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SOIL MINERAL CONCENTRATIONS AND SOIL MICROBIAL ACTIVITY IN GRAPEVINE INOCULATED WITH ARBUSCULAR MYCORRHIZAL (AM) FUNGUS IN CHILE

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Abstract

BENNEWITZ, E., GARRIDO, E., FREDES, C., GUTIERREZ, L., LOŠÁK, T.: *Soil mineral concentrations and soil microbial activity in grapevine inoculated with arbuscular mycorrhizal (AM) fungus in Chile.* Acta univ. agric. et silvic. Mendel. Brun., 2008, LVI, No. 5, pp. 13–17

A two year-experiment was carried out to study an effect of root inoculation with arbuscular mycorrhizal (AM) fungus on soil mineral concentrations and soil microbial activity in grapevine (*Vitis vinifera*) cv. "Cabernet Sauvignon" cultivated in Chile. Plants were inoculated with a commercial granular inoculant (Mycosym Tri-ton®) and cultivated in 20 L plastic pots filled with an unsterilized sandy clay soil from the Vertisols class under climatic conditions of Curicó (34°58´ S; 71°14´ W; 228 m ASL), Chile.

Soil analyses were carried out at the beginning of the study and after two years (four samples of rhizospheric soil for each treatment) to assess the effects of mycorrhizal infection on soil mineral concentration and physical properties. Soil microbial activity was measured by quantifying the soil production of CO_2 in ten replications of 50 g of soil from each treatment. Root mycorrhizal infection was assessed through samples of fresh roots collected during 2005 and 2006. Fifty samples for each treatment were analyzed and the percentage of root length containing arbuscules and vesicles was assessed.

During both years (2005 and 2006) all treatments showed mycorrhizal infection, even the Control treatment where no AM was applied. Mycorrhizal colonization did not affect the soil concentrations of N, P, K, Ca, Mg, K, Ca, Mg, Mn, Zn, Cu, Fe, B, organic matter, pH/KCl and ECe. Soil CO_2-C in vitro production markedly decreased during the period of the study. No significant differences where detected among treatments in most cases.

VA-mycorrhiza, *Vitis ×vinifera*, inoculation, soil microbial activity.

The study and management of microbial interactions in the soil–plant interfaces plays a key role in modern low-input-based agro-technologies (Barea et al., 2002). Among these new technologies we can find the use of microbial inoculants like arbuscular mycorrhizal (AM) fungus, which can be applied as bioprotectors, phytostimulators or biofertilizers (Gianinazzi et al., 2002). Grapevines form mutualistic symbioses with arbuscular mycorrhizal (AM) fungi. The fungal symbiont becomes a major interface between the soil and plant, playing an essential role in plant nutrient acquisition/development and in plant/soil protection against environmental stresses (Gianinazzi et al. 2002). The beneficial effects of arbuscular mycorrhizal inoculation with respect to grapevine growth, yield and the nutritional status have been reported by several authors (Caglar and Bayram 2006, Karagiannidis and Nikolaou 1999, Biricolti et al., 1997, Karagiannidis et al., 1995). Mycorrhizal fungi have shown to be specially important in sustainable agriculture systems like organic or integrated production. In these kinds of systems effective production requires the crop to have access to nutrient and water and to be maintained relatively free of the adverse impact of pests and diseases, but with minimal recourse to added inputs (Atkinson et

al., 2002). Microbial activity can play a key role in sustainable agriculture systems due its potential to recycle nutrients, improve plant nutrition, and reduce or substitute the application of industrial fertilizers (Alarcón et al., 2002; Velasco et al., 2001). Microbial activity is regulated among other factors by the nature and composition of the microbial community of soils (Alexander, 1991).

The objectives of the investigation were to study, for grapevine (*Vitis vinifera*) cv. "Cabernet Sauvignon", the effects of root inoculation with mycorrhizal fungi (genus *Glomus*) on soil mineral concentrations and soil microbial activity in Chile.

