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NUCLEAR TECHNIQUES TO STUDY THE ROLE OF MYCORRHIZA IN INCREASING FOOD CROP PRODUCTION

PROCEEDINGS OF A CONSULTANTS' MEETING
ON THE USE OF NUCLEAR TECHNIQUES
TO STUDY THE ROLE OF MYCORRHIZA
IN INCREASING FOOD CROP PRODUCTION
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION OF ISOTOPE AND RADIATION APPLICATIONS
OF ATOMIC ENERGY FOR FOOD AND AGRICULTURAL DEVELOPMENT
HELD IN VIENNA, 16–20 NOVEMBER 1981

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FOREWORD

A group of consultants, whose names are listed at the end of this publication were invited by the FAO/IAEA Division to Vienna from 16-20 November 1981 to review, together with the Division's staff, the state-of-the-art regarding Vascular-arbuscular-mycorrhizal symbiosis with various food crops, to assess the useful role of the association in food crop production, and to recommend inputs that the Joint FAO/IAEA Division could make to promot research which might lead to the exploitation of VAM for increased crop production. The reports presented at the meeting covered several topics, including the ecology of the VAM fungus, mechanism of VAM infection, factors affecting the establishment of an effective symbiosis with food crops, mechanisms for enhanced nutrient availability to mycorrhizal plants, increased tolerance of mycorrhizal plants to adverse environmental conditions, inoculum production and field inoculation procedures. These reports, together with the experimental plans and recommendations made at the meeting, are embodied in this unpriced Technical Document. It is hoped that the free distribution of this publication will ensure a wide circulation among interested scientists, particularly those in the developing countries.

Dr. S.K.A. Danso of the Soil Fertility, Irrigation and Crop Production Section was the Scientific Secretary for the meeting, and has compiled this publication.

EDITORIAL NOTE

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THE MYCORRHIZAL RESPONSE

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Abstract

Vesicular-arbuscular mycorrhizal (VAM) infection has been shown to stimulate growth of a wide range of plant species, particularly in nutrient deficient soils and in soils with various deleterious effects, such as high salt content or root pathogens. VAM are therefore of economic and 'strategic' importance in their potential for maximizing food productivity on poor or marginal soils. There are however a number of factors which affect the persistence or abundance of the fungus in soil, as well as in the successful establishment of an effective symbiosis with higher plants. These factors, together with the potential role of isotopes in their study, are discussed.

INTRODUCTION

The papers at this meeting will deal with a number of aspects of the stimulation of plant growth by vesicular-arbuscular mycorrhizas (V.A.M.) and factors affecting the response. In this paper I indicate a few of the broad soil-plant factors affecting response but focus particularly on the nature of the response and indicate some of the roles of isotopes in these studies. I shall illustrate my points with only one or two pertinent examples in each case; this is neither a comprehensive documentation of the literature nor a detailed philosophical discourse. However, I certainly hope my general philosophy on V.A.M. and its potential for increasing plant productivity in a number of situations will be clear.

With the *general* exception of Chenopodiaceae and Cucurbitae (see Gerdemann 1975, Hirrell *et al.* 1978) most economic plant species are capable of infection with v.a. endophytes (v.a.e.). These include

agricultural plants such as wheat, barley, maize (and other grasses) and legumes, horticultural species such as tomatoes, citrus, avocado, apple and tea, many forest species such as *Araucaria*, *Casuarina*, *Alnus*, and leguminous trees and other plants such as oil palm and rubber. Thus most economic plants of developing countries may benefit from the association, one important exception being padi rice which is rarely infected although dryland rice is infected.

We must realize that productivity is determined not by the two membered system of plant and soil alone but by a more dynamic three membered system of soil plant species - v.a.e. Many soils already contain some v.a.e. but often these are low in population or are not the most efficient fungi which could be obtained for a crop. Many, if not most crops may therefore be benefiting to some extent from mycorrhizal infection already, and the important question is the extent to which agricultural science can increase this. The scientists' tasks are to

1. Identify the factors involved in plant response.
2. Identify the soil-plant systems most likely to benefit consistently from the association, and
3. Assess the possibilities and strategies to manage the symbiosis in order to optimize the plant response.

The first two are examined in this paper and basic aspects of the third are examined in my paper on epidemiology (pp. 85-102).

PLANT RESPONSES

Research over the last 15 years (much at the glasshouse level and in soils sterilized to remove indigenous mycorrhiza fungi) has shown that VAM can stimulate growth of a very wide range of important economic plants in nutritionally poor soils. Some reviews are those by Tinker (1975), Gerdemann (1975), and Bowen (1980).

Most emphasis has been on phosphate deficient soils but there is increasing demonstration that VAM may increase absorption of many ions, including Zn^{++}, NH_4^+, $SO_4^=$, Cu^{++} and K^+ (Gilmore, 1971; Tinker 1975; Bowen and Smith, 1981; Rhodes and Gerdemann, 1978; Powell, 1975). One of the earlier studies on phosphate was that by Hayman and Mosse (1971) on onions in sterilized soils with a range of available phosphate (Table 1). The response occurred over a range of available soil P and in most cases the response was eliminated by adding phosphate, thus indicating the nature of the response in these soils.

TABLE 1

Response of onions to v.a. endophyte inoculation
(Hayman and Mosse, 1971)

Soil P. (ppm)[1]	Dry wt. of shoots		
	Uninoculated	Uninoculated + Phosphate[2]	Inoculated
11.6	0.05	0.33	0.21
8.4	0.02	0.06	0.15
14.0	0.03	0.57	0.28
6.8	0.02	0.28	0.28
25.4	0.08	0.37	0.31
82.0	0.02	0.35	0.17
40.2	0.01	0.06	0.01

1 $NaHCO_3$ extractable P

2 0.4g $CaH_2\ PO_4\ H_2O$ per kg. soil.

The nature of the response is further indicated in Fig. 1 (from Abbott and Robson, 1977) in a pot study using sterilized soil for growing subterranean clover. In the absence of phosphate in this very phosphate deficient soil, a mycorrhizal response was obtained but

clover growth was still very poor. Maximum clover growth was obtained with mycorrhizal inoculation with (in this case) about half to two thirds of the phosphate required for maximum growth in non-mycorrhizal plants. Although this principle is the same from soil to soil, the exact relationship will vary with the fungus-plant-soil combination. Note also that mycorrhizal fungi differ in the response they give.

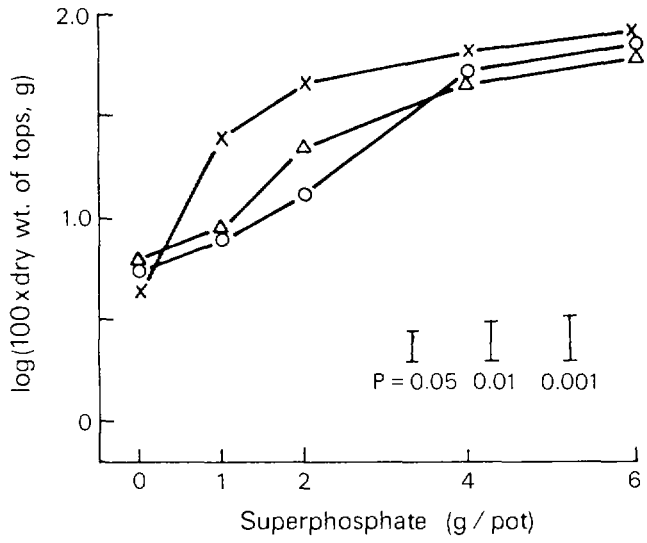

Fig. 1. Effect of v.a.m. on growth and phosphate in tops of subterranean clover at different phosphate levels in sterilized soil. X and Δ = different mycorrhizal fungi. 0 = uninoculated. (from Abbott and Robson, 1977)

Although most of the early studies on responses to v.a.m. were performed as glasshouse experiments there are an increasing number of field studies. Some of the logistic problems associated with field studies were indicated by Owusu-Bennoah and Mosse (1979) and are related principally to the difficulty of producing large amounts of inoculum for studies with large plots. It is necessary that more and more field studies be carried out to identify the potential responses in the field as a function of soils, plant species, inoculated fungus and the natural mycorrhizal population. Owusu-Bennoah and Mosse indicated a number of field responses and we may assume these will be quite common with particular crops. Some of the responses in the field (in small scale trials) include: a 1.5 to 4-fold increase in

growth of citrus in fumigated nursery soil (Hattingh and Gerdemann, 1975), a 6-fold increase in lucerne growth and a 30 percent increase in barley growth in untreated field soil (Owusu-Bennoah and Mosse, 1979), 35 per cent increase in barley ear weight (Clarke and Mosse, 1981), a 2½ fold increase in white clover growth (Hayman and Mosse, 1979) and a 27 percent increase in barley yield (Powell, 1981). A field study by Owusu-Bennoah and Mosse (Table 2) embodies much of the knowledge of response as we presently know it. In this case the results are drawn from different replicates in a variable field soil and I have used it only to illustrate principles established from other work, i.e. I have assumed the differences are real.

TABLE 2

Responses of lucerne and barley to mycorrhizal inoculation (from Owusu-Bennoah and Mosse, 1979)

Soil and Plant Species	Shoot Dry Wt (gm^{-1})[1] Inoculum Nil	A[2]	B[2]
Replicate I 9 ppm P[3]			
Lucerne	0.63	0.90	4.52
Barley	3.91	7.34	9.64
Replicate III 13 ppm P			
Lucerne	2.95	5.22	18.8
Barley	13.70	13.71	12.74

[1] Harvested at 13 weeks
[2] Inoculum A - mixed inoculum, including *Glomus mosseae*
B - *Glomus caledonius*
[3] $NaHCO_3$ extractable P

(1) Barley responded to mycorrhizal inoculation at the lower soil phosphate level but not at the higher, whereas lucerne responded at both fertility levels. This is not uncommon, and is related to the different rooting intensity of each species and their relative development

of root hairs (eg. see data of Crush, 1974). Grasses tend to have a very fibrous root system with well developed root hairs and therefore use the soil resources of poorly mobile ions such as phosphate, ammonium, zinc, copper and potassium more effectively than can plants such as legumes, some herbs and tree species which often have less intensive rooting systems and poorly developed root hairs. Thus Powell (1979) recorded a 48 percent increase in shoot growth of ryegrass from inoculation but a 91 percent increase in clover growth in the same soil. Infection with v.a.m. also makes the legume a better competitor in a mixed pasture. In the extreme, some plants have very coarse rooting systems and no root hairs - these have been termed 'magnolioid' by Baylis (1975) - and such species tend to respond well to mycorrhizal infection over a very wide fertility range. Indeed some such plants are often considered to be obligately mycotrophic, i.e. they may need mycorrhizal fungi to develop their full potential. Gerdemann (1975) defined 'mycorrhizal dependency' as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at *a given level of fertility*. Mycorrhizal dependency can vary between plant genotypes and Newcomb (1975) observed that following soil fumigation (which removes indigenous mycorrhizal fungi) Rough lemon and Troyer citrange were little affected but sweet orange frequently grew to only 40-50 percent of their normal size. Menge *et al.* (1978) found the ranking of mycorrhizal dependency of citrus cultivars inoculated with *Glomus fasciculatus* changed with the soil fertility. The mycorrhizal fungus used will also affect the ranking of mycorrhizal dependency; this may be associated with the development of the fungus in the host under different conditions (see Bowen, pp. 85-102). As information from experiments accumulates, it may be possible to compile a 'directory' of mycorrhizal dependency of various species and cultivars but soil conditions, and strain of fungus endophyte will affect this.

Among the economic plants with root systems approaching the magnolioid type (and hence potentially having large response to mycorrhizas) are citrus, avocardo (?rubber, oil palm, coffee and cassava) and some forest tree genera such as *Araucaria* and *Podocarpus*.

(2) At 9ppm phosphate inoculum A gave little stimulation to lucerne over that given by the natural population of v.a. endophytes, but *Glomus caledonius* (inoculum B) gave a very large stimulation. However, on barley at this fertility level *both* inocula caused large (but probably different) stimulations of growth. That is:

(a) plant-fungus species combinations vary in their response and

(b) introduced inocula *may* be more efficient than indigenous mycorrhizal fungi(see Bowen, pp. 85-102).

(3) A soil fertility factor also affects the plant-fungus response: on lucerne in replicate I (9ppm available phosphate) inoculum A was little better than the indigenous v.a.e. but it was considerably better than the indigenous inocula in replicate III (13 ppm available phosphate) Unrecorded soil factors other than phosphate could also have been involved.

Records of plant species x fungus species x soil fertility interactions are increasing. They emphasize not only the need for more testing of plant-fungus-soil combinations but also a scope for increasing productivity through selection of the best combination. Intra-specific variations in the fungus eg. Abbott and Robson 1978 and in plant susceptibility to infection and response also occur. I have referred to citrus variation above and Table 3 (from Azcón and Ocampo, 1981) indicates large differences in infection of different wheat cultivars with *Glomus mosseae* (under the particular growth conditions). It may be, that agronomists should select plant varieties for ready infection by v.a.e.

TABLE 3

Cultivar differences in wheat mycorrhizal infection
(Azcón and Ocampo, 1981)

Cultivar	Percent Root Infected (± SE)
Lozano	33 (9.9)
Cocorit	12 (1.6)
Champlein	0
Boulmiche	3 (0.9)

MODES OF RESPONSE

In order to predict the range of soil conditions in which we might expect mycorrhizal infection to benefit the plant it is necessary to know something about the reasons for mycorrhizal response. Although the emphasis has been on increases in uptake of nutrients from nutrient poor soils, there is increasing evidence that this is too restrictive a view; there are other situations in which nutrient uptake is enhanced and there are also possibilities of physiological effects on the plant.

The Nutrients Absorbed

Most emphasis has been on increased absorption of phosphate from soils, but as indicated above increases in absorption of other ions, including potassium, sulphate, copper, zinc and probably ammonium, and other ions also occur. Much, if not all, of this increased uptake can be explained by the growth of fungal hyphae into soil from the root, the absorption of nutrients by the hyphae, their translocation to the plant and release of nutrients to the plant (e.g. Hattingh *et al.* 1973).

The growth of the fungi into soil can be regarded as an extension of the rooting system which will especially benefit plants with poorly developed root systems over a wide range of fertility. However, it should be remembered that species with roots of the graminoid type

have low rooting intensities also both under nutrient poor conditions and other conditions. The close relation between rooting intensity and ion uptake holds particularly for poorly mobile ions and immobile ions. Phosphate, and some elements such as copper and zinc are poorly mobile or immobile under most soil conditions and v.a.m. would enhance uptake of these under all conditions. Mobility of some other ions, normally diffusing to roots more rapidly can be depressed considerably by moderate soil moisture deficiencies, eg. potassium and sulphate; uptake of these will be considerably enhanced by mycorrhizal fungi under such conditions. Hyphal growth into soil may extend the 'rooting length' at a much less energy cost than producing and maintaining roots.

Phosphate plays an important role in uptake of other nutrients, for high phosphate levels may depress mycorrhizal development, and with this decreased mycorrhizal uptake of other elements occurs. This is indicated in Table 4 (from Timmer and Leyden, 1980).

TABLE 4

Effects of phosphate on mycorrhizal stimulation of copper uptake by sour orange seedlings
(Timmer and Leyden, 1980)

P level[1]	Seedling Ht. (cm)		Foliar Cu (ppm)	
	M [2]	NM [2]	M [2]	NM [2]
0	31.5	9.1	7.0	1.7
1	37.5	20.9	5.3	3.8
2	39.3	29.1	4.3	2.0
8	44.5	51.1	3.0	3.3

[1] = mg P applied.l^{-1} soil

[2] = NM ≡ non-mycorrhizal, M ≡ inoculated with *Glomus fasciculatus*

Note in (Table 4) a greatly enhanced absorption of Cu in mycorrhizal plants with no added phosphate, which was depressed markedly at high phosphate levels due to inhibition of mycorrhizal development.

One corollary of the increased absorption of immobile ions such as copper and zinc by growth of mycorrhizal fungi into soil is that under certain conditions increased absorption *may* occur also of potentially harmful ions such as cadmium. *If* this occurs it would be important only with some crops and under special conditions but it is worthy of study.

Emphasis on mycorrhizal response has been on less mobile ions and the extension of the rooting system *via* hyphal growth. Although mycorrhizal infection can also increase the absorption capacity /$cm^2$of the root for many ions, it is usually the physical factor of 'root' length increase which is important. Absorption capacity, however, is important in uptake of highly mobile ions such as nitrate. Mycorrhizal fungi prefer ammonium sources of N, but many can use nitrate (Bowen and Smith, 1981).

Forms of nutrient

Several studies indicate that the forms of inorganic phosphate absorbed from soil by mycorrhizal fungi are the same as those used by the plant alone (Hayman and Mosse, 1972; Barrow *et al.* 1977)

Mycorrhizal fungi can use many organic forms of nitrogen and phosphate, as can higher plants given the opportunity. These organic nutrients are usually not available in soil, as they are readily used by other soil micro-organisms both for nutrition and energy. However mycorrhizal fungi may compete effectively with such soil micro-organisms as they penetrate litter well (the sites of nutrient release and turnover). This requires further study. Stribley and Read (1980) have shown that another type of mycorrhiza (ericaceous) can use amino acids as effectively as ammonium as a source of nitrogen.

Mixed ecosystems

Our concepts of mycorrhizal function have been almost entirely derived with monocultures in mind. However, most natural ecosystems and many important agricultural systems are mixed ecosystems. Two examples of these are: mixed cropping such as maize (or sorghum) interplanted with grain legumes, and mixed pastures, usually of a grass and legume. I have referred above to the greater mycorrhizal response of sparsely rooted species, e.g. legumes, than of grasses and it is highly likely that mycorrhizal infection is an important factor in competition between plants in mixed systems. Hall (1978) found growth of mycorrhizal white clover in ryegrass was 40 times that of non-mycorrhizal clover in a phosphate deficient soil. In mixed ecosystems the sharing of soil resources both for poorly mobile ions and for highly mobile ions such as nitrate is proportional to the root length of competing species, which is 'enhanced' by the growth of the mycorrhizal hyphae from roots into soil. There is evidence that mycorrhizal fungi play a part in competition for soil resources (Fitter 1977), but much more research needs to be done in this area. Because some plant species respond more to one fungus than another, there *may* be considerable scope in a planted system to manipulate the competitiveness of each plant species in a mixture, by manipulation of the mycorrhizal fungi infecting them.

Responses in other than nutrient deficient soils

The thrust of mycorrhizal research has been on nutrient uptake and therefore thinking has been directed primarily to nutrient deficient soils. Nutrient deficiency can also be 'induced' by factors which restrict the rooting of a species even in soils which are regarded normally as adequate in nutrients. Thus low rooting intensity occurs not only in nutrient poor soils but also in many deleterious conditions as diverse as high salinity, high acidity, high soil temperature and

root disease. I have suggested (Bowen, 1980) that our view of possible mycorrhizal function is too narrow i.e. 'tunnel vision', and that *mycorrhizas should be regarded as an alternate root strategy for many soil situations leading to poor root growth*, which will lead to reduced uptake of ions such as phosphate. The many mycorrhizal fungi which can associate with a wide range of plants constitute a large gene pool and there are probably many mycorrhizal fungi with greater tolerance of an adverse soil condition than the plant species, eg. high v.a.e. populations can be found in highly saline soils (Bowen, 1980). Thus if the root can be infected by a mycorrhizal fungus compatible with the soil conditions it can compensate, at least partly, for the deficit in roots caused by the deleterious conditions. Indeed this may be an important supplementary approach to breeding plants for tolerance of deleterious soil conditions.

I have given below two examples of this compensatory effect. The first, Table 5 (from Davis and Menge, 1981) indicates mycorrhizal infection can compensate partly for loss of roots due to the root disease organism *Phytophthora parasitica*. In this case the compensation is by an organism (the v.a.e.), not susceptible to that disease.

TABLE 5

V.A. mycorrhiza - *Phytophthora* interaction in sour orange (from Davis and Menge, 1981)

Inoculum	Total D.W. (g)	
	- Phytophthora	+ Phytophthora
0	5.3	3.8
Glomus fasciculatus	16.9	9.0
G. mosseae	11.9	7.0
G. fasciculatus + *G. constrictus*	12.6	10.5

The second example (Fig. 2) is from Graw (1979) in which mycorrhizal infection of *Tagetes minuta* substantially assisted the plant under highly acid soil conditions (pH 4.3). It is further interesting that this did not happen with *Guizotia abyssinica* at pH 4.3, which had little or no mycorrhizal infection at that pH.

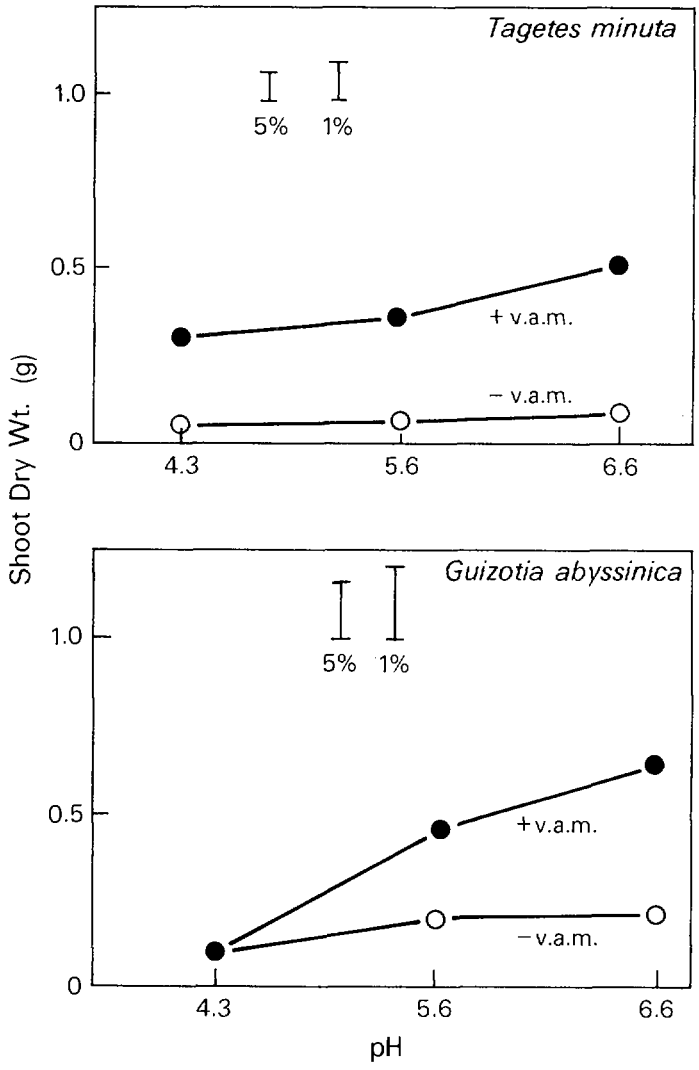

Fig. 2. Effects of v.a.m. on plant growth in acid soil (from Graw 1979).

There are an increasing number of cases of mycorrhizal enhancement of plant growth in adverse conditions (see Bowen 1980) which can be explained in large part, if not entirely by the addition of mycorrhizal fungal properties to those of the root. *This area of research needs much more study; it gives a new dimension to the possibility of growing plants satisfactorily in adverse soils.*

Only a nutrient uptake phenomenon?

The main mycorrhizal response is a nutrient uptake one but are there other types of responses? Many claims have been made that the physiology of a mycorrhizal plant is 'different' from that of a non-mycorrhizal plant but in almost every case, the physiology of a large, nutrient-sufficient mycorrhizal plant is compared with that of a small, nutrient-deficient non-mycorrhizal plant. That is not valid; it is necessary to compare the physiology of mycorrhizal plants with matched (fertilized) non-mycorrhizal plants of the same size or nutrient content.

There is increasing evidence, however, that mycorrhizal effects on plants other than nutrient uptake may occur. A record of greater production of cytokinin type compounds in mycorrhizal ***Bouteloua gracilis*** (Allen et al. 1980) is tantalizingly interesting because of the involvement of cytokinins in such properties as leaf longevity, which will affect assimilation of carbon dioxide by the tops. Unfortunately, no indication was given that mycorrhizal and non-mycorrhizal plants were matched. Levy and Krikun (1980), using mycorrhizal and non-mycorrhizal citrus with the same growth rate, found mycorrhizal plants had higher transpiration flux and photosynthesis rates during recovery from water stress, but no statistical analysis of the data was presented. Further suspicion that factors other than growth of fungi into soil are involved in the mycorrhizal response comes from anecdotal suggestions (most unpublished so far) that differences in mycorrhizal response with different v.a.e. are not always related to mycelial growth into soils. Further suggestion of physiological effects comes from studies on v.a.m. -nematode interactions (see below). Clearly there is scope for more study of possible 'physiological' effects due to mycorrhizas.

Interactions with pests and diseases

I have indicated a possible mycorrhizal compensation to root growth impairment caused by diseases or pests such as nematodes. A

number of studies have shown that other factors operate also in the case of nematode-v.a.m.interactions – factors which for the want of a better label we may call 'physiological', although they may be related to nutrition in some way. Thus Table 6 from studies of Bagyaraj *et al.* (1979), shows infection of tomato by *Glomus fasciculatus* to reduce numbers of nematode galls on the root significantly. The roots were similar in dry weight but mycorrhizal plants had a somewhat higher phosphate concentration. Infection of roots by nematodes increased sporulation of the mycorrhizal fungus - a phenomenon which could increase mycorrhizal infection and response in subsequent crops.

TABLE 6

V.a.m. - nematode interactions on tomato
(Bagyaraj *et al.* 1979)

Inoculum	Gall counts/plant 30 days	45 days
Meloidogyne incognita	510	1204
M.i. + Glomus fasciculatus	253	872
P = 0.05	188	
Meloidogyne javanica	483	617
M.j. + Glomus fasciculatus	378	40.2
P = 0.05;	152	

Interaction with disease can occur in several ways (discussed by Bowen, 1980). In brief: direct antagonism of the v.a.e. to the root disease organism is unlikely in most instances but the mycorrhizal development may (i) compensate for loss of roots (ii) allow a mycorrhizal segment which has been cutinized or suberized, to continue ion absorption (via the fungus) while retaining the protection afforded by the suberization or cutinization, and (iii) directly affect susceptibility of the plant, possibly *via* the effect of nutrient level in the plant.

Soil Structural Effects

There is increasing evidence that envelopment of soil and sand by hyphae of v.a.m. penetrating soil assists in building soil structure. Thus Sutton and Sheppard (1976) showed by inoculating pots of sterilized dune sand with *Glomus*, mycorrhizal *Phaseolus vulgaris* had five times the weight of sand aggregates/kg as uninoculated pots. Tisdall and Oades (1979) showed the root system of ryegrass was more efficient than that of white clover in stabilizing aggregates of loam because ryegrass supported a larger population of v.a.m. hyphae in soil. Electron microscope studies showed the hyphae were covered with a polysaccharide gel which probably helped the binding process.

An importance in nitrogen fixation

There is no evidence that mycorrhizal fungi can fix atmospheric nitrogen, despite various claims for this. To find nitrogen fixation in a eucaryotic organism would be most unusual. However v.a.m. do

TABLE 7

Effect of v.a.m. on legume growth and nodulation
(from Crush, 1974)

Plant Species and Treatment	Plant Fresh Wt. (g)	Nodulation[1] Ranking	Mycorrhizal Infection % [2]
Centrosema pubescens			
Mycorrhizal Inoculated	3.88	5	86
Uninoculated	1.67	1	0
Uninoculated plus phosphate	4.95	5	0
Stylosanthes guyanensis			
Mycorrhizal Inoculated	1.63	5	74
Uninoculated	0.47	0	0
Uninoculated plus phosphate	0.91	5	0

1 All plants were inoculated with appropriate rhizobia. Nodulation was ranked 0-5, 5 = intensely nodulated.

2 Percentage of the root mycorrhizal.

have a large impact on symbiotic nitrogen fixation by legumes and by non-legumes such as *Casuarina* (a non-leguminous tree, fixing nitrogen in symbiosis with the actinomycete *Frankia*), by their enhancement of phosphate uptake and other nutrients such as Zn (and possibly Mo) which affect nitrogen fixation. The importance of v.a.m. in nodulation is indicated in Table 7 (from Crush, 1974).

CONCLUSION

This talk has emphasized the potential for mycorrhizal fungi to stimulate growth of a wide range of plant species in many soil conditions, particularly in nutrient deficient soils but also in soils with other deleterious factors. Their economic and 'strategic' importance in helping conserve supplies of fertilizer is obvious as is their potential for maximizing food productivity on poor or marginal soils. They give plant scientists another tool to manage to increase productivity and may be a powerful supplementary tool in designing plants for 'difficult' soils.

There is increasing evidence from field studies, of the potential of v.a.m. for increasing productivity. However, there are a number of factors governing response to inoculation: the plant species, the soil, the efficiency of the fungus strain compared with that of the native v.a.e. population (if there is one) and the ability for an introduced v.a.e. to establish and persist. There is a clear need to define the soil-plant systems most likely to give a response in the field. Initial studies should focus on the major economic plant species and the major soil types. As a first step in predicting the value of v.a.m. inoculation, it should be possible to produce a series of curves relating species response to soil phosphate, climatic conditions and v.a.e. used. This calls for much routine testing, but it is necessary. There may be some scope for using isotopes in such studies to monitor uptake of soil nutrients readily.

There is much yet to be learnt about the nature of the mycorrhizal response, in order for us to fully appreciate its potential. I have indicated v.a.m. are probably important in many situations other than in nutrient poor soils, for example in situations involving competition in mixed agriculture and in compensating for reduced root growth in adverse soil conditions. It is in such areas that the use of isotopes in research will be invaluable, not only for defining mechanisms but in comparing the efficiency of different fungi in such situations. Initially such studies will be performed in controlled glasshouse conditions but they also must be performed in the field; in both cases much isotope based research will be highly advantageous.

In later papers I discuss the need to study the distance for which hyphae of different fungi grow from the root into soil and also the 'energy' cost of the symbiosis, i.e. the diversion of assimilate to the mycorrhiza. Obviously, the use of $^{14}CO_2$ and other radioactive isotopes in such studies is a powerful tool.

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EFFECT OF PLANT SPECIES, SOIL AND ENVIRONMENTAL FACTORS ON VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) INFECTION AND NUTRIENT UPTAKE

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Abstract

The vesicular-arbuscular mycorrhizal (VAM) system should meaningfully be considered as a 3-way interaction between plant, soil and fungus. By disassembling the complex VA mycorrhizal symbiosis and considering each component in turn, it has become evident that many factors, such as plant species, soil and environmental conditions can affect the overall balance of the complete system. For the plant to gain maximum benefits from the association, the best possible contribution of plant, fungus and environmental conditions need to be identified and maintained.

Introduction

The last decade has seen a wealth of papers on VA mycorrhizal infections and their role in nutrient uptake as affected by plant species, soil and environmental factors. This is such a broad topic that only the main principles and a limited number of specific examples can be discussed here.

At the heart of the vesicular-arbuscular mycorrhizal (VAM) system is the 3-way interaction between plant, soil and fungus. Each interaction is affected by soil type and fertility, plant species and cultivar, and fungal species or strain. External factors in the environment govern the degree to which each interaction is expressed. In this review, each component will be considered in turn within the overall symbiotic framework.

Susceptibility of different plant species to VA mycorrhizal infection

It is not understood why VAM fungi have such broad host ranges, yet some plant families are almost immune.

The ability of a single VAM isolate to infect roots of unrelated plants such as strawberry, ash, various grasses and legumes, and even hazel (which is also ectomycorrhizal) (14) suggests its nutritional requirements are not very specific. Nevertheless, an isolate with such a broad host range will not grow in pure culture on any nutrients tested so far. It needs a susceptible root. In addition there is recent evidence that plant species within a single family can vary in their susceptibility as much as plant species in very distinct families. Even varieties or cultivars of the same species can be infected to different extents. As yet, there is no well-substantiated hypothesis to explain these phenomena.

In natural ecosystems virtually every individual plant can be mycorrhizal, often with a third or more of its root system infected (14). Agricultural species including maize and many legumes, can also be heavily infected. However, the amount of infection can range widely because it is affected by soil and environmental factors, so controlled inoculation studies are necessary to reveal the true compatibility in any plant-fungus pair.

Sugar beet and the brassicas contrast with most other crops because they are almost immune to mycorrhizal infection. Why is this so ? Failure to infect some brassicas has been attributed in part to toxic root exudates. For example in mixed cropping systems in Pakistan, mycorrhizal infection in wheat was inhibited in the presence of mustard but not in the presence of another, sulphur-free, brassica (11). The

mustard was believed to exude fungitoxic sulphur compounds.

Results different to those with mustard were obtained at Rothamsted in a series of experiments involving 5 different host:non-host combinations (17, 18). Instead of the non-host plant inhibiting infection in the host plant, the supposed non-host actually developed some infection when inoculated and grown with a host plant. Cabbage, for example, remained uninfected when grown alone, but developed infection in 1 to 5% of its root length when grown with lettuce which became heavily mycorrhizal. Slight infection was also observed in oilseed rape grown with barley, swede with onion, kale with potato, and sugar beet with maize. However, no arbuscules were observed, only intercellular hyphae and vesicles so they were not vesicular-arbuscular infections in the strict sense. In addition some VAM fungi have been reported to grow around the roots of pine, an ectomycorrhizal host, but without actually penetrating them (3). These examples argue against any general role of root exudates in the lack of susceptibility of certain plants to mycorrhiza. Rather it seems that resistance of so-called non-hosts may involve compatibility at the tissue or cellular level.

With susceptible plant species, there is some host-endophyte specificity. This is not as pronounced as soil-endophyte specificity, but certain plant-fungus pairs have been noted recently in which the development of infection is favoured compared to other combinations where it is rather limited.

Influence of soil type and fertility levels on VA mycorrhizal infection

Soil type influences VAM infections indirectly through the vegetation it supports. Thus a north-temperate podzol, supporting almost pure stands of ectomycorrhizal trees such as pines, spruces and firs, will contain mainly ectomycorrhiza-forming basidiomycetes and ascomycetes, with the phycomycetous VAM fungi appearing only where sporadic pockets of their host species occur. An acid heathland dominated by Vaccinium, Erica and Calluna species will contain mainly ericaceous endomycorrhizal fungi like Pezizella ericae. In most other habitats including diverse types of grassland, savannas, scrub, open woodlands, dense rain forests and semi-deserts, the VA mycorrhizal fungi are usually predominant. Soil disturbance can greatly reduce VAM populations and old mine spoils, for example, may be initially recolonized by plants that do not depend on mycorrhiza for nutrient uptake.

VA mycorrhizal populations of agricultural soils are affected by cultivation practices, notably fertilizer additions, pesticide applications and crop rotations. Both mycorrhizal infection and spore numbers were decreased by N and P fertilizer in wheat plots in Broadbalk field at Rothamsted (5). One of the four species of VAM fungi present, viz. Glomus sp "white reticulate", was especially sensitive to added fertilizer. In wheat plots in Little Knott field at Rothamsted (5), nitrogen fertilizer had a marked negative effect on the number of Glomus caledonius spores and amount of mycorrhizal infection.

The effects of phosphate fertilizer on spore numbers and mycorrhizal infection in barley and potato plots at Rothamsted were not directly related, probably due to effects on root growth and hence total mycorrhizal biomass (7). The highest infection levels occurred in soil given least phosphate but there were most spores in soil containing intermediate levels of phosphate.

One paradox with mycorrhiza and soil fertility is that there is often no consistent relationship between the two when different sites are compared, despite the examples quoted previously. Therefore other major factors are involved in the distribution patterns of VA mycorrhiza, including what can only be termed "random variation". The picture is further complicated by the fact that some soils contain non-sporing endophytes and there are other infective progagules in soil besides spores. Thus in some surveys there is little correlation between spore numbers and soil infectivity (10).

Finally there is the question of soil-endophyte specificity. Some endophytes infect best in acid soils, e.g. Glomus fasciculatus 'E3'. Others are more infective in neutral or alkaline soils, e.g. Glomus mosseae 'YV'. Often the endophytes indigenous to a particular infertile soil are more sensitive to added fertilizer than is an inoculant strain, from a culture collection, which is adapted to higher soil nutrient levels. This has been shown clearly in upland acid peat soils where lime and fertilizer have been added in land improvement schemes (9).

Influence of environmental factors on VA mycorrhizal infection

Low temperatures can markedly decrease the rate of infection, particularly by endophyte strains or species isolated from warm areas. Hence temperature optima of VAM fungi must be considered when selecting inoculum for experiments. Poor light conditions can also lead to poor infection. This is expressed not so much in terms of per cent root length infected but more in terms of the appearance of the infection. Thus arbuscular development by *Glomus mosseae* in onions was greatest under high light and almost lacking in heavily shaded plants given short daylengths, even though hyphal spread was little affected (6).

Season also affects the development of VAM infections. They tend to follow a 3-phase pattern with an early lag phase in the spring, a phase of rapid colonization of the root cortex during much of the summer, and a plateau in the autumn (5, 20). However this pattern is rendered less simple by the fact that different endophyte species infect at different rates. This is an important consideration in practice because a growth-stimulating endophyte would be no good as inoculum in a soil or on a crop if it were to infect too slowly for the plant to benefit adequately during early growth when plant demand for phosphate is greatest.

Differences between plant species in their dependence on VA mycorrhiza

As well as differing in their susceptibility to mycorrhizal infection, plants also differ in how they

respond to mycorrhiza in terms of nutrient uptake and growth. Some plants show considerable mycorrhiza-dependence, or mycotrophy, in soils poor in phosphate. This can be very striking in natural ecosystems. For example several of the main plant species from a deciduous woodland grew poorly in their native soil when it had been sterilized to remove the indigenous VAM fungi, but adding inoculum greatly increased plant P-uptake and growth (14). This was especially true for violet and strawberry, whereas blackberry grew well without mycorrhiza. Likewise, in agricultural soils, forage legumes, onions, maize and some fruit tree seedlings can benefit considerably from VA mycorrhiza whereas other crops may have no need of mycorrhiza even though they become infected, e.g. ryegrass. Occasionally some plants grow less well if they are mycorrhizal and the soil is not P-deficient. At the other extreme a few plants appear to be obligately mycorrhizal, e.g. Stylosanthes and cassava. Under controlled growing conditions responsive plants such as lucerne generally show decreasing benefits from mycorrhiza at increasing levels of soluble soil P, while exceptionally responsive plants like Stylosanthes continue to be mycotrophic even at moderately high P levels. In the tropics Stylosanthes is usually heavily mycorrhizal from indigenous endophytes, which is presumably why it is able to grow in the very P-deficient soils there.

How can we explain the enormous range in the dependence of different plant species on mycorrhiza ? The best answer is to be found in the studies of Baylis (2).

His observations led him to propose that plants with coarse relatively hairless roots are most likely to benefit from mycorrhiza, while plants with many fine roots and long root hairs tend to benefit least. Thus root geometry is an important criterion when a particular plant is being assessed for the likelihood of it responding to mycorrhiza. Nevertheless, some woody plants with coarse roots and few root hairs are not very mycorrhiza-dependent because they grow so slowly that their demand for P is low.

The influence of soil on mycorrhizal effects on nutrient uptake by plants

The ways in which soil affects plant responses to VAM endophytes is one of the most important areas of current mycorrhizal research. The key role of soil phosphate in the mycorrhizal system is well known. Generally, if a plant will respond to added phosphate in a particular soil, it will also respond to a suitable VAM infection. Hence soil fertility plays a major role in governing potential mycorrhizal benefits. There is an approximate, but by no means precise, inverse relationship between the amount of labile (soluble) P in a soil and the size of a growth response to mycorrhiza.

Table 1 shows the broad relationship between soil P and mycorrhizal benefits in lucerne and onion, but the figures for white clover show that such benefits are never completely predictable. In an infertile grassland peat, partially sterilized by γ-irradiation (0.8 Mrad) which killed the native endophytes, mycorrhiza increased clover growth fourfold but did not affect the P con-

Table 1. Effects of VA mycorrhiza on some temperate crops.

Crop	Soil P status[a]	Dry Weight (g)		%P in shoot	
		mycorrhizal	control	mycorrhizal	control
Onion[b]	12	0.21	0.05	0.19	0.14
	25	0.31	0.08	0.29	0.09
	high	0.17	0.13	0.39	0.22
Lucerne[c]	8	1.03	0.03	0.17	0.09
	26	3.15	2.46	0.16	0.08
	40	4.53	3.39	0.18	0.18
White clover[c]	low[d]	1.01	.27	0.09	0.09
	low[e] + RP[f]	1.99	1.87	0.16	0.14
	12	1.94	1.63	0.19	0.10
	12 + RP	2.10	1.48	0.19	0.12

[a] the figures are ppm $NaHCO_3$-soluble P; [b] Hayman & Mosse, 1971; [c] Hayman, unpublished; [d] peat from hill grassland; [e] sandy loam from lowland grassland; [f] rock phosphate, 45 mg/pot.

centration of the plant tissues; adding rock phosphate greatly increased both growth and P content. In the unsterile peat the native endophytes seemed reasonably effective. By contrast, in another grassland soil (sandy loam) with pH and soluble P similar to the peat, rock phosphate had no effect on either plant growth or shoot P content, whereas mycorrhiza increased per cent P without increasing growth. The native VAM population of the unsterile loam seemed ineffective. Thus one can obtain quite different results and draw different conclusions according to the soil used. This cautions against overly ambitious interpretations of the function of VA mycorrhiza based on experiments in only one soil.

It is clearly of great interest to know where the mycorrhizal plants get their extra phosphate from.

Do they tap sources of phosphate not available to non-mycorrhizal plants ? We (8) attempted to answer this question by using ^{32}P to label the soluble P fraction, i.e. the P in solution and on the exchangeable sites, in a range of soils, and then comparing the specific activities of the P taken up by mycorrhizal and non-mycorrhizal onion plants. In this system the insoluble organic P would be virtually unlabelled and the insoluble inorganic P only weakly labelled. Hence, if the P taken up by the mycorrhizal plants had a lower specific activity (^{32}P/total P), this would be evidence that the mycorrhizal infection was enabling the roots to obtain relatively more of the insoluble soil P. However, in seven different soils from which the total P taken up by mycorrhizal plants ranged from about 1 to 20 times as much as in the non-mycorrhizal plants, mycorrhiza made no difference to the specific activity of this P. Similar results have been obtained by other workers. All the data indicate that the mycorrhizal infection was acting primarily by enabling the plants to take up more P from a source which was available to all roots, mycorrhizal or not. Furthermore, radishes (non-hosts) grew better after non-mycorrhizal strawberry plants than after mycorrhizal ones, again suggesting more absorption from the labile pool by mycorrhizal plants so as to leave less for any plants subsequently grown in that soil, rather than any mycorrhizal solubilization of insoluble P to increase the pool of available P in soil.

From these and other experiments, and Baylis's "root hair hypothesis", it is concluded that the main benefit conferred by VA mycorrhiza on a plant is an

extension of the nutrient-absorbing system. Thus the hyphae of VA mycorrhizas physically increase the amount of root-soil contact. In a typical soil containing 10^{-6}M or less P in the soil solution, a phosphate-depletion zone arises around a root because the root absorbs phosphate much faster than phosphate ions diffuse through the soil to the root surface. The mycorrhizal hyphae by-pass this depletion zone and tap the undiluted P in the soil solution beyond. They translocate this P back to the root cortex. Hence the continuity between the root-based and soil-based mycelium via the appressoria on the root surface is critical to the functioning of a mycorrhizal infection. The use of ^{32}P point sources has enabled the demonstration of hyphal translocation of P across at least 7cm of soil (19).

Subsequent experiments (15) with the grass Melinis minutiflora in ^{32}P-labelled tropical soils containing little available P (2 to 3ppm $NaHCO_3$-soluble P) also showed that the additional P taken up by mycorrhizal plants had the same specific activity as the P taken up by non-mycorrhizal plants. However another grass, Paspalum notatum, grown in a similar ^{32}P-labelled P-deficient soil, failed to take up any phosphate unless it was mycorrhizal - the non-mycorrhizal controls remained unlabelled. This is surprising because Melinis and Paspalum have similar fine, hairy roots. It therefore seemed that there was a threshold below which Paspalum plants could only take up phosphate if they were mycorrhizal. Thus the explanation of mycorrhizal function in terms of

physically extending the root absorbing surface is not the complete answer, even though it is the major one.

If mycorrhizal hyphae enable a root to remove extra P from the available pool rather than to dissolve some of the insoluble P, there is a danger of the soil becoming depleted of soluble P which will be replenished only slowly from the insoluble P. To prevent this, there should be some phosphate input into the system. In practice one could try and emulate a crop's growth response to standard dressings of superphosphate by giving the plant an efficient mycorrhizal infection coupled with either smaller than standard applications of superphosphate or with alternative forms of phosphate fertilizer such as rock phosphate.

The better utilization of rock phosphate by plants when they are mycorrhizal was first shown by Murdoch, Jackobs and Gerdemann (16). Although the possibility of solubilization of insoluble P by VAM was implied, a more likely explanation is that the VAM hyphae make better contact than plant roots with the particles of rock phosphate and thus mop up more of the ions on the surfaces of the sparingly soluble particles. More ions would then come into solution by chemical dissociation.

There is some evidence that a combination of mycorrhiza and phosphate-solubilizing bacteria may enhance plant uptake of P from soil. In one of two alkaline Spanish soils tested, growth of maize was increased more by a combination of bacteria and VAM fungi than by either alone (1). A synergistic effect was implicated. Thus soil can influence mycorrhizal function through interactions

with other components of the soil microflora. Also the bacteria survived longer around roots that were mycorrhizal, but whether this was due to changes in root exudation or the physical surface provided by the hyphae, for example, was not examined. In other experiments there appeared to be a slight utilization of rock phosphate when both phosphobacteria and Glomus mosseae were present, even though these were high pH soils and plants are not normally known to utilize rock phosphate in alkaline soils.

Another way in which soil affects the VA mycorrhizal system is by its influence on the fungal endophytes. Some soils may support a very efficient native endophyte population. Figure 1 shows the positive effect on onions of the endophytes native to a barley field which had never grown onions. Thus the VAM population was adapted to the soil rather than to the host plant. In other soils the native endophytes may infect without stimulating growth or they may be too sparse to produce an adequate level of infection. Many tests with different endophyte species have shown that the most effective endophyte in one soil is not necessarily the most effective on the same plant in another soil. In fact, there is considerable soil-endophyte specificity. Part of this is due to soil pH, part to other factors which may include fertility levels, soil structure and organic matter content.

It should be clear from the foregoing that we must regard the vesicular-arbuscular mycorrhizal symbiosis not just as a fungus-plant system but as a complex system incorporating the soil as well. The overall growth response of the plant is determined by a 3-way interaction between

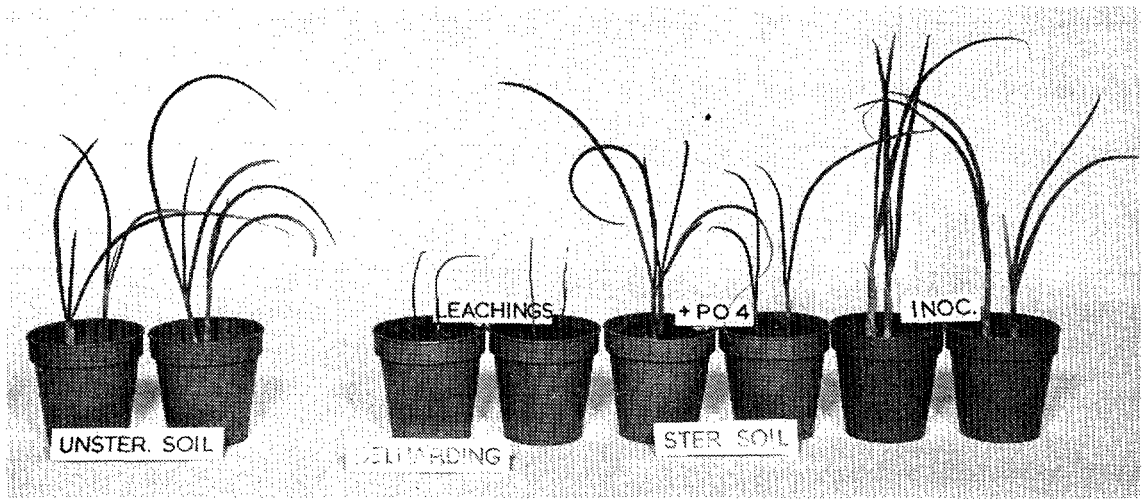

Figure 1. Onions grown in low-phosphate soil from a barley field Note the good growth of onions in unsterile soil (collected from Delharding field at Rothamsted) in contrast to poor growth in the same soil gamma-irradiated to kill the indigenous mycorrhizal fungi (STER. SOIL - "LEACHINGS"). In the "LEACHINGS" treatment, plants were given washings from the inoculum to supply contaminating micro-organisms but not the mycorrhizal endophytes. The addition of phosphate or mycorrhizal inoculum to the gamma-irradiated soil restored good growth.

soil, plant and fungus. In this tripartite system the main flow of nutrients is that of phosphate from the soil and the fungus to the plant, and carbohydrate from the plant to the fungus. The effects of the endophyte on the flow of trace elements such as zinc and copper from the soil to the plant are also important.

Influence of environmental factors on mycorrhizal activity

Plant protection chemicals now form part of the agricultural environment and can adversely affect mycorrhiza. These effects cannot always be detected by examining infections because sometimes the mycorrhizal function may be impaired

with no obvious decrease in infection levels. This occurred when the systemic fungicides benomyl and thiophanate methyl were added to soil in which strawberry and onion plants were growing (4). Phosphate uptake was not affected by the fungicides in the non-mycorrhizal plants but was markedly decreased in the mycorrhizal plants, even though infection remained at a fairly high level.

Light is an important environmental factor which can affect mycorrhiza through the supply of photosynthate to the fungus in the root. In controlled environment cabinets I have observed equal growth responses of onion in a low-P soil to either phosphate or mycorrhiza in full light, but in shaded conditions plants responded similarly to phosphate as in full light whereas they did not respond so much to mycorrhiza. This can be interpreted as decreased light permitting less photosynthesis so that there was less carbohydrate available to the fungus to provide energy for phosphate uptake. In other words, the effect of shading probably operated indirectly through phosphate nutrition rather than directly by causing a carbon drain on the plant by the mycorrhizal fungus during limited photosynthesis. Similar results were obtained by decreasing daylength instead of light intensity (6) where the 30% reduction in growth in less light in mycorrhizal but not phosphate-treated plants seemed too much to be accounted for by a drain of photosynthate to a fungus comprising approximately 5% or so of the root biomass. Recently the plant has been shown to compensate for carbon going to the fungus by an increased rate of photosynthesis (12).

Mycorrhizal activity also varies with temperature. The ability of mycorrhizal plants of the tropical species Eupatorium odoratum to utilize sparingly soluble forms of phosphate reached a maximum at 30°C, whereas the ability of plants without mycorrhiza to utilize these phosphates was poor and unaffected by temperature (13). With readily available phosphate non-mycorrhizal plants performed similarly to mycorrhizal plants.

The time factor is important in mycorrhizal studies. The relative growth effect of VA mycorrhiza varies with time and this can be an important consideration when comparing the effectiveness of different endophytes. Where responses build up more slowly with some endophytes than with others, the time of harvest will be critical in making plant growth comparisons.

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EFFECT OF PHOSPHATE FERTILIZATION ON THE DEVELOPMENT OF ROOT INFECTION IN SOME CROPS GROWN IN THE WEST ASIAN AND NORTH AFRICAN REGIONS AND THE INFLUENCE OF PRECEEDING CROPS ON THE DEVELOPMENT OF NATIVE MYCORRHIZAL FUNGI

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Abstract

Root infection by native mycorrhizal fungi was investigated in twelve different food, forage legume and cereal crops under 3 (0, 30 and 60 kg P_2O_5/ha) phosphorus regimes. With the exception of Vicia faba, all the crops examined in Tel Hadya soils, had very high mycorrhizal infection. Forage legume crops, in general, had greater % infection of roots than the food legume and cereal crops. Although the addition of 60 kg P_2O_5/ha inhibited mycorrhizal infection in some of the crops at the early vegetative stage, this influence was not noticed during the later growth stages. The incidence of native mycorrhizal population varied considerably in different locations. However, at each particular location, the development of mycorrhizal fungi was greatly influenced by the preceeding crops.

INTRODUCTION

The important role of vesicular-arbiscular (VA) mycorrhizal fungi in the phosphate nutrition of different crops has been demonstrated by several workers (Crush, 1974; Islam et al 1980; Hatting and Gerdemann, 1975; Mosse 1973; Mosse 1975; Mosse 1977a; Mosse, 1977b). Although many of these studies were conducted in pots **of sterilized or unsterilized soil inoculated with a specific spore type, and under controlled conditions, the results gave strong indications** that VA-mycorrhizal fungi in association with plants, could play a vital role in increasing food production through the uptake of phosphorus especially in phosphate deficient soils. More studies on this association need to be conducted under field conditions, so as to increase production of different crops under minimal phosphate fertilizer application.

Many soils in the ICARDA-region* are deficient in available phosphorus, in particular, below the surface layer. Rock phosphates are locally available in many countries in North Africa and the Middle East. Because of their low solubility, rock phosphates, in calcareous soils, are however, not very efficient sources of phosphorus for crops. Only commercial P-fertilizers, such as single and triple super phosphate, are capable of supplying high levels of available P in these soils.

Given the importance of P in plant nutrition, ICARDA is intensifying its efforts to effectively utilize VA mycorrhizal infection to increase the production of several crops grown in this region. As a result, some studies were initiated during the 1980-81 growing season on ICARDA's farm at Tel Hadya in Northern Syria. The major objectives of these studies were as follows:

1. To study the nature and incidence of root infection by the native endophytes in several crops.
2. To identify the most efficient mycorrhizal crops for low phosphate soils in the dry areas.
3. To study the influence of phosphate fertilizer application on mycorrhizal development in different crops grown in fields in the dry areas.
4. To study the influence of different crops on the build-up of native mycorrhizal population mainly to predict phosphorus fertilizer requirement for the subsequent crops.

Some results obtained from these studies are briefly presented.

MATERIALS AND METHODS

The field experiment for studying mycorrhizal incidence in the roots was planted in late November 1980. Twelve different crops, which consisted of, 3 food legumes, 5 forage legumes and 4 cereal crops (Table - 1) were planted in soils supplied with 0, 30 and 60 kg P_2O_5/ha. There were 4 rows for each crop, each measuring 5 m long. The row to row distance depended on the crop.

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* ICARDA-region is spread over 22 countries of West Asia and North Africa including Afghanistan in the North East and Morocco in the South.

Each phosphate treatment was replicated four times, and these were randomized within the blocks and also within each phosphate treatment the different crops were randomized. Triple super phosphate was used as a phosphorus source and the amounts required for individual plots were weighed seperately and applied by broadcasting at the time of sowing. The cereal crops received 60 kg N/ha as ammonium nitrate at planting time while both the food and forage legume crops were inoculated with appropriate Rhizobium bacteria. The initial P available in the soils ranged between 3-4 ppm. Approximately 350 mm of rainfall was received during the growing period and no supplementary irrigation was provided.

Plants were examined for mycorrhizal infection at early vegetative, early flowering and mid pod-filling stages. The plants were dug very carefully to get most of the fine roots. Root samples were stained according to methods described by Phillips and Hayman (1970), and mycorrhizal infection was estimated by following the same method as used by Islam et al (1980). Shoot dry weight was obtained on oven dried samples (dried at 80°c).

In order to establish the presence of spores and mycelia, soil samples were collected from the different fields during the dry season. These fields had been planted to barley or wheat in the previous season. To study the influence of different crops on mycorrhizal development, soil samples were collected from the ICARDA Tel Hadya farm 15-20 days after the specific crops had been harvested. From each location 20 to 30 samples (depending on the field size) were collected from the 0-25cm layer of soil. These samples were bulked and mixed gently.

Five hundred grams of soil from the bulked sample was wet-sieved very carefully (Gerdemann and Nicolson, 1963) and passed through sieves of 500, 250, 150, 100 and 50μ pore dimension to collect the different endogone spores and mycelial fragments (Mosse, 1968). The number of spores present in each sieved portion suspended in water was then counted under a light microscope. The results obtained were expressed on soil dry weight basis.

RESULTS

Mycorrhizal infection:

Figure 1. gives the mean % root infection by native endophtes for the different food, forage legume and cereal crops under 3 phosphate regimes during early vegetative, early flowering and mid pod-filling stages respectively. Considerable mycorrhizal infection developed in the roots even in the early vegetative stage. i.e. more than 35% roots were infected. Infection later increased with plant age, reaching more than 90% for some crops. Forage legume crops, in general, had more infection in the roots than either food legume or cereal crops. Addition of triple superphosphate fertilizer up to 60 kg P_2O_5/ha did not produce much influence on the mycorrhizal **infection, contrary to results obtained in many pot experiments in which small amounts of soil were used.**

Table I LIST OF THE CROPS AND CULTIVARS USED IN THE EXPERIMENTS

	Crop	Common Name	Cultivar Used
Group	Botanical Name		
Food Legume	*Lens culinaris*	Lentil	Syrian Local Large
	Cicer arietinum	Chickpea	ILC-263
	Vicia faba	Fababean	Syrian Local Large
Forage Legume	*Vicia narbonensis*	Vetch	IFVI 588
	Lathyrus sativus	Lathyrus	IFLA 165
	Pisum sativum	Pisum	IFPI 35
	Medicago littoralis	Annual medic	IFMA 3
	Medicago truncatula	Annual medic	IFMA 1
Cereal	*Triticum aestirum*	Bread wheat	S311 X Nortino
	Hordeum vulgare	Barley	Martin
	Triticale	Triticale	Beagle
	Triticum durum	Durum	Stork

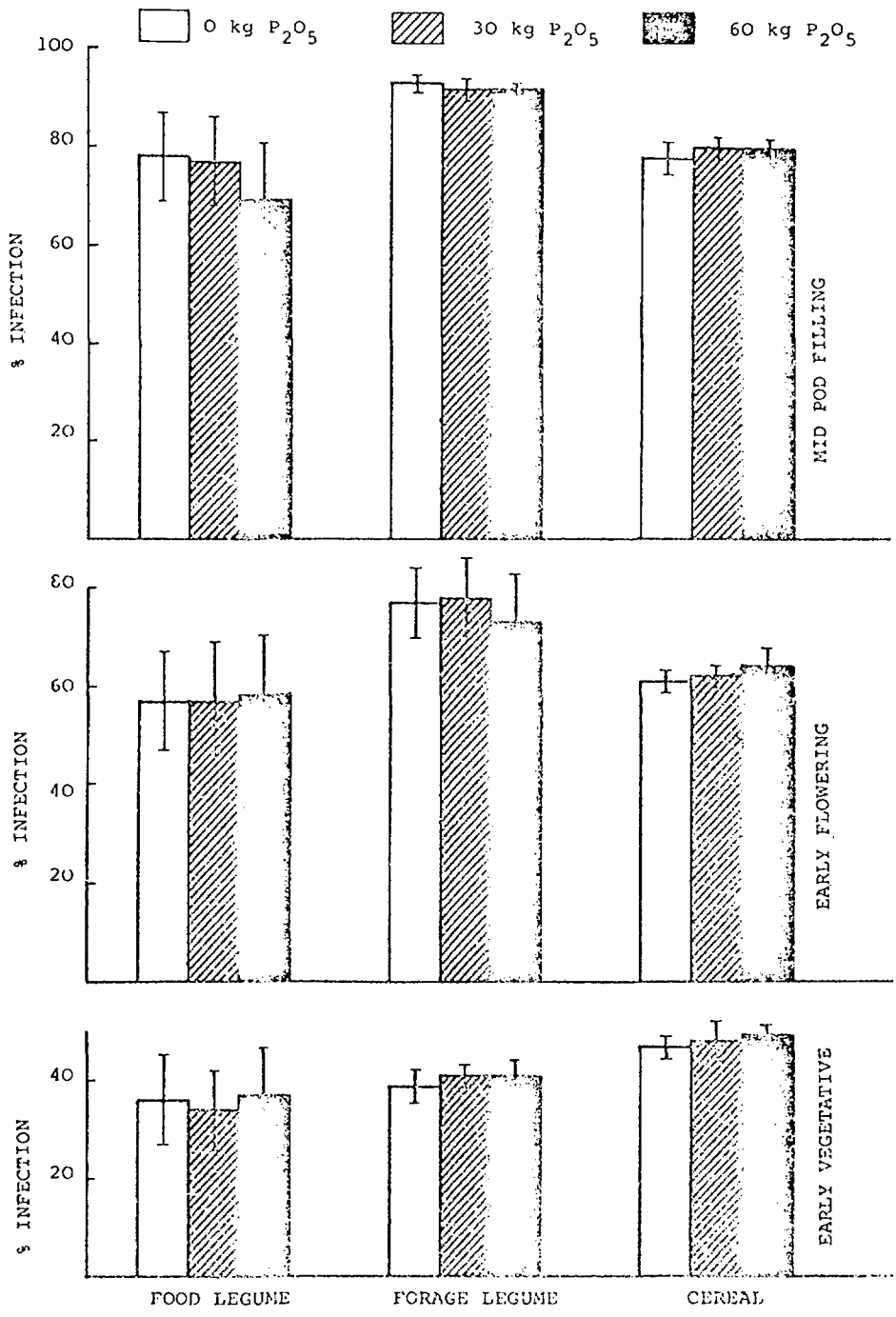

Fig. 1 THE MEAN % ROOT INFECTION BY THE NATIVE ENDOPHYTES IN THE DIFFERENT FOOD, FORAGE LEGUME AND CEREAL CROPS UNDER 3 PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA SOIL, 1980-81.

Of the three food legume crops, lentil had the highest proportion of infected roots at the final harvest, followed by chickpea and then fababean (Figure 2). Addition of 60 kg P_2O_5/ha inhibited mycorrhizal infection in lentil at early vegetative stage but did not do so at later stages of growth. Infection of chickpea and fababean roots was however, adversely affected by 60 kg P_2O_5/ha application, especially at the mid pod-filling stage (Figure 2)

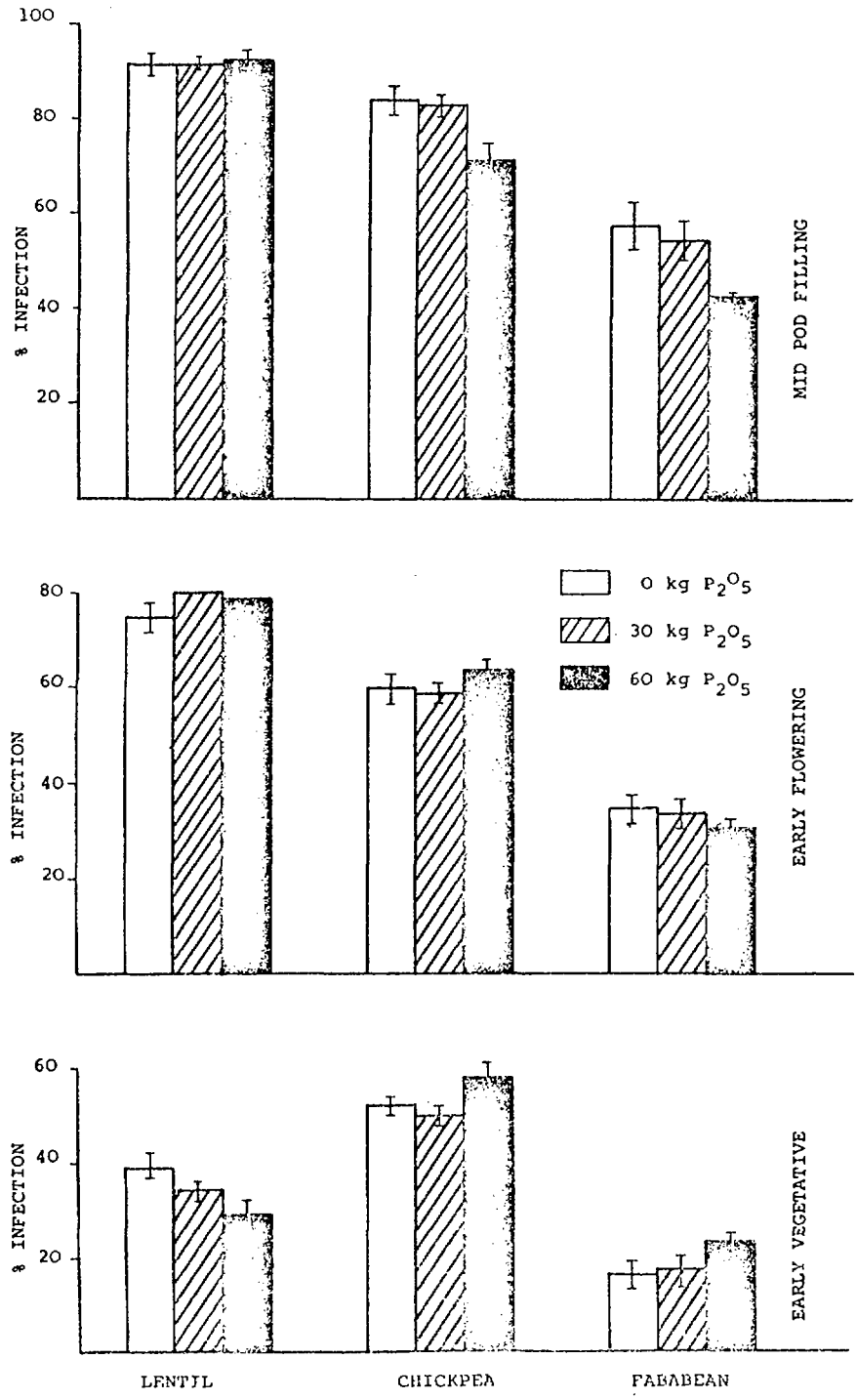

Fig. 2 THE PERCENTAGE ROOT INFECTION BY THE NATIVE ENDOPHYTES IN THE DIFFERENT FOOD LEGUME SPECIES UNDER 3 PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA SOIL, 1980-81.

Mycorrhizal incidence was not studied for M. littoralis and M. truncatula at early vegetative and early flowering stages because of extreme slow growth which did not allow enough roots to be obtained for staining. All the five forage legume crops had very high incidence of infection in the roots, each registering more than 80% roots infected at grain filling stage (Figure-3). Pisum sativum, however, had less proportion of infected roots than the other forage legume species. Phosphate fertilizer addition up to 60 kg P_2O_5/ha had very little influence on the % infection.

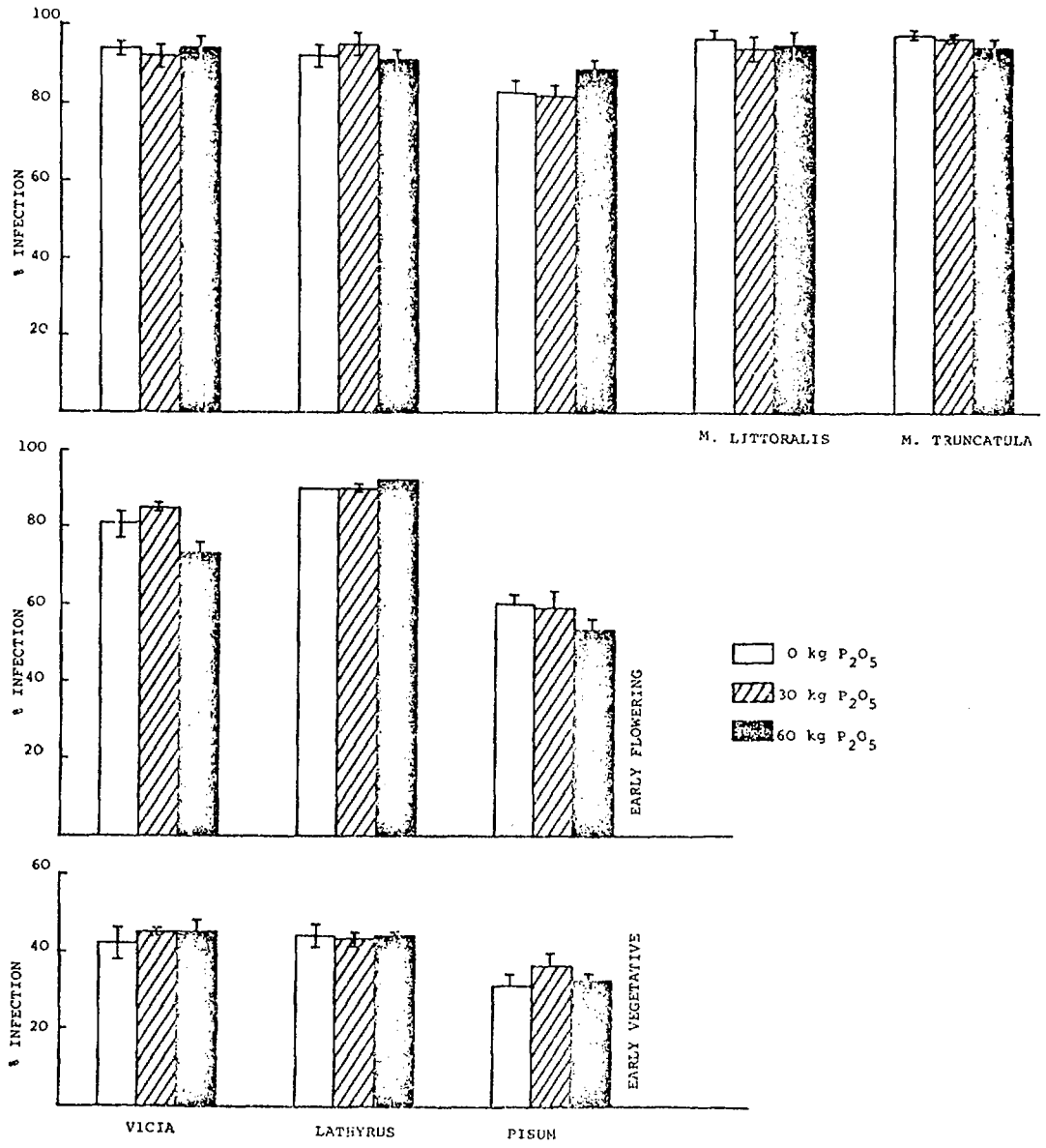

Fig. 3 THE PERCENTAGE ROOT INFECTION BY THE DIFFERENT FORAGE LEGUME SPECIES UNDER 3 PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN THE TEL HADYA SOIL 1980-81.

Of the 4 cereal crops, barley and breadwheat had slightly less infected roots than durum wheat and triticale (Figure-4). Again 60 kg P_2O_5/ha phosphate fertilizer addition did not produce detectable influence on the development of mycorrhiza in any of these cereal crops.

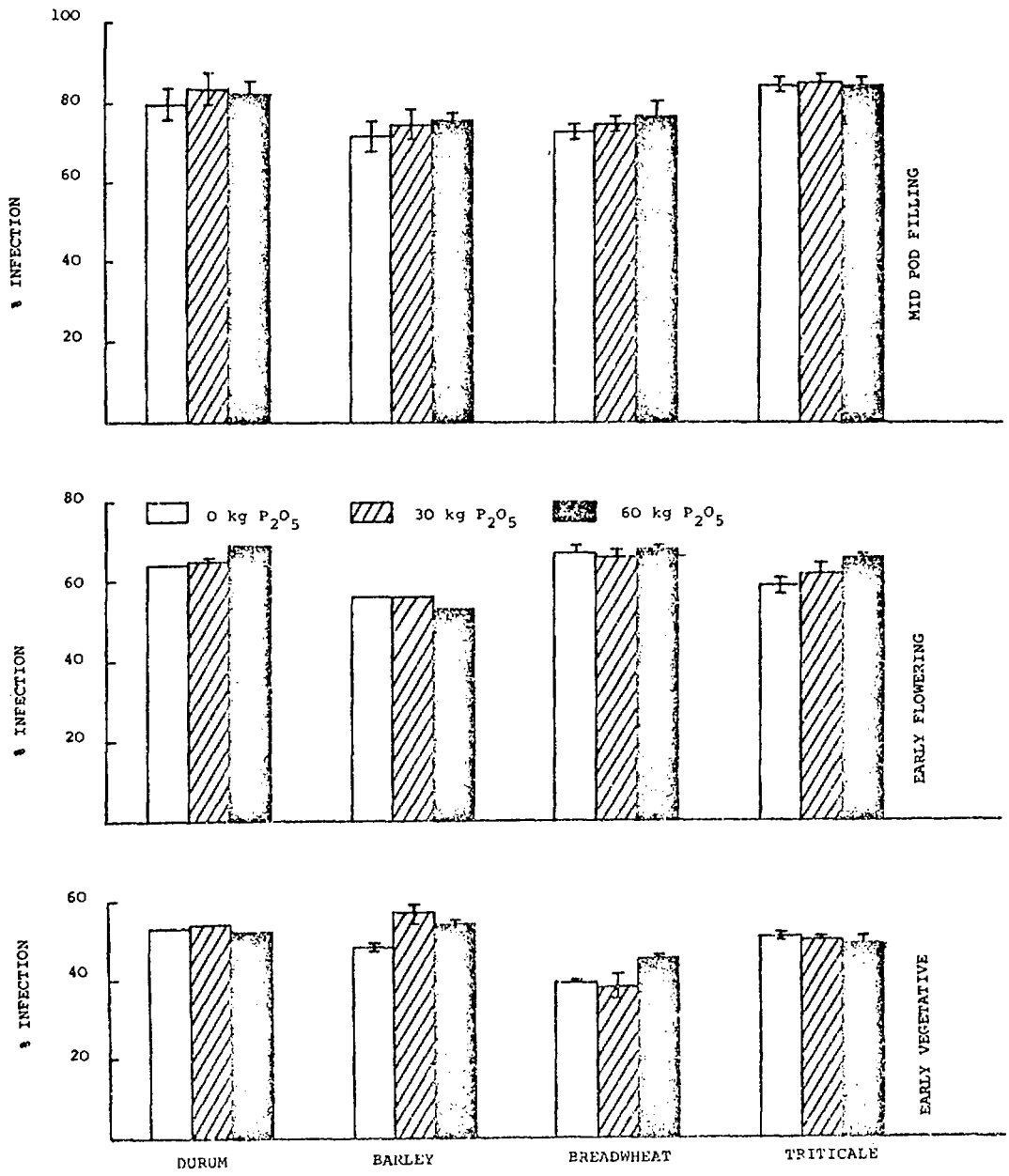

Fig. 4 THE PERCENTAGE ROOT INFECTION BY THE NATIVE ENDOPHYTES IN THE DIFFERENT CEREAL SPECIES UNDER 3 PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA SOIL, 1980-81

Dry Matter Production:

Tables 2,3, and 4 give the shoot dry weight obtained for the different food, forage legume and cereal crops at different stages of growth under the 3 phosphate regimes. Shoot dry matter production for

all the crops increased with plant age. The rate of increase was much higher between flowering and grain-filling stages in comparison to the vegetative and flowering stages. Fababean, Pisum and durum wheat produced the most dry matter amongst the different food, forage legume and cereal crops. Addition of phosphate fertilizers increased shoot dry matter production for some crops only, but the others failed to respond to phosphate fertilizer application.

TABLE 2 PRODUCTION OF SHOOT DRY MATTER BY THE THREE FOOD LEGUME SPECIES UNDER THREE PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA, 1980-81.

Crop	Rate of P_2O_5 (kg/ha)	Shoot Dry Wt. (g/plant)		
		Early veg.	Early flowering	Mid Pod Filling
Lentil	0	0.06	1.14 ± 0.04	11.06 ± 1.12
	30	0.06	1.10 ± 0.01	12.08 ± 0.94
	60	0.07	1.08 ± 0.02	13.21 ± 1.16
Chickpea	0	0.28	0.51 ± 0.01	9.11 ± 0.22
	30	0.32	0.76 ± 0.09	8.71 ± 0.17
	60	0.31	0.84 ± 0.05	8.95 ± 0.43
Fababean	0	0.71	1.99 ± 0.17	28.47 ± 3.26
	30	0.76	2.25 ± 1.10	32.45 ± 2.05
	60	0.72	2.35 ± 0.14	34.68 ± 2.48

TABLE 3 PRODUCTION OF SHOOT DRY MATTER BY THE FIVE FORAGE LEGUME SPECIES UNDER THREE PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA, 1980-81.

Crop	Rate of P_2O_5 (kg/ha)	Shoot Dry Wt (g/plant)		
		Early veg.	Early flowring	Mid. Pod Filling
Vicia	0	0.21	3.93 ± 0.50	16.87 ± 0.47
	30	0.23	6.19 ± 0.62	20.63 ± 0.62
	60	0.24	6.86 ± 0.67	20.33 ± 0.59
Lathyrus	0	0.13	2.24 ± 0.14	17.28 ± 0.73
	30	0.15	2.01 ± 0.04	15.50 ± 1.52
	60	0.13	2.64 ± 0.05	16.00 ± 1.16
Pisum	0	0.17	1.03 ± 0.02	22.90 ± 2.31
	30	0.16	0.95 ± 0.12	17.43 ± 0.98
	60	0.16	0.88 ± 0.07	26.22 ± 1.75
M. littoralis	0	*N.D.	N.D.	3.11 ± 0.19
	30			3.78 ± 0.13
	60			7.88 ± 0.98
M. truncatula	0	N.D.	N.D.	3.55 ± 0.18
	30			5.98 ± 0.53
	60			5.65 ± 0.59

*N.D. Not Determined

TABLE 4 PRODUCTION OF SHOOT DRY MATTER BY THE FOUR CEREAL SPECIES UNDER THREE PHOSPHATE REGIMES AT DIFFERENT STAGES OF GROWTH IN TEL HADYA, 1980-81

Crop	Rate of P_2O_5 (kg/ha)	Shoot Dry Wt (g/plant)		
		Early veg.	Early flowering	Mid Pod Filling
Durum	0	0.10	0.46 ± 0.00	5.88 ± 0.34
	30	0.09	0.47 ± 0.03	5.82 ± 0.50
	60	0.10	0.51 ± 0.04	5.88 ± 0.38
Barley	0	0.06	1.66 ± 0.11	3.65 ± 0.34
	30	0.08	1.96 ± 0.08	4.39 ± 0.24
	60	0.07	1.38 ± 0.07	4.27 ± 0.38
Breadwheat	0	0.05	0.71 ± 0.04	5.15 ± 0.39
	30	0.05	0.66 ± 0.02	5.68 ± 0.35
	60	0.05	0.78 ± 0.05	5.81 ± 0.42
Triticale	0	0.10	2.50 ± 0.27	3.08 ± 0.30
	30	0.10	2.47 ± 0.46	5.08 ± 0.04
	60	0.13	4.36 ± 0.38	5.21 ± 0.27

Development of Mycorrhiza:

The development of native endophytes at several locations in Syria within different rainfall zones were evaluated under cereal crops. The results (Table 5) show that the incidence of spores and mycelia varied considerably in the different locations. Hayyan and Breda soils had much more spores and mycelia than the other locations. **Tel Hadya soils had the least number of spores. The amount of rainfall** does not seem to have any influence on spore development.

TABLE 5 DEVELOPMENT OF MYCORRHIZAL SPORES AND MYCELIA UNDER CEREAL CROPS AT SEVERAL LOCATIONS IN SYRIA.

Location	Rainfall range (mm)	Mycelial status	No. of spores/100g dried soil
Jenderis	450	+	14.1 ± 1.5
Sayetezar	450	++	16.8 ± 1.0
Tel Hajjar	300-450	++	34.7 ± 0.8
Hayyan	300-450	+++	> 250
Tel Hadya	300-450	+	9.1 ± 0.8
Nasriyeh	300-450	+	15.3 ± 1.3
Breda	200-300	+++	> 150
Ghererife	200-300	++	20.9 ± 0.9

+++ Abundant ++ Moderate + Scanty

In seperate studies at Tel Hadya farm the development of native endophytes under different food, forage legume and cereal crops were evaluated. Table 6 gives the status of mycelia and number of spores present in 100g. of dried soil. Genreally forage legume crops (i.e. Lathyrus and Vicia spp.) stimulated the development of more mycorrhiza than the food legume and cereal crops. Most of the spores were recovered from the 100-150 μ sieve fraction. The majority of spores were either white reticulate or honey coloured types. However, these need further classification.

TABLE 6 DEVELOPMENT OF MYCORRHIZAL SPORES AND MYCELIA UNDER DIFFERENT CROPS IN TEL HADYA SOILS.

Crop	Mycelia Status	No. of Spores/100g dry soil
Lentil	++	24.0 ± 2.2
Fababean	++	9.6 ± 1.4
Chickpea	++	8.8 ± 1.2
Vicia	++	63.4 ± 8.0
Lathyrus	+++	73.2 ± 8.2
Breadwheat	++	9.6 ± 0.8
Barley	+	6.0 ± 0.6
Triticale	++	18.0 ± 2.2

+++ Abundant ++ Moderate + Scanty

DISCUSSION

The results described above reveal that with the exception of fababean, there was a high incidence of mycorrhizal infection by the native endophytes. The different forage legume species seem to benefit more from this association than the food legume and cereal crops. This gives an indication that the forage legume species will perform better in phosphate deficient soils. Faba bean on the other hand may benefit from phosphate fertilizer addition more than from the mycorrhizal association. The shoot dry matter production figures strongly support these views.

Addition of phosphate fertilizers up to 60 kg P_2O_5/ha had very little influence on the overall development of mycorrhizal infection in the roots for the majority of the crops. Some crops even failed

to produce more dry matter under higher phosphate treatments (i.e. at 60 kg P_2O_5/ha). These results are quite contrary to many of the findings obtained for pot experiments.

Again the development of mycorrhiza was greatly influenced by the different soil types and crops grown. These raised several questions:

1. Whether the applied phosphate fertilizers are effectively used by the crops.
2. Whether a cereal crop planted after fababean will need more phosphate fertilizer than a cereal crop planted after Lathyrus.
3. What will be the best way to determine the phosphate fertilizer requirement for a particular crop in a particular location.
4. Whether soils having more native mycorrhizal population will need less fertilizer than those having lower native population.

It is very essential that the efficiences of some of these native endophytes are compared with some of those which are known to be highly effective in low phosphate soils.

The presence of a large number of mycorrhizal spores and mycelia in Hayyan and Breda soils,gives a strong indication that rock phosphate fertilizers could be as efficient as triple super phosphate fertilizers in supplying P. Further studies are planned to answer some of these basic questions.

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SOME FACTORS AFFECTING THE ABUNDANCE OF MYCORRHIZAS IN GRASSLAND

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Abstract

Nearly one quarter of the world's land surface is occupied by permanent grassland, and possible ways of increasing the productivity of grassland deserve attention. Since permanent grasslands rarely receive phosphorus fertilizer, mycorrhizas could be important.

Research in Britain has shown that mycorrhizal abundance is related to grazing intensity and to the percentage of ground area covered by vegetation. Infection levels in a plant are also influenced by what other species are growing nearby.

Knowledge of factors affecting mycorrhizal abundance could allow grasslands to be managed for greater abundance of indigenous mycorrhizas.

INTRODUCTION

About one quarter of the world's land surface is classed by FAO as "permanent meadows and pastures", whereas only one tenth is arable (Table 1). For the developing and developed countries separately the percentage figures are much the same. Since grazing animals are a major protein source, ways of increasing the productivity of grassland clearly deserve attention. It is rarely economic to apply artificial fertilizers to permanent grassland, and this suggests that mycorrhizas could be important.

TABLE 1

Percentage of land area occupied by arable crops and by permanent meadows and pastures. Data for 1978, from FAO Production Yearbook 1979.

	Developing countries	Developed countries	Whole world
Arable	8.9	11.8	10.1
Permanent meadows and pastures	24.8	23.2	24.1

Although man's control over permanent grassland is less than over arable, even in countries with limited technology some management of grassland is possible. The most widespread management practices are burning and control of the timing and intensity of grazing. If we know how mycorrhizal abundance is affected by various environmental factors, it may be possible to manage grassland for greater abundance of indigenous mycorrhizas.

Here I present some examples, from the work of my own research group, of field and greenhouse studies of the relationship of mycorrhizal abundance in some British grassland plants to factors of the environment and the vegetation.

A FIELD SURVEY

In September 1978 Plantago lanceolata was collected from 40 sites in England and Wales, most of them grassland but some severely disturbed. P. lanceolata, although not a grass, is readily eaten by cattle and sheep; it was used here as a convenient indicator of how mycorrhizal infection can vary in relation to factors of soil and vegetation. Measurements were made of various features of the site, soil and vegetation. N, P and K concentrations in the Plantago leaves were determined. Percentage of root length infected with vesicular-arbuscular mycorrhiza was determined by the method of Phillips and Hayman (1970). For further details of technique see Newman, Heap and Lawley (1981).

Among the 40 sites mycorrhizal infection ranged from 29 to 90%. The results were subjected to multiple regression, to find out which factors were most closely related to mycorrhizal infection. The statistically significant factors were:

(1) vascular plant cover (negative relation), $P < 0.01$;
(2) Plantago leaf length (positive relation), $P = 0.01$;
(3) soil organic matter (positive relation), $P = 0.04$.

Fig. 1 shows the relationship of mycorrhizal infection to vascular plant cover when vegetation height was 13 cm or less. If the effect of organic matter is allowed for the relationship is clear. At one of the sites an experiment was carried out in which all the vegetation was removed from around selected Plantago lanceolata individuals (see Christie, Newman and Campbell (1978) for details). The removal resulted in an increase of mycorrhizal infection in the Plantago roots much in line with the predictions of Fig. 1. Thus denser vegetation cover seems to reduce mycorrhizal abundance.

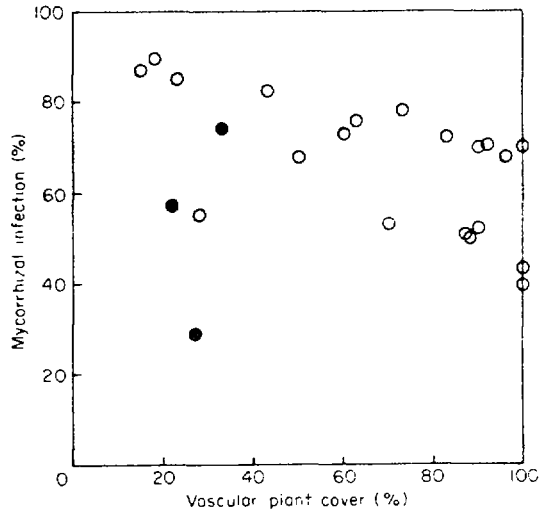

FIGURE 1

Mycorrhizal infection of Plantago lanceolata, in relation to vascular plant cover, at sites where vegetation height was 13 cm or less. ●, soil organic matter less than 3%.

The relation of mycorrhiza to Plantago leaf length probably indicates an effect of grazing. Taking sites with vegetation cover 90% or more, Plantago in grazed sites averaged 62% mycorrhizal infection, in ungrazed sites 72%. The greater infection at ungrazed sites is in a sense surprising, since the plants were more shaded by other species, and shading has been shown to reduce infection in some species (Furlan and Fortin, 1977; Daft and El-Giahmi, 1978). Daft and El-Giahmi (1978) found that clipping reduced mycorrhizal infection of several species, but Wallace (1981) found that in the Serengeti Plains more intensely grazed sites tended to have more infection. Clearly the influence of grazing and cutting on mycorrhizas in grassland deserves further research.

The relation of mycorrhizas to organic matter in the survey could suggest a promoting effect of animal dung on mycorrhizas. Previous evidence on this has led to varying conclusions (see several papers in Sanders, Mosse and Tinker, 1975).

SPECIFIC EFFECTS OF NEIGHBOURING SPECIES

We have seen that the density of vegetation around a Plantago influences its mycorrhizas. But what if the density is kept the same but the species composition changed? This has been investigated in pot experiments, always keeping the total number of plants per pot the same, but sometimes having them all of the same species, sometimes a mixture of two species. Table 2 shows some results with grassland species. Sometimes each species maintains

TABLE 2

Abundance of mycorrhizal infection in plants grown separately or in two-species mixtures. Within each group of four figures (a species-pair separate and together) any two not followed by the same letter are significantly different ($P < 0.05$). For further details see Christie et al. (1978) and Lawley et al. (1982).

(a) Neutral grassland species. Mycorrhizal intensity scored on scale 0-4.

	separate	together
Lolium perenne	0.60a	0.62a
Trifolium repens	1.07b	1.09b
Anthoxanthum odoratum	0.39a	0.54a
Trifolium repens	1.07c	0.90b
Lolium perenne	1.09ab	0.99a
Plantago lanceolata	1.18b	0.99a

(b) Acid grassland species. Percentage of root length infected.

	separate	together
Agrostis tenuis	74.0a	75.5a
Festuca ovina	87.5b	75.8a
Deschampsia flexuosa	66.0a	85.0b
Festuca ovina	87.5b	88.0b

in the mixture virtually the same mycorrhizal abundance as in monoculture, but more often at least one of the species shows a significant change. There does not seem to be any clear pattern of change. Sometimes infection is higher in mixture than monoculture, sometimes lower. A particular species (see Lolium perenne, Festuca ovina) can alter the infection of one species it grows with, yet have no effect on another species. Experiments by Ocampo, Martin and Hayman (1980) with mixtures of crop species also showed effects of one species on another's mycorrhiza which seemed to be specific to the particular species combination.

CONCLUSION

If grassland is to be managed for higher mycorrhizal abundance, we need to know how various environmental and vegetation factors influence mycorrhizas. So far research has concentrated on factors such as addition of mineral nutrients which are of little relevance to permanent grassland. Factors relevant to grassland, about which we need more information, include burning, grazing, trampling, cattle dung and plant species composition.

ACKNOWLEDGEMENTS

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EFFECTS OF VESICULAR-ARBUSCULAR MYCORRHIZA AND "PHOSPHATE-SOLUBILIZING BACTERIA" ON THE UTILIZATION OF ROCK PHOSPHATE BY PLANTS IN NEUTRAL-ALKALINE SOILS

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Abstract

Autoradiographic techniques using ^{32}P labelled soils have provided evidence that phosphate-depletion zones develop around plant roots and that mycorrhizas are able to take and translocate phosphate ions to the plant by exploring soil beyond the P-depleted rhizosphere. Besides, such mycorrhizal uptake is highly efficient. Thus, mycorrhizas not only enlarge the P-depleted zones but also these zones are even more exhausted in available phosphate. The use of sparingly soluble rock phosphate (RP) to restore the phosphate stock has been assayed and the interaction between RP and the mycorrhizal effects studied.

There is evidence to show that mycorrhizas achieve a better exploitation of RP because the hyphae make a closer contact with phosphate particles than roots, thus absorbing any soluble ions as they are chemically (or biochemically) dissociated from RP. However, such phosphate release is rather limited in non-acidic soils.

This paper reports on situations in which VA mycorrhizal fungi and phosphate solubilizing bacteria (PSB) seem to cooperate to improve the utilization of added RP by plants growing on neutral to alkaline soils. The combined VA-fungi + PSB inoculum increased significantly plant growth and P-uptake above that achieved by either separately. The effectiveness of PSB whether native or inoculated into soil is dependent on the presence of available carbon sources. Despite the general scarcity of energy-providing substrates in the soil as a whole, it is feasible that PSB could act by releasing phosphate ions from RP in discrete microhabitats endowed with the necessary pre-requisites for P-solubilization. The phosphate ions hydrolysed by the bacteria from RP could enter the soil solution. As mycorrhizal plants can explore microhabitats outside the rhizosphere and translocate these ions to the plant, re-fixation of the solubilized ions by soil components (clay minerals, Ca^{++}, Fe^{+++} and Al^{+++} ...), may be reduced, explaining the benefit of PSB + VAM inoculation.

INTRODUCTION

It is now well established by autoradiographic techniques using ^{32}P labelled soil, that a zone of phosphate depletion usually develops around plant roots. This zone is about 1 mm wide and coincides with the rhizosphere. The existence of this zone is a key factor in plant nutrition, because P plays a vital role in plant physiology and biochemistry (3,8).

The reasons for the development of such phosphate-depleted zones are also well established. Although plants require large amounts of phosphate, its concentration in the soil solution is very low, as most of the soil phosphorus (95-99%) is in chemical forms that are not directly available for plant root uptake. Besides, phosphate ions are fairly immobile in soil, being readily precipitated or absorbed in soil colloids. Diffusion of phosphate ions towards plant roots is therefore very slow. The rate at which plants take up phosphate ions is quicker than their diffusion from the non-rhizosphere soil to the absorption zones, at root surface, thus resulting in the development of a P-depleted zone. However, the external hyphae of VA mycorrhiza are able to absorb and translocate phosphate to the root beyond the P-depleted zone, and it is generally accepted that although a mycorrhizal network of hyphae (values of 1.34 m hyphae per cm of root infected have been described) obtains most of the phosphate from the same source used by non-mycorrhizal roots (i.e. the labile pool of this element in soil), mycorrhiza are more efficient in phosphate uptake. Physiological studies reviewed by Smith (11) indicated that the apparent Michaelis constant (Km) of phosphate uptake is lower in mycorrhizal than in non-mycorrhizal roots. This implies that mycorrhiza possess a pathway with a higher affinity for phosphate, explaining why the threshold for effective phosphate uptake is lower in mycorrhizal than in non-mycorrhizal roots.

As can be deduced from the above statements, mycorrhiza not only enlarge P depleted zones, but also, these zones are even more exhausted in phosphate ions. This could produce an impoverishment of phosphate ions present in soil after several harvests. The phosphate stocks must be replenished in such cases either by applying soluble phosphate fertilizers or by using less expensive, but sparingly soluble forms of phosphorus (2, 7).

In the case of soluble fertilizers, it is striking that high doses of soluble P inhibit mycorrhizal formation, and reduce the expected benefits that plants obtain from mycorrhizal infection. Consequently, studies on a more

rational use of P fertilizers and its compatibility with VA mycorrhiza will be useful not only from the economic point of view, but also from the ecological standpoint. These statements are strengthened if we think of the rapid fixation of soluble fertilizers in most soils, and that no more than 25% of the P fertilizers is taken up by the crop in its year of application (2, 3).

With respect to the second possibility of using sparingly soluble phosphate, there is some evidence indicating that plants benefit from VA mycorrhiza in the presence of insoluble phosphate. It was therefore suggested that mycorrhizal plants might be able to utilize non labile forms of soil phosphate which non-mycorrhizal plants cannot use. However, production of surface phosphatases in VA mycorrhiza has not been demonstrated, and the mechanism based on the exudation of chelating hydroxyacids by hyphae seems to be of little significance. Assays in ^{32}P labelled soils indicate that mycorrhizal and non-mycorrhizal plants take up phosphate from the same source. Hence, a more efficient absorption of labile P rather than phosphate solubilization accounts for the mycorrhizal effects. Mycorrhiza achieves a better exploitation of the sparingly soluble phosphate because the hyphae make a closer contact with phosphate particles where the soluble ions are being chemically (or biochemically) dissociated than do roots (2, 3, 5).

For the utilization of a non-labile phosphate source by mycorrhiza to occur, at least a slow liberation of some phosphate ions must take place.

Undoubtedly, rock phosphate has been the most utilized source of sparingly soluble fertilizer, with the aim of studying its role in restoring the depleted phosphate stock in soil. The general conclusion obtained indicates that in acid soils, rock phosphate application improves growth of both mycorrhizal and non-mycorrhizal plants, but the inoculation with appropriate VA endophytes greatly enhances its utilization. However, in neutral and alkaline soils, RP remains unavailable for both mycorrhizal and non-mycorrhizal plants (1, 4, 5, 6, 7, 9, 10, 12).

The above statements are particularly important in Spain, as agricultural soils in southern Spain are neutral to alkaline (calcareous) and fix phosphate. Hence great amounts of soluble P fertilizers are often required to maintain the original levels of fertility in these soils. Thus, we have continued with the study of rock phosphate utilization by plants growing in neutral-alkaline soils. The aim of this communication is to show some results we have obtained.

RESULTS AND DISCUSSIONS

In the experimental situations examined, the results showed that rock phosphate in general is of limited availability in calcareous soils. For example, Table 1 shows that in two alkaline soils, rock phosphate did not significantly increase the available phosphate content. Medicago sativa was grown in these soils, and it was found that mycorrhizal infection did not apparently increase the availability of rock phosphate to M. sativa. Table 2 shows the results obtained in soil no. 9. Mycorrhizal infection by native or introduced VA endophytes was little affected by added rock phosphate, but soluble phosphate added as a control treatment significantly depressed the degree of infection in all cases (Figure 1).

TABLE 1

Available phosphate content of two alkaline soils after 2 weeks incubation with rock phosphate (1.33 g/Kg soil).

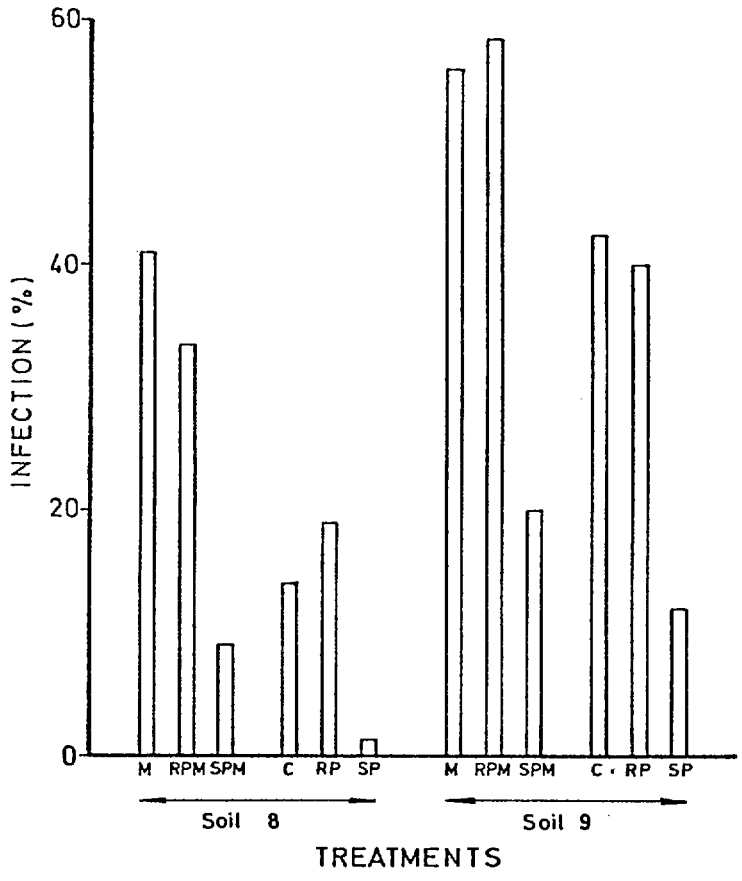
Treatment	0.5 M $NaHCO_3$ soluble P (ppm)	
	Soil 8	Soil 9
Control (C)	20.8	16.6
Rock P (RP)	21.4	15.6

TABLE 2

Effect of VAM fungus (*Glomus mosseae*) and rock phosphate on growth and nutrition of *Medicago sativa* grown in unsterilized soil No. 9.

Treatment	Shoot Dry Weight (mg)	Phosphorus %	Phosphorus Total
Control	398[a]	0.29	1.15
Mycorrhiza (M) (YV)	667[b]	0.31	2.07
Rock P (RP)	409[a]	0.28	1.14
RP + M	577[c]	0.30	1.73

Values in a column followed by the same letter are not statistically significant at P = 0.05.



M = Mycorrhiza inoculated
RP = Rock Phosphate
SP = Soluble Phosphate
C = Control (native mycorrhiza)

Figure 1

However, in another experiment carried out using different soils, we found a situation in which plants were able to use rock phosphate (Table 3).

TABLE 3

The role of VA mycorrhizal inoculation and Rock phosphate (RP) addition (0.1%) on tomato plants growing in unsterile neutral-alkaline soils.

	1		2		3		4	
	-	RP	-	RP	-	RP	-	RP
Shoot dry weight(g):								
Control	1.29[a]	1.31[a]	0.65[a]	0.70[a]	1.03[a]	1.12[a]	1.10[a]	1.35[a]
YV-inoculated	1.85[b]	1.93[b]	1.70[b]	1.85[b]	1.60[b]	1.65[b]	1.80[b]	2.20[c]
P content(% D.M.):								
Control	0.10	0.10	0.12	0.11	0.10	0.12	0.12	0.12
YV-inoculated	0.14	0.15	0.17	0.16	0.17	0.17	0.19	0.20
VA infection(%):								
Control	23[a]	27[a]	17[a]	19[a]	26[a]	25[a]	11[a]	12[a]
YV-inoculated	48[b]	41[b]	30[b]	37[b]	41[b]	40[b]	50[b]	48[b]

For each soil and parameter, values followed by the same letter are not statistically significant at 5% level.

Plants did not utilize RP in soils 1, 2 and 3, but in soil no. 4, RP improved plant growth in Glomus-inoculated plants and uninoculated controls.

The analytical characteristics of the test soils (Table 4) did not indicate anything special to justify the different behaviour of the plants in soil no. 4, except its neutral pH and its higher organic matter content. Nevertheless, soils differred in biological composition. The number of phosphate solubilizing bacteria (PSB) able to dissolve RP present in soil no. 4 were significantly higher than in the others (Table 5). It is noteworthy that a great number of PSB was stimulated in the root zone of Glomus-inoculated plants. If these bacteria had solubilized RP in soil, the

dissociated ions would be taken up by mycorrhiza. These facts could account for the results obtained in soil no. 4.

TABLE 4

Some analytical characteristics of the test soils.

	1	2	3	4
pH(water)	7.4	7.6	7.6	7.0
Soluble P (Olsen)	7	3.5	5	6
Total P	617	650	723	901
Organic Matter (%)	1.23	1.15	1.28	1.94

TABLE 5

Number of Rock-phosphate solubilizing bacteria ($x10^3$/g dry soil) at harvest of tomato plants growing in the test soil.

Treatment	1		2		3		4	
	-	RP	-	RP	-	RP	-	RP
Control	3.0	3.1	0.7	0.8	6.5	7.5	170	250
VV-inoculated	3.5	3.6	0.8	0.8	8.2	8.1	460	730

RP = Rock phosphate

This possibility was tested in a new experiment using the rock-phosphate responsive soil (no. 4) and an unresponsive one (no. 3). The experiment was designed with the aim to study the interaction between VA mycorrhizal fungi and PSB in enhancing the utilization of rock phosphate by plants.

Table 6 shows results obtained in soil no. 4. As can be realized, all the inoculated treatments enhanced the utilization of RP by the test plants. A cooperation of the two microbial inoculants was evident.

TABLE 6

Influence of inoculation of tomato plants with VA mycorrhizal fungus (M) and phosphate solubilizing bacteria (PSB) on Rock phosphate (RP) utilization in soil No. 4.

Treatments	Shoot Dry Weight (g)		P content (% Dry m.)		VA Infection (%)	
	-	RP	-	RP	-	RP
Control	1.10	1.31	0.10	0.10	12	10
PSB	1.30	1.71	0.17	0.17	21	22
M	1.37	1.80	0.19	0.18	47	48
PSB + M	1.45	2.07	0.20	0.24	54	55
L.S.D. (5%)	0.12	0.17				

In soil no. 3, although the plants did not utilize rock phosphate when either bacteria or mycorrhizal fungi were inoculated separately, the combined inoculum of both types of microorganisms significantly improved the utilization of RP by the plants (Table 7).

TABLE 7

Influence of inoculation of tomato plants with VA mycorrhizal fungus (M) and phosphate solubilizing bacteria (PSB) on Rock phosphate (RP) utilization in soil No. 3.

Treatment	Shoot Dry Weight (g)		P content (% Dry m.)		VA Infection (%)	
	-	RP	-	RP	-	RP
Control	1.20	1.21	0.10	0.12	28	24
PSB	1.30	1.35	0.16	0.18	37	39
M	1.42	1.40	0.16	0.18	51	49
PSB + M	1.50	1.80	0.21	0.22	65	61
L.S.D. (5%)	0.15	0.16				

These experiments were conducted with tomato as test plants. Additional studies were also carried out with clover.

Nevertheless, the effectiveness of phosphate solubilizing bacteria, whether native or inoculated into the soil has been a topic of much controversy. This is because, although it has been demonstrated that some soil bacteria can hydrolyse insoluble phosphate in vitro, including rock phosphate, there are some problems that make it very difficult for a significant amount of phosphate solubilization to occur in soil. These difficulties are associated with the scarcity of available energy substrates as is demonstrated in Scheme 1.

SCHEME 1

MICROBIAL SOLUBILIZATION OF PHOSPHATES IN SOIL

REQUISITES:

* Microhabitats with appropriate physicochemical and biological conditions.
* Available carbon substrate as energy source for:
  - Growth
  - Organic acid (chelating) production

PROBLEMS:

* In rhizosphere microhabitats:
  (substrates in root exudates and sloughed out cells)
  - Competition for substrates
  - Microbial antagonism
* In non-rhizosphere microhabitats:
  - Lack of available carbohydrates
  - Difficulties with the translocation of solubilized $PO_4^{\equiv}$, if any.

Inspite of the above statements, the results obtained in the experiment described seem to indicate that PSB acted by releasing phosphate ions from rock phosphate. To clarify these findings, another experiment was planned in an effort to answer some of the unanswered questions concerned with aspects of

mycorrhizal stimulation of the bacterial population in the rhizosphere and the possibility that phosphate-solubilizing bacteria and Glomus inoculated together might enhance the ability of a plant to use P from sparingly soluble rock phosphate added to soil.

These studies were carried out in soil number 3 amended with 0, 0.1 and 0.5% rock phosphate.

Table 8 gives dry weights of shoots and roots and root/shoot ratios for the different treatments.

TABLE 8

Dry weights and root/shoot ratios of lavender plants given different inoculation treatments and three concentrations of rock phosphate. After Azcon et al. 1976 (1).

Inoculation treatment	% Rock phosphate added	Dry weight/3 plants (mg) Shoots	Roots	Root/Shoot ratio
	0	180	110	0.61
C (washing)	0.1	170	112	0.65
	0.5	180	118	0.65
LSD (5%)		NSD	NSD	
	0	200	200	1.00
B=Bacteria	0.1	250	185	0.74
	0.5	380	300	0.79
LSD (5%)		40	45	
	0	320	125	0.39
E3	0.1	480	210	0.43
	0.5	490	210	0.43
LSD (5%)		67	39	
	0	580	300	0.51
E3 + B	0.1	640	310	0.48
	0.5	870	340	0.39
LSD (5%)		60	45	
	0	1550	450	0.29
YV	0.1	1710	420	0.25
	0.5	1950	440	0.22
LSD (5%)		153	59	
	0	1670	500	0.30
YV + B	0.1	1500	450	0.30
	0.5	1800	460	0.25
LSD (5%)		170	61	

NSD = No Significant Difference

Higher additions of rock phosphate did not increase growth of control plants. In contrast, shoot dry weights of the inoculated plants clearly increased with additions of rock phosphate as shown in the B, E_3, $B + E_3$ and YV treatments, although root dry weights were less affected. This indicates that microbial inocula, in some way, had to increase the accessibility to the plant of this sparingly soluble form of phosphate.

These results show that the combined inoculum of E_3 + B significantly increased plant growth above that achieved with either of the two alone. The combined inoculum of YV plus bacteria did not significantly increase plant growth above YV inoculation alone. YV-inoculated plants grew best of all, regardless of whether bacteria or rock phosphate were added. However, one month before harvest, plants with YV plus bacteria were about 50% taller than plants with YV only; during the last month of assay, plants with YV alone grew rapidly and at harvest shoot and root dry weights were similar for YV-inoculated plants, with or without bacteria. Nevertheless, plants took up more phosphate in the YV plus bacteria treatment (Table 9). This suggests the possibility of some solubilization of rock phosphate by the bacteria. In fact, the bacteria markedly increased the P content of non-mycorrhizal plants as well as plants inoculated with either YV or E_3.

Root/shoot ratio increased with bacterial inoculation and this could be due to the production of plant growth substances, mainly auxins, by these bacteria. However, mycorrhizal plants had smaller R/S ratios than the controls.

TABLE 9

P content (%P) and total P taken up (mg) by lavender plants given different inoculation treatments and three concentrations of rock phosphate. After Azcón et al. 1976 (1).

Inoculation treatment	% Rock phosphate added	Shoots % P	Roots % P	Total P (mg) taken up per 3 plants
	0	0.061	0.071	0.187
C (washing)	0.1	0.060	0.079	0.189
	0.5	0.062	0.080	0.204
LSD (5%)		NSD	NSD	NSD
	0	0.120	0.128	0.496
B=Bacteria	0.1	0.121	0.130	0.560
	0.5	0.121	0.131	0.852
LSD (5%)		NSD	NSD	0.098
	0	0.160	0.170	0.724
E3	0.1	0.156	0.174	1.113
	0.5	0.125	0.135	0.895
LSD (5%)		0.022	0.024	0.165
	0	0.178	0.170	1.542
E3 + B	0.1	0.162	0.166	1.650
	0.5	0.140	0.142	1.701
LSD (5%)		0.026	0.024	0.108
	0	0.148	0.106	2.771
YV	0.1	0.134	0.107	2.740
	0.5	0.127	0.122	3.035
LSD (5 %)		0.021	0.017	0.186
	0	0.188	0.148	3.879
YV + B	0.1	0.210	0.166	3.897
	0.5	0.214	0.162	4.597
LSD (5%)		0.030	0.021	0.241

NSD = No Significant Difference

Figure 2 shows that inoculation with Glomus, especially YV, favoured the early eatablishment of phosphate-solubilizing bacteria. After two months of plant growth, this effect disappeared, and the number of inoculated bacteria declined in all treatments.

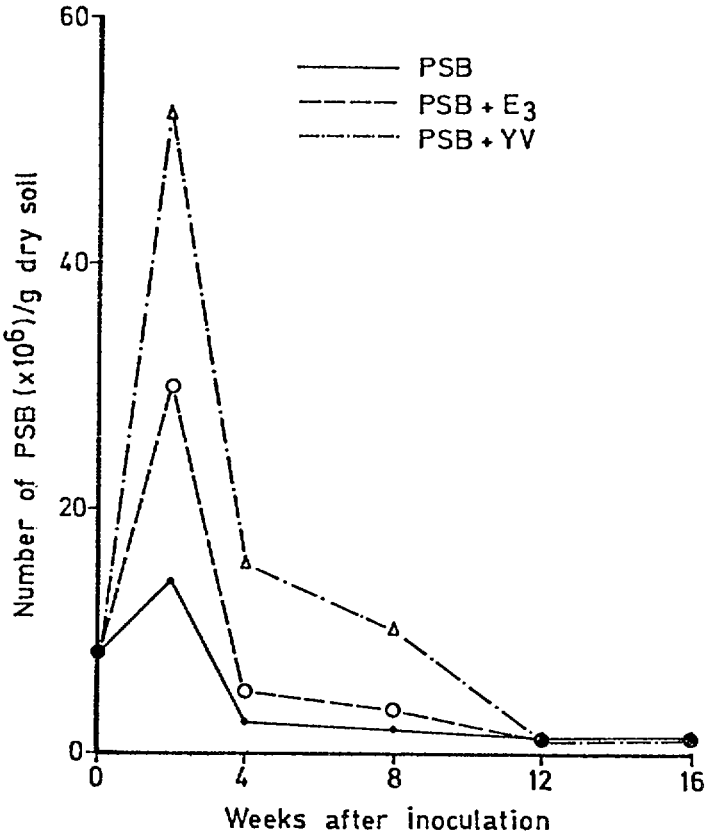


Figure 2

PSB stimulated mycorrhizal infection in E_3 inoculated plants (Table 10) but not in the case of YV.

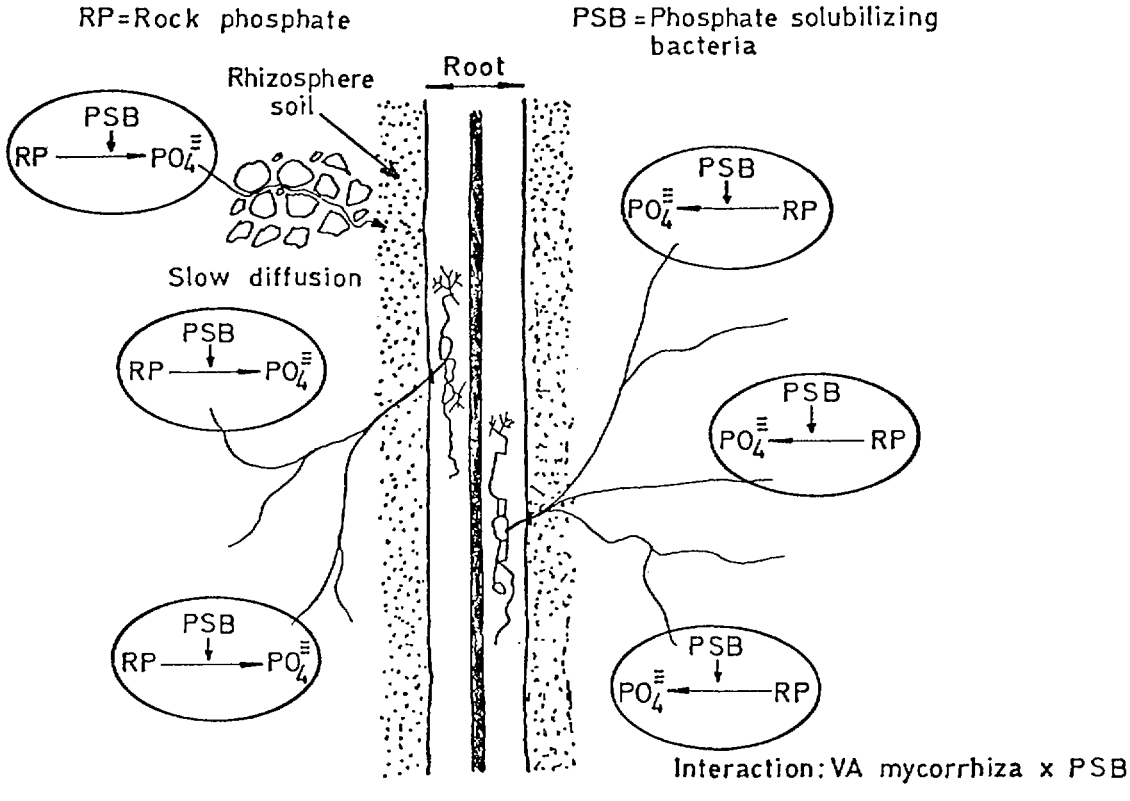
TABLE 10

Mycorrhizal infection of lavander as affected by phosphate solubilizing bacteria inoculation.

Treatment	VA Infection (%)
E_3	25-45
E_3 + PSB	35-60
YV	60-85
YV + PSB	60-80

Dry weights of plants inoculated with bacteria only increased steadily with rock phosphate concentration. The % P was the same, irrespective of the rock phosphate concentration, although this could be a growth-dilution effect. Two possible explanations are: (i) the bacteria might affect plant growth by the plant hormones that they synthesise. Such substances could influence the early stages of plant growth so that more actively growing roots were able to explore a greater volume of soil and (ii) some solubilization of phosphate by the bacteria. In this case, small amounts of phosphate hydrolysed by the bacteria from rock phosphate could enter the soil solution and then be taken up by plants. As mycorrhizal plants can explore the different microhabitats in non-rhizosphere soil better than non-mycorrhizal plants, they are less affected by chemical fixation by soil components. Mycorrhizal plants could therefore gain more benefit from the presumed activity of PSB. Alternatively, these extra phosphate ions could come from dead bacteria, since more bacteria grow and subsequently die around the mycorrhizal than non-mycorrhizal roots, at least, in the earlier stages of plant growth (Scheme 2 depicts hypothesis).

SCHEME 2



It is planned to use ^{32}P in our projects, to try and establish directly if bacteria really hydrolyse some phosphate ions from rock phosphate under the conditions employed in the present study. Whatever the mechanisms involved, this study may have practical significance for alkaline soils because of the general insolubility of rock phosphate fertilizer in such soils, in contrast to its availability to plants in acid soils.

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FUNGUS AND EPIDEMIOLOGICAL FACTORS IN THE MYCORRHIZAL RESPONSE

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In an earlier paper(pp.7-28)I dealt with some plant responses to vesicular arbuscular mycorrhizas, emphasizing soil-plant factors and the mechanisms of the response. In this paper I focus particularly on fungal factors and the development of the infection. Such factors are important in deciding whether it is necessary to inoculate in a potentially responsive soil-plant combination and if so, what organism one should select. This leads into the topic of 'management' of the symbiosis.

A number of important points about the mycorrhizal response emerging from several studies are embodied in Tables 1 and 2 from Abbott and Robson (1978) and Daniels and Menge (1981) respectively. Table 1 shows:

(i) At low P levels, very large differences may occur in mycorrhizal stimulation of growth, both between fungus species and between strains of the one species. I conclude that selection at the species level alone is probably not sufficient if one is selecting for highly efficient fungi.

(ii) The indigenous population may not be as efficient as introduced organisms. That is, one should not assume that the indigenous population is the most efficient for a site - the indigenous population probably has been selected for survival *under the existing soil conditions*, but even this may change if those soil conditions are changed, e.g. by fertilizing.

(iii) (Following the comment above) note that at high P, infection by two of the inocula (including the indigenous strain) was depressed considerably whereas the two *G. monosporus* isolates showed little sensitivity to high P. These two fungi still gave a significant (but reduced) mycorrhizal response even in the presence of fertilizer. Some fertilizer will often be needed to maximize productivity even with the addition of mycorrhizal fungi, and the 'appropriate' fungus should be matched to the agricultural management system, in this case added fertilizer.

(iv) Response need not be directly related to the extent of the infection by different fungi (see low P treatment) although often this does happen, i.e. often differences in response are related to the extent of infection (see results of Mosse, 1972).

TABLE 1

Response of *Trifolium subterraneum* to mycorrhizal fungi

Plants were grown in unsterilized field soil in pots for 7 weeks.
(Abbott and Robson 1978)

Inoculum	Phosphate Level			
	P_1[1]		P_8[1]	
	Fresh Wt. Tops g.	Infection[2]	Fresh Wt. Tops g.	Infection[2]
Nil	4.34	57	17.69	20
Glomus monosporus #1	8.88	70	20.30	54
G. monosporus #2	11.13	82	21.7	69
G. fasciculatus	6.42	78	18.36	31
L.S.D. (P = 0.05)	1.94		L.S.D. (P = 0.05) 1.94	

1 Soil $NaHCO_3$ extractable P = 1 ppm.; P_1 = 1 g superphosphate/3kg soil. P_8 = 8 g superphosphate/ 3 kg soil.

2 Percent of the root volume which was mycorrhizal.

Table 2 shows that although infection may occur in most plant-fungus combinations (all were infected) significant increases in growth may occur only in some fungus-species combinations under any one situation. However, Daniels and Menge pointed out this may sometimes be related to extent of the infection, higher inoculum densities being needed with small spored fungal species.

TABLE 2

V.a. mycorrhiza stimulation of different plant species
(from Daniels and Menge 1981)

Plants were grown in sterile soil for 4-5 months

Plant Species	Inoculum (all *Glomus* species)			
	G. epigaeus	*G. mosseae*	*G. fasciculatus*	*G. constrictus*
Tomato	+ *	+	-	-
Citrus	+	+	+	-
Maize	+	-	-	+
Sudan Grass	+	-	-	-
Asparagus	+	-	-	-
Cotton	-	+	-	-
Olive	-	-	-	-

* + indicates significant growth responses at P = 0.05.

RELATIONS BETWEEN SOIL POPULATIONS AND PLANT RESPONSES

For symbionts such as mycorrhizal fungi we need to know the levels of populations in soil for which management techniques must aim to maintain in order to optimize response. The population in question may be the indigenous population or an inoculated one. Figure 1 shows response of subterranean clover in one soil as a function of number of propagules of the v.a. mycorrhizal fungi in the soil (Bowen 1980).

The heavy line was derived experimentally and the lighter lines are hypothesized for different levels of soil phosphate. Because of differences in efficiency between mycorrhizal fungi, the curve will probably vary between fungi but it should be possible to predict the family of curves similar to Fig. 1 for a known fungus-soil combination.

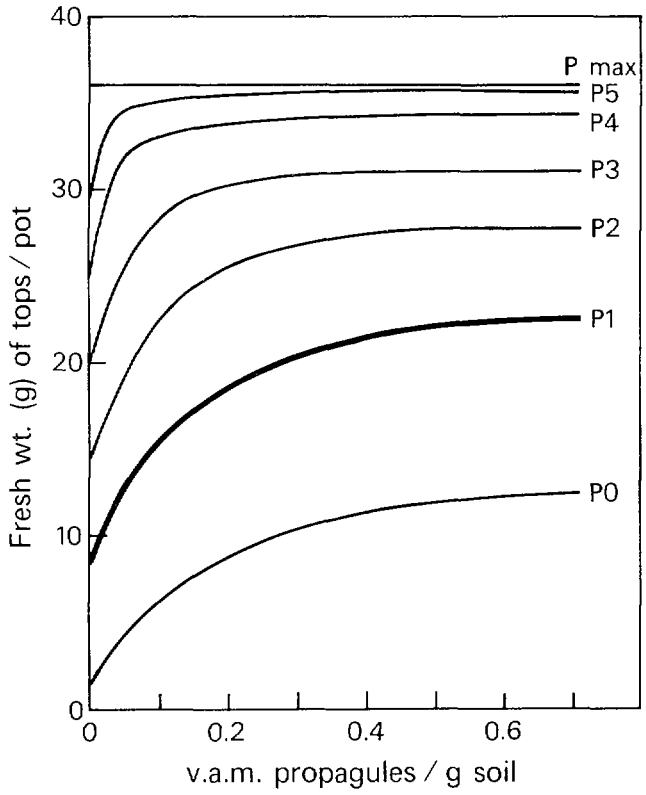

Figure 1 Response curves to varying populations of v.a. fungus spores at a range of P levels. The dark line was derived experimentally and the light lines are hypothesized. The plant was *Medicago truncatula* (Bowen, 1980).

Despite emphasis by research workers on spores which can be wet sieved from soil, it is becoming increasingly obvious that these are not the only infective propagules for often soils are highly infective although very few spores can be extracted. This may be partly due to the fact that the wet sieving method usually used (e.g. Gerdemann and Nicolson, 1963) recovers only spores above a certain size and smaller

ones are lost. Indeed, some v.a. endophytes (v.a.e.) may rarely spore. Another most common cause of the non-relation between infectiveness of a soil and the spores recovered from sieving is that there are other propagules, most likely infected root fragments (e.g. containing vesicles). The infectiveness of a soil can be obtained by a dilution to extinction assay (e.g. Porter, 1979) often this involves diluting the soil with aliquots of sterile sand but if, instead of sand one uses aliquots of the same soil which have been sterilized (Smith and Bowen 1979), one obtains a response curve of the type indicated in Fig. 1. as well as a propagule count.

THE FUNGUS - PLANT COMPONENTS OF THE RESPONSE

The components of the response are:

. 1 Infection of the root

. 2 Spread of the infection in the root and

. 3 Growth of the fungus into soil (and litter), and consequent nutrient uptake.

Infection of the root

Infection of the root by soil organisms is the resultant of 3, probably 4 processes (Bowen 1979).

(i) Germination of the organisms in soils

(ii) Growth to the root

(iii) [Sometimes growth in the rhizosphere].

and (iv) Infection itself.

Methods have been developed (see Bowen 1979) to examine the impact of soil factors on each of these, independent of each other. Soil factors may affect any one or each of these to such an extent that infection is seriously affected or even prevented. Such inhibiting factors can be physical, chemical or biological - 'biological control' of symbionts can be as real a phenomenon as is biological control of plant pathogens. In some soils, some v.a.e. can be introduced with

ease only after the general soil microflora has been removed by sterilization (Bowen, unpublished).

One example of the effect of a soil parameter on infection is indicated in Table 3, showing the overall important effect of soil moisture on the infection (Reid and Bowen, 1979). Similarly, Smith and Bowen (1979) reported marked effects of soil temperature on the infection. These represent the summation of effects on the three processes below.

TABLE 3

Effect of soil water potential on v.a. infection of *Medicago truncatula* (Reid and Bowen 1979)

Water Content %	Water Potential -M Pa	Infection Points cm^{-1} root
28	0.10	1.7
22	0.19	4.1
15	0.43	1.3
11	0.91	0.5
9	1.4	0.1

Spore Germination Spore germination is affected by a number of factors of which moisture is probably the most important. The soil moisture effects on germination (Table 4) however are not identical to those of Table 3 so those effects can not be explained completely as effects on germination alone.

TABLE 4

Effects of soil moisture on spore germination (*G. mosseae*) (Bowen, unpublished).

Moisture		Germination Percentage	
Percent	Potential - M Pa	11 Days	30 Days
9	1.4	11	14
12.5	0.65	17	18
17	0.32	28	56
22	0.19	11	11

Spores of different species of v.a. mycorrhizal fungi vary considerably in their time for germination as indicated by the data of Table 5 (Powell, 1976).

There is even variation between 'ecotypes' of the same species as shown in Table 6 (from Daniels and Duff, 1978)

TABLE 5

Germination of v.a.m. fungal spores in soil (from Powell, 1976)

Time (days)	Percent Spores Germinaten		
	Glomus mosseae	*Acaulospora laevis*	*Glomus fasciculatus*
16	60	5	0
21	50	14	0
43	42	21	0
56	42	56	40

Growth in the rhizosphere and infection There are apparently no definitive studies on growth of v.a. mycorrhizal fungi in the rhizosphere of plants (as opposed to growth and infection). Ratnayake *et al.* (1978) considered that increased leakage of carbohydrates and other substrates from phosphate deficient plants assists the growth of the mycorrhizal fungus but no rhizosphere growth studies appear to have been made.

The most studied environmental factor affecting infection processes is phosphate which, at high levels, first distorts infection in the root and finally inhibits it. That different v.a.e. vary in their reaction to this was seen in Table 1. That the phosphate inhibition operates through the plant is seen from Table 7 (from Lambert *et al.* 1979) where maize had a more depressive effect than soybean.

TABLE 7

Effect of added phosphate on v.a.m. infection (Lambert *et al.* 1979)

Soil P. added (ppm)	Percent Root Infected	
	Soybean	Maize
0	86	66
25	81	64
75	79	50
200	75	21

Plants resistant to infection, e.g. *Arabidopsis thaliana* and *Pinus radiata* allow growth of the fungi in the rhizosphere (Bevege and Bowen 1975) and such plants may be extremely useful for studying growth in the rhizosphere. There is little, if any, evidence however that extensive growth in the rhizosphere is necessary before infection of susceptible hosts and this needs further clarification.

Spread of infection in the root

Very large differences exist between fungus-plant combinations in the development of the infection (Table 8 from Bevege and Bowen; (1975). More study is needed on the relation of spread in the root to the effectiveness of the association; Sanders *et al.* (1977) indicated their least effective fungus on onions developed poorly in the root.

TABLE 8

Development of v.a. infection with *Glomus mosseae* (from Bevege and Bowen, 1975)

Character	Days after Infection	
	Clover	Onion
Arbuscule formation	1	4
Senescent arbuscules	7-9	10-15
Vesicle formation	1	14
Production of external hyphae	1-2	0
Internal spread	Rapid	Slow

Growth of fungi into soil from the root

It is generally accepted that the major component of the mycorrhizal response is the growth of hyphae into soil, the absorption of nutrients and their translocation back to the plant (see Bowen, this meeting).

Much has been made sometimes of a lack (when it occurs) of strict correlation between infection level and response. Infection level has been expressed usually as infection (or hyphal connections) cm^{-1} or volume of root infected. However, if we accept that the mycorrhizal response is due mainly to growth of hyphae into soil we should interpret mycorrhizal response according to Fig. 2. That is, depending on the mobility of the ion, there is a 'plateau' in the root length needed to

meet the plant's needs. Thus the extension of absorbing length by the mycorrhiza, takes the 'rooting intensity' from A up to B. Depending on the nutrient status of the soil (and the plant species) relatively little infection or relatively large infection may be required to reach the 'plateau' level. Thus in some soils thirty percent infected root may be all that is required to achieve this level and one may find little difference between an organism giving sixty percent infection and one giving thirty percent.

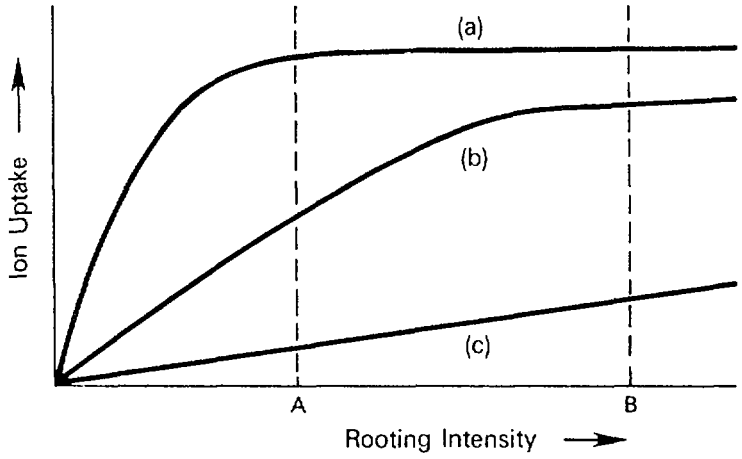

Fig. 2. Relation between rooting density and ion uptake for (a) highly mobile ions; (b) poorly mobile ions and (c) immobile ions (After Barley, 1970).

However, even this assumes all fungi will grow to similar extents into the soil and this may be a quite wrong assumption. It is somewhat paradoxical that despite the consensus agreement (at present) that the important thing in the mycorrhizal response is fungus growth into soil, there has been almost no study of factors affecting growth of fungi into soil and the extent to which different fungi do this. In studies by Sanders *et al.* (1977), three endophytes produced a similar amount of external mycelia but one had little external mycelium (and little spread of infection in the root). They calculated that 80 cm of external hyphae were produced per cm of infected root. Hattingh *et al.* (1973) recorded hyphal extension 1-2 cm into soil and in some

tracer uptake studies there is evidence their extension several centimetres into soil, e.g. Rhodes and Gerdemann, 1978; 4.5cm).

From data on ectomycorrhizal associations (Skinner and Bowen 1974) it is highly likely that different soils and soil conditions will affect fungus growth into soils considerably (Table 9).

TABLE 9

Effect of soil type on penetration of soil by ectomycorrhizal hyphal strands (Skinner and Bowen, 1974)

Soil	Fungus Growth (mg. dw/soil core)[1]
Lateritic podzolic	0.7
Lateritic podzolic	51.3
River sand	5.9
Rendzina	26.5

[1] Growth of fungus into a core of the test soil, 2.4cm diameter and 4 cm long.

SPEED OF INFECTION

The infection factors above influence the speed of infection and the time taken for the mycorrhizal response to occur. Under ideal conditions infection can occur on a root segment only a few days old, but under less ideal conditions the infection may take some three to four weeks (Furlan and Fortin 1973) to develop. Under some conditions mycorrhizal responses are first seen at some 3-4 weeks of age although increases in other properties, may occur much earlier e.g. phosphate stimulated nitrogenase activities of nodulated legumes (Smith and Daft, 1977). Rich and Bird (1974) reported the occurrence of mycorrhizas on the radicle of cotton five days after seedling emergence and a significant positive correlation under field conditions between early-season mycorrhizal infection and vegetative growth and development of cotton.

MANAGEMENT

Studies by Dr. Hayman (e.g. Hayman 1975) have shown a very great influence of fertility and crop rotations on infection levels of roots and on spore production. Sparling and Tinker (1978) recorded that the *Glomus tenuis* was predominant in low phosphate soils they studied but was eliminated by addition of basic slag to the soil. Similarly Abbott and Robson (1977) found some endophytes occurred predominantly in cultivated and fertilized soils, and that soil pH played an important role in cultivated soils in determining the major spore type present.

Schenck and Kinlock (1980) studied changes in root infection by v.a.e. and spore production for 6 agronomic crops grown in monoculture for 7 years on a newly cleared woodland site. Not only did spore production differ markedly between crops, but also particular crops heavily favoured sporulation of particular species. Furthermore, there were large yearly variations in the spore numbers produced - an examination of their data suggests a relation between high root infection one year and high sporulation (i.e. inoculum density) in the previous year. Ocampo and Hayman (1981) have shown non-host plants may also stimulate infection in subsequent crops.

Such studies (and others not reported here) show that there is considerable scope for management methods either to enhance natural populations of particular v.a.e. known to be present and highly effective or to ensure the persistence of an inoculated fungus at a high enough level to ensure its maximum effect on plant production.

INOCULATION

A consideration of inoculation methods is beyond the scope of this paper. However, this is an area of increasing research. I suggest the most immediately applicable areas are those in which plants are outplanted from nurseries, where intensive input is practicable.

Indeed, production of inoculum commercially for use with plants such as avocardo and citrus already occurs in the U.S.A. The logistic problem is not so easy with inoculation on large acreages of sown crops. In field experiments of limited size infected soil and seeds pelletted with inoculum have been used successfully (Hall 1979). It may be, that the future inoculation in broad note agriculture lies in 'seeding' a certain proportion of a crop with inoculum and then employing management techniques to build up large population.

CONCLUSION

I have indicated some of the fungus factors concerned in the mycorrhizal response, in the development of the infection and the relation between soil population and infection. We know a great deal about the mechanisms of plant response (although much more needs to be known) but our knowledge of epidemiology and the ecology of the mycorrhizal fungi and their management is fragmentary. Obviously there is a need for much more study of compatible (effective) fungus-plant-soil combinations. Much of this will be on *ad hoc* basis, but will lead to an accumulation of knowledge from which to draw general predictions. Already there are a number of apparent generalizations in this area emerging. However, detailed epidemiological studies are also necessary to understand the impacts of management and soil conditions on the response, the factors for which a successful v.a.e. should be selected, and strategies in manipulation of the symbiosis.

Finally, one may consider the characteristics a 'desirable' mycorrhizal fungus should have. Each scientist will probably have his own list but my list reads something like this:

1. The fungus must be potentially efficient in relieving the limiting soil factor at a site. This has often implied phosphate nutrition but there may be many roles of mycorrhizal fungi other than in phosphate

uptake (see Bowen, this meeting). This efficiency will be affected by the plant-fungus combination and by several soil factors.

2. The potential of the fungus must be able to be expressed

This will include

(i) rapid infection under a wide range of conditions.

(ii) 'balanced' rapid development in the host, i.e. it should not be an unduely large assimilate sink.

(iii) it must be capable of extensive growth into soil in a wide range of soil physical, chemical and microbiological conditions.

and (iv) it should reproduce well in a wide range of conditions to assist in its persistance.

3. The fungus should be able to be "managed", i.e. inoculum should be able to be produced readily, introduced to soil successfully, and managed to produce high inoculum potential and persistence in soil.

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MYCORRHIZAS IN RELATION TO MINERAL NUTRITION OF GRASSLAND PLANTS

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Abstract

Previous pot experiments have indicated that growth of grasses is increased by mycorrhizal infection only on extremely phosphorus-deficient soils. Mycorrhizas can be important in promoting growth of legumes, especially when they are competing against grasses. Experiments described here show that mycorrhizas can substantially increase phosphorus transfer between grassland plants (grasses or non-grasses). In permanent grassland nutrient cycling is crucial and mycorrhizas may play an important role in this.

INFLUENCE OF MYCORRHIZAS ON GROWTH OF GRASSES

It is well established that mycorrhizas promote the growth of many species, but there is uncertainty about how often they promote the growth of grasses. Table 1 shows, as an example, some results from experiments by Sparling and Tinker (1978a, b). They grew five grass species and one clover species, mycorrhizal and non-mycorrhizal, on three nutrient-deficient British soils in pots. There is some suggestion that on the most phosphorus-deficient soil Agrostis tenuis grew better when mycorrhizal, but that it was actually harmed by mycorrhizas on the Brickfield soil. However, for none of their five grasses, in three experiments, did Sparling and Tinker find statistically significant promotion of growth by mycorrhizas, and occasionally there was significant inhibition. In contrast, growth of Trifolium repens on one of these soils was greatly increased by mycorrhizas (Table 1). Other experiments comparing growth of mycorrhizal and

TABLE 1

Growth of two grass species and clover, mycorrhizal (M) and non-mycorrhizal (NM), in three soils. Data of Sparling and Tinker (1978a, b).

Soil:		Brickfield	Roddlesworth	Malham
Olsen P (ppm)		2.6	1.2	0.5
Dry weight (g) of grasses after 19 months				
Agrostis tenuis	M	4.2	4.4	6.8
	NM	6.2	4.0	5.6
Festuca rubra	M	6.2	4.9	4.1
	NM	6.4	5.0	4.2
Dry weight (mg) of clover after 2 months				
Trifolium repens	M			67.2
	NM			2.9

non-mycorrhizal grasses have been conducted by Crush (1973), Ali (1976), Koucheki and Read (1976) and Hall (1978). These papers lead to the conclusion that only on extremely phosphorus-deficient soils is growth of grasses increased by mycorrhizal infection, and on many soils mycorrhizas either have no effect on growth or actually decrease it.

One can criticise such experiments on technical grounds. In order to compare mycorrhizal and non-mycorrhizal plants the soil must be sterilized; this releases extra ammonium and often soluble phosphate, which could reduce both the amount of subsequent mycorrhizal infection and the plants' response to it. This effect is likely to be particularly marked with grassland soils because of their high organic matter content. Nevertheless, it is clear from results such as those in Table 1 that grasses are less influenced than some other species by mycorrhizal inoculation. This is often attributed to their profuse root system, which may allow them to exploit most of the available soil phosphorus even without the aid of mycorrhizal hyphae.

Growth promotion of non-grasses, especially legumes, in grassland by mycorrhizas is likely to be very important. Table 2 illustrates how the effect can be enhanced when grasses and non-grasses are competing. Here both Lolium and Trifolium grew well when non-mycorrhizal provided they were not competing, but competition resulted in strong suppression of Trifolium if non-mycorrhizal. Mycorrhizas increased Trifolium growth 3-fold if it was competing against the grass but only 1.6-fold when on its own.

TABLE 2

Fresh weight (g) of Lolium perenne and Trifolium repens, growing either separately or together, in soil sterilized and then either reinoculated with mycorrhizal spores (M) or not (NM). Data of Crush (1974).

	Separate		Together	
	NM	M	NM	M
Lolium	9.9*	10.4*	9.4*	8.9*
Trifolium	9.8*	15.7†	0.8*	2.3†

Percentage of roots mycorrhizal: * $<$ 2%, † $>$ 80%.

ROLE OF MYCORRHIZAS IN NUTRIENT CYCLING

I suggest that the importance of mycorrhizas for grasses has been greatly underestimated because in nearly all experiments the grasses have had to take up all their nutrients from the soil. In permanent grassland nutrient cycling is the key process. In the absence of fertilizer application, there is very little phosphorus input to a grassland ecosystem - no gaseous form to be fixed, and usually less than 1 kg/ha/year in rain. Thus phosphorus cycling is almost entirely internal, and productivity is likely to depend on how much of the phosphorus is in the biomass (rather than chemically fixed in the soil) and on how fast it cycles.

In permanent grassland half or more of the primary production is commonly below ground (Marshall 1977); therefore roots provide a large part of the dying plant material each year and hence of the nutrients available for recycling. It is thus important to know how mycorrhizas are involved in transfer of phosphorus from dying to living roots. This was investigated by my research assistant Alison Heap (Heap and Newman 1980a, b).

Several plants were grown in each pot of grassland soil; the plants were either all of the same species or of two different species. By prior sterilization of the soil and reinoculation it was arranged that the plants were mycorrhizal in some pots but not in others. The shoots of some plants ('donors') were injected with ^{32}P as carrier-free H_3PO_4. After a week their shoots were cut off and discarded; their roots then began to die and decompose. After a further week the shoots of the remaining plants ('receivers') were harvested and their ^{32}P content determined by digesting them in acid, followed by liquid scintillation counting. Table 3 shows some of the results. There was some transfer of phosphorus from donor to receiver when the plants were non-mycorrhizal, but a marked increase when they were mycorrhizal. This occurred whether donor and receiver were of the same or different species. It occurred with the grass Lolium perenne at least as much as with the non-grass Plantago lanceolata. For further details of these experiments see Heap and Newman (1980b).

TABLE 3

Amount of ^{32}P transferred to receiver shoot, expressed as $\frac{^{32}P \text{ in receiver shoot}}{^{32}P \text{ in donor root}} \times 100$

Expt No.	*Donor sp.	Receiver sp.	% of P transferred NM†	M	Statistical significance of difference
1	Lolium	Plantago	1.0	2.2	< 0.05
	Lolium	Trifolium	0.067	0.53	< 0.001
2	Lolium	Plantago	0.65	2.1	< 0.02
	Lolium	Lolium	2.4	8.6	< 0.05
	Plantago	Plantago	1.5	4.1	< 0.05

*The species were Lolium perenne, Plantago lanceolata, Trifolium repens.

†NM = non-mycorrhizal, M = mycorrhizal

Whittingham and Read (1982) have performed similar experiments on ^{32}P transfer between large and small plants of Festuca ovina and Plantago lanceolata. They too found enhancement of transfer between mycorrhizal plants even though their donor plants were not detopped. Evidently death of the root system is not essential for this transfer, though it may well be necessary for the two plants to differ in their 'physiological state'. Whittingham and Read also showed, in a sand-culture experiment, that there could be sufficient nutrient transfer to increase growth of the receiver plant.

The transfer of phosphorus from one root to another, on different plants, could occur by three mechanisms. (1) The phosphorus could pass in soluble form from the donor root into the soil solution, move by diffusion or mass flow to the receiver root and be taken up by it. (2) Phosphorus could pass into the soil solution as before, be taken up by mycorrhizal hyphae attached to the receiver and be translocated by them into the receiver root. (3) If mycorrhizal hyphae form connections between the two root systems the phosphorus could pass into the fungus within the donor root and be translocated into the receiver root without ever being in the soil solution. In our experiments some ^{32}P was transferred between non-mycorrhizal plants, so clearly mechanism (1) can operate. The increased transfer between mycorrhizal plants indicates that either mechanisms (2) or (3), or both, also operate. Alison Heap was able to show that mycorrhizal hyphae do interconnect roots of Lolium perenne and Plantago lanceolata, as well as different roots of each of these species (Heap and Newman 1980a), so mechanism (3) is at least a possibility.

DISCUSSION

The profuse root systems of grasses, with numerous, long root hairs, make them efficient at phosphorus capture from soil even in the absence of mycorrhizas. However, if a major phosphorus source is dying roots this will be much more localized than soil phosphorus. Furthermore, there will be many saprophytic microorganisms growing on and in the decaying roots and these will also be taking up phosphorus. Living roots will, in a sense, be competing with these and also with chemical fixation sites in soil. The living roots will usually be further away from the dying roots than will these 'competitors'. Mycorrhizal hyphae, whether they can take phosphorus from within the dying root or by ramifying just outside it, would be well placed to compete for phosphorus and hence to increase the proportion transferred to living plants.

Other principal sources of phosphorus for cycling in grassland are dying stem and leaf material and animal dung. Again these are likely to be localized sources, and mycorrhizas could assist plants to capture nutrients released from them. In forests mycorrhizal hyphae ramify over the surfaces of leaves in the litter, and may take up nutrients as soon as they are released; a similar process could occur in grassland.

This paper has emphasised phosphorus transfer, but mycorrhizas may enhance transfer of other elements, e.g. K, S, even N, whose uptake from soil they do not usually promote. They may enhance transfer of N from legumes to non-legumes. The role of mycorrhizas in nutrient cycling clearly merits further research.

ACKNOWLEDGEMENT

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IMPLICATIONS OF SOME EXTRA-CELLULAR PRODUCTS OF SOIL MICRO-ORGANISMS ON PLANT INFECTION BY VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

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Abstract

Vesicular-arbuscular mycorrhizal fungi have not yet been successfully cultured axenically. Knowledge of the biosynthetic abilities of these fungi and of their requirements for suitable growth and development would help to unravel the interactions taking place between plants and these fungi, why and how the infection occurs and the nature of host dependence. Hence, progress in the study of the biology of mycorrhizal formation is difficult.

This paper reviews the related literature and summarises the experimental work carried out by the authors. The results obtained indicate that soil microorganisms can assist mycorrhizal infection and the above mentioned mechanisms seem to be involved in the "stimulation" of the VA fungi in the rhizosphere and/or in the formation of "entry points" in susceptible plant roots.

Vesicular-arbuscular mycorrhizal fungi, members of the Endogonaceae (Phycomycetes), are quantitatively important constituents of soil microbiota, being among the most common of all soil-borne fungi. As these microorganisms have not yet been successfully cultured axenically, they must actually be considered as obligate symbiotic biotrophs. Although hyphal growth can be obtained from germination of either untreated or surface-sterilized spores, it has not been possible to isolate and reproduce these mycelia in viable condition. Consequently, progress on the study of the biology of these fungi is difficult.

Knowledge of the biosynthetic abilities of these fungi and of their requirements for suitable growth and development would help us

to study the interactions that take place between plants and fungi, why and how the infection occurs and perhaps the nature of host dependence.

The assays carried out by Hepper (1979) on the germination and growth of surface-sterilized Glomus spores indicated that synthesis of the protein required for germination is programmed by stored m-RNA or new m-RNA that is synthesised by the spores. These results suggest that the endophytes resemble saprophytic fungi more than obligate biotrophs. During growth, lipids were not exhausted and the addition of sugars was ineffective, indicating that carbon supply was not the limiting factor.

The studies by Powell (1976) show that neither spore germination nor the inital direction of hyphal growth were influenced by the presence of host roots. Hyphae from spores were not attracted to the roots until they approached them closely. Firstly, the stimulated germ tube formed a fan-like structure of mainly septate hyphae, from which the infective aseptate hyphae developed later. Since hyphae from root segments did not form preinfection structures to infect a new root, it was suggested that these fan-like structures had a function of absorbing nutrients or hormones from root exudates.

Studies of mycorrhizal infection in root organ cultures also indicated the lack of apparent attraction of germ tubes towards the root until they grew very close to it. When a fungal hypha becomes attached to the root surface, it may or may not penetrate the root. Young lateral roots seemed to exert more stimulatory effects on the fungus. Once an infection is established, the root becomes more prone to further penetration. This behaviour could be due to the fungus being invigorated and/or that some changes occur in the root as a consequence of the establishment of the first successful "entry

point". As root infection by VA fungi has many common features with those of parasitic fungi, Mosse and Hepper (1975) suggested that changes in hormonal balance and in the permeability of host cells occur in some of these fungal infections.

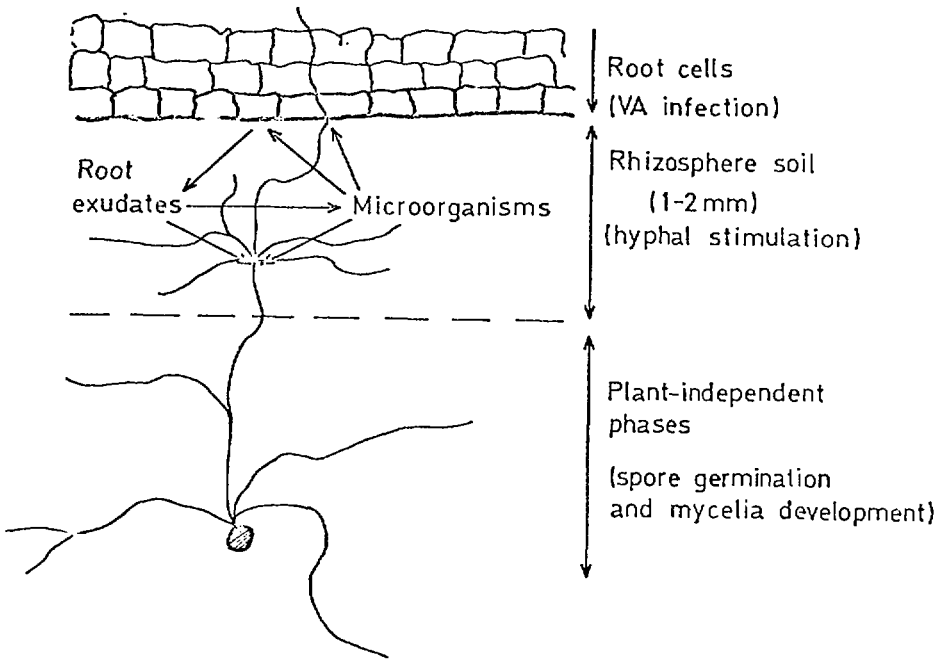
In summary, there are four key facts in VA mycorrhiza formation: 1) a plant-independent spore germination and mycelia development; 2) a stimulation of the germ tubes when close to the root surface; 3) attachement of the infective hyphae to the root surface and 4) root penetration.

With respect to the stimulation of the hyphae in the rhizosphere it is obvious to think about the role of root exudates, the main cause of the "rhizosphere effect". In fact, Ratnayake et al. (1978) found a positive correlation between VA mycorrhizal infection and the degree of root exudation, which in turn, correlated with an increased permeability of root membranes. Azcon and Ocampo (1981) associated the absence of mycorrhizal infection in some wheat cultivars with the scarce exudation from the roots of these plants.

Powell (1976) also argued that plants stimulated fungal hyphae by a nutrient or hormone present in the root exudates. However, there is another important factor that differentiates rhizosphere from non-rhizosphere soil. This is the presence of active populations of microorganisms. These are, in turn, also able to produce substances which are known to influence the development of plants and microorganisms. Among these substances we have plant hormones and compounds increasing cell permeability (CICP) that would increase rates of root exudation (Bowen, 1980). The role of these CICP of microbial origin that help hyphal penetration by relaxing root cell

wall can also be given as a hypothesis. In fact VA infection in axenic conditions is rather difficult.

Figure 1 illustrates the situation described above, emphasizing the possible role of microorganisms.



VAM Formation: Hypothetical role of soil microorganisms

Figure 1

The role of extracellular products of rhizosphere microorganisms on VA mycorrhiza formation has been a matter of interest to us. Thus, several experiments were developed in our Laboratory in order to assess the influence of some soil microorganisms, or certain metabolites contained in cell-free supernatants of their cultures, on some stages of VA fungi development. Several types of rhizosphere inhabiting microorganisms were studied: Rhizobium, Azotobacter, phosphate-solubilizing bacteria and some fungi.

We shall consider first, the effect of Rhizobium cultures.

Figure 2 shows that there is something in cell-free supernatants of Rhizobium which stimulates % VA infection and root development. The next logical step was to investigate the presence of functionally important metabolites of Rhizobium sp. and their effect on VA mycorrhiza formation.

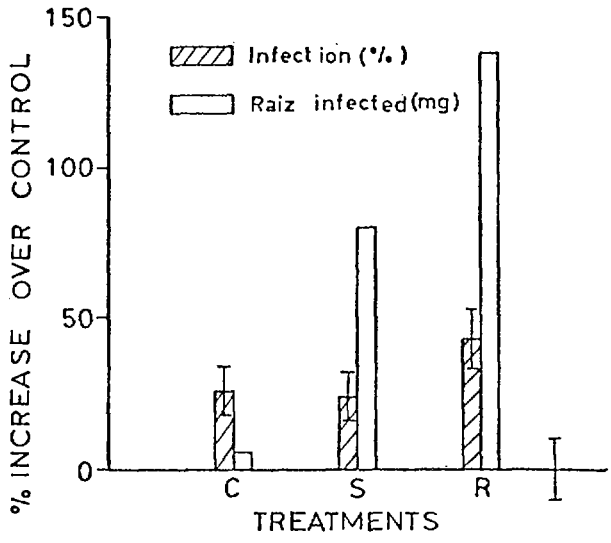


Figure 2

C = Rhizobium cells (supernatant culture - free)

S = Cell-free supernatants

R = Whole Rhizobium culture

In the selection of such metabolites, it is necessary to consider the most important events in the pre-infection of a legume root by its specific Rhizobium. Figure 3 illustrates these events.

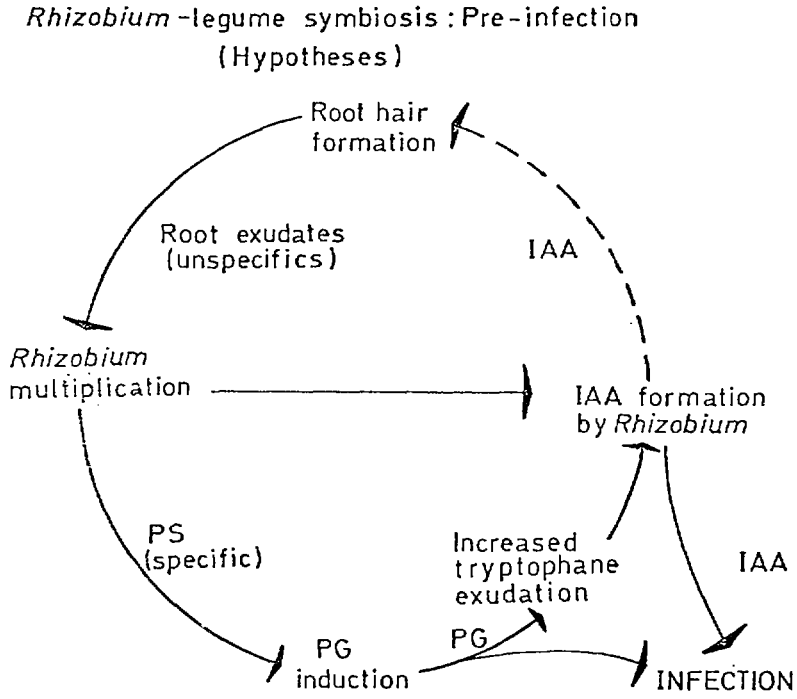


Figure 3

PG = Polygalacturonase

IAA = Indol acetic acid

PS = *Rhizobium* polysaccharides

Some time ago, Ljunggren and Fahraeus (1959) proposed a "polygalacturonase" hypothesis to explain the entry of Rhizobium into the root hair. According to it, these bacteria induce the production of pectolytic enzymes by the plant cells, which then alter the cell wall to permit the penetration of Rhizobium with the subsequent formation of the infection thread. This induction seems to be specific, occurring only between a particular legume and its homologous Rhizobium. These same authors reported that extracellular polysaccharides produced by Rhizobium are the inducers of the pectolytic enzymes. This fact, and the "polygalacturonase" hypothesis, have remained controversial due to the inability of several workers to confirm the results of Ljunggren and Fahraeus (1959).

However, other authors have found a close relationship between the presence of extracellular polysaccharide (PS) preparations in the nutrient mineral solution where plants grow, and the level of root exudation. This effect indicates that the cell wall has been modified to allow the liberation of part of its internal nutrient pool. Such an effect, although specific, may be taken advantage of by other soil microorganisms which can then proliferate around the root and eventually enter the root (Olivares, Montoya and Palomares, 1977).

Some assays were therefore conducted to study the action of PS from R. melliloti on the formation of VA mycorrhiza in M. sativa and G. mosseae.

The experiments were carried out in long test tubes filled to half height with a mixture of soil and sand. Once filled and watered, the tubes were steam sterilized and then each received aseptically the

seedlings raised from surface-sterilized seeds of Medicago sativa, and about 50 surface-sterilized spores of Glomus mosseae.

The PS of R. meliloti was obtained as described by Amarger, and was sterilized by tyndalization. An appropriate range of aqueous dilutions were made to give the concentrations of PS indicated in Table 1.

TABLE 1

Effect of different concentration of *Rhizobium* polysaccharides on VA mycorrhizal infection under sterile conditions.

PS Concentration (mg/ml)	Total infection (%)	"Root infected" (mg)
0	2.1 ± 0.1	9.3
0.3	17.1 ± 3.1	83.7
1.0	18.1 ± 1.9	106.2
3.0	28.7 ± 3.1	180.5
7.0	21.1 ± 2.8	125.7

The alfalfa plants were grown in a glass-house with their lower parts protected from direct light. Plants were aseptically fed with a sterile nutrient solution, lacking phosphate. The results clearly show that PS affects the formation of VA mycorrhiza, and that there is an optimal concentration (Table 1).

There are two possible explanations of the results:

a) The PS acted in the same way as Rhizobium, according to the PG hypothesis of Ljunggren and Fahreus (1959), hence improving the formation of the VA symbiosis through the establishment of entry points (we know that the formation of the first "entry point" is a critical stage in mycorrhiza establishment).

b) The PS could act by increasing root exudation and, thus, improving the external phase of the VA fungus, to facilitate its penetration.

These possibilities are now being tested in our Laboratory.

The results obtained agree with an early observation by Mosse (1962). She found that, under axenic conditions, the fungus failed to attach and penetrate plant roots unless a soil microorganism, Pseudomonas, was also added. This bacterium possesses pectolytic activity and the most likely explanation that the author suggested is, that compounds secreted by the bacterium act on the structure of the cell wall, affecting its plasticity. This can affect the susceptibility of plant root to fungal infection.

Other functionally important constituents of culture supernatants of Rhizobium (and of many soil microorganisms) are the plant hormones. The effects of these on VA mycorrhizal formation were also studied by us. The role of plant hormones, mainly auxins, in the formation of sheathing mycorrhiza is well known (Slankis 1974).

The growth and infection levels in mycorrhizal Lavandula, Lycopersicum and Medicago plants were compared after treatment with preparations from Rhizobium, Azotobacter and phosphobacteria (a Pseudomonas). The bacterial cultures are known to contain growth substances.

The assessement of mycorrhizal infection is given in Table 2. Plant hormones increased the total percentage infection in all cases tested. Cell-free supernatants of phosphate-solubilizing bacteria (Sp), the whole culture of Azotobacter (A) and phosphobacteria (P) and

TABLE 2

Effect of different bacterial and hormone treatments on the total % infection of Lavandula, Lycopersicum and Medicago (Azcón, Azcón-G. de Aguilar and Barea, 1978).

Treatment*	Lavandula Soil 3	Lavandula Soil 8	Lycopersicum Soil 8	Medicago Soil 8
Control	33	32	30	29
S_A	39	36	-	-
S_P	38	37	42	46
S_R	-	-	-	37
A	42	37	40	-
P	40	39	40	38
R	-	-	-	42
PH	42	38	41	-
LSD				
P = 0.05	5.8	5.4	5.7	5.7

* See text

the plant hormones (PH) gave similar levels of mycorrhizal infection in tomato plants. Infection of lavender plants was similar in both soil 3 and soil 8. The supernatant from Azotobacter and complete cultures of Azotobacter and Pseudomonas, together with the hormone treatment, significantly increased mycorrhizal infection in soil 3. In soil 8 the complete culture of Pseudomonas and the hormones enhanced the infection also. Mycorrhizal infection was increased by all the treatments given to alfalfa. These results are in agreement with those of Bagyaraj and Menge (1978), who found that Azotobacter enhanced root infection of tomato by Glomus.

The conclusion that can be decuced from the reported data is that cell-free supernatants of the bacterial cultures tested, behaved in a manner similar to the applications of pure plant hormones at similar doses to those found in such supernatants.

The applied treatments contained auxins, gibberellins and cytokinins. As it is known, among several other activities, auxins control root formation and relax the cell wall; gibberellins increase leaf area and root growth and cytokinins are involved in many basic processes of plant growth. All these activities could be related to the formation of the mycorrhiza. Under the conditions of the experiments reported in this investigation, it is perhaps not surprising that the mycorrhizal infection is affected in plants stimulated by the application of growth substances at a critical stage of their development. A direct effect, however, of the plant hormones on the external phase of the fungus, or on the establishment or/and the activity of the symbiosis cannot be excluded.

Once the effect of a mixture of growth substances was established, the role of the different substances involved, when applied separately, was subsequently studied. A series of experiments was designed with this purpose, all of which used M. sativa as test plants and G. mosseae as the VA fungus.

Table 3 shows the results of the first assay, which indicate a positive effect of auxin and cytokinins on VA infection. However, these results could be, in part, misleading, because only single PH concentrations were applied.

TABLE 3

Effect of auxins, gibberellins and cytokinins on VA mycorrhiza formation.

Treatments (0.1 µg/plant)	Infection (%)	"Root infected" (mg)
Control	25.81 ± 2.8	19.38
Auxins	33.92 ± 3.2	28.45
Gibberellins	22.97 ± 2.5	20.09
Cytokinins	43.14 ± 3.9	42.06

We studied later the effect of several hormone concentrations on VA mycorrhiza formation, with the aim of investigating a possible direct effect on fungal development. The PHs were given at different stages (time) of establishment of the Glomus-Medicago symbiosis. For each one of these series the effect of five different doses of the hormone were studied (0.001, 0.01, 0.1, 1.0 and 10.0 ug/plant). These doses were applied to the host's root system, but the time of application was different for each one of the three series of plants.

Series 0: pH doses were applied simultaneously to the inoculation of Glumus spores.

Series 1:pH doses were applied one week after spore inoculation, and growth of the plants.

Series 2:pH doses were applied two weeks after spore inoculation, and plant growth.

There were also untreated control plants, but these were Glomus-inoculated.

For each treatment and series, there were two harvest times. Figure 4 shows the results obtained with auxins, at the second harvest. As can be observed, the exogenous IAA clearly affects endomycorrhizal formation, irrespective of the time of its application.

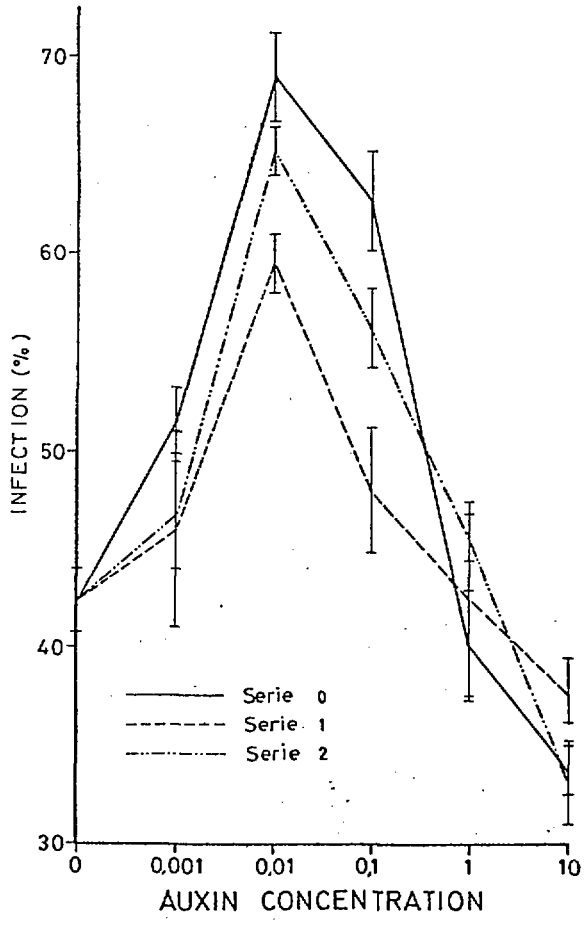


Figure 4.

In general, typical dose-response curves to auxins were obtained and the maximum effect was achieved by applying about 0.01 ug/plant.

Once the maximum was reached, higher doses of IAA tended to inhibit root growth. Since certain IAA levels cause root tissue to evolve ethylene, and since it is known that low ethylene concentrations inhibit the elongation of root, this substance has been proposed as responsible for root growth inhibition. In this context, the effect on root growth caused by the "supraoptimal" IAA

concentrations described in this assay could be accounted for, by the release of ethylene. In addition, ethylene could interfere with VA fungal development, because of the suggested fungistatic properties of this gas. This would agree with other studies in which we found an inhibitory effect of ethylene on VA endomycorrhizal establishment as shown in Figures 5 and 6.

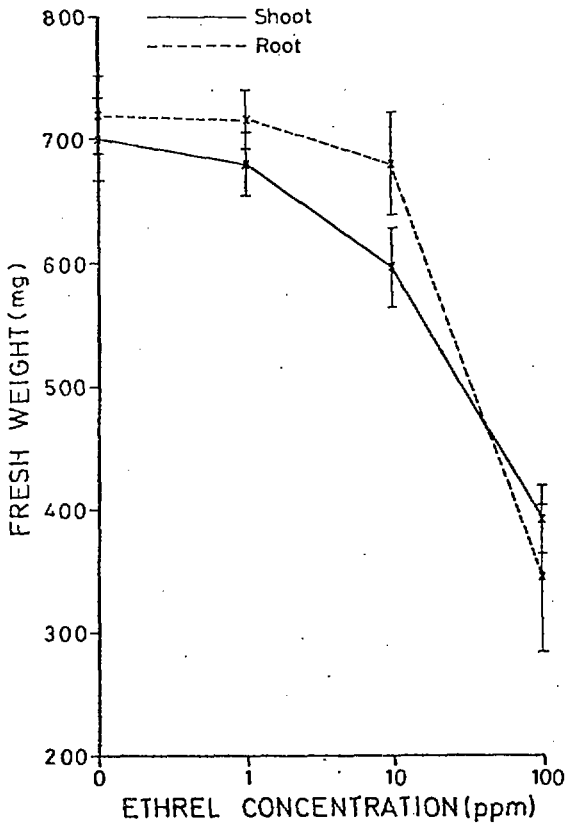


Figure 5

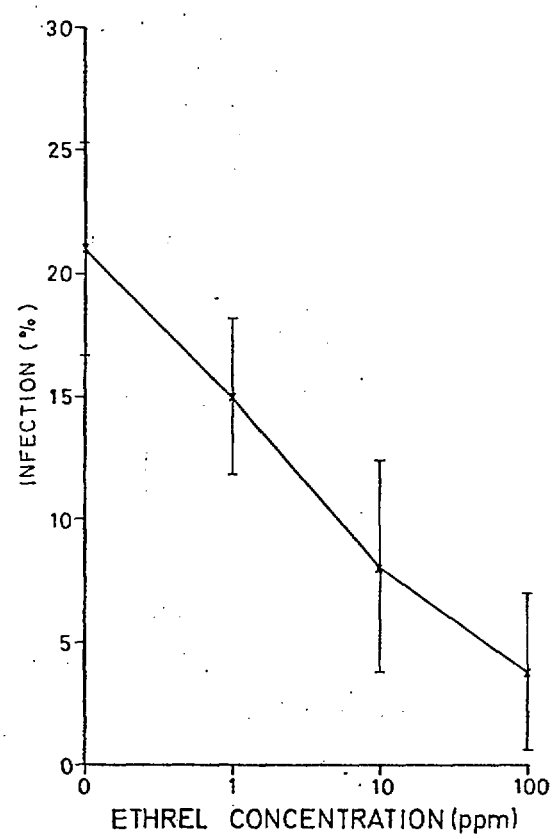


Figure 6

Our results did not indicate a direct effect of IAA on fungal development. However, data obtained (Table 4) in a plate assay using surface-sterilized spores suggest a possible enhancement of hyphal growth (but not on spore germination). The variability in the behaviour of single spores did not allow any statistical analysis, so that, the validity of this conclusion is not very strong. The possible role of auxin on mycelial development would be supported by the fact that, in spite of the chitinous nature of these fungi, they

have also a considerable amount of cellulosic material. The role of auxins in relaxing the cellulosic component of plant cell envelopes is very well established. This could explain the action of the hormone, either synthetic or contained in microbial culture, on the root penetration process of Endogonaceae hyphae.

TABLE 4

Effect of auxins on the growth of phyphae from *Glomus mosseae* spores in axenic culture.

Auxins concentration (µg/ml)	Mean length of hyphal per germinated spore (mm) (days)			
	3	6	10	12
0	1.6	4.1	5.9	8.3
0.01	1.5	5.2	7.5	10.1
0.1	2.1	5.2	7.7	10.3
1	1.9	5.6	8.1	12.1

The influence of gibberellins (Figure 7) and cytokinins (Figure 8) also looks positive, but the absence of any effect on spore germination or hyphal growth in plate studies does not support direct effect on fungal development. However, more work is necessary on this in order to get conclusive ideas on the possible implications of plant hormones. We know that the activity of a given hormonal substance is not only dependent on its concentration but also on the presence of the other active substances. It is obvious that hormones, both from plants and from bacteria, interact in the rhizosphere and that the proportion of the different kinds of substances really interacting is unknown in most of the experimental designs.

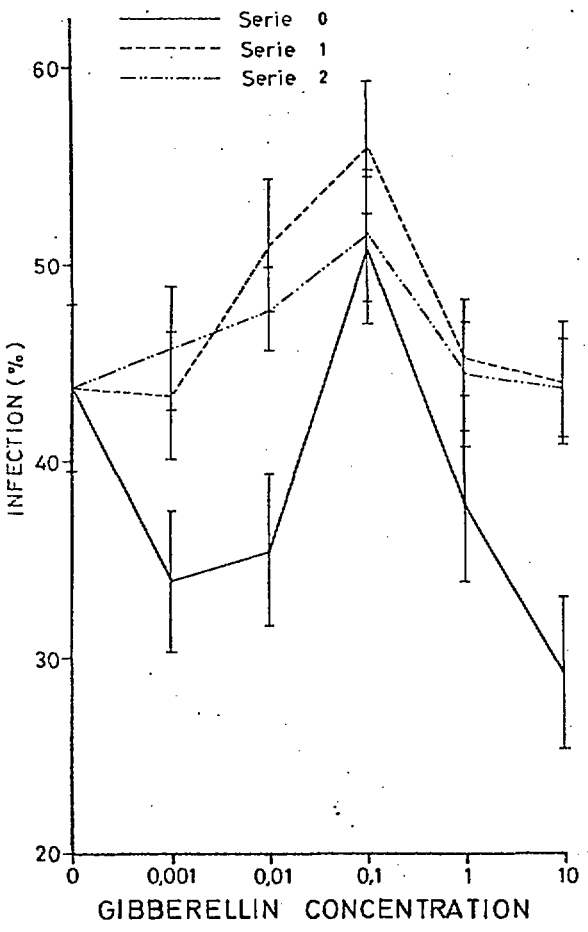


Figure 7.

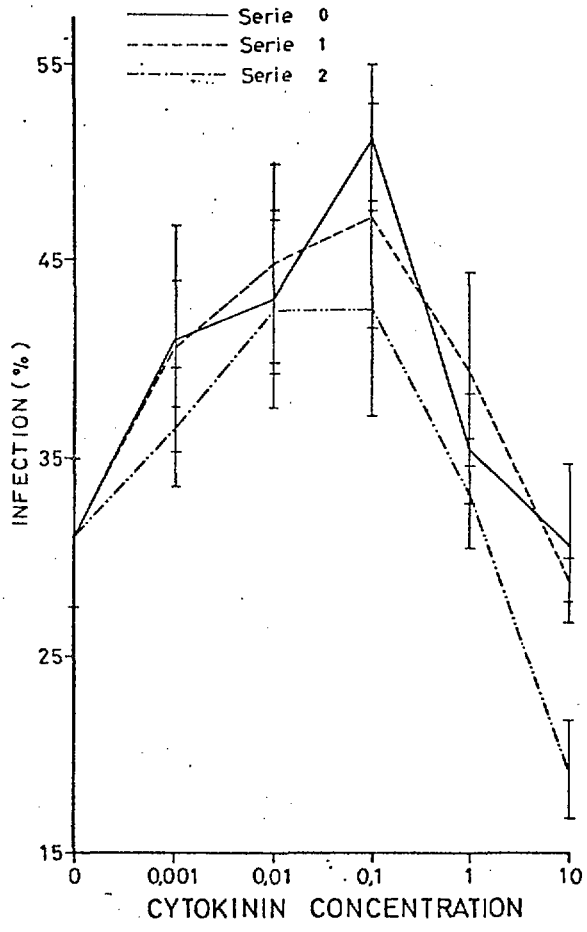


Figure 8

The situation is more complicated if the responsible VA fungi are also able to produce PH, as fungi of sheathing mycorrhizas do. In this context, Mosse (1962) found that in axenically established VA mycorrhiza, once the penetration started, the fungus stimulated the growth of infected roots, which also became more profusely branched (a typical hormonal effect). Recently, Allen et al. (1980) demonstrated that VA mycorrhizal infection increases the level of cytokinins in the host plant. However, VA endomycorrhizal fungi had not been proved to synthesize these substances because of the difficulties in obtaining axenic cultures. We have recently described a method using a liquid medium (water), by which spores of G. mosseae (Nicol. and Gerd.) could be readily germinated axenically. This liquid in which spores had

been germinated has been shown to contain substances that behave as auxins, gibberellins and cytokinins, in specific bioassays. (Azcon-Aguilar and Barea, 1978; Barea and Azcon-Aguilar, 1982).

In conclusion, we could say that mutualistic symbiosis involving plants and microorganisms, depend, both for their formation and function, on a series of interactions between the constituent partners. The implication of plant hormones, either synthesized by the host, by the endophytes, or by rhizosphere microorganisms, on the establishment and development of these biotrophic associations has been demonstrated.

Furthermore, we are also studying the influence of free-living rhizosphere microorganisms on VA fungi in pure culture. The preliminary results have shown that several fungi stimulate G. mosseae "growth"in culture. The rate and extent of spore germination were not affected but the length of the hyphae and the number of vegetative spores per resting parent spore were greatly increased by most of the tested organisms. Such effect was achieved even in the absence of any physical contact between G. mosseae and the free-living microorganisms. Obviously, the effect of rhizosphere microorganisms to stimulate mycelial development in absence of any host plant, reinforces the idea of a possible contribution of these microorganisms in the stimulation of VA hyphae in the rhizosphere. Moreover, studies aimed at helping the axenic growth of VA fungi have a great interest since obtaining axenic cultures of these fungi is vital to the production of high quality inoculants that will allow the application of mycorrhiza on a large scale.

Whatever the mechanisms involved, our results support the hypothesis that extracellular products of rhizosphere microorganisms

seem to be involved in the growth of VA fungi and in the processes of VA mycorrhiza formation.

Nuclear techniques could be an invaluable research tool for studying the implications of root exudates and soil microorganisms in VA infection. In fact, the use of ^{14}C allows the best appreciation of the amounts of substrates coming from the roots. Changes in root membrane permeability can be estimated by measuring the loss of ^{86}Rb (as a tracer for K^+). Isotopes, such as ^{32}P, could be useful in speeding up assessment of effectiveness of a "rhizosphere product" on VA mycorrhiza formation, because ^{32}P could give a rapid indication of when a mycorrhizal infection begins to function, as a consequence of successful root penetration.

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WATER RELATIONS AND DROUGHT TOLERANCE OF VASICULAR-ARBUSCULAR MYCORRHIZAL PLANTS

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Abstract

It is well documented that if mycorrhizal plants are growing under nutritional conditions such that they are substantially larger than their non-mycorrhizal plants, there will be differences in water relationships. Mycorrhizal plants will usually have higher transpirational rates, stomatal conductances, hydraulic conductivities, and water potentials than their smaller non-mycorrhizal counterparts. Addition of nutrients, particularly phosphorus, to non-mycorrhizal plants, in order to eliminate mycorrhizal growth enhancement will usually eliminate most of the differences in water relationships with the possible exception of stomatal conductance and CO_2 fixation. Nutrition cannot be eliminated as a cause of increased stomatal conductance of mycorrhizal plants compared to approximately similar sized non-mycorrhizal plants compared to approximately similar sized non-mycorrhizal plants without measuring plant nutrient concentrations. This is because low plant nutrient concentrations are known to inhibit stomatal functioning.

The demonstration that mycorrhizal plants are more drought tolerant than non-mycorrhizal counterparts and that in some cases addition of large amounts of phosphorus to the soil will not eliminate the benefits of mycorrhizal infection is very exciting. It provides additional stimulus for studying the potential use of mycorrhizal fungi especially in arid or semi-arid ecosystems where their presence may prove to be of immense economic benefit.

Introduction

Research efforts on the water relations and drought tolerance of vesicular arbuscular (VA) mycorrhizae have increased in recent years. At least two reviews of this subject have been written (Reid, 1979; Safir and Nelsen, 1981). This review will present an interpretation and up to date review of the published and some unpublished research results in this area. I will also

include a discussion of how phosphorus (P) nutrition may alter the effects of VA mycorrhizae on plant water balance.

Well Watered Conditions

The first reports of altered water relations of plants infected by VA mycorrhizal fungi were those of Safir et al. (1971, 1972). They found, under well watered conditions, and low soil nutrition, that mycorrhizal soybean plants had hydraulic conductivities 40% higher than nonmycorrhizal controls. These differences in hydraulic conductivity were eliminated by the addition of Hoagland's nutrient solution (500 ml) to the soil at the time of seeding. Two methods were employed for the above determinations with similar results obtained. First, conductivities (hydraulic) were calculated using a method described by van den Honert (1948), which requires measurements of soil and plant water potentials, as well as transpiration rates on a leaf area basis. The second method required an assumption that conductivities would be related to the rates of leaf recovery from moderate water deficit upon rehydration of the soil (Boyer, 1969). The results of Safir et al. (1971, 1972) are supported by the work of Nelsen and Safir (1982a) using onion plants, in that mycorrhizal plants had higher hydraulic conductivities than nonmycorrhizal controls when low levels of phosphorus (P) were present in the soil at seeding time. The differences in conductivity were much larger using onions (approximately 4 fold) than soybean and the differences were eliminated by the addition to the soil of P to nonmycorrhizal plants at levels which eliminated growth differences between mycorrhizal and nonmycorrhizal plants. Levy and Krikun (1980) also found that similar sized mycorrhizal and nonmycorrhizal rough lemon seedlings had similar conductivities, transpiration rates, and water potentials, when both were supplied with a commercial nutrient solution.

It was suggested by Safir et al. (1972) that the roots were the sites of the increased hydraulic conductivities of mycorrhizal plants based on comparisons of recovery rates from moderate water stress of whole plants and plants with severed roots. Leyton (1981) calculated root hydraulic conductivities from rates of water forced through detopped root systems using a pressure bomb. The mycorrhizal roots were much more conductive than were nonmycorrhizal roots, thus, indirectly supporting the results of Safir et al. (1972). The site of the increased root conductivities of mycorrhizal roots, if present, is still uncertain, however, since other studies by Duniway (1980 personal communication), using pressure bomb techniques, showed that mycorrhizal safflower roots had lower hydraulic conductivities than nonmycorrhizal roots and that the conductivities decreased as mycorrhizal infection increased.

It is possible that the mycorrhizal hyphae in the soil would enable mycorrhizal plants to take up water. Sanders and Tinker (1973) calculated potential inflow rates of water through VA mycorrhizal hyphae. These calculations indicated that if the increased transpirational water flux of mycorrhizal plants was conducted to the roots through the hyphae the hyphal flux rates per strand (hyphal) would be unrealistically high. Their calculations did not argue

against a small amount of water moving through the hyphae to the roots. Nelsen and Safir (unpublished) used a split plate system which separated the hyphae from the roots. After allowing the leaves to dehydrate slightly by withholding water from the soil, small portions of soil containing only hyphae were rehydrated. Leaf water status was monitored in a thermocouple psychrometer designed for intact leaves. Soil rehydration did not affect leaf water potentials for periods up to 10 hours, after which the experiments were terminated. Also, results of experiments with onions, using tritrated water, did not show hyphal translocation. These experiments suggest that hyphal transport of water to roots, if occurring, is not capable of altering the water balance of leaves. Indirect evidence of some hyphal water movement to the roots has been provided by Cooper and Tinker (1981). They showed that high transpirational rates could increase P translocation by the fungus 2-3 times the rates found at low transpiration rates.

Another possibility for the increased conductivity of mycorrhizal roots is that the mycorrhizal hyphae within the root could provide a low resistance pathway for water movement. The results of Safir et al. (1972) argue against this possibility. They reported that the addition of PCNB to the soil had no effect on the differences in hydraulic conductivity between mycorrhizal and nonmycorrhizal soybean plants. PCNB has been shown to eliminate the increased uptake and hyphal translocation of P of mycorrhizal plants when compared to nonmycorhizal plants (Gray and Gerdemann, 1969). If the hyphae within the root were providing low resistance channels, the conductivities of mycorrhizal roots should have decreased after application of PCNB.

The available evidence strongly suggests that hydraulic conductivities of mycorrhizal plants will be higher than those of nonmycorrhizal plants at the time of growth stimulation, if infection substantially increases growth over that of nonmycorrhizal plants. The higher conductivities of mycorrhizal plants should cause leaf water potentials to be higher than those of nonmycorhizal controls under moderate to high evaporative demand conditions. Stomatal conductances for vapor flux and transpiration rates should also be higher for mycorrhizal plants under the same conditions if leaf water status is at least partially controlling stomatal aperture. The work of Nelsen and Safir (1982a) supports the above hypothesis in that mycorrhizal onions had higher hydraulic conductivities, lower leaf water potential and higher transpiration rates than nonmycorrhizal controls when mycorrhizal growth stimulation was present. The application of sufficient P to the soil of nonmycorrhizal plants to enable the nonmycorrhizal and mycorrhizal plants to be similar sized eliminated differences in transpiration, water potential, and hydraulic conductivity. These same parameters were similar for similar sized mycorrhizal and nonmycorrhizal rough lemon seedlings (Levy and Krikun, 1980).

All mycorrhizal plant types may not behave in a similar manner since Christensen and Allen (1979,1980) have shown that mycorrhizal Bouteloua gracilis (blue grama) plants had lower stomatal conductances than nonmycorrhizal controls

under well watered conditions. They found differences in hormone levels in mycorrhizal and nonmycorhizal plants, and postulated that these differences may have caused the above differences in stomatal conductance. Also, mycorrhizal safflower plants had slightly lower stomatal resistances when compared with similar sized nonmycorrhizal under conditions of ample water supply (1980, J.M. Duniway, personal communication).

Limited Water Conditions

Safir et al. (1971, 1972) demonstrated that mycorrhizal soybean leaves recovered from moderate water stress faster than nonmycorrhizal controls after soil rehydration. These differences were eliminated when high nutrient levels were applied to the soil of both mycorrhizal and nonmycorrhizal plants at seeding time. Levy and Krikun (1980) found that mycorrhizal rough lemon plants had higher transpiration rates, stomatal conductances, and photosynthetic rates than similar sized controls upon recovery from water stress. They did not find differences in water potential between mycorrhizal and nonmycorrhizal plants during recovery from water stress, and suggested that hormonal differences were responsible for the larger stomatal conductances of mycorrhizal plants during recovery. Christensen and Allen (1979, 1980) showed that stomatal conductances of mycorrhizal blue grama plants decreased at a slower rate, and transpiration rates remained higher than smaller nonmycorrhizal plants as soil water potentials decreased. This indicates that stomatal conductances and hydraulic conductivities may be higher for desert adapted mycorrhizal blue grama plants over a wide range of soil water potentials.

Short term studies are of limited value in determining the effects of any variable on long term plant parameters such as growth, phenological development or yield. For this reason, Nelsen and Safir (1982b) exposed onion plants (mycorrhizal and nonmycorrhizal) to 7 cycles of water stress. The regime began 4 weeks after seeding and lasted 8 weeks in order to determine the long term effects of water stress and to simulate, to some extent, field conditions. Mycorrhizal plants at 8 and 12 weeks, which were given low levels of P, were more drought tolerant than were non-mycorrhizal onions given high levels of P (=114 kg/ha). The levels of P given to the non-mycorrhizal plants were sufficient to enable these plants to be the same size as mycorrhizal plants under well watered conditions (Nelsen and Safir, 1982a). Drought tolerance of mycorrhizal plants was shown by greater fresh and dry weights and by higher tissue P concentrations. The P concentrations of the nonmycorrhizal plants were at levels (0.1-0.3% dry weight) known to retard the growth of onion plants (Stribley et al., 1980a, b) as well as other plants (Epstein, 1972). Phosphorus was not limiting in the mycorrhizal plants. Water stress limited the growth of both mycorrhizal and non-mycorrhizal plants, however, since leaf water potentials and transpiration rates for mycorrhizal and nonmycorrhizal plants were similar at 8 and 12 weeks. It was concluded that P deficiency was

responsible for the decreased growth of nonmycorrhizal plants when compared to mycorrhizal plants.

It is not surprising that mycorrhizal plants are able to absorb P during drought considering their extensive fungal network in the soil. It has also been shown that nonmycorrhizal onions are reduced in their capacity to absorb P when water stressed (Dunham and Nye, 1976). This inability to absorb P is probably magnified by the decreased diffusion rate of P in dry soil (Viets, 1972).

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THE ROLE OF VASICULAR-ARBUSCULAR MYCORRHIZA IN N_2-FIXED BY LEGUME-*Rhizobium* SYