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Synergistic Effect of Vesicular-Arbuscular-Mycorrhizas and *Azotobacter chroococcum* on the Growth and the Nutrient Contents of Tomato Plants

By

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With 3 Figures

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Summary

EL-SHANSHOURY A. R., HASSAN M. A. & ABDEL-GHAFFAR B. A. 1989. Synergistic effect of vascular-arbuscular mycorrhizas and *Azotobacter chroococcum* on the growth and the nutrient contents of tomato plants. – Phyton (Austria) 29 (2): 203–212, with 3 figures. – English with German summary.

Tomato plants (*Lycopersicon esculentum* MILL. cv. Luxor) were grown in sterilized sandy soil, low in plant-available N and P. The soil was inoculated or uninoculated with a vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum, Azotobacter chroococcum,* or both the two tested organisms together. Inoculation of tomato with *A. chroococcum* enhanced root infection with *G. fasciculatum,* stimulated the plant growth and resulted in increased shoot-N, -Ca, -Mg and -K compared to the other treatments and root-N, -P, -Na, -Ca and -Fe compared to uninoculated plants. Inoculation with *G. fasciculatum* recorded the highest levels of P in both roots and shoots, and resulted in increased root-Na, -K, -Ca, -Mg and -Fe. *A. chroococcum* displayed a synergistic effect with *G. fasciculatum* in tomato by enhancing mycorrhizal infection, the plant growth and the levels of N and P.

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Zusammenfassung

El-Shanshoury A. R., Hassan M. A. & Abdel-Ghaffar B. A. 1989. Synergistische Wirkung von vascular-arbuscularer Mycorrhiza und *Azotobacter chroococcum* auf Wachstum und Nährstoffgehalt von Tomatenpflanzen. – Phyton (Austria) 29 (2): 203–212, mit 3 Figuren. – Englisch mit deutscher Zusammenfassung.

Tomatenpflanzen *(Lycopersicon esculentum* Mill. cv. Luxor) wurden in sterilisierter, sandiger, an pflanzenverfügbarem N und P armer Erde kultiviert. Die Erde wurde mit dem vesicular-arbuscularen Mycorrhizapilz *Glomus fasciculatum,* mit *Azotobacter chroococcum* oder mit beiden beimpft, Kontrollen blieben ungeimpft. Impfung mit *A. chroococcum* stimulierte das Wachstum der Pflanzen und erhöhte im Vergleich mit den übrigen Ansätzen den Gehalt der Sprosse an N, Ca, Mg und K, im Vergleich zu den ungeimpften Pflanzen war der Gehalt der Wurzeln an N, P, Na, Ca und Fe erhöht. Impfung mit *G. fasciculatum* führte zu den höchsten P-Gehalten in Sproß und Wurzel und erhöhte in den Wurzeln den Gehalt an Na, K, Ca, Mg und Fe. *A. chroococcum* wirkt mit *G. fasciculatum* hinsichtlich der Erhöhung der Infektion mit dem Mycorrhizapilz, des Wachstums der Pflanze und ihrer N- und P-Gehalte synergistisch.

Introduction

Microorganisms in the rhizosphere can increase or decrease the absorption of inorganic nutrient by plant roots and these appear to be significant interactions that occur between these organisms under certain conditions. Changes in the rhizosphere might affect the rhizosphere microflora and in turn, plant growth (Bagyaraj & Menge 1978). It has been clearly shown that vesicular arbuscular (VA) mycorrhiza can improve plant growth through increased uptake of phosphorus, especially in low fertility soils (Daft & Nicolson 1966, Mosse & al. 1973, Gerdemann 1975).

Azotobacter chroococcum is known to improve plant growth through nitrogen fixation or through production of plant growth regulators (Azcon & al. 1973, Barea & Brown 1974, Azcon & Barea 1975, El-Shanshoury 1979, El-Shourbagy & al. 1979).