MATERIALS AND METHODS

Location

The study was carried out between 2005 and 2006 at the Experimental Station of the Department of Agronomy and Forestry, Universidad Católica del Maule, located in central Chile (34º 58´ S; 71º 14´ W; 228 m ASL). An unsterilized sandy clay soil from the Vertisols class with a total humus content of 6.31 %, ECe: 0.173 dS m^{-1} and pH/H_2O 5.62 was used for the study. The soil was sieved (2 mm) and mixed with sand at a ratio of 1:1.20 kg of the resulted substrate was employed for every plant. The mineral content of the soil (mg kg^{-1}) was: P-Olsen (35.0), K (149.0), Ca (672.0), Mg (102.0), Fe (113.5), Mn (3.18), Cu (2.78) and B (1.24). Organic matter: 6.31 %, ECe: 0.173 dS m^{-1}. The soil was not fertilized. Vines were planted during late March 2005 in 20 L plastic pots. 10 plants cv. "Cabernet Sauvignon" (one-year-old) were utilized for every treatment. Plants were inoculated with a commercial granular inoculant (Mycosym Tri-ton®). The inoculant was composed of AM from the *Glomus* genus and was made with spores and small pieces of mycorrhizal roots fixed on a mix of perlite and sand. The inoculant was mixed with the soil at different depths to assure a direct contact with the roots. The following treatments were applied: Control, T1.– Inoculant 2.5 ml/plant, T2.– 5.0 ml/plant, T3.– 10.0 ml/plant.

Measurements

1. **Root mycorrhizal infection**. Samples of fresh roots were taken on 28. 09. 2005, 28. 11. 2005 and 16. 01. 2006 and cut into 1.0 cm segments. All segments of each treatment were massed together, cleared with 2% KOH, and stained with tripan blue (Phillips and Hayman, 1970). Fifty samples for each treatment were analyzed and the percentage of root length containing arbuscules and vesicles was assessed by the gridline-intersect method (Giovanetti and Mosse, 1980), mounted on microscope slides and estimated by observation with 200 X magnification.
2. **Soil chemical and physical analyses.** Soil analyses were carried out at the beginning of the study and after two years (four samples of rhizospheric soil for every treatment) to assess the effects of mycorrhizal infection on soil mineral concentration and physical properties. Analysis included: N total (micro-Kjehldahl with salicylic acid), P ($NaHCO_3$-extractable, 0.5M, pH 8.5), exchangeable K, Ca and Mg (CH_3COONH_4, pH 7), Zn, Fe, Cu, Mn (DTPA method), soluble B in boiling water, organic matter (H_2SO_4 and $K_2Cr_2O_7$ oxidation), pH/KCl and electrical conductivity of the saturated paste extract (ECe).
3. **Soil microbial activity.** Microbial activity in the soil was measured by quantifying the soil production of CO_2 in ten replications of 50 g of soil from each treatment. The soils were deposited in glass flasks with screw-on covers and incubated at 25 °C for four weeks. The soil moisture was adjusted to 35 % at the beginning of the assay and after each reading. CO_2 was measured every week. 5 ml NaOH 1N and a small strip of filter paper were used for the absorption of CO_2. These were placed in a test tube inside the glass jars. New solution and paper were used for each determination. Carbonate was precipitated with 2% barium chloride, and the excess of NaOH was titrated with hydrochloric acid, using phenolphthalein as indicator (Pochon and Tardieux, 1962). The quantity of CO_2 produced was obtained using the formula of Stotzky (1965) and was expressed in mg of C-CO_2 for g^{-1} of soil. Based on the C-CO_2 produced and the percentage of organic matter of every sample, the rate of mineralization for every week was calculated.

Experimental design and statistical analysis.

A completely randomized experiment was designed for the study. Four different treatments were carried out. Every treatment consisted of ten plants, with each single plant as replication. The obtained results were subjected to analysis of variance, and Tukey's multiple range test was employed in the case of significant differences. All statistical analyses were performed using the JMP Statistical Software.

RESULTS AND DISCUSSION

Root mycorrhizal infection

During both years (2005 and 2006) all treatments showed mycorrhizal infection, even the Control treatment where no AM were applied (Tab. I). These results reflected the presence of native mycorrhizas in the soil of the study, with activity in roots of grapevines. The greater degree of root infection was observed in treatment T3 (Highest doses of innoculum). The percentage of root length infected increased markedly during the second year in most of the cases, even in the control treatment (6.0 % – 16.8 %). These results indicated that the natural population of mycorrhiza of the soil may increase from year to year if appropriate conditions are given (minimal soil disturbance, no application of soil herbicides, adequate fertilization among others). According to the results of this study the AM fungus contained in the inocu-

lant seems therefore to be able to infect grapevine roots, and the responses are significantly greater than the control without application. One application of the inoculant at the moment of plantation appears to be sufficient, if colonization has ocurred successfully, to secure mycorrhizal root colonization, but no great differences (increase or decrease of the infection) occur from year to year once the products have been applied.

