3 days when the water content of the pot medium was dropped below 70 % of field capacity.

II - INOCULUM - INOCULATION

For the inoculum preparation, some years ago soil samples were collected from three different vineyards of the Thessaloniki region. AM fungi spores were collected from soil samples by the wet sieving GERDEMANN and NICOLSON (1963). Spore morphology

Table I - Physicochemical properties of the soil used as pot medium.

Propriétés physiques et chimiques du sol utilisé comme milieu des pots

Soil type	pH (1:1)	$CaCO^3$ %	$ECX10^3$ (25 °C) (µS/cm)	Org. matter %	P Olsen (mg.Kg^{-1})	K exch. (mg.Kg^{-1})	B	Zn	Mn (mg.Kg^{-1})	Fe	Cu
SCL*	7.20	1.3	0.890	1,35	7.5	335	1.08	1.12	35.90	8.08	3.8

*SCL = sandy clay loam

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was ascertained under a compound microscope and identification was made using the keys of HALL and FISH (1979); TRAPPE (1982); SCHENCK and PEREZ (1988). The fungal species *Glomus mosseae* was most frequently associated with grapevine roots. Young maize plants were inoculated by *Glomus mosseae* spores and served as inoculum.

At transplanting, half of the plants were inoculated with Glomus mosseae mycorrhizal inoculum consisting of 80 cm^3 per pot mycorrhizal root pieces and rooting medium, derived from the stock of mycorrhizal maize plants. The inoculum was placed in the planting hole beneath the vines.

III - SOIL CHEMICAL ANALYSES

From experimental soil a sub-sample was air dried, sieved (< 2 mm) and pH was measured in water (soil/water: 1/2). Organic matter, EC (electrical conductivity) and $CaCO_3$ were determined according to JACKSON (1960). Extractable P was assessed according to OLSEN *et al.* (1954). The soil nutrients Zn, Fe, Mn and Cu were determined with DTPA extraction procedure (LINDSAY and NORVEL (1978), B with hot water and K by acetic acid (JACKSON, 1960).

IV - LEAF ANALYSES

For leaf analysis, each sample (blade + petiole) was made up of three leaves from each pot, opposite to the first cluster at veraison stage, during the second and third experimental year. The leaves were then washed to remove dust and pesticide residues, dried at 72 °C, ground and stored in plastic bags. One g of dry matter was ashed at 550 °C, dissolved in 6N HCl and diluted with distilled water. Nitrogen was determined by the Kjeldahl procedure, phosphorus spectrometrically, potassium photometrically, calcium and magnesium volumetrically with 0.01N EDTA, manganese, zinc, iron and copper by atomic absorption spectrophotometry (JACKSON, 1960). Boron was determined by the curcumin method.

V - FRUIT ANALYSIS

The 3rd year of experimentation, grape samples were collected at maturity and soluble solids were measured as °Brix by means of a hand-held temperature compensation refractometer. Juice pH was measured by electronic pH meter. Titratable acidity was measured by titrating 10 ml juice against 0.1N NaOH to pH 8.2.

VI - MYCORRHIZAL ANALYSIS

Soil samples were taken from each pot at the last week of September and were maintained in closed plastic bags at 4 °C until processing, but not later than 15 days after sampling. In the laboratory, the samples were carefully homogenized and the mycorrhizal fungal spores were isolated from 100 g soil by the wet sieving and decanting technique followed by sucrose centrifugation (GERDEMANN and NICOLSON, 1963 ; SIEVERDING, 1991). The isolated spores that appeared healthy and without microbial attack were counted using a dissecting microscope (60 x).

From each pot, fine (sub-lateral) roots were collected and stained in trypan blue according to PHILLIPS and HAYMAN (1970). Percentage infection was assessed by the grid line method of GIOVANNETTI and MOSSE (1980). A minimum of 100 intercepts were scored for each sample.

VII - DATA ANALYSIS

The experimental design was a 2 x 2 x 5 factorial with 4 pots per experimental unit. Analysis of variance was done by MSTAT statistical programme, and the means were compared by Least Significant Differences (LSD).

Table II - Root colonization (%) and number of spores per 100 g soil of mycorrhizal Victoria grapevine plants grafted onto two rootstocks, grown with different P- forms in soil

Colonisation (%) des racines et nombre des spores pour 100 g de sol du cépage de vigne Victoria mycorhizé, greffé sur deux porte-greffes et cultivé avec différentes sources de P

	Victoria /3309C		Victoria /110R	
	% colonization	number of spores	% colonization	number of spores
O	36.67	87.67	38.33	74.33
CaDP	25.33	23.00	27.33	47.00
tCaP	66.00	346.33	78.33	406.67
AlP	69.33	314.33	80.67	407.00
FeP	70.67	323.67	84.00	384.33
LSD (0.05)	10.35	93.20	10.35	93.20

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RESULTS

I - MYCORRHIZAL COLONIZATION

As shown in table II, percent root colonization and spore count were significantly higher in the treatments that have received insoluble P-forms compared to the soluble ones or to the control. In the tCaP, AlP, and FeP treatments the percentage colonization was increased up to 1.9 and 2.2 times for the 3309C and the 110R rootstock respectively as compared to the control. On the other hand the spore number per 100 g, was also increased up to 3.69 and 5.17 times respectively, compared to those of the control. Moreover, decreased mycorrhizal colonization and spore number were observed, in the CaDP treatment. In the treatments with insoluble P-forms, root colonization and spore numbers were higher in 110R rootstock than in the 3309C, whereas no differences were found between the control and the soluble P sources.

An increased number of arbuscules and cysts was also detected in the three treatments with insoluble P-forms, whereas these mycorrhizal parameters were strongly reduced in CaDP application (data not shown). In some tested root fragments of this last treatment not-one simple cyst could be found.

II - SHOOT GROWTH

Figure 1 shows the influence of mycorrhizal colonization on the pruning weight of vine plants grown in pots as related to P sources. It can be seen that non mycorrhizal plants had a reduced pruning weight, compared to mycorrhizal plants. No differences were observed between the two rootstocks as well as among the P-form treatments in non mycorrhizal vines. Root mycorrhizal colonization improved pruning weight of the vine plants from 2 to 9 times as compared to control plants. Table III presents the M/NM (mycorrhizal/non mycorrhizal) ratios of the pruning weight, node number and soluble solid content : Increased values are observed in the insoluble-P treatments with the exception of total soluble solids. Analysis of variance showed a significant interaction between rootstock variety and P-form. Thus, 110R rootstock had the highest pruning weight in tCaP treatment whereas 3309C rootstock showed the highest pruning weight with AlP fertilizer. The M/NM values of this parameter were greater than 1.0 in both rootstocks as well as in all the P treatment, being highest in both tCaP and AlP treatments.

The effect of the rootstock on the node number per plant of Victoria variety is presented in figure 2. The three factors used in this trial had significant influence on the number of nodes. Mycorrhizal plants had greater node number per plant than non-mycorrhizal ones. In general, the 110R had greater node number in the most of the cases. In both rootstocks, the highest number of nodes was observed in tCaP and AlP treatments. The M/NM values for the node number varied from

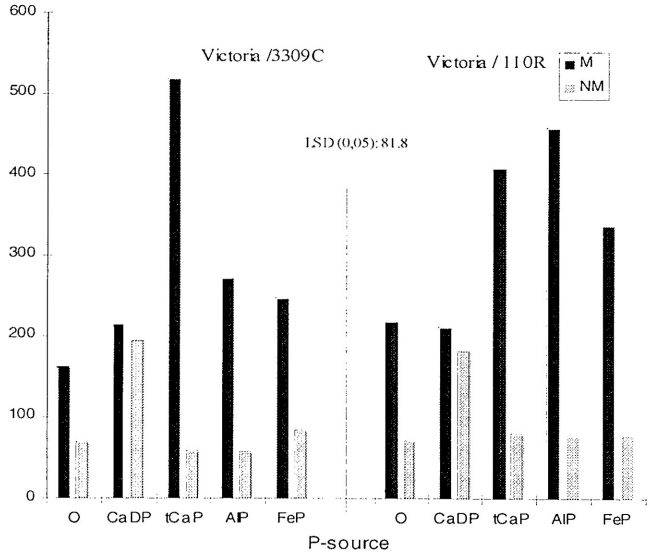


Fig. 1 - Pruning weight of mycorrhizal (M) and non-mycorrhizal (NM) Victoria grapevine plants grafted onto two rootstocks, grown in soil supplemented with different P forms.

Poids de bois de taille du cépage de vigne Victoria mycorhizé et non-mycorhizé greffé sur deux porte-greffes et cultivé sur un sol ayant reçu différentes sources de P.

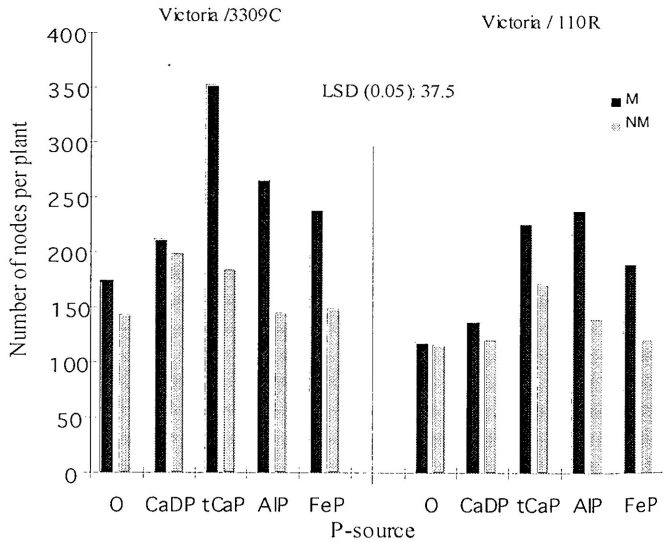


Fig. 2 - Number of nodes of mycorrhizal (M) and non-mycorrhizal (NM) Victoria grapevine plants grafted onto two rootstocks, grown in soil supplemented with different P forms.

Nombre des nœuds du cépage de vigne Victoria mycorhizé et non-mycorhizé greffé sur deux porte-greffes et cultivé sur le sol ayant reçu différentes sources de P.

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Table III - The M/NM ratios of pruning weight, number of nodes and berry soluble solids of mycorrhizal (M) and non-mycorrhizal (NM) grapevine plants, grown at different P forms supplements in soil.

Les valeurs M/NM pour le poids de bois de taille, le nombre des neuds et des solides solubles totaux des fruits des plants de vigne mycorhizés (M) et non- mycorhizés (NM) cultivés avec des sources différentes de P

Treatments	Pruning weight	Node number	Soluble solids
	Victoria/3309C		
O	2,3	1.2	0.9
CaDP	1.0	1.0	0.7
tCaP	9,1	1.9	0.7
AlP	4,9	1.9	0.7
FeP	3,0	1.6	0.7
	Victoria/110R		
O	3.1	1.0	0.7
CaDP	1.2	1.1	0.7
tCaP	5.1	1.3	0.7
AlP	6.0	1.7	0.7
FeP	4.2	1.6	0.6

1 to 1.9 in 3309C and from 1 to 1.7 in 110R, being highest in insoluble P form treatments (table III).

III - FRUIT COMPOSITION

Total soluble solids in fruits at harvesting were higher in the treatments with tCaP, AlP and FeP, when compared to control and CaDP for both factors, mycorrhizal infection and rootstock (figure 3). In non mycorrhizal plants concentrations of total soluble solids in fruits were increased. The M/MN ratios remained stable (0.7) irrespective of the P-form (table III). Total acidity varied from 3 to 3.5 without significant differences between all treatments.

IV - NUTRIENT LEAF CONCENTRATIONS

Leaf (blade + petiole) N, P, K, Ca and Mg concentrations were higher in mycorrhizal plants with non significant interaction between mycorrhizal colonization and P-forms as well as between mycorrhizal colonization and rootstock variety. M/NM values of the macronutrients varied from 1.1 to 1.8 (table IV). Leaf P concentrations increased in mycorrhizal plants varying from 0.17 % to 0.23 % and from 0.19 % to 0.26 % (d. m. basis) in 3309C and 110R rootstocks, respectively. In the non-mycorrhizal plants the leaf-P concentration ranged from 0.11 % to 0.18 % and from 0.14 % to 0.18 % respectively.

The highest values of M/NM ratios (1.8) were observed for leaf P concentrations in tCaP treatment.

Among the micronutrients, B showed the same tendencies as the macronutrients. The M/NM ratios varied between 1.2 and 1.6.

Mycorrhizal plants showed lower leaf concentrations for the other micronutrients, when compared to the non-mycorrhizal plants. The M/NM ratios were always lower than 1.0.

DISCUSSION

The root colonization by AM fungi was significantly higher in insoluble P form treatments. A similar tendency was also shown for the spore densities. This indicates that the soluble phosphate fertilizers in high doses inhibited the growth of mycorrhizal symbiosis. The reduction of mycorrhizal colonization by soluble-P forms has been reported many times (AMIJE *et al.*, 1990; DIEDERICHS, 1990). MOSSE (1973), stated that vesicles are eliminated and arbuscules become smaller and fewer with increasing soil phosphate concentrations. The small total number of spores in control and soluble P treatments would alone account for the low level of colonization produced in this plots. Considering that growth of colonized vines was negatively affected by the presence of superphosphate, it can be assumed that mycorrhizae are sensitive to increasing levels of soluble P.

Inoculation by *Glomus mosseae* fungi significantly improved the pruning weight of the vines. This is supported by the results of a number of workers who reported that added inoculum enhanced plant growth (AMES and BETHLENFALVAY, 1987; BOLAN *et al.*, 1997; DIEDERICH, 1991). The increased shoot growth of mycorrhizal vines in insoluble P-forms may be due to higher P uptake and growth response. It has been reported that growth response of mycorrhizal plants to rock phosphate fertilizers, is due to a more efficient absorption of phosphate ions as they become chemically dissociated from particles of the fertilizer (PARUNAN *et al.*, 1980). In our experiment, mycorrhizal plants which received insoluble P, showed higher pruning weight than those received soluble phosphate salt (CaDP). Phosphorus fertilizers of different solubility had different effect on vine growth. Similar results have been reported by BJÖRN *et al.* (1989) and DIEDERICHS (1990) for other plant species.

WALKER and SIVAK (1986), reported that improved P nutrition should increase the supply of soluble carbohydrates within the plants, which should have a positive effect on fruit yield and shoot growth.

Different growth response was found between the two rootstocks used in this trial. For the mycorrhizal plants, those grafted on 3309C achieved the maximum

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Table IV - Macronutrient (N, P, K, Ca, Mg) concentrations of leaves (% d.m.) and the ratio M:NM in mycorrhizal (M) and non-mycorrhizal (NM) grapevine plants, grown at different P forms supplements in soil.

Concentrations en macro-éléments (N, P, K, Ca, Mg) des feuilles (% m.s) et les valeurs M/NM pour les plants de vigne mycorhizés(M) et non- mycorhizés(NM) cultivés avec différentes sources de P.

	Victoria / 3309C			Victoria / 110R		
P-source	M	NM	M/NM	M	NM	M/NM
N %						
O	2.21	1.97	1.1	2.22	1.98	1.1
CaDP	2.36	1.82	1.3	2.20	1.97	1.1
tCaP	2.29	1.90	1.2	2.13	2.00	1.1
AlP	2.36	2.01	1.2	2.13	1.77	1.2
FeP	2.35	2.02	1.2	2.13	1.89	1.1
LSD(P:0.05)	0.132			0.132		
P %						
O	0.17	0.13	1.3	0.26	0.18	1.4
CaDP	0.23	0.18	1.3	0.21	0.17	1.2
tCaP	0.20	0.11	1.8	0.25	0.14	1.8
AlP	0.20	0.13	1.6	0.22	0.14	1.6
FeP	0.20	0.15	1.3	0.19	0.16	1.2
LSD (P:0.05)	0.031			0.031		
K %						
O	1.95	1.45	1.3	2.45	1.72	1.4
CaDP	2.07	1.55	1.3	2.09	1.68	1.2
tCaP	1.88	1.40	1.3	2.55	2.04	1.3
AlP	1.96	1.56	1.2	2.46	1.76	1.4
FeP	1.83	1.41	1.3	2.44	1.88	1.3
LSD(P:0.05)	0.361			0.361		
Ca %						
O	2.78	2.28	1.2	2.58	2.11	1.2
CaDP	2.86	2.07	1.4	2.57	1.89	1.4
tCaP	2.59	2.14	1.2	2.26	1.65	1.4
AlP	2.50	2.00	1.3	2.26	1.96	1.2
FeP	2.42	2.19	1.1	2.46	1.98	1.2
LSD (P:0.05)	0.310			0.310		
Mg %						
O	0.50	0.33	1.5	0.66	0.47	1.4
CaDP	0.63	0.44	1.4	0.71	0.56	1.3
tCaP	0.57	0.50	1.1	0.64	0.49	1.3
AlP	0.57	0.54b	1.1	0.66	0.49	1.3
FeP	0.55b	0.54b	1.0	0.66	0.44	1.5
LSD (P:0.05)	0.042			0.42		

pruning weight in the presence of tCaP rock phosphate whereas those grafted on 110R in the presence of AlP. Considerable differences have been reported between the host plant species when using the same fungus (DIEDERICHS, 1987). The increased shoot growth of mycorrhizal plants was a result particularly of an increased node number per plant. Improved P uptake in mycorrhizal vines increased the node formation and, subsequently enhanced plant growth. Nevertheless no differences were found for inter node length among the different treatments (data not shown).

Several changes in plants are known to result from colonization and these may have direct or indirect effect on growth pattern; morphogenetic analysis of vine plants showed that infected plants developed a denser root system, with a higher number of lateral roots (BERTA *et al.*, 1993; SCHELLENBAUM *et al.*, 1991). On the other hand changes in the distribution and levels of both cytokinins and auxins, are known to occur in mycorrhizal plants. EDRISS *et al.* (1984) found elevated cytokinin levels in infected Citrus plants. Rootstock also had a considerable effect on growth

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after bud burst. A close positive dependence has been reported between the cytokinin concentration in the roots and the shoots, considerably affecting the shoot growth (LILOV and ANDONOVA, 1976 ; NIKOLAOU *et al.*, 2000). Besides that, RICHARDS and ROWE (1977) supported that the controlling influence of the root on the growth of the shoot is mediated by its cytokinin production.

The macronutrients N, K, Ca and Mg were mostly significantly affected by the different treatments. AM fungi increased the leaf concentrations of these macronutrients. The high plant availability of these nutrients in mycorrhizal plots is in agreement with previous findings (BAVARESCO and FOGER, 1996 ; KARAGIANNIDIS *et al.*, 1995). Increased leaf boron concentration was also found in mycorrhizal vines, with higher values in 110R rootstock. Similar results for boron were also obtained by BAVARESCO and FOGHER (1996). The leaf P concentration in mycorrhizal plants was significantly greater than in non-mycorrhizal plants. Increased P content due to mycorrhizal colonization has been reported for several plant species and also for the vine (NAPPI *et al.*, 1985 ; SCHUBERT and CRAVERO, 1985 ; KARAGIANNIDIS *et al.*, 1995 ; BAVARESCO and FOGHER, 1996). However other findings indicated that from practical standpoint, especially in soils with very low P, mycorrhizal infection will not be beneficial unless the host plant is provided with superphosphate fertilizer for initial root establishment and growth of plants (RAJU *et al.*, 1987). In our pot trial this initial root and plant growth was successful because of a low but sufficient medium P content (7.5 ppm, table I). On the other hand, the plants in pots have a limited volume according to pot volume, thus a part of the initial needs in nutrients can be provided by tissue nutrient content. The main contribution of mycorrhizal fungi to the host is to reach and translocate phosphate through their exraradical hyphae which extend up to 9 cm in the rhizosphere (SYLVIA, 1998), which would otherwise not be available to the host. Phosphatase activity in the rhizosphere play an important role for the acquisition of P by plant roots. TARAFDAR and MARSCHNER (1994) reported that mycorrhizal roots produced increasing amounts of phosphatase particularly released by extraradical mycelium. They also suggested that phosphatase activity was strongly correlated with hyphal length which was highest within 10 mm from the root surface.

Mn, Zn, Fe and Cu concentrations decreased in the leaves of mycorrhizal plants. In the present study findings agree with those of WEISSENHORN *et al.* (1995). GILDON and TINKER (1983) however suggested that mycorrhizal fungi improve the heavy metal uptake and as a result increase its accumulation in the plants. However MANJUNATH and HABTE (1988) who studied the Cu uptake by plants suggested that under conditions of high soil Cu solubility, this metal is accumulated in roots and not translocated to the shoots. It is possible that a similar accumulation occurs for the other heavy metals. In addition, at a high supply, heavy metals may be absorbed at hyphal cell walls or within the hyphal cells and fixed by polyphoshate granules (GADD, 1993; GALLI, 1994).

Phosphorus concentration in the leaves seems to depend on the forms of P supply but also to rootstock variety. In 3309C, phosphorus leaf concentrations of the mycorrhizal plants fertilized with the insoluble tCaP was higher than those treated with soluble form (CaDP) (table IV). This can be attributed to an increased mycorrhizal activity observed in this treatment (table I). Similar results indicating the ability of mycorrhizae to mobilize phosphorus from different P sources were also obtained with maize by DIEDERICHS (1991). However using other sparingly soluble phosphate (AlP, FeP) the leaf P concentrations were comparable to the tCaP values, particularly in 3309C rootstock.

The rootstock 110R showed a higher uptake of P and B. It has been reported that genetically different rootstocks influence scion nutrient status, growth, and yield. GRANT and MATTHEWS (1996) reported that different rootstocks absorb different amounts of P and that shoot and leaf growth resulted from a different P uptake. They also demonstrated that under P sufficiency,

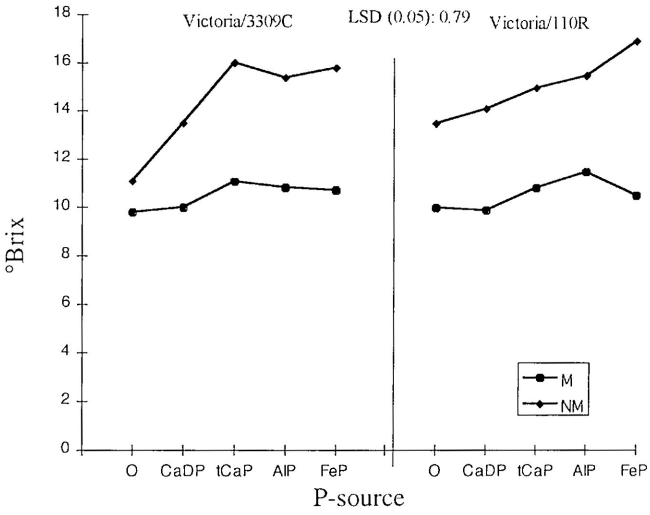


Fig. 3 - Total soluble solids (°Brix) of mycorrhizal (M) and non-mycorrhizal (NM) Victoria grapevine plants grafted onto two rootstocks, grown in soil supplemented with different P forms

Fig. 3 - Solides solubles totaux du cépage de vigne Victoria mycorhizé et non-mycorhizé greffé sur deux porte-greffes et cultivé sur un sol ayant reçu différentes sources de P.

J. Int. Sci. Vigne Vin, 2002, **36**, n°4, 195-204
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Table V - Micronutrient (B, Zn, Mn, Fe, Cu) concentrations of leaves and the ratio M/NM in mycorrhizal (M) and non-mycorrhizal (NM) grapevine plants, grown at different P forms supplements in soil.

Concentrations en oligo-éléments (B, Zn, Mn, Fe, Cu) des feuilles et les valeurs M:NM pour les plants de vigne mycorhizés (M) et non- mycorhizés (NM) cultivés avec différentes sources de P.

	Victoria / 3309			Victoria / 110R		
P-source	M	NM	M/NM	M	NM	M/NM
			B mg/kg			
O	15.7	11.3	1.4	25.0	18.7	1.3
CaDP	12.7	11.0	1.2	24.7	16.7	1.5
tCaP	19.3	12.3	1.6	18.0	11.3	1.6
AlP	16,7	13.3	1.3	22.0	15.0	1.5
FeP	15.7	12,7	1.3	18.7	14.0	1.3
LSD (P:0.05)	3.615			3.615		
			Mn mg/kg			
O	69.3	120.3	0.6	54.3	103.3	0.5
CaDP	77.3	162.0	0.5	59.7	118.7	0.5
tCaP	81.0	153.7	0.5	28.0	69.3	0.4
AlP	101.7	152.0	0.7	22.0	104.0	0.2
FeP	86.0	146.7	0.6	22.7	65.3	0.3
LSD (P:0.05)	21.89			21.89		
			Zn mg/kg			
O	28.0	41.7	0.7	23.0	32.7	0.7
CaDP	24.7	33.	0.7	19.0	31.7	0.6
tCaP	22.0	34.3	0.6	17.3	23.3	0.7
AlP	29.0	38.3	0.8	17.3	29.7	0.6
FeP	26.3	31.7	0.8	17.0	26.7	0.6
LSD (P:0.05)	6.19			6.19		
			Fe mg/kg			
O	96.7	103.7	0.9	99.3	132.3	0.8
CaDP	86.7	158.0	0.5	100.3	135.3	0.7
tCaP	88.3	119.7	0.7	109.7	136.0	0.8
AlP	88.3	106.0	0.8	104.3	134.0	0.8
FeP	99.0	118.0	0.8	119.0	136.0	0.9
LSD (P:0.05)	15.32			15.32		
			Cu mg/kg			
O	4.0	10.5	0.4	2.7	7.0	0.4
CaDP	6.3	10.3	0.6	2.0	3.7	0.5
tCaP	5.5	12.3	0.4	2.5	3.5	0.7
AlP	6.7	9.2	0.7	3.0	3.7	0.8
FeP	6.2	8.8	0.7	3.5	3.8	0.9
LSD (P:0.05)	1.89			1.89		

vines grafted on 110R had higher petiole P concentrations than vines grafted onto other rootstocks (Freedom, A X RG1, du Lot).

An increased total soluble solid content of the berries was found (figure 3) in the insoluble P treatments. This is a considerable factor for the grape and wine quality. Mycorrhizal infection induced an increased shoot growth with a greater number of leaves per plant. This may improve total photosynthesis per plant and grape maturity.

These findings lead to the assumption that both mycorrhizal efficiency and root colonization could be closely correlated with the presence of hardly soluble P as it has been reported in the previous work (KARAGIANNIDIS *et al.*, 1997).

CONCLUSION

The results of the present study illustrate that insoluble P-forms could be used in vine cultivation as phosphorus fertilizer under edaphic conditions which

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improve mycorrhizal activity in the rhizophere. Under such condition mycorrhizal fungi could help plants to acquire phosphorus from the important but unavailable phosphate reserves of the soil accumulated after successive P-fertilization by superphosphates. It could be interesting to assess the use of the rock phosphate under root mycorrhizal colonization As this pot trial gave promising results, further experimentation under field conditions particularly on indigenous mycorrhizal fungi - rootstock interaction as well as on artificial field inoculation is needed.

REFERENCES

AMES R.T. and BETHLENFALVAY G. J., 1987. Localized increase in nodule activity but no competitive interaction of cowpea rhizobia due to pre-establishment of vesicular-arbuscular mycorrhiza. *New Phytol.*, **106**, 207-215.

AMIJE, F., STRIBLEY D.P. and TINKER P.B., 1990. Soluble carbohydrates in roots of leek (*Allium porrum*) plants in relation to phosphorus supply and VA mycorrhizas. *Plant and Soil*, **124**, 195-198.

BAVARESCO L. and FOGHER C., 1996. Lime-induced chlorosis of grapevine as affected by rootstock and root infection with arbuscular mycorrhiza and Pseudomonas fluorescens. *Vitis*, **35**, n°3, 119-123.

BERTA G., FUSCONI A. and TROTTA A., 1993. VA mycorrhizal infection and the morphology and function of root system. *Environ. Exp. Botany*, **33**, 159-173.

BJÖRN F., MOAWAD A.M. and ACHTNICH W., 1989. Effect of VA mycorrhiza on dry weight and phosphorus content in shoot of cereal crops fertilized with rock phosphates at different soil pH and temperature levels. *Z. Pflanzenernähr. Bodenk.*, **152**, 255-259.

BOLAN N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil*, **134**, 189-207.

BOLAN N.S., ROBSON A.D. and BARROW N.J., 1997. Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plant and Soil*, **99,** 401-410.

CABALA-ROSAND P. and WILD A.,1982. Direct use of low grade phosphate rock from Brazil as fertilizer. II. Effects of mycorrhizal inoculation and nitrogen source. *Plant and Soil*, **65**, 363-373.

DIEDERICHS C., 1987. Interaction between five endomycorrhizal fungi and the root knot nematode Meloidogyne javanica on chickpea under tropical conditions. *Trop. Agric.*, **64**, n°4, 353-355.

DIEDERICHS C., 1990. Improved growth of *Cajanus cajan* (L.) Millsp. in an unsterile tropical soil by three mycorrhizal fungi. *Plant and Soil*, **123**, 261-266.

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

EDRIS M.H., DAVIS R.M. and BURGER D.W.,1984. Influence of mycorrhizal fungi on cytokinin production in sour orange. *J. Am. Soc.Hortic. Sci.*, **109**, 587-590.

GADD G.M., 1993. Interactions of fungi with toxic metals. *New Phytol.*, **124**, 25-60.

GALLI U., SCH?EPP H. and BRUNOLD, C., 1994. Heavy metal binding by mycorrhizal fungi. *Physiol. Plant.* **92**, 364-368.

GEBBING H., SCHWAB A. and ALLEWELDT G., 1977. Mikorrhiza der Rebe. *Vitis*, **16**, 279-285.

GERDEMANN J.W. and NICOLSON T. H., 1963. Spore of mycorrhizal endogen species extracted from soil by wet sieving. *Trans. Brit. Mycol. soc.*, **46**, 234-235.

GIANINAZZI-PEARSON V.,1986. Mycorhizae: A potential for better use of phosphate fertilizer. *Fertilizers Agriculture*, **92**, 3-12.

GILDON A. and TINKER, P. B., 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytol.*, **95**, 247- 261.

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

GRANT S. R. and MATTHEWS M.A., 1996. The influence of phosphorus availability and rootstock on root system characteristics, phosphorus uptake, phosphorus partitioning, and growth efficiency. *Am. J. Enol. Vitic.* **47**,403- 409.

HALL I.R. and FISH B.J., 1979. A key to the Endogonaceae. *Trans. Brit. Mycol. Soc.*, **73**, 261-270.

JACKSON M. L., 1960. *Soil chemical analysis*. Constable and Ltd., London. p. 498.

JOHANSEN, A., JAKOBSEN, I. et JENSEN, E.S., 1993. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 3. Hyphal transport of 32P and 15N. *New Phytol.*, **124**, 61-68.

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

KARAGIANNIDIS N., VELEMIS D. and STAVROPOULOS N., 1997. Root colonization and spore population by VA-mycorrhizal fungi in four grapevine rootstocks. *Vitis*, **36**, 57-?.

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KARAGIANNIDIS N. and NIKOLAOU, N., 1999. Arbuscular mycorrhizal root infection as an important factor of grapevine nutrition status. Multivariate analysis application for evaluation and characterization of the soil and leaf parameters. *Agrochimica*, **42**, n°3-4, 151-165.

LILOV D. and ANDONOVA T., 1976. Cytokinins, growth, flower and fruit formation in *Vitis vinifera*. *Vitis*, **15**,160-170.

LINDSAY W.L. and NORVELL W.A., Development of a DTPA soil test for Zn, Fe, Mn and Cu. *Soil Sci. Soc. Am. Proc.* **42**, 421-428.

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

MOSSE B.,1973 b. Plant growth response to vescular mycorrhiza. IV. In soil given additional phosphate. *New Phytol.*, **72**, 127-136.

NADIAN H., SMITH S.E., ALSTON A.M. and MURRAY, R. S., 1996. The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonisation. *Plant and Soil*, **182**, 39-49.

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

NIKOLAOU N. KOUKOURIKOU, M. and KARAGIANNIDIS N., 2000. Effects of various rootstocks on xylem exudate cytokinine content, nutrient uptake and growth patterns of grapevine *Vitis vinifera* L.cv. Thompson seedless. *Agronomie,* **20**, 363-373.

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

PARUNAN A.K., ROBSON A.D. and ABBOTT L.K., 1980.The effectiveness of vesicular-arbuscular mycorrhizas in increasing growth and phosphorus uptake of subterranean clover from phosphorus sources of different solubilities. *New Phytol.*, **84**, 327-338.

PHILLIPS, J.M. et HAYMAN, D.S.,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.

RAJU P.S., CLARK R.B., ELLIS J.R. et MARANVILLE J.W., 1987. Vesicular-arbuscular mycorrhizal infection effects on sorghum growth, phosphorus efficiency, as mineral element uptake. *J. Plant Nut.*, **10**, n°9-16, 1331-1339.

RICHARDS D. et ROWE R.N., 1977. Root-shoot interactions in peach: the function of the root. *Ann. Bot.*, **41**, 1211-1216.

SCHELLENBAUM L., BERTA G., RAVOLANIRINA F., TISSERANT B., GIANINAZZI S. and FITTER A.H., 1991. Influence of endomycorrhizal infection on root morphology in a micro propagated woody plant species (*Vitis vinifera* L.). *Ann. Bot.*, **67**, 135-141.

SCHENCK N.C. and PEREZ Y., 1988. *Manual for Identification of VA Mycorrhizal Fungi*, 2nd edn. University of Florida, Gainesville.

SCHUBERT A. and CRAVERO, M.C., 1985. Occurrence and infectivity of vesicular-arbuscular mycorrhizal fungi in north-western Italy vineyards. *Vitis*, **24**,129-138.

SIEVERDING E., 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. *Deutsch Gesellschaft für Technische Zusammenarbeit,* Eschborn, Germany .

SYLVIA D.M., 1998. Activity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *Soil Biol. Biochem.*, **20**, n°1, 39-43.

TARAFDAR J.C. et MARSCHNER H., 1994. Phosphatase activity in the rhizosphere and hyphosphere of mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.*, **26**, 387-395.

THOMPSON J.P., 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum usitatissimum* L.) by improving P and Zn uptake. *Soil Biol. Biochem.*, **26**, 1133-1143.

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

WALKER D.A. et SIVAK M.N., 1986. Photosynthesis and phosphate: A cellular affair. *Trends Biochem. Sci.*, **11**, 176-179.

WEISSENHORN I., AENCH M. et LEYVAL C., 1995. Biovailability of heavy metals and arbuscular mycorrhizae in a sewage-sludge-amended sandy soil. *Soil Biol. Biochem.*, **27**, n°3, 287-296.

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