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ASSESSMENT OF FIELD SITUATIONS FOR THE FEASIBILITY OF VESICULAR—ARBUSCULAR MYCORRHIZAL INOCULATION, USING A FORAGE LEGUME AS TEST PLANT[1]

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ABSTRACT

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This paper reports experiments aimed at predicting ecological situations where vesicular—arbuscular mycorrhizal (VAM) inoculation would be beneficial. Twelve unsterile soils were assayed and *Hedysarum coronarium*, a forage legume, was the test plant. Three vesicular—arbuscular endophytes, namely *Glomus mosseae*, *G. fasciculatus* and a *Glomus* sp. were tested, and in all cases *G. mosseae* was the more efficient, with indications of some plant—fungus specificity. A considerable degree of dependency on mycorrhizas, for suitable growth and N_2-fixation, was also demonstrated for the tested legume—*Rhizobium* sp. system. The studied soils varied in their concentration of plant-available phosphorus and in the amount, infectivity and effectiveness of indigenous VAM propagules; however, no correlation between these parameters could be found. The level of success of the introduction of *G. mosseae* in the presence of naturally existing endophytes was evaluated by the extent of the plant response in terms of growth, nodulation and N and P uptake in each soil. The inoculation of *G. mosseae* was effective in 7 out of the 12 soils; however, it was not possible to find a correlation between any of the soil parameters studied (i.e. soluble P concentration, amount of mycorrhizal root pieces and VAM mycelium and spores) and the plant response to *G. mosseae* inoculation. Thus, it is necessary to emphasize the need for simple techniques to screen stiuations where it would be worth attempting VAM inoculation, since each particular soil must be checked for each given plant—fungus combination.

INTRODUCTION

A selective pressure as exerted by soil fertility, agrochemical amendment and the vegetation type upon naturally occurring vesicular—arbuscular mycorrhizal (VAM) propagules has, in many instances, led to some ecological situations where these fungi, though adapted to survive in the environment, exhibit a low efficiency in terms of improving plant growth and nutrition. In addition, other situations occur where the efficient mycosymbionts

[1] The authors wish to dedicate this article to Professor L. F. Leloir, I.I.B. Buenos-Aires, Argentina, on his 80th anniversary.

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TABLE I

Analytical characteristics of the test soils

Soil No.	pH (H_2O)	Organic matter (%)	Total N (p.p.m.)	Total P (p.p.m.)	Total K (p.p.m.)	Soluble P (p.p.m.)[a]
1	7.9	2.2	1500	925	575	22.5
2	7.7	1.8	1300	1200	365	10.0
3	7.5	1.7	1275	450	317	8.0
4	7.9	1.5	1100	325	250	3.7
5	7.2	1.3	1100	290	63	10.0
6	6.5	1.5	1000	295	87	2.5
7	7.7	1.5	1120	620	260	8.0
8	7.0	2.2	1350	910	321	33.0
9	6.9	1.8	1200	980	370	17.0
10	7.8	1.7	1150	1350	275	12.0
11	7.6	1.0	925	320	270	5.0
12	6.9	1.1	1010	495	255	7.0

[a]0.5 M $NaHCO_3$ soluble P (Olsen et al., 1954).

such as vesicular–arbuscular mycelium and infected root fragments (Mosse, 1977; Hayman, 1983) were also estimated under the dissecting microscope.

Plant growth conditions and measurements

Unless otherwise stated, the soils used were untreated, i.e. unsterilized. They therefore retained their biological and chemical characteristics, but were homogenized, sieved (2 mm) and mixed with nutrient-free sand (5:2 v/v) in order to compensate for the pot-imposed compaction. The experimental soils were distributed in 500-ml-capacity pots, where 3 plants per pot were grown for 10 weeks in a glasshouse at 19–25°C. Two-day-old surface-sterilized seedlings of *H. coronarium*, inoculated with a selected, specific *Rhizobium* sp. (Azcón-Aguilar et al., 1982b), were used for transplanting. There were 5 replicate pots per treatment, and mycorrhizal inocula were mixed with the soil in the pot. These inocula consisted of standardized aliquots from specific mycorrhizal plants maintained in a culture collection. During the growth experiments, plants were watered from below and fed with Long Ashton nutrient solution (Hewitt, 1952) lacking N and P. At harvest, plants were weighed, their shoots analysed for N and P and the development of VAM and root nodules quantified as previously described (Azcón-Aguilar et al., 1982a).

Selection of VAM fungi for H. coronarium

Three VAM fungi, i.e. *Glomus mosseae*, *Glomus fasciculatus* and *Glomus* sp., from the collection of this laboratory, were tested together with an uninmight be sparsely distributed (Bowen, 1980; Abbott and Robson, 1982; Hayman, 1982b; Barea and Azcón-Aguilar, 1983). Since VAM in plant ecology and plant nutrition is potentially very important, suitable VAM endophytes might be "artificially" incorporated to help plant growth in the above-mentioned situations.

Several experiments are being carried out in small but representative plots to establish the ecological and physiological bases for future mycorrhizal inoculation-programmes under field conditions. Accordingly, two types of study, each being relevant in terms of successful field inoculation, must be undertaken: firstly, the assessment of field situations where it would be worth attempting VAM inoculation; secondly, the selection of suitable fungi, on the basis of their infectivity, effectiveness for a given plant—soil combination, ability to spread and the persistence of their effects in the soil. The final response will depend on a series of factors known to determine plant benefits from VAM, including: (i) the degree of mycorrhizal dependence of the host plant; (ii) the size and effectiveness of the native mycorrhizal population; (iii) the level of soil fertility and, above all, the concentration of assimilable P in the test soil (Abbott and Robson, 1982; Hayman, 1982a, 1984).

On the basis of such premises, the aim of this paper is to test several of these factors for 12 field soils using a legume as the test plant. Legume—*Rhizobium* systems have, in general, a considerable mycorrhizal dependence, as is well known from their high P demand for nodulation and N_2-fixation (Barea and Azcón-Aguilar, 1983).

In order to find criteria that determine the responses of the test crop to VAM inoculation in the field, the relationships between the available P in the soils, the indigenous VAM propagules (type, number, infectivity and effectiveness), the establishment of the introduced fungi and the level of the plant response (growth, nodulation, VAM formation and N and P uptake) have been investigated.

MATERIAL AND METHODS

Host plant and test soils

Hedysarum coronarium L., a forage legume, was the test plant.

Twelve field soils from the Granada province of Spain were tested. Since none of them had ever supported the growth of *H. coronarium*, inoculation with its specific *Rhizobium* sp. was required. Some analytical characteristics of these soils are given in Table I. The population of VAM propagules in the 12 soils was evaluated from samples (3 per soil) each comprising several bulked sub-samples obtained at random from the top 15 cm of soil. Spores were recovered and counted from 50-g portions of each thoroughly mixed sample as described by Hayman and Stovold (1979). The presence and quantity of other possible forms of mycorrhizal inoculum in the soil sievings, oculated control. Untreated soils Nos. 1–6, were used, and the compatibility and interactions of each of the inoculated fungi with the indigenous vesicular–arbuscular endophytes in each soil were investigated by estimating both the intensity of root colonization and the effects on plant growth and nutrient uptake.

Evaluation of the Relative Mycorrhizal Dependency degree (RMD index) for H. coronarium

Since the calculation of the RMD index must be carried out by comparing the yields of mycorrhizal plants vs. non-mycorrhizal plants (Menge et al., 1982; Plenchette et al., 1983), suitable non-mycorrhizal control soils were needed. These were achieved by steam-sterilization at 100°C for 1 h on 3 consecutive days and then re-inoculating with a filtrate from samples

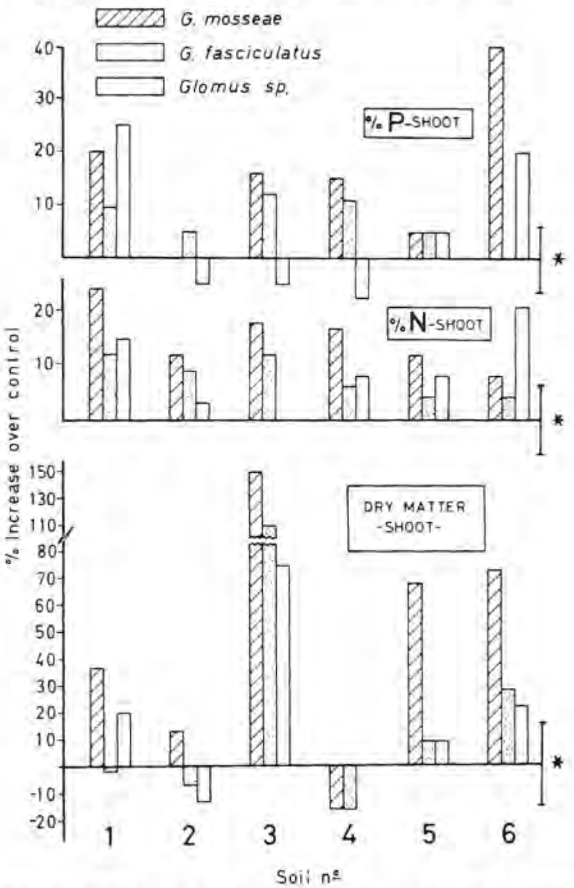

Fig. 1. Selection of successful inoculant VA mycorrhizal fungi for *H. coronarium* (+ *Rhizobium* sp.). The controls are the effect of naturally existing VA propagules in each soil.

of the same, untreated soil which possesses its natural microbiota, except for mycorrhizal propagules (Smith and Smith, 1981).

The experiment was undertaken using Soils 7—12. *G. mosseae* was the fungus assayed. Shoot dry weight and N and P concentration in the plant shoots were the basic parameters used to calculate the RMD Index (%) for *H. coronarium*, as affected by soil properties.

Assessment of field sites where VAM inoculation may be beneficial

G. mosseae was the selected endophyte. The effect of the mycorrhizal inoculation was studied in the 12 test soils, the control being the natural fertility of each one of these soils.

RESULTS

Selection of the VAM fungi for H. coronarium

Figure 1 shows that *G. mosseae* was more effective than the other endophytes tested in terms of mycorrhizal efficiency, and was therefore selected for the inoculation experiments. There were no significant differences in the degree of VAM in the different treatments. The percentage of mycorrhizal roots ranged from 60 to 80% in all cases. Native fungi found in the soils resembled species of *Glomus*, with *G. mosseae* being present in most cases. Soils 4 and 6 possessed a fine-hyphaed endophyte. An indication that introduced fungi became established could be deduced, in some instances, from observation, i.e. the presence of many prominent oval vesicles

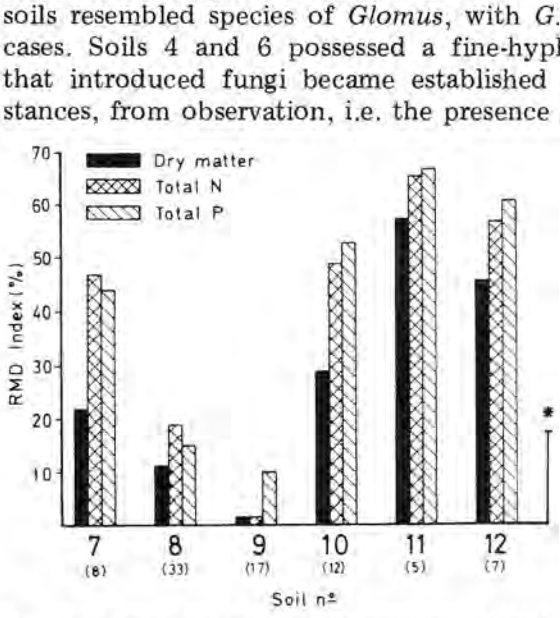

Fig. 2. Relative Mycorrhizal Dependency (RMD) for *H. coronarium* (+ *Rhizobium* sp.) grown in several soils. Numbers in parentheses indicate the plant-available soil phosphorus (Olsen et al., 1954). (*) indicates the least significant difference over the control at $P = 0.05$ for the dry matter yield.

in the roots inoculated with *G. fasciculatus* and *Glomus* sp., which made these fungi easily distinguishable in cases of mixed infection with the native fungi, and the increased number of the typical sporocarps of *G. mosseae* associated with the external mycelium in the roots which were inoculated with this species.

Relative Mycorrhizal Dependency degree for H. coronarium

Figure 2 summarizes the calculations of the RMD Index of *H. coronarium* for several soils, applying the formula proposed by Plenchette et al. (1983).

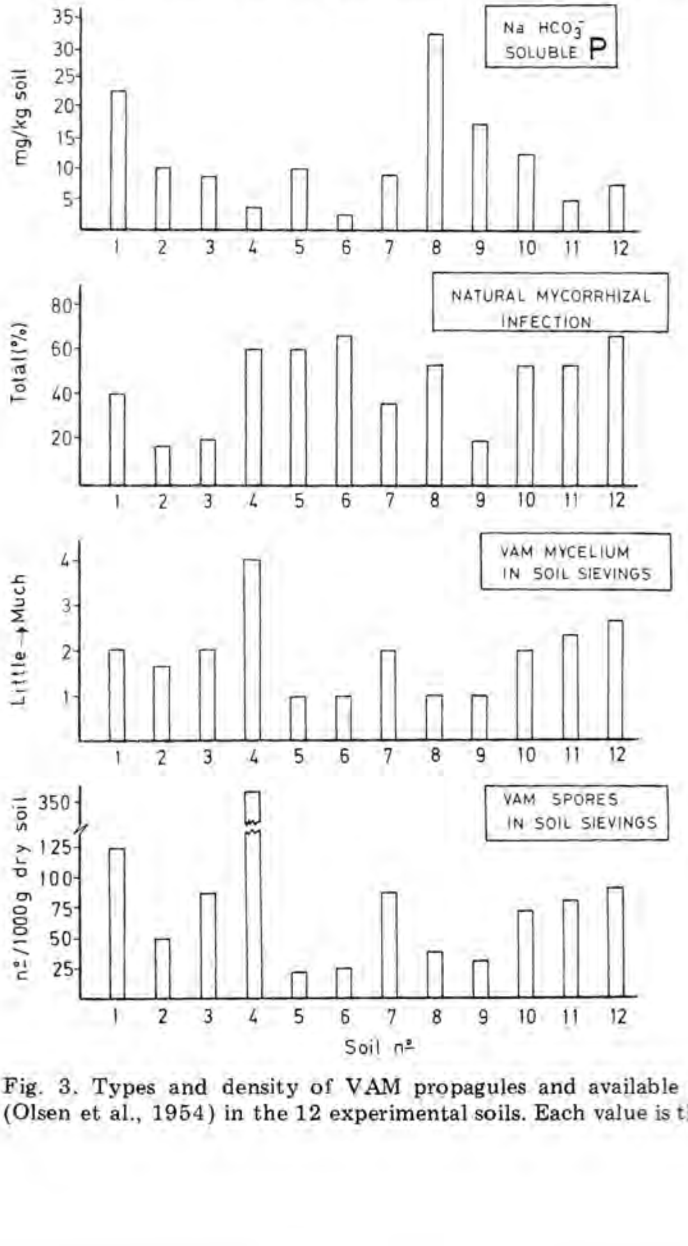

Fig. 3. Types and density of VAM propagules and available phosphate concentration (Olsen et al., 1954) in the 12 experimental soils. Each value is the average of 3 replicates.

It is apparent that the RMD Index (%) of the test plant was affected by soil fertility.

Assessment of field sites where VAM inoculation may be beneficial

Figure 3 shows a clear relationship between spore concentrations and the quantities of vesicular—arbuscular mycelium in the soil sievings ($r = 0.897$). However, the density of these forms of VA propagules was not related to the amount of natural root infection ($r = 0.199$), nor to the concentration of assimilable P in the soils ($r = 0.265$).

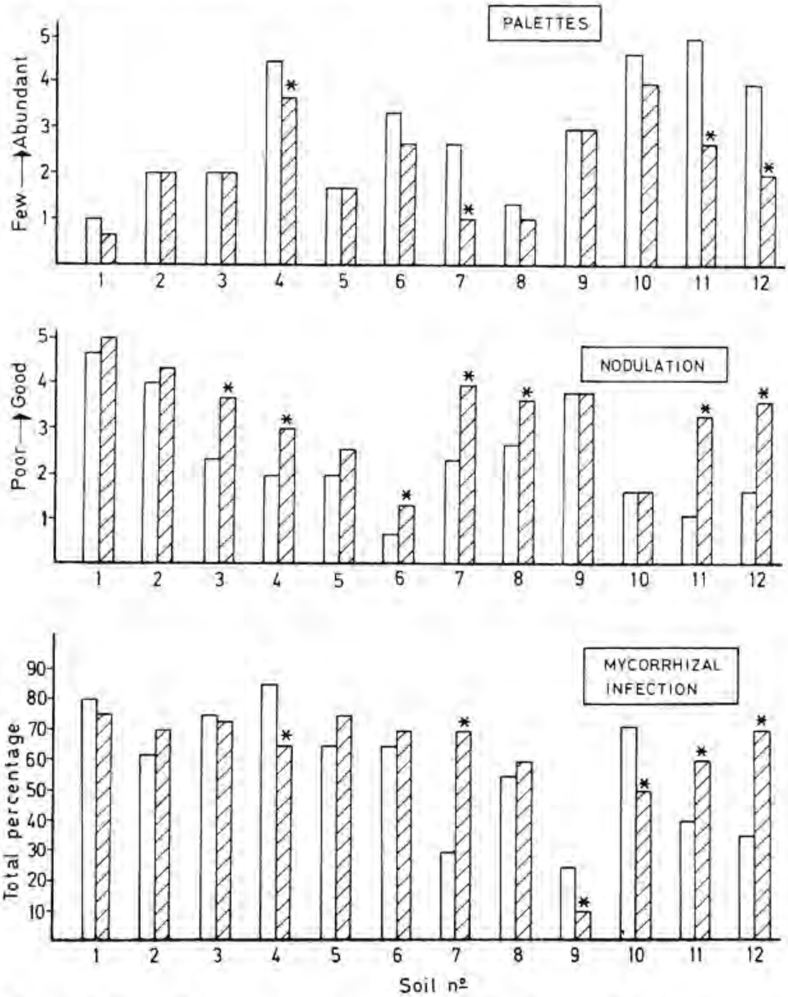

Fig. 4. Effect of *G. mosseae* plus natural endophytes (shaded columns) and the control natural endophytes (unshaded columns) on the formation of "palettes", nodules and VAM on the root system of *H. coronarium* in the 12 tested soils. (*) indicates significant differences at $P = 0.05$.

The natural infectivity and effectiveness of the 12 experimental soils can be evaluated from data given in Figs. 4 and 5 (unshaded columns). The corresponding analysis indicates a lack of correlation between these parameters (mycorrhizal infection vs. dry matter yield, $r = -0.386$; mycorrhizal infection vs. shoot P concentration, $r = 0.111$). Since natural infectivity was consistently high in most of the soils, the introduced *G. mosseae*

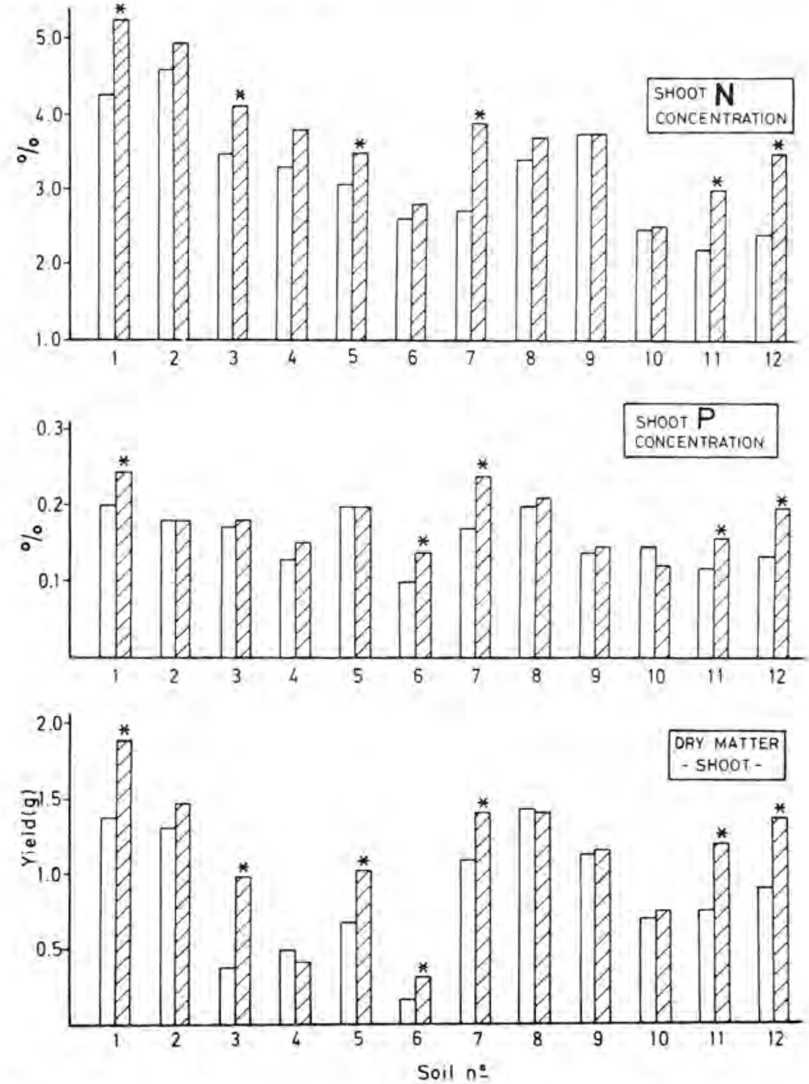

Fig. 5. Effect of *G. mosseae* plus natural endophytes (shaded columns) and the natural endophytes in the controls (unshaded columns) on the growth and nutrition of *H. coronarium* (+ *Rhizobium* sp.) in the 12 tested soils. (*) indicates significant differences at $P = 0.05$.

affected the infection levels only very slightly (Fig. 4). Only in the low natural-infectivity Soils 7, 11 and 12 did *G. mosseae* significantly enhance the percentage of VAM. In Soils 4, 9 and 10, the native and introduced endophytes competed for root colonization, and in Soil 9, mycorrhizal infection by either native or introduced fungi was inhibited.

Despite the absence of differences in the extent of vesicular–arbuscular infection, *G. mosseae* improved growth, nodulation and nutrient uptake of the plants (Figs. 4 and 5). These results, which revealed the effectiveness of *G. mosseae* (Fig. 6), clearly indicate the soils where mycorrhizal inoculation seems to be beneficial.

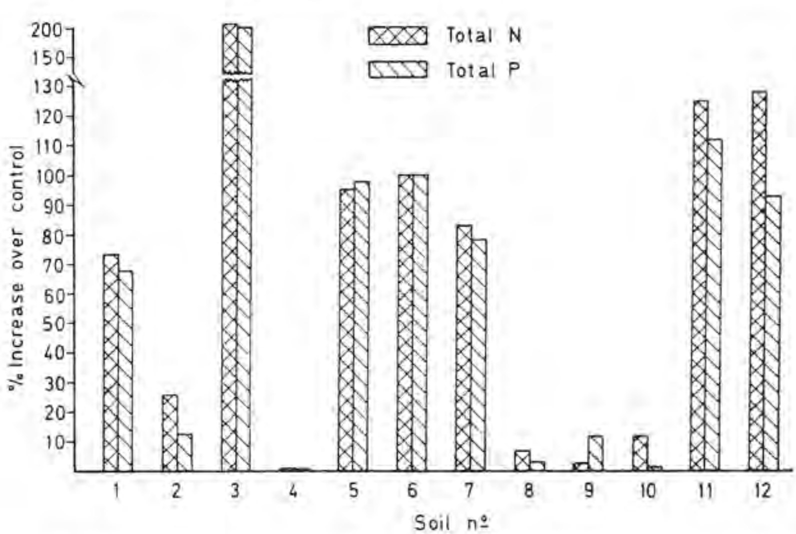

Fig. 6. Transformed data from Fig. 5 to show the effect of the inoculation of *G. mosseae* on nutrient content of *H. coronarium* (+ *Rhizobium* sp.) in the 12 unsterile test soils. The control is the effect of naturally existing VAM fungi in each soil.

The P concentration in the plant shoots was inversely related (r = -0.822) to the presence of "palettes" (modified roots typical of this legume), as has been previously described (Azcón-Aguilar et al., 1983). Nodulation (Fig. 4) and the percentage P in plant shoots (Fig. 5) were directly interrelated (r = 0.667), as was expected. The percentage N in shoots (Fig. 5) paralleled (r = 0.904) the degree of nodulation (Fig. 4), indicating the validity of the method used to measure it (which includes number, size, colour and disposition of the nodules in the root system).

There was an interesting lack of correlation between any of the soil parameters studied (i.e. soluble P concentration, amount of mycorrhizal root pieces, VAM mycelium and spores (Fig. 3)) and the plant response to *G. mosseae* inoculation, as dry matter yield (Fig. 5); r = -0.338, r = -0.137, r = -0.124 and r = -0.285, respectively.

DISCUSSION

The reported experiments, aimed ultimately at predicting ecological situations where VAM inoculation would be beneficial, have allowed us to select some suitable locations to carry out field tests using the same test legume. However, since exceptions are all too common, it is not possible to generalise, and each particular site needs particular study; hence, rapid and simple techniques to screen a great number of situations must be developed in order to choose responsive field sites.

The first point of interest is the selection of successful inoculant fungi, and in agreement with several authors (Powell, 1982; Abbott and Robson, 1982; Hayman, 1982a and 1984), the present paper emphasizes as selection criteria the production of shoot forage and the nutritional status of this material. In addition, the preference of *H. coronarium* for *G. mosseae* in all the tested soils should be pointed out. This indicates a degree of specificity between fungi and host-plant which is less documented than the one existing between fungi and soil (Hayman, 1983).

On the other hand, in spite of the fact that plant growth dependancy on mycorrhizas is a characteristic of the plant (Baylis, 1975), the level of the mycorrhizal response is affected by the level of soil fertility (Gerdemann, 1975). Environmental influences condition the so-called RMD index, which can be measured by comparing the yield with mycorrhiza to the yield without under different growth conditions (Menge et al., 1982; Plenchette et al., 1983). Available evidence suggests that when a single plant species is grown in a number of soils, the level of plant-available P in these soils is a critical factor affecting the RMD index of the test plant. This is corroborated by the reported results, since the correlation coefficient between the available P (Olsen et al., 1954) and the RMD index in the tested soils is $r = 0.690$.

It is obvious that *H. coronarium* is dependent on mycorrhiza, although this plant is able to thrive with an adequate phosphate supply. However, mycorrhiza are needed in order to achieve maximum yield, as shown by the phosphate-response curves of mycorrhizal and non-mycorrhizal plants (Azcón-Aguilar et al., 1983).

The lack of general correlations between the density of mycorrhizal propagules, soluble P concentration in soil, natural infectivity and effectiveness, etc., reported in this paper, is an example of how soil fertility, the cropping history of the locations and management practices can affect the ecology and symbiotic activity of the naturally existing VAM fungi (Abbott and Robson, 1981a; Hayman, 1982b). Indeed, studies in naturally infected plants show little relationship between growth responses to mycorrhiza and the level of available P in the soil (Stribley et al., 1980), or between plant yield and the total percentage of root infected by indigenous endophytes (Abbott and Robson, 1981b).

Once the need for individual case studies to estimate pre-conditions determining responses to VAM inoculation is recognized, it becomes clear that some soils, i.e. 1, 3, 5, 6, 7, 11 and 12, seem to be suitable for the introduction of the more effective vesicular—arbuscular fungus *G. mosseae*. All of these except Soil 1 have a low available P concentration and an intermediate level of VAM propagules, but they are quite varied in the degree of infectivity. Thus, the reason for their responsiveness to the introduction of *G. mosseae* could be accounted for by the low infectivity and/or effectiveness of their naturally occurring endophytes.

In contrast, the inoculation by *G. mosseae* was ineffective in Soils 2, 4, 8, 9 and 10. Some possible causes of ineffectiveness are efficient indigenous endophytes, a high density of ineffective mycorrhizal propagules which probably preclude the establishment of *G. mosseae*, and a high available P concentration.

A criticism of assays such as this is that glasshouse experiments are not absolutely representative of field conditions; however, as Hayman (1982b) points out, glasshouse trials can be used as a guide to soil—plant—endophyte compatibility in order to reduce the amount of screening necessary in the field.

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REFERENCES

Abbott, L.K. and Robson, A.D., 1981a. Infectivity and effectiveness of five endomycorrhizal fungi: Competition with indigenous fungi in field soils. Aust. J. Agric. Res., 32: 621—630.

Abbott, L.K. and Robson, A.D., 1981b. Infectivity and effectiveness of vesicular—arbuscular mycorrhizal fungi: Effect of inoculum type. Aust. J. Agric. Res., 32: 631—639.

Abbott, L.K. and Robson, A.D., 1982. The role of vesicular—arbuscular fungi in agriculture and the selection of fungi for inoculation. Aut. J. Agric. Res., 33: 389—408.

Azcón-Aguilar, C., Barea, J.M., Azcón, R. and Olivares, J., 1982a. Effectiveness of *Rhizobium* and VA *Mycorrhiza* in the introduction of *Hedysarum coronarium* in a new habitat. Agric. Environ., 7: 199—206.

Azcón-Aguilar, C., Barea, J.M. and Olivares, J., 1982b. Mycorrhizal infection of *Hedysarum coronarium* as affected by rhizobial strain and combined nitrogen. In: S. Gianinazzi, V. Gianinazzi-Pearson and A. Trouvelot (Editors), Les Mycorhizes: Biologie et Utilisation. INRA Publ., 1982 (Les Colloques de l'INRA, No. 13), pp. 195—198.

Azcón-Aguilar, C., Azcón, R., Barea, J.M. and Olivares, J., 1983. The role of mycorrhiza at enhancing P-uptake by the forage legume *Hedysarum coronarium* L. under different phosphate regimes. In: Proc. 3rd Int. Congr. Phosphorus Compounds, 1—4 October 1983, Brussels, Belgium, pp. 619—626.

Barea, J.M. and Azcón-Aguilar, C., 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. In: N.C. Brady (Editor), Advances in Agronomy. Academic Press, New York, pp. 1—54.

Baylis, G.T.S., 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: F.E. Sanders, B. Mosse and P.B. Tinker (Editors), Endomycorrhizas. Academic Press, London, pp. 373—389.

Bowen, G.D., 1980. Misconceptions, concepts and approaches in rhizosphere biology. In: D.C. Ellwood, J.N. Hedger, M.J. Latham, J.M. Lynch and J.H. Slater (Editors), Contemporary Microbial Ecology. Academic Press, London, pp. 283—304.

Gerdemann, J.W., 1975. Vesicular—arbuscular mycorrhizae. In: J.G. Torrey and D.T. Clarkson (Editors), The Development and Function of Roots. Academic Press, London, pp. 575—591.

Hayman, D.S., 1982a. Practical aspects of vesicular—arbuscular mycorrhiza. In: N.S. Subba Rao (Editor), Advances in Agricultural Microbiology, Oxford and IBH Publishing, New Delhi, pp. 325—373.

Hayman, D.S., 1982b. Influence of soil and fertility on activity and survival of vesicular—arbuscular mycorrhizal fungi. Phytopathology, 72: 1119—1125.

Hayman, D.S., 1983. The physiology of vesicular—arbuscular endomycorrhizal symbiosis. Can. J. Bot., 61: 944—963.

Hayman, D.S., 1984. Methods for evaluating and manipulating vesicular—arbuscular mycorrhiza. In: J.M. Grainger and J.M. Lynch (Editors), Microbiological Methods for Environmental Biotechnology, Society for Applied Bacteriology, Academic Press, New York, London, pp. 95—117.

Hayman, D.S. and Stovold, G.E., 1979. Spore populations and infectivity of vesicular—arbuscular mycorrhizal fungi in New South Wales. Aust. J. Bot., 27: 227—233.

Hewitt, E.J., 1952. Sand and Water Culture Methods Used in the Study of Plant Nutrition. Tech. Commun. No. 22, Commonw. Agric. Bur. Farnham Royal, Gt. Britain, 547 pp.

Menge, J.A., Jarrell, W.M., Labanauskas, C.K., Ojala, J.C., Huszar, C., Johnson, E.L.V. and Sibert, D., 1982. Predicting mycorrhizal dependency of Troyer citrange on *Glomus fasciculatus* in California citrus soils and nursery mixes. Soil Sci. Am. J., 46: 762—768.

Mosse, B., 1977. Plant growth responses to vesicular—arbuscular mycorrhiza. X. Responses of *Stylosanthes* and maize to inoculation in unsterile soils. New Phytol., 78: 277—288.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. Circular No. 939, U.S. Dep. Agric. Washington, DC, 19 pp.

Plenchette, C., Fortin, J.A. and Furlan, V., 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil, 70: 199—209.

Powell, C.Ll., 1982. Selection of efficient VA mycorrhizal fungi. Plant Soil, 68: 3—9.

Smith, F.A. and Smith, S.E., 1981. Mycorrhizal infection and growth of *Trifolium subterraneum*: use of sterilized soil as a control treatment. New Phytol., 88: 299—309.

Stribley, D.P., Tinker, P.B. and Snellgrove, R.C., 1980. Effect of vesicular—arbuscular mycorrhizal fungi on the relations of plant growth, internal phosphorus concentration and soil phosphate analyses. J. Soil Sci., 31: 655—672.