Soil chemical and physical properties

Mycorrhizal colonization did not affect the soil concentrations of N, P, K, Ca, Mg, K, Ca, Mg, Mn, Zn, Cu, Fe and B (Tab. II and III).

These results agree with those of Xinshu and Runjin (1990), who worked with an unsterilized soil cultivated with *Malus hupehensis* seedlings and inoculated by AM mycorrhizal fungi (*Glomus versifome* and *Glomus macrocarpum*). Mycorrhizal inoculation also did not influence most of the minerals concentration in the soil of this study.

I: *Mean mycorrhizal infection in roots of grapevine cv. "Cabernet Sauvignon" (various dates)*

Treatments	Date of evaluation		
	28.09.05	28.11.05	16.01.06
Control	6.0 b	8.0 c	16.8 bc
T1: 2,5 ml/plant	6.0 b	17.2 b	14.8 c
T2: 5,0 ml/plant	14.4 a	29.6 a	23.2 b
T3: 10 ml/plant	17.6 a	24.0 ab	34.8 a

Values marked by the same letters in column are not statistically different ($P \leq 0.05$) according to Tukey's test.

II: *Mineral content (macroelements) in the experimental soils*

Treatments	Macroelements (mg kg^{-1})				
	N	P	K	Ca	Mg
Control	8.6 a	34.0 a	125.6 a	1160 a	134.2 a
T1: 2,5 ml/plant	13.6 a	34.0 a	141.0 a	1200 a	146.4 a
T2: 5,0 ml/plant	12.3 a	33.6 a	152.3 a	1180 a	134.2 a
T3: 10 ml/plant	12.3 a	31.6 a	147.0 a	1240 a	146.4 a

Values marked by the same letters in column are not statistically different ($P \leq 0.05$) according to Tukey's test.

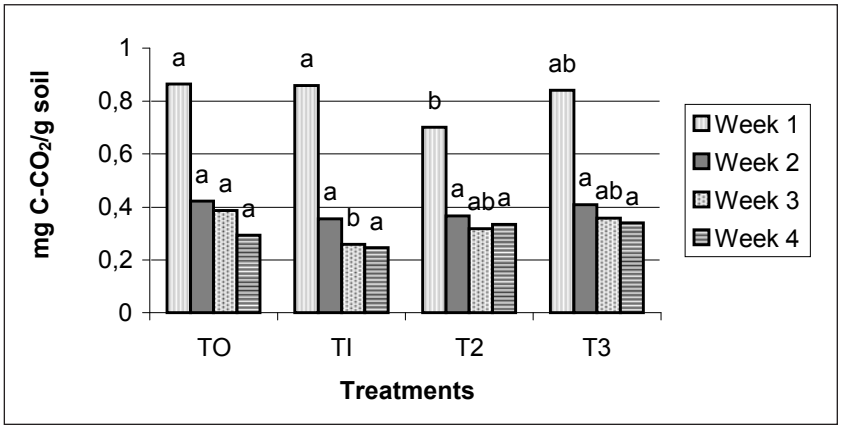
III: *Mineral content (macroelements) and some physical properties in the experimental soils*

Treatments	Microelements (mg kg^{-1})					Physical properties		
	Mn	Zn	Cu	Fe	B	O.M.	pH	CE (dS m^{-1})
Control	2.9 a	7.3 a	3.0 a	119.3 a	1.2 a	5.8 a	5.6 a	0.1 a
T1: 2,5 ml/plant	3.8 a	9.4 a	2.8 a	111.3 a	1.1 a	5.5 a	5.7 a	0.1 a
T2: 5,0 ml/plant	3.8 a	9.8 a	2.9 a	110.1 a	1.2 a	5.5 a	5.6 a	0.1 a
T3: 10 ml/plant	3.3 a	8.9 a	2.8 a	107.5 a	1.2 a	5.9 a	5.7 a	0.1 a

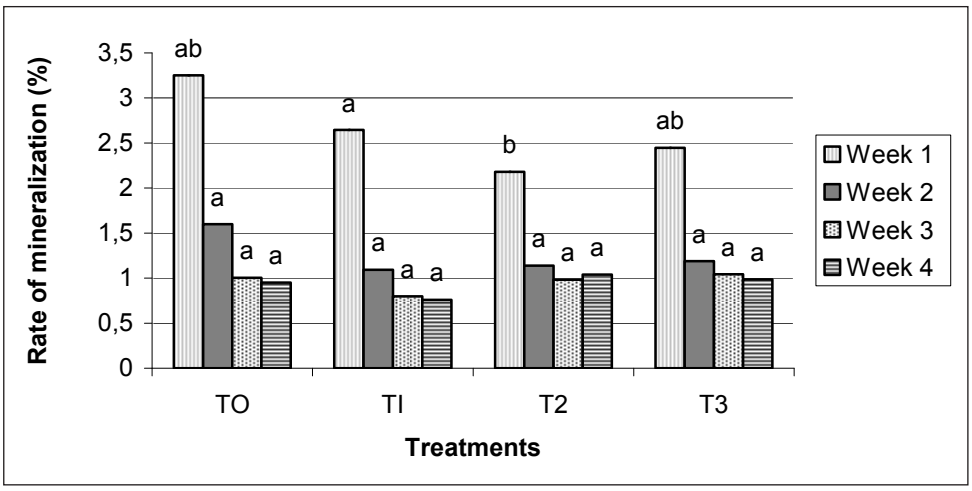
Values marked by the same letters in column are not statistically different ($P \leq 0.05$) according to Tukey's test.

Microbial activity

Results are presented in figures 1 and 2. Soil CO_2-C in vitro production markedly decreased during the period of the study. No significant differences were detected among treatments in most of the cases. In some cases Soil CO_2-C production was lower in soils from inoculated plants. These results are difficult to interpret. More research is needed in this area.



1: *Soil CO_2-C in vitro production of the soils of the study*



2: *Rate of mineralization in soils of the study*

CONCLUSIONS

The studied biopreparation has the capacity to colonize and persist in the roots of grapevine (*Vitis vinifera*) cv. "Cabernet Sauvignon". Mycorrhizal colonization have no effect on soil concentrations of the studied elements and the aforementioned physical properties of the soil. In most cases inoculation did not influence soil CO_2-C in vitro production and the rate of mineralization.

SOUHRN

Obsahy minerálních živin v půdě a půdní mikrobiální aktivita u vinné révy naočkované arbuskulární mykorhizní houbou (AM) v Chile

Dvouletý experiment byl uskutečněn s cílem zjistit efekt inokulace kořenů arbuskulární mykorhizní houbou (AM), její vliv na koncentraci živin v půdě a půdní mikrobiální aktivitu u vinné révy (*Vitis vinifera*) cv. 'Cabernet Sauvignon' pěstované v Chile. Rostliny byly naočkované granulovaným inokulantem (Mycosym Tri-ton®) a kultivovány ve 20L plastových nádobách s nesterilizovanou písčito-jílovitou půdou z třídy vertisolů v klimatických podmínkách města Curicó (34º 58´ j.š.; 71º 14´ v. d.; 228 m n. m.), Chile.

Analýza půdy byla provedena na počátku pokusu a po dvou letech (čtyři vzorky rhizosferní půdy z každé varianty) z důvodů zjištění vlivu mykorhizní infekce na obsah živin v půdě a některé fyzikální vlastnosti. Půdní mikrobiální aktivita byla určena pomocí měření produkce půdního CO_2, a to desetkrát z 50 g půdy z každé varianty. Infekce kořenů mykorhizou byla zkoumána ze vzorků čerstvých kořenů odebraných během let 2005 a 2006. Bylo analyzováno padesát vzorků z každé varianty a byla procentuálně vyhodnocena délka kořenů mající arbuskuly a vesikuly.

Během obou let (2005 a 2006) byla prokázána mykorhizní infekce, i přes to, že u kontrolního vzorku nebyla mykorhiza aplikována. Mykorhizní kolonizace neovlivnila koncentraci živin v půdě – N, P, K, Ca, Mg, K, Ca, Mg, Mn, Zn, Cu, Fe, B, organické hmoty, pH/KCl and ECe. Půdní CO_2-C in vitro produkce se viditelně snížila během průběhu pokusu. Ve většině případů nebyly pozorovány signifikantní diference mezi variantami.

VA-mycorhiza, *Vitis ×vinifera*, inoculace, půdní mikrobiální aktivita

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