Danish Research Service for Plant and Soil Science **Report no. 1705**
Research Centre for Agriculture
State Laboratory for Soil and Crop Research
Soil Chemistry Department
DK-2800 Lyngby

The effect of inoculating lucerne (*Medicago sativa*) with vesicular-arbuscular mycorrhizal fungi and *Rhizobium meliloti*

Virkningen på lucerne af podning med vesikulær-arbuskulær mykorrhiza og Rhizobium meliloti

Anni Jensen[1] and Jørgen Dissing Nielsen

Summary
Lucerne plants were inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungi, *Glomus caledonius* and *Glomus mosseae* and/or *Rhizobium meliloti.* The plants were grown in pots in voliere in a soil which had received either no phosphorus fertilizer or 20 kg P/ha/year since 1964.

Inoculation with VAM fungi increased yield and P and N uptake of shoots. However VAM infection was no higher at the end of the experiment than that established by naturally occurring fungi in uninoculated soil. Roots were nodulated and inoculation with *Rhizobium meliloti* influenced nutrient uptake and yield only slightly.

Key words: *Glomus caledonius, Glomus mosseae,* lucerne, P-uptake, *Rhizobium meliloti,* root length, VA-mycorrhiza.

Resumé
Interessen for endotrof mykorrhiza hos landbrugsplanter har i de senere år været stigende som følge af muligheden for ved podning at kunne spare på P-gødning. Ved denne undersøgelse blev lucerne podet med de 2 vesikulær-arbuskulær mykorrhiza (VAM) dannende svampe, *Glomus caledonius* og *Glomus mosseae* samt med lucerne rodknold-bakterier *Rhizobium meliloti.* Lucerne blev dyrket i karforsøg i 2 jorde, som fra 1964 henholdsvis ikke var tilført P (jord 1) og gødet med 20 kg P/ha årlig (jord 2).

Podning med VAM øgede tørstofudbyttet, P-optagelsen og N-optagelsen, selv om VAM infektionen ved forsøgets afslutning ikke var højere end VAM infektionen etableret af naturlig forekommende VAM svampe i upodet jord. I alle forsøgsled var der dannet rodknolde på lucernen, og podning med lucernebakterier havde kun en svag virkning på næringsoptagelse og tørstofudbytte.

Nøgleord: Lucerne, lucerne rodknold-bakterier, P-optagelse, VA-mykorrhiza.

---

[1] Present address: Pajbjergfonden, Dyngby, DK-8300 Odder.

Introduction

In several years the P-balance-sheet of Danish agricultural soils has been positive and the increase of inorganic P during 25–50 years on an average 150 kg P/ha for the topsoil (20 cm). In spite of the increased P-content, yield increases are still found after fertilization with P (*Skriver,* 1980). It was shown that the uptake ratio of P-fertilizer during one growing season was approximately 10 to 20% (*Lamm,* 1961). Inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi in irradiated soil increased the uptake ratio of fertilizer-P for lucerne (*Nielsen & Jensen,* 1983).

VAM increases P-uptake and growth in a number of agricultural crops, especially when grown in soils low in available phosphorus (*Mosse,* 1973; *Gerdemann,* 1975; *Tinker,* 1975). In legumes it is found, that VAM not only increases P-uptake but also stimulates nodulation and N_2 fixation by increasing their P-content (*Mosse et al.,* 1976; *Carling et al.,* 1978; *Hayman & Mosse,* 1979; *Smith et al.,* 1979; *Robson et al.,* 1981).

VAM inoculation increased P-uptake and growth of lucerne in irradiated soil (*Smith & Daft,* 1977; *Nielsen & Jensen,* 1983). In a field experiment 2 different VAM-inocula increased lucerne yields 8 and 6 times respectively compared to uninoculated plants infected by the native VAM endophytes (*Owusu-Bennoah & Mosse,* 1979). Similar results were obtained by *Barea et al.* (1980).

This paper reports the results of inoculating lucerne with *Rhizobium meliloti* and VAM-fungi in a soil which had received either no phosphorus fertilizer or 20 kg P/ha/year since 1964.

Materials and methods

The soil specimens were from an experimental field: soil 1 had received no phosphorus fertilizer since 1964 while soil 2 during the same period had received 20 kg P/ha/year. Data of the soils are listed in Table 1. The treatments were: untreated, inoculated with *Rhizobium meliloti,* inoculated with *R. meliloti* + 400 mg $CaHPO_4$-P/pot, inoculated with a mixture of *Glomus caledonius* (Gerdemann and Trappe) and *Glomus mosseae* (Gerdemann and Trappe), inoculated with a mixture of *G. caledonius* and *G . mosseae* + 4 × 840 mg NH_4NO_3-N/pot, inoculated with *R. meliloti* + *G. caledonius* and *G. mosseae.* Plants were grown in pots containing 20 kg of a soil-sand mixture 1:1 and each treatment was replicated 4 times. In the N-fertilized treatment, N-fertilization was carried out by adding 840 mg NH_4NO_3-N/pot on 16 June, 12 July, 27 July and 17 August (total amounts equals 400 kg N/ha). P-fertilizer was added at experimental start (equals 50 kg P/ha). All pots recieved 1.6 g K_2SO_4-K/pot (equals 200 kg K/ha) at experimental start.

Inoculation with VAM fungi was carried out by mixing 600 spores and infected root pieces of *G. caledonius* and 4400 sporocarps and infected root pieces of *G. mosseae* per pot in a 5–6 cm soil layer 3 cm below the soil surface. VAM free inoculum leachings <38 μm were added to all pots. Seeds of lucerne, *Medicago sativa* cv. Vela, were planted 14 May, 1982. After germination plants were thinned to 40 per pot.

The pots were placed in voliere, and plants in soil 1 and soil 2 were harvested after 10, 12, 17 and 22 weeks and 9, 11, 14 and 22 weeks respecti-

Table 1. Soil data prior to the experiment.

Soil	mg P/100 g soil				Texture per cent					
	Total-P	P-H_2SO_4[1]	Org.-P	P-$NaHCO_3$[2]	Rt	Clay	Silt	Fine sand	Coarse sand	Humic
1	39.0	10.2	28.8	1.1	6.0	15.1	19.0	37.6	25.7	2.6
2	46.8	19.2	27.6	3.4	6.2	16.3	18.5	37.0	25.2	3.0

[1] P extracted with 0.2 n H_2SO_4

[2] P extracted with 0.5 n $NaHCO_3$

vely and allowed to regrow after each harvest. Dry weight of plant shoots was recorded and plants were analysed for P, N, K, Ca, Mg, Zn and Cu.

For determination of root length and VAM frequency root samples were taken after 22 weeks by removing about 700 g of soil in cores, each 2.5 cm in diameter and 20 cm deep. Roots were washed in water, cleared in 10% KOH and stained in trypan blue in lactophenol (*Phillips & Hayman,* 1970). Root length per g dry weight of soil was determined by examining roots from the whole sample, while VAM frequency was determined by a line intersection method observing 100 intersection points per sample (*Ambler & Young,* 1977).

Presence or absence of nodules was examined for each pot after 7 weeks growth and in one pot per treatment at the end of the experiment.

Results

Root length and VAM frequency are shown in Table 2. Application of N-fertilizer increased root length, whereas root length was unaffected by inoculation with VAM, inoculation with *R. meliloti* and application of P-fertilizer.

VAM inoculation did not change VAM frequency in roots from that established by naturally occurring VAM fungi. P-fertilization decreased VAM frequency, whereas N-fertilization did not change the proportion of root length infected but increased infected root length as a result of increased root growth. Inoculation with *R. meliloti* did not influence VAM frequency.

Nodules were observed in all treatments except in the controls after 7 weeks growth, and at the end of the experiment nodules were observed in all treatments. These data are not presented in tabular form.

Yields of shoots are shown in Table 3. The effect of VAM and *R. meliloti* can be seen by comparing control with M, TR with M + R or control with R, M with M + R respectively. The introduced VAM species increased yield of shoots compared to yield of shoots with roots infected by naturally occuring VAM fungi in both soils and this effect was most pronounced at the first 2 harvests. Inoculation with *R. meliloti* increased yield significantly for some of the harvests in soil 1 and for first harvest in soil 2. Both P- and N-fertilization increased yield significantly except for P-fertilization in one harvest.

Table 2. Root length and VAM frequency.

	Root length* mm/g dw of soil		VAM frequency* % infected root length		VAM frequency* mm infected root length/g dw of soil	
Treatment	Soil 1	Soil 2	Soil 1	Soil 2	Soil 1	Soil 2
Control	39.5 a-c	38.2 a	68 c-e	62 bc	27.0 b-d	23.4 bc
R	37.4 a	36.0 a	71 d-f	62 bc	26.5 b-d	22.3 a-c
R + P	37.3 a	39.0 a-c	55 b	41 a	20.4 ab	15.8 a
M	32.8 a	45.8 bc	82 g	68 cd	27.1 b-d	30.8 de
M + N	63.6 d	65.2 d	79 fg	64 cd	50.2 g	41.6f
R + M	46.7 c	40.4 a-c	77 e-g	70 c-f	36.1 ef	28.2cd

Soil 1: Unfertilized with P since 1964. Soil 2: Fertilized with 20 kg P/ha yearly since 1964.
Control: Uninoculated
R: Rhizobium inoculated
M: VAM-inoculated
P: P-fertilized
N: N-fertilized

* Values with the same letter are not significantly different at the 5% level (one-way anova followed by multiple range test, LSD procedure, *Nie et al.*, 1975).

Table 3. Yield of shoots (g dry weight per pot).

Soil	Harvest	Control	R	R+P	M	M+N	R+M	LSD
1	1	8.06	15.59	20.10	12.92	24.43	17.17	1.84
1	2	7.85	9.64	16.16	9.60	16.11	10.86	1.98
1	3	16.18	17.35	18.76	16.16	23.13	18.89	3.09
1	4	4.23	5.01	12.82	4.39	9.01	6.64	1.74
	total	36.32	47.59	67.84	43.07	72.67	53.56	5.32
2	1	10.02	18.00	26.42	16.23	23.94	20.52	3.23
2	2	9.40	13.48	19.19	17.97	24.74	14.30	3.38
2	3	16.62	15.52	22.15	19.96	25.09	22.65	4.40
2	4	14.08	11.30	15.21	15.57	23.04	11.86	4.88
	total	50.12	58.30	82.96	67.73	96.81	67.83	10.15

Abbreviations as in Table 2.

Concentrations of N, P, Ca, Mg, Zn and Cu only showed small variations except for an in-crease in P-concentration after P-fertilization. Uptake of K , Ca, Mg, Zn and Cu in shoots followed the same pattern as yield. These data are not presented in tabular form.

Table 4 shows P-uptake in shoots. Both P-uptake in shoots in all but 4. harvest and total shoot-P-uptake was significantly increased by VAM inoculation in soil 2, while only P-uptake in shoots at 1. harvest was significantly increased in soil 1. Inoculation with *R. meliloti* increased P-uptake significantly at 1. harvest in both soils and in soil 1 also total P-uptake. Application of P-fertilizer increased P-uptake significantly at all harvests in both soils, as a result of increased P-concentration in shoots and increased yield of shoots. Treatments supplemented with N had significantly higher P-uptake for all but one harvest in both soils due to the increased yield of shoots.

Table 5 shows N-uptake in shoots. Inoculation with *R. meliloti* increased N-uptake in shoots significantly at 1. harvest and total uptake for soil 1. However for soil 2 inoculation with *R. meliloti*

Table 4. P-uptake in shoots (mg P per pot).

Soil	Harvest	Control	R	R+P	M	M+N	R+M	LSD
1	1	18.2	29.9	55.3	23.9	43.3	33.5	5.5
1	2	27.2	28.5	47.0	29.6	44.9	32.9	5.0
1	3	30.7	33.0	52.9	33.1	36.5	35.7	7.0
1	4	9.5	9.8	26.8	10.6	16.5	11.0	4.0
	total	86.0	101.2	182.0	97.1	141.2	113.0	12.8
2	1	22.4	40.9	83.6	37.3	50.9	49.7	7.6
2	2	32.8	37.3	76.7	58.1	69.0	46.2	13.8
2	3	49.3	46.9	75.9	65.3	74.8	75.1	9.0
2	4	38.5	27.1	42.6	41.0	57.5	28.8	13.4
	total	143.0	152.2	278.8	201.7	252.2	199.7	27.3

Abbreviations as in Table 2.

Table 5. N-uptake in shoots (mg N per pot).

Soil	Harvest	Control	R	R+P	M	M+N	R+M	LSD
1	1	223	554	766	443	672	607	62
1	2	388	457	688	443	699	505	94
1	3	573	661	765	566	674	641	120
1	4	116	165	257	122	387	156	54
	total	1300	1836	2472	1573	2431	1909	206
2	1	264	662	995	612	746	747	129
2	2	337	395	861	659	1052	609	166
2	3	719	678	932	892	901	955	179
2	4	413	220	337	344	565	252	199
	total	1733	1956	3135	2507	3264	2564	458

Abbreviations as in Table 2.

only significantly increased N-uptake at 1. harvest.

Inoculation with VAM fungi only increased N-uptake in one case in soil 1 but in most cases in soil 2. N-fertilization significantly increased N-uptake in both soils because of increased yield. The concentration of N in shoots was not changed by N-fertilization. Treatments supplemented with P had significantly higher N-uptake for all but one case.

Table 6 shows that the P-content in soil had generally decreased during the growth period and mainly in the VAM inoculated treatments.

Discussion

In this experiment inoculation with VAM fungi increased yield and P- and N-uptake of lucerne, although % VAM infected root length and mm VAM infected root length was no higher at the final harvest than VAM frequency established by

Table 6. The influence from treatment and cropping on P-content in soil.

	mg P/100 g soil			
	$P\text{-}H_2SO_4$[1]		$P\text{-}NaHCO_3$[2]	
	Soil 1	Soil 2	Soil 1	Soil 2
Prior to the experiment	10.2	19.2	1.1	3.4
After the experiment, treatments:				
Control	8.1	16.1	1.1	2.3
R	8.6	14.7	1.1	2.2
R+P	8.7	16.1	1.5	2.4
M	7.9	14.0	0.9	2.2
M+N	7.7	15.0	0.9	1.9
R+M	7.4	13.8	1.1	1.9
LSD	1.14	1.86	0.17	0.31

[1] P extracted with 0.2 n H_2SO_4

[2] P extracted with 0.5 n $NaHCO_3$

Abreviations as in Table 2.

naturally occurring fungi. Whether this was due to a higher efficiency of the introduced VAM species or to an increased number of VAM propagules cannot be stated from this experiment. Differences in efficiency of different VAM species have earlier been found in lucerne by *Owusu-Bennoah & Mosse* (1979) and increasing growth response with increasing inoculum potential has also been found earlier by *Powell* (1981) in *Trifolium repens.*

The decreasing effect of VAM inoculation during growth period indicates that the increase in VAM inoculum potential was important. Although the final VAM infection was not influenced by inoculation the increased inoculum potential can have caused an earlier establishment of infection and a better developed infection at the earlier stages of plant growth. The decreasing effect of VAM inoculation during growth period can also be explained by a less competitive ability of effective introduced VAM fungi compared to the naturally occurring VAM fungi.

Nodules were found in all treatments and inoculation with *R. meliloti* did not have a major effect on yield and uptake of P or N. From this it can be concluded that these experimental soils contained an inoculum potential of *R. meliloti* high enough to establish infection. Inoculation with *R. meliloti* had a smaller effect in soil 2 than in soil 1. Although nodulation was not quantified, plants un-inoculated with *R. meliloti* in soil 2 appeared to have more nodules than the corresponding plants grown in soil 1. This might explain the above mentioned difference.

In both soils P-content was decreased more in the VAM inoculated treatments than in treatments without VAM inoculation. This corresponds with the increased P-uptake in plants after VAM inoculation.

This experiment shows that inoculation with VAM compared to naturally occurring VAM fungi can increase growth of lucerne and uptake of nutrients mainly P even in a soil fertilized with 20 kg P/ha yearly since 1964; but inoculation on field scale will only be possible when VAM fungi can be multiplicated in culture.

Acknowledgements

The authors wish to express their gratitudes to *Jytte Toft* and *Grete Rasmussen* for careful technical assistence and to *T. Vincents Nissen,* M.Sc., for valuable proposals and comments on the experimental plan. Thanks are due also to the Botanical Institute of Århus University for providing facilities for measuring root length and of VAM infection.

A grant was received from Heye's Fond and the authors are very grateful for this financial support.

Literature

Ambler, J. R. & Young, J. L. (1977): Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Sci. Soc. Am. J. 41, 551–556.

Barea, J. M., Escudero, J. L. & Azcon-Aguilar, C. (1980): Effects of introduced and indigenous VA mycorrhizal fungi on nodulation, growth and nutrition of *Medicago sativa* in phosphate-fixing soils as effected by P fertilizers. Plant and Soil 54, 283–296.

Carling, D. E., Riehle, W. G., Brown, M. F. & Johnson, D. R. (1978): Effects of a vesicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soyabeans. Phytopathology 68, 1590–1596.

Gerdemann, J. W. (1975): Vesicular-arbuscular mycorrhizae. IN: The development and functin of roots (Ed. by *J. G. Torrey & D. T. Clarkson*). Academic Press, London, 575–591.

Hayman, D. S. & Mosse, B. (1979): Improved growth of white clover in hill grasslands by mycorrhizal inoculation. Ann. Appl. Biol. 93, 141–148.

Lamm, C. G. (1961): A study of isotopic exchanges and chemical conversions between fertilizer-phosphorus and soil-phosphorus. Acta Agric. Scand. 11, 95–114.

Mosse, B. (1973): Advances in the study of vesicular-arbuscular mycorrhiza. Ann. Rev. Phytopat. 11, 171–196.

Mosse, B., Powell, C. Ll. & Hayman, D. S. (1976): Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76, 331–342.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H. (1975): Statistical package for the social sciences. McGraw-Hill, New York. 398–433.

Nielsen, J. D. & Jensen, A. (1983): Influence of vesicular-arbuscular mycorrhiza fungi on growth and uptake of various nutrients as well as uptake ratio of fertilizer P for lucerne (*Medicago sativa*). Plant and Soil 70, 165–172.

Owusu-Bennoah, E. & Mosse, B. (1979): Plant growth responses to vesicular-arbuscular mycorrhiza. XI Field inoculation responses in barley, lucerne and onion. New Phytol. 83, 671–679.

Phillips, J. M. & 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, 158–160.

Powell, C. Ll. (1981): Effect of inoculum rate on mycorrhizal growth responses in pot grown onion and clover. Plant and Soil 62, 231–239.

Robson, A. D., O'Hara, G. W. & Abbott, L. K. (1981): Involvement of phosphorus in nitrogen fixation by subterranean clover. (*Trifolium subterraneum* L.) Aust. J. Plant Physiol. 8, 427–436.

Skriver, K. (1980): Økonomiforsøg med fosfor og kalium. In: *Olesen, J.* Oversigt over landsforsøgene, 1979, 139–140.

Smith, S. E. & Daft, M. J. (1977): Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa.* Aust. J. Plant Physiol. 4, 403–413.

Smith, S. E., Nicholas, D. J. D. & Smith, F. A. (1979): Effects of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum* L. Aust. J. Plant Physiol. 6, 305–316.

Tinker, P. B. (1975): Effects of vesicular-arbuscular mycorrhizas on higher plants. In: 29th Symposium of the Society of Experimental Biology (Ed. by *D. G. Jennings* and *D. L. Lee*). Cambridge University Press, London, 325–349.

Manuscript received 11 July 1983.