The synergistic effect on plant growth following inoculation with VA mycorrhizal fungi and phosphate solublizing bacteria has been recorded by Barea & al. 1975, they concluded that the synergistic effect might be due to plant growth substances produced by the bacteria rather than the soluble phosphate ion released.

Information on the interaction between *Azotobacter* and VA mycorrhizae is lacking. However, Bagyaraj & Menge 1978 recorded a synergistic interaction between *Azotobacter* and VA mycorrhizae on enhancing dry weight of tomato plants.

This paper presents results of a study conducted to determine the effect of interaction between *Azotobacter chroococcum* and *Glomus fasciculatum* on the growth response, mineral content and mycorrhizal infection levels in tomato plant (*Lycopersicon esculentum* Mill.).

Materials and Methods

Tomato (*Lycopersicon esculentum* MILL., cv. Luxor) seeds (30 mg) were surface sterilized by 1% sodium hypochlorite and planted as described in PACOVSKY & al. 1986. One week-old seedlings were transplanted to 12-cm pots containing 2 kg steam sterilized soil. The pots were kept in a glasshouse with a temperature range of 23–33°C. Four groups of pots were established: the first one was inoculated with *Azotobacter chroococcum*, the second was inoculated with VA mycorrhizal fungus *Glomus fasciculatum*, the third was inoculated with the two tested organisms together and the last one remained without any inoculation (control). All treatments received 150 ml of N- and P-free nutrient solution (modified from Hewitt nutrient solution) once weekly. Each pot was watered to field capacity every second day. When the plants were 30 days old, all were fertilized with a single dose of 7.3 mg KH_2PO_4 per pot.

To inoculate tomato seedlings with the test organisms, the following procedure was employed: *A. chroococcum* was grown on solid nitrogen free medium modified by EL-SHANSHOURY 1979 which composed of (g/l) sucrose 30.0, K_2HPO_4 0.16, NaCl 0.2, $MgSO_4 . 7 H_2O$ 0.2, $CaCO_3$ 2.0, $Fe_2(SO_4)_3$ 0.005, $NaMoO_4 . 2 H_2O$ 0.005, $NaBO_4$ 0.005, tryptophan 10 mg, and agar 20; pH 7.0. Seven-day-old culture of *Azotobacter* was scraped into sterile saline to give a suspension containing 2×10^9 cells/ml. 3 ml of this suspension were added to the root region at the time of transplanting.

Plants that received the VA mycorrhizal fungus *G. fasciculatum* (THAXTER sensu GERD) GERD & TRAPPE were inoculated with 3 ml containing between 200 and 250 fungal spores. This inoculum was collected from the rhizosphere of *Zea mays* pot culture as described by BAGYARAJ & MENGE 1978. Control was made by adding 3 ml sterile distilled water around the roots.

Plants were harvested 16-weeks after transplanting. The mycorrhizal status of the roots were assessed according to the method described by PHILIPS & HAYMAN 1970. The growth terms of root depth, shoot height, fresh and dry weigths of root and shoot were recorded. Root/shoot ratios of fresh and dry weigths were computed. Nutrients of dry root and shoot systems were determined according to ALLEN & al. 1974. Mineral elements (Na, K, Ca, Mg and Fe) were determined by the acid digestion method using Shimadzu Atomic Absorption Flame Spectrophotometer (Modell AA-640-12). Phosphorus contents were estimated by the Molybdenum Blue method using Novaspec spectrophotometer. The micro-Kjeldahl method was used to determine total nitrogen.

Data were computed statistically to find out any significant difference between the treatments. For this prupose, the one-way analysis of variance "t-test" and confidence limits at 5 % level of significance were carried out according to STEEL & TORRIE 1980.

Results and Discussion

1. Effect of inoculation upon plant growth

As shown in Table 1, the plants inoculated with both *Azotobacter* and *Glomus fasciculatum* together recorded a higher percentages of mycorrhizal infection than those inoculated singly with *G. fasciculatum* ($P = 0.05$). This observation was in agreement with BAGYRAJ & MENGE 1978 who indicated

Table 1.

The percentage of mycorrhizal infection of 16-weeks old tomato plant.

Inoculation	% Root infection
Control	0
Azotobacter	0
Glomus	38^{+} (P = 0.05)
Azotobacter + *Glomus*	79^{++} (P = 0.01)

that *A. chroococcum* enhanced infection and spore production by the mycorrhizal fungus. Similar interactions have also been observed between *A. paspali* and VAM fungi in *Paspalum* (BAREA & al. 1973) and between *A. chroococcum* and *G. fasciculatum* in tall fescue *(Festuca arundinacea)* (HO & TRAPPE 1980).

The inoculation with *Azotobacter* alone significantly increased the root depth, shoot height, fresh and dry weights of roots and shoots (P = 0.05) and root/shoot of fresh and dry weights (Figure 1). The beneficial effect of *Azotobacter* on tomato plants might be due to nitrogen fixation and secretion of a high quantity of plant growth regulators (AZCON & BAREA 1975, EL-SHOURBAGY & al. 1979, EL-SHANSHOURY 1979). Plants inoculated with either a combination of *Azotobacter* + *Glomus* or *Glomus* alone resulted in an increase of the shoot mean growth, but they reduced the root mean growth, therefore the root/shoot ratios of the fresh weight $(R/S)_f$ and dry weight $(R/S)_d$ were reduced. There is much evidence in the literature that the presence of mycorrhizae decrease the root/shoot ratio by increasing the aboveground production and possibly by reducing the need for belowground production in agreement with our finding. However, CRUSH 1974, DAFT & EL-GAIAHMI 1974, 1975, ASIMI & al. 1980 and REDENTE & REEVES 1981 reported a reduction of root/shoot ratios when *Glomus* was present, as compared with *Rhizobium*-inoculated plants. HALL & al. 1984 reported a reduction of root/shoot ratios of *Lolium* mycorrhizal pots.

2. Nitrogen and phosphorus content

The data represented in Figure 2 confirmed the previous observations, where the inoculation of tomato plants with both *Glomus* and *Azotobacter* either individually or in combination, significantly increased nitrogen and phosphorus concentrations in the root, shoot and whole plant. This increase may be attributed to N-fixation or glutamate synthetase activity (AZCON & BAREA 1975, SMITH & al. 1985).

Nitrogen levels in root materials and whole plant recorded the highest value in *Glomus* + *Azotobacter*-inoculated plants. Single inoculation with either *Glomus* or *Azotobacter* increased root- and whole plant-N content more than uninoculated control plants, but less than the dual inoculation

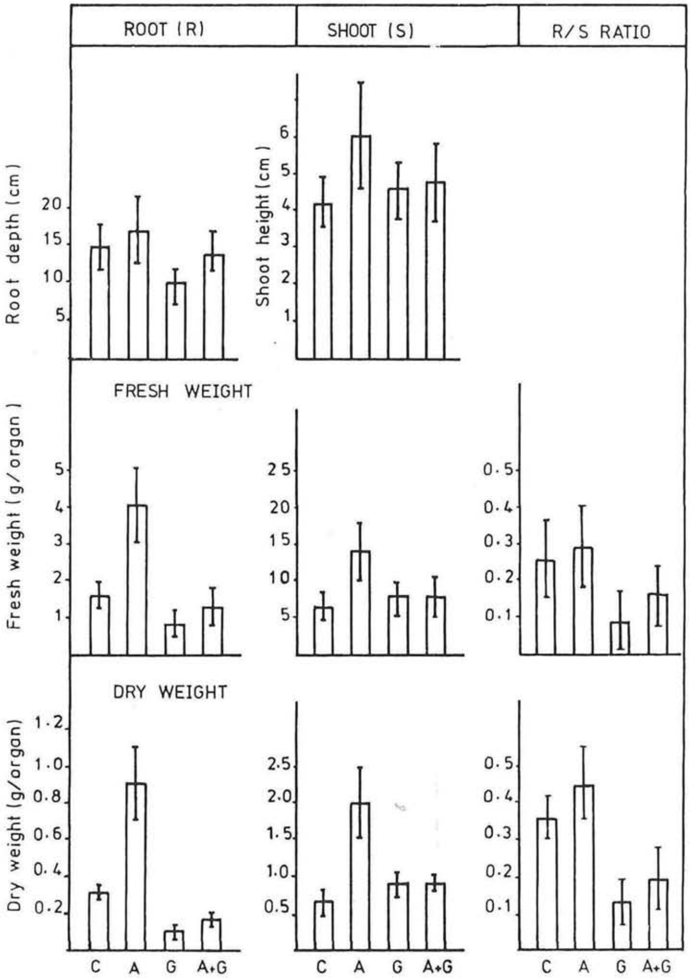

Fig. 1. Growth response of tomato inoculated with *Azotobacter chroococcum* (A), *Glomus fasciculatum* (G) and *Azotobacter* + *Glomus* (A+G). C = uninoculated control.

with both organisms together. This indicate that there is a synergistic or additive beneficial effect of the two organisms on tomato plant. In this connection, BAGYARAJ & MENGE 1978 and REDENTE & REEVES 1981 recorded a synergistic effect of mycorrhizal fungi *Glomus fasciculatum* and a nitrogen fixing *(Azotobacter* or *Rhizobium)* with respect to nitrogen fixation rates of tomato and sweetvetch.

The response of inoculated plants to the accumulating P is also recorded in Figure 2. Plants inoculated with either *Glomus* alone or in a combi-

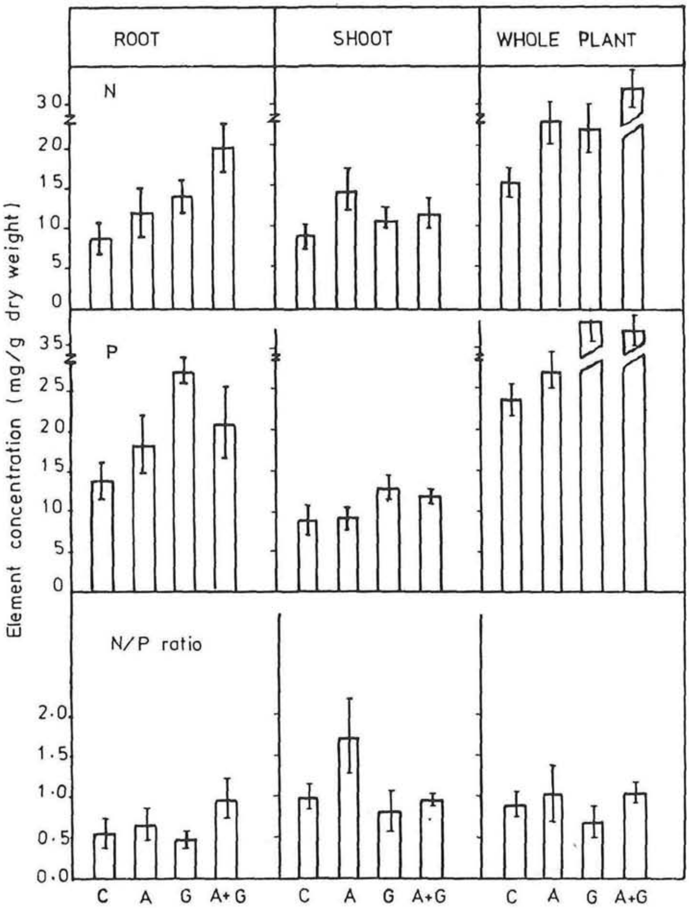

Fig. 2. N– and P contents and N/P ratio of tomato plants as influenced by inoculation with *Azotobacter chroococcum* (A), *Glomus fasciculatum* (G) and *Azotobacter* + *Glomus* (A+G). C = uninoculated control.

nation with *Azotobacter* resulted in the highest P levels. The explanation for the increased P-uptake following VA mycorrhiza inoculation probably is that P is absorbed in VA mycorrhizal hyphae and transported across roots to plants shoots. These results are supported and confirmed by the findings reported by HATTINGH & al. 1973, PEARSON & TINKER 1975, RHODES and GERDEMANN 1978, REDENTE and REEVES 1981 and BLOSS & PFEIFFER 1983.

As a general, the presence of *Azotobacter* increased N content rather than P, on the contrary the inoculation with mycorrhizal fungi increased P content rather than N. So, the N/P ratios increased in *Azotobacter*-inoculated plants but decreased with *Glomus*. Dual inoculation increased N/P ratio in the root material and they had no influence on shoot-N/P ratio (Figure 2).

These results agree with the findings reported by AZCON-AGUILAR & BAREA 1981, POWELL 1981, ABBOUT & ROBSON 1982 and HALL & al. 1984.

3. *Other nutrient contents*

The results (Figure 3) showed that the inoculation of tomato plant with *Azotobacter* or *Glomus* either individually or in combination increased the

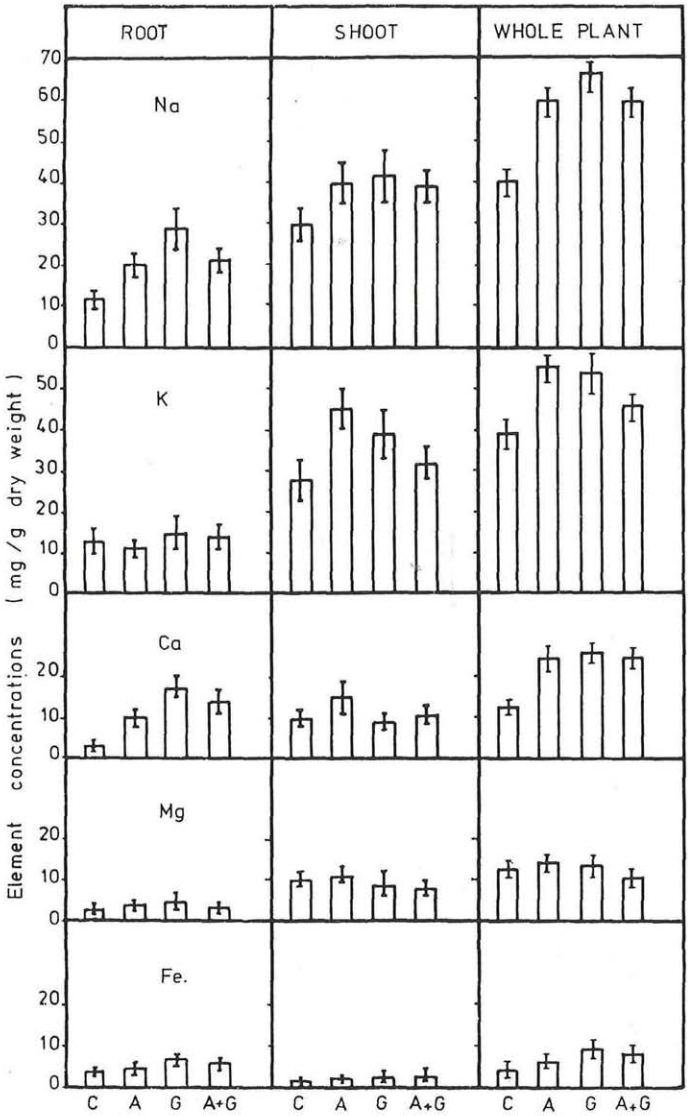

Fig. 3. Concentration of Na, K, Ca, Mg and Fe in tomato plants as influenced by inoculation with *Azotobacter chroococcum* (A), *Glomus fasciculatum* (G) and *Azotobacter* + *Glomus* (A+G). C = uninoculated control.

nutrient content (Na, K, Ca, Mg and Fe) in the root, shoot and whole plant materials. Plants infected with *Glomus* had the highest levels of root-Na, -K, -Ca, -Mg and -Fe; shoot-Na and -Fe and whole plant-Na, -Ca and -Fe; whereas plants inoculated with *Azotobacter* had the highest levels of shoot-K, -Ca and -Mg and whole plant contents of K and Mg. The accumulation of these minerals may be attributed to secretion of growth regulators which accelerate the uptake of these minerals in accordance with NICKELL 1982 who stated that uptake of K by wheat is accelerated by gibberellic acid. Clear evidence for mycorrhizal involvement in uptake of nutrients other than phosphate is scanty. BLOSS & PFEIFFER 1983 reported that inoculated guayule with two *Glomus* species had greater concentrations of Ca, Mg, Mn and Fe than did uninoculated plants. NIELSEN & JENSEN 1982 reported that VAM inculation tended to increase Na- and K-uptake and decreased Ca-, Mg- and Zn-concentrations in shoots. In our finding the presence of *Glomus* depressed shoot-Mg concentration and the inoculation with *Azotobacter* in the presence of *Glomus* depressed Mg concentrations in shoot and whole plant. HALL & al. 1984 recorded that *Glomus* depressed Mg concentration of rye grass.

References

ABBOUT L. K. & ROBSON A. D. 1982. The role of vescicular-arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. – Austral. J. Agricult. Res. 33: 389–408.

ALLEN S., GRIMSHAY H. M., PARKINSON J. A. & QUARMBY C. 1974. Chemical Analysis of Ecological Materials. – Blackwell Scientific Publications, Osney, Oxford, London, pp. 565.

ASIMI S., GIANINAZZI-PEARSON V. & GIANINAZZI S. 1980. Influence of increasing soil phosphorus levels on interactions between vescicular-arbuscular mycorrhizae and *Rhizobium* in soybeans. – Can. J. Bot. 58: 2200–2205.

AZCON R. & BAREA J. M. 1975. Synthesis of auxins, gibberellins and cytokinins by *Azotobacter vinelandii* and *Azotobacter Beijerinckii* related to effects produced on tomato plants. – Plant and Soil 43: 609–619.

AZCON-AGUILAR C. & BAREA J. M. 1981. Field inoculation of *Medicago* with V. A. mycorrhiza and *Rhizobium* in phosphate-fixing agricultural soil. – Soil Biology and Biochemistry 13: 19–22.

AZCON R., BAREA J. M. & CALLAO V. 1973. Selection of phosphate-solubilizing and nitrogen-fixing bacteria for using as biological fertilizers. – Cuad Circular of Biology 2–1: 23–30.

BAGYARAJ D. J. & MENGE J. A. 1978. Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. – New Phytologist 80: 567–573.

BAREA J. M. & BROWN M. G. 1974. Effect on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. – Appl. Bacteriol. 37: 583–593.

BAREA J. M., BROWN M. E. & MOOSE B. 1973. Association between VA *mycorrhiza* and *Azotobacter*. – Rothamsted Report 1972, part 1, pp. 81–82.

– , AZCON R. & HAYMAN D. S. 1975. Possible synergistic interaction between endogone and phosphate-solubilizing bacteria in low-phosphate soils. In: SANDERS F. E., MOOSE R. & TINKER P. B. (eds.), Endomycorrhizas, pp. 409–417. – Academic Press London, New York.

BLOSS H. E. & PFEIFER C. M. 1983. Latex content and biomass increase in mycorrhizal guayule *(Parthenium argentatum)* under field conditions. – Annual Applied Biology 104: 175–183.

CRUSH J. R. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. – New Phytologist 73: 743–749.

DAFT M. J. & EL-GIAHMI A. A. 1974. Effect of endogone mycorrhiza on plant growth. VII. influence of infection on the growth and nodulation in french bean *(Phaseolus vulgaris)*. – New Phytologist 73: 1139–1147.

– & EL-GIAHMI A. A. 1975. Effect of *Glomus* infection on three legumes. In: SANDERS F. E., MOOSE B. & TINKER P. B. (eds.), Endomycorrhizas, pp. 584–592. – Academic Press London, New York.

– & NICOLSON T. H. 1966. Effect of *Endogone* mycorrhizae on plant growth. – New Phytologist 65: 343–350.

EL-SHANSHOURY A. R. 1979. Production of plant growth hormones by certain microorganisms. – M. Sc. Thesis, Faculty of Science, Tanta Univesity, Egypt.

EL-SHOURBAGY M. N., EL-SAYED M. A. & EL-SHANSHOURY A. R. 1979. Inoculation of soil with *Azotobacter chroococcum*. – Egypt. J. Bot. 22 (3): 205–214.

GERDEMANN J. W. 1975. Vesicular-arbuscular mycorrhizae. In: TORREY J. G. & CLARKSON D. T. (eds.), The Development and Function of Roots, pp. 575–591. – Academic Press Lóndon, New York.

HALL I. R., JOHNSTONE P. D. & DOLBY R. 1984. Interactions between endomycorrhizas and soil nitrogen and phosphorus on the growth of ryegrass. – New Phytologist 97: 447–453.

HATTINGH M. J., GRAY L. E. & GERDEMANN J. W. 1973. Uptake and translocation of ^{32}P-labelled phosphate to onion roots by endomycorrhizal fungi. – Soil Sci. 116: 383–387.

HO I. & TRAPPE J. M. 1980. Nitrate reductase activity of non mycorrhizal Douglas-fir rootlets and some associated mycorrhizal fungi. – Plant and Soil 54: 395–398.

MOSSE B., HAYMAN D. S. & ARNOLD D. J. 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P. – New Phytologist 72: 809–815.

NICKELL L. G. 1982. Plant Growth Regulators-Agricultural Uses, pp. 59–62. – Springer-Verlag Berlin, Heidelberg, New York.

NIELSEN J. D. & JENSEN A. 1982. Influence of vesicular-arbuscular mycorrhiza fungi on growth and uptake of various nutrients as well as uptake of fertilizer P for lucerne *(Medicago sativa)* . – Plant and Soil 70: 165–172.

PACOVSKY R. S., PAUL E. A. & BETHLENFALVAY G. J. 1986. Response of mycorrhizal and phosphorus-fertilized soybeans (*Glycine max* cultivar Amsoy – 71) to nodulation by *Bradyrhizobium* or ammonium nitrate. – Crop Science 26: 145–150.

PEARSON V. & TINKER P. B. 1975. Measurement of phosphorus fluxes in the external hyphae of endomycorrhizas. In: SANDERS F. E., MOOSE R. & TINKER P. B. (eds.), Endomycorrhizas, pp. 227–287. – Academic Press London, New York.

PHILIPPS J. M. & HAYMAN D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – Transactions of British Mycological Society 55: 158–161.

POWELL C. L. I. 1981. Effect of inoculum rate on mycorrhizal growth responses in pot grown onion and clover. – Plant and Soil 62: 231–239.

REDENTE E. F. & REEVES F. B. 1981. Interactions between vesicular-arbuscular mycorrhizae and *Rhizobium* and their effect on sweetvetch growth. – Soil Sci. 132: 410–415.

RHODES L. H. & GERDEMANN J. W. 1978. Translocation of calcium and phosphate by external hyphae of vesicular-arbuscular mycorrhizae. – Soil Sci. 136: 125–126.

SMITH S. E., ST. JOHN B. J., SMITH F. A. & NICHOLOAS D. J. D. 1985. Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneum* and *Allium cepa:* Effects of mycorrhizal infection and phosphate nutrition. – New Phytologist 99: 211–228.

STEEL R. G. D. & TORRIE J. H. 1980. Principles and Procedures of Statistics. – McGraw-Hill New York, London.