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NUTRITIONAL STATUS, VEGETATIVE AND GENERATIVE BEHAVIOUR OF APPLE TREES AFTER THE APPLICATION OF TWO BIOPREPARATIONS

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Abstract

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A two year-experiment was carried out to study the effect of root inoculation with two biopreparations, Vambac® (VA-mycorrhiza genus *Glomus*, *Gigaspora* and the rhizospheric bacteria *Agrobacterium radiobacter*) and Amalgerol® (composed of vegetative and sea-algae oils and extracts) on leaf mineral concentration, vegetative and generative behaviour in two year old apple trees cv. 'Jonagold'/M.9. Trees were grown in 10 l plastic pots in unsterilized sandy clay soil (Fluvisols) under climatic conditions of South Moravia (49°,25´ N; 16°,55´ E), Czech Republic. Leaves were sampled eight times during the experiment (four times per year) for analysis of: N, P, K, Ca, Mg, Zn, Fe, Cu, and Mn. Mycorrhizal infection was determined with the gridline-intersection method. Inoculated plants showed higher VA root infection during both years (best results were 46% on the first year and 41.7% on the second year) and higher leaf P concentrations, in particular when biopreparations were applied together (P = 0.23% DW), than non inoculated (4% of root infection during year one and 15% during year two; P = 0.183% DW). N, K, Ca and Mg were not significantly affected by treatments. In many cases application of biopreparations decreased Fe, Mn and Zn leaf levels. Shoot length was strongly enhanced in inoculated plants only during the first year (46% more than non inoculated). No significant differences were detected between inoculated and non inoculated plants for: trunk diameter, spur number, root volume, leaf dry weight, number of blossoms and yield. It is concluded that the studied biopreparations have the capacity to colonize and persist in the roots of apple trees (VA-mycorrhizal fungus), enhance the uptake of phosphorus and vegetative growth but they could decreased the uptake of Fe, Mn and Zn.

VA-mycorrhiza, *Malus × domestica*, *Agrobacterium radiobacter*, inoculation, nutrient uptake

Biopreparations include microorganisms and their metabolites that are capable of enhancing soil fertility, crop growth, and/or yield. These include both indigenous microbes and microbial inoculants, that is, microorganisms that replace fertilizers or increase a crop's fertilizer use efficiency as an alternative to the use of chemicals. They could provide an alternative to agricultural chemicals as a more sustainable and ecologically sound practice to increase fruit trees productivity.

Mycorrhizas are the most widespread associations between microorganisms and higher plants (Marschner, 1995). Apple trees show a strong dependency on mycorrhizae (Koch et al., 1982), and in orchards they form symbioses with the naturally-occurring VAM (Morin et al., 1994). Mycorrhizae benefits apple plants by improving growth and nutrition (Geddeda et al., 1984; Hoepfner et al., 1983; Plenchette et al., 1981), involving mainly P (Marschner, 1995), even at high soil P levels (Morin et al., 1994) and, in some cases, other immobile nutrients such as Zn and Cu (Gnekow and Marschner, 1989). The favourable effect of *Agrobacterium radiobacter* on apple tree growth and the incidence of diazotroph bacteria in the apple rhizosphere after the inoculation of seedling roots or rootstocks has been described (Čatská, 1988; Čatská and Hudská, 1993; Čatská and Taube-Baab, 1994). In the case of Amalgerol® sufficient quantities of biodegradable material of microbial and plant origin are returned to the soil, increasing or at least maintaining its fertility and the biological activity within it. As a result the bacterial activity could be increased as well as the mobility of important nutrients, reducing deficiency deseases and the need of fertilizers.

The objectives of the investigation were to study, in apples cv. 'Jonagold'/M.9: 1. – the effects of the use of two biopreparations on leaf concentration of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn; 2. – the vegetative and generative responses after inoculation; and 3. – the ability of the mycorrhizal fungus contained in Vambac® and its interactions with Amalgerol® to colonize the roots, the amount of this colonization and plant responses to colonization.

MATERIALS AND METHODS

Location and soil

The study was carried out between 1998 and 2000 at the Department of Agrochemistry and Plant Nutrition of the Mendel University of Agriculture and Forestry Brno. Brno is situated in South Moravia, Czech Republic (49°,25´ N; 16°,55´ E; 250 m. a s. l.). Brno has a moderate central european climate with annual average temperatures of + 9.4 °C and average annual precipitation of 505 mm. An unsterilized soil from the Research Station of the Mendel University in Žabčice was used for the study. It corresponds to a sandy clay soil from the Fluvisols class with 25% of particles < 0.01 mm, a total humus content of 2.2 % and pH in KCl 5.7. The soil was sieved (2 mm) and not sterilized. 10 kg of soil was employed for every plant. The mineral content of the soil (mg kg^{-1}) according to Mehlich II procedure was: P (43), K (200), Ca (4287), Mg (497), Fe (5213), Mn (612) and Cu (15.6).

Plant material

Two years old Jonagold/M.9 apple trees from the orchards of the agricultural cooperative "Malé Haná" in Vanovice, Blansko Region, Czech Republic were used for the experiment. Trees were planted during 1998 (March 30^{th}–31^{st}) in 10 l plastic pots and headed at 50 cm height, lateral shoots were cut away to establish a functional framework. Ten plants were used for every treatment with a single plant as replicate. During winter, plants were protected from frost in special compartments under soil level.

Biopreparations

Vambac®: is an commercial biotechnological soil inoculant composed of VAM from the *Glomus* and *Gigaspora* genus and rhizogenic bacteria such as *Agrobacterium radiobacter* immobilized on inorganic bearers.

Amalgerol® is a product composed in base of vegetative and sea algae oils and extracts, vegetative ethers and waxes which promote the action of the soil organisms and organic matter.

Experimental treatments

Treatments were: 1. – Control, 2. – V1: Vambac® 20 g $tree^{-1}$, 3. – V2: Vambac® 40 g $tree^{-1}$, 4. – V3: Vambac® 60 g $tree^{-1}$, 5. – A1: Amalgerol® 1%, 6. – A2 2%, 7. – A3 3%, 8. – (V+A)1: Vambac® 20 g $tree^{-1}$ + Amalgerol® 1%, 9. – (V+A)2: Vambac® 40 g $tree^{-1}$ + Amalgerol® 2%, 10. – (V+A)3: Vambac® 60 g $tree^{-1}$ + Amalgerol® 3%.

Measurements

1. **Foliar analysis.** Included: N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn. Four leaf samplings (After blooming, end of shoot growth, flower buds differentiation and leaf fall) were carried out annually (1998, 1999). Each sample consisting of 5 leaves per tree; 50 leaves for every treatment (taken from the mid-third region of the current year's extention growth).
2. **Chemical methods.** Leaf samples were cleaned, owen dried at 60 °C and reduced in particle size. Wet digestion was utilized for organic matter destruction in the case of N, P, K, Ca, Mg and dry ashing for Fe, Mn, Cu and Zn. N (Kjeldahl), P (Colorimetric method), K (Flame photometer), Ca, Mg, Zn, Fe, Cu, Mn (Atomic absorption spectrophotometer, AAS-Philips PU 9200 x).
3. **Vegetative and generative responses**. Measurements included: shoot length, trunk diameter, spur number, root volume, leaf dry weight, flower number and yield.
4. **Mycorrhizal infection.** Fresh roots samples were taken from each plant during spring (both years) and cut into 1.0 cm segments. 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 vesicles was assesed by the gridline-intersect method (Giovanetti and Mosse, 1980), mounted on microscope slides and estimated by observation with 400 X magnification.

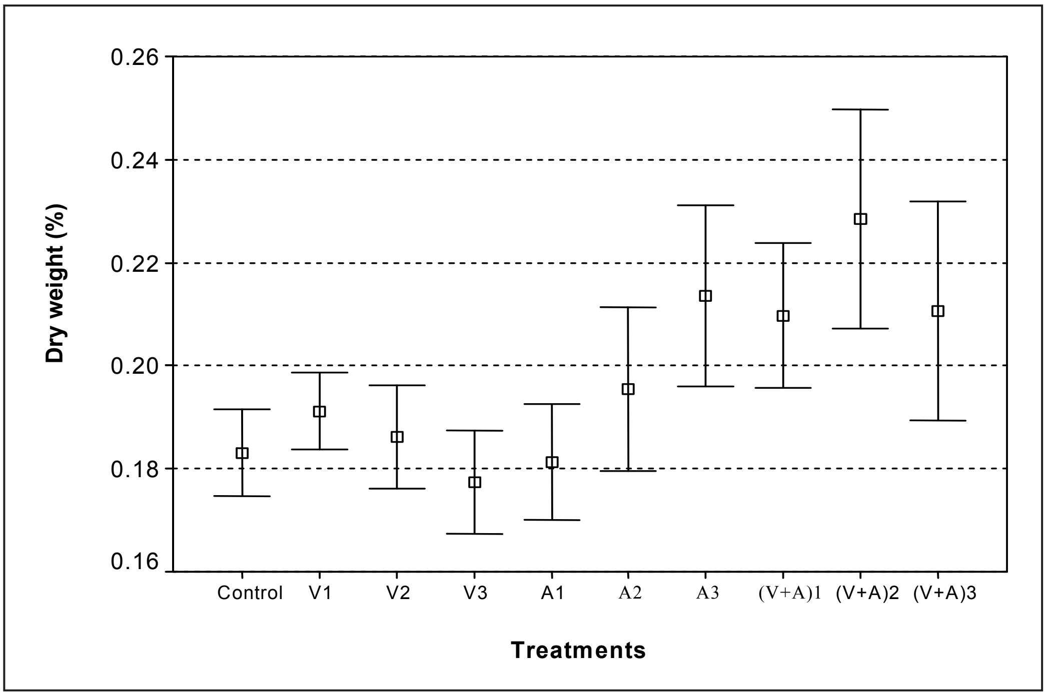
Experimental design and statistical analysis

A completely randomized factorial experiment was designed for the study. Factors were: Treatment (10 different treatments), date of sampling (After bloom, end of shoot growth, flower buds differentiation and leaf fall), year (1998, 1999). Every treatment consisted of 10 trees, with each single tree as replication. Results were subjected to ANOVA; Tukey's multiple range test was employed in the case of significant differences.

RESULTS AND DISCUSSION

Foliar mineral content

1. **Phosphorus.** The foliar elemental composition was influenced by treatment, date of sampling and year. Values were significantly higher in the case of treatments A3, (V + A)1 and (V + A)2 (Fig. 1).



1: *Leaf concentration of phosphorus. Average of two years (1998, 1999) and four dates of sampling (After bloom, end of shoot growth, flower buds differentiation and leaf fall). *A3, *(V+A)1, *(V+A)2=significant at $p \leq 0.05$*

2. **N, K, Ca and Mg.** They were not significantly affected by treatments. Foliar elemental composition was influenced only by date of sampling and year (Fig. 2).
3. **Cu.** Was influenced only by the year (Fig. 3)
4. **Fe, Mn and Zn.** In many cases, the application of the biopreparations decreased Fe, Mn and Zn leaf concentrations (Fig. 3, 4 and 5).

Vegetative and Generative Responses

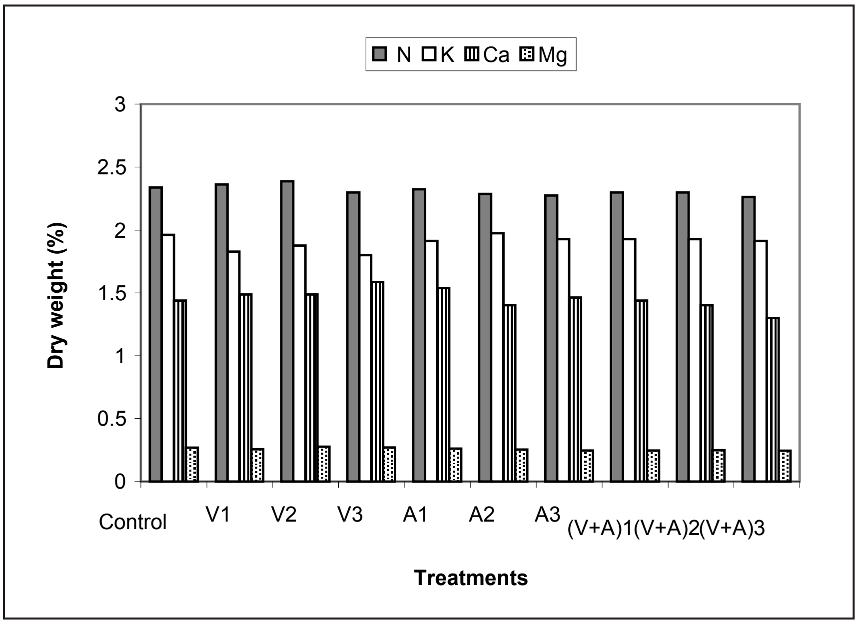
1. **Shoot lenght**. Seasonal shoot growth in the first year was significantly greater than control in the case of: V2 with (20% increase), V3 (28% increase), A1 (46% increase) and (V+A)1 (18% increase). On the second year no differences for shoot growth were detected (Data are not shown). These results agree with many others where growth stimulation of VAM in apple trees in different stages of development has been observed (Plenchette et al., 1981; Morin et al., 1994).
2. **Leaf Fresh and Dry Weight, Trunk Diameter, Spur Number and Root Volume**. No significant differences were detected (Data are not shown).
3. **Number of blossoms.** Significant differences were detected between treatments but not different from the Control treatment. During the first year trees bloomed only in those treatments where Vambac® was employed.
4. **Number of fruits**. Trees began to bear in the second year of the study but in an irregular way. Treatment V3 resulted significantly greater than the others.

Mycorrhizal colonization status

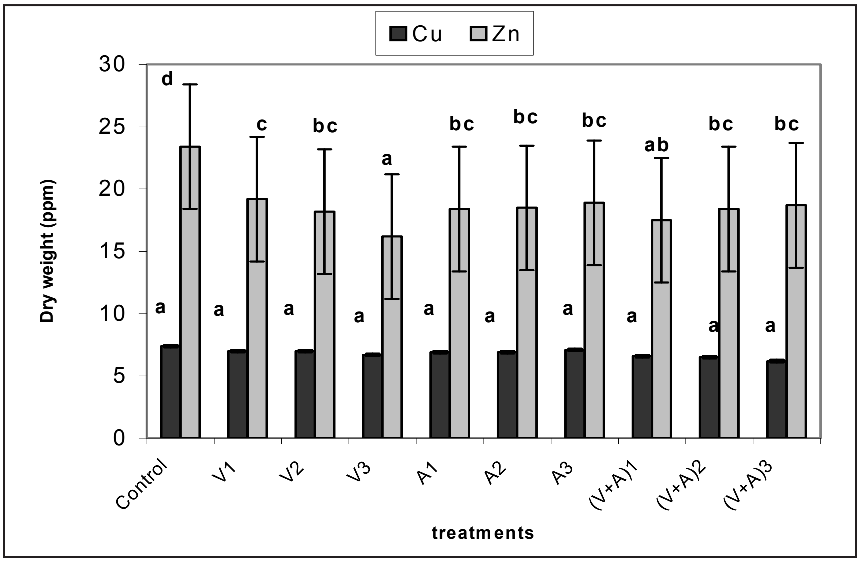
Inoculated plants showed higher VA root infection percentage during both years (46% during the first year and 41.7% during the second in the best results) During the first year (1999) all treatments showed mycorrhizal infection, even the Control treatment and treatments where no VAM were applied. This results reflects the presence of native mycorizas in the soil of the study, with activity in apple roots. The second year (2000) treatments were delimited to the followings for this evaluation: Control, V3, A3 and (V + A)3. All of them showed again mycorrhizal infection. The greater degree of root infection was observed the same as in the case of the first year in treatments (V + A)3 and V3. The percentage of root lenght infected increased markedly during the second year in the case of the control treatment (4%–15%) and in the other treatments the percentages of infection remained in similar values. This results showed that the natural population of mycorrhhiza of the soil can increase from year to year if appropriate conditions are given (minimal soil disturbance, no application of soil herbicides, adequate fertilization among others).

CONCLUSIONS

The studied biopreparations have the capacity to colonize and persist in the roots of apple trees (VA-mycorrhizal fungus), enhance the uptake of phosphorus and vegetative growth but they could decreased the uptake of Fe, Mn and Zn. These results obtained in experimental conditions and young



2: *N, K, Ca and Mg concentration in leaves (Average values of two years and four dates of sampling)*



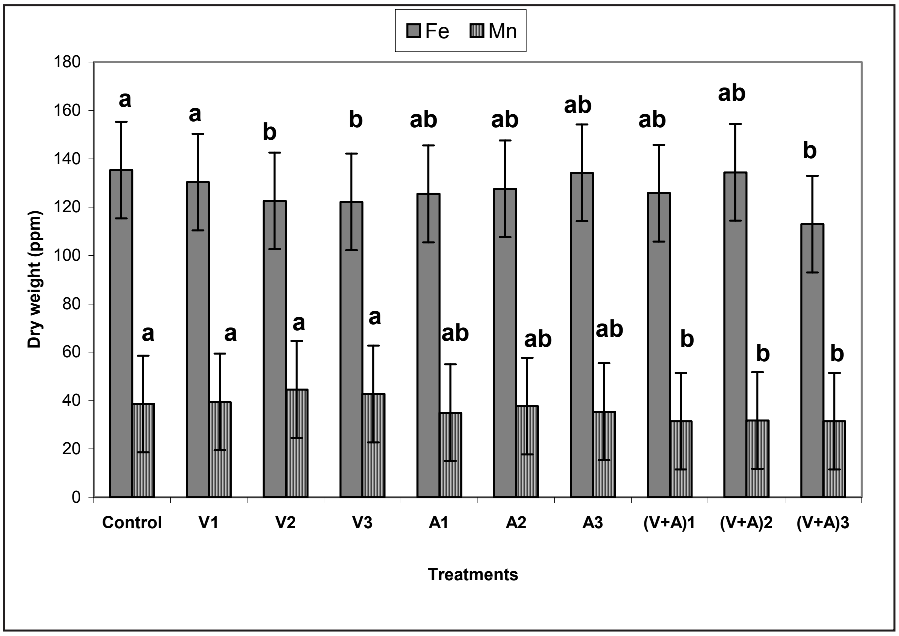
3: *Cu and Zn concentration in leaves (Average of years and date of sampling). Values marked by the same letters in column are not statistically different (P ≤ 0.05) according to Tukey's test*

apple trees have a practical importance. The use of the studied biopreparations could be a good alternative for apple trees especially in the nursery, where moderate amounts of colonization could be achieved and after the transfer of these plants to a low-nutrient environment, the studied products could spread and enhanced plant growth and production.

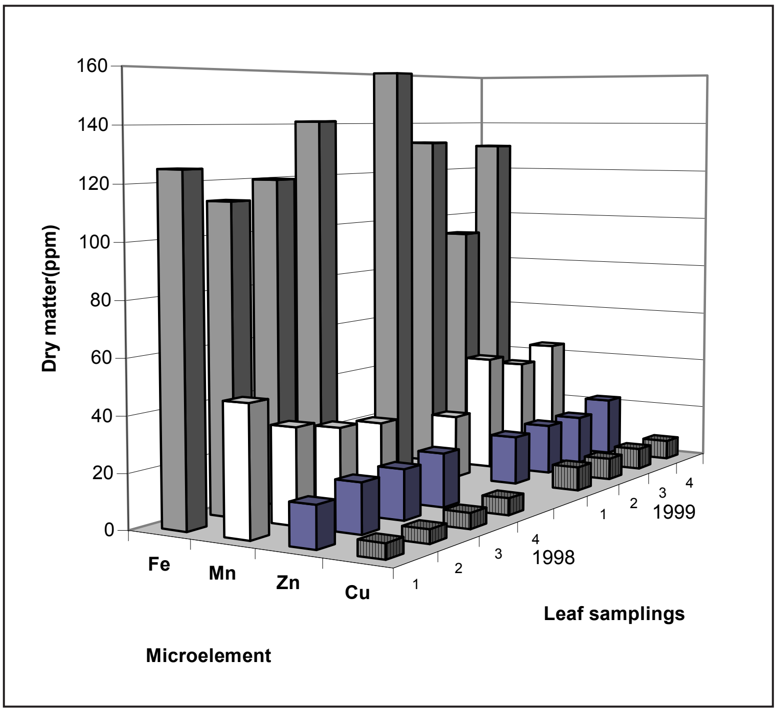
SOUHRN

Živinný stav, vegetativní a generativní projevy u jabloní po použití dvou biopreparátů

Pokus byl uskutečňován po dobu dvou let s cílem zjistit vliv naočkování kořenů dvěma biopreparáty Vambac® (VA-mykorhiza rodu Glomus a rhizosferních bakterií) a Amalgerol® (jehož základem jsou oleje a extrakty z rostlin a mořských řas) na obsah živin v listech, vegetativní a generativní projevy



4: *Fe and Mn concentration in leaves (Average of years and date of sampling). Values marked by the same letters in column are not statistically different (P ≤ 0.05) according to Tukey's test*



5: *Microelement leaf concentration. For each year (1998, 1999) average values of four dates of sampling (After bloom (1), end of shoot growth (2), flower buds differentiation (3) and leaf fall (4))*

u dvouletých jabloní `Jonagold´/ M.9. Stromky byly pěstovány v desetilitrových plastových nádobách ve vegetační hale Ústavu agrochemie, půdoznalství, mikrobiologie a výživy rostlin MZLU v Brně (49°,25´ sš; 16°,55´ vd). Pro chemickou analýzu bylo odebráno osm vzorků listů během pokusu (čtyřikrát za rok) pro analýzu: N, P, K, Ca, Mg, Zn, Fe, Cu, Mn. Mykorhizní infekce se zjišťovala metodou průsečku na mřížce. U naočkovaných rostlin bylo prokázáno vyšší procento VA kořenové infekce bě-

hem obou let (nejlepší výsledky byly 46 % v prvním roce a 41,7 % v druhém roce) a vyšší koncentrace P, zvláště když byly oba preparáty aplikovány společně (P = 0,23 % v sušině), narozdíl od nenaočkovaných rostlin (4 % kořenové infekce v prvním roce a 15 % v druhém; P = 0,183 % v sušině). N, K, Ca a Mg nebyly aplikací nijak znatelně ovlivněny. Aplikace biopreprátů v mnoha případech snížila koncentraci Fe, Mn, a Zn v listech. V prvním roce po naočkování byla délka jednoletých výhonů u naočkovaných rostlin významně delší (o více než 40 %), avšak ve druhém roce se tento rozdíl již neprojevil. Kromě délky výhonů se naočkované rostliny nelišily od kontrol v žádné další sledované charakteristice (průměr kmene, počet plodonošů, objem kořenů, hmotnost sušiny listů, počet květů a výše sklizní). Z výsledků lze učinit závěr, že studované biopreparáty mají schopnost kolonizovat kořeny jabloní mykorhizními a rhizosferními mikroorganismy, které zde přetrvávají a zvyšují příjem fosforu a vegetativní růst stromků. Naproti tomu snižují příjem Fe, Mn a Zn.

VA-mykorhiza, *Malus ×domestica*, *Agrobacterium radiobacter*, naočkování, příjem živin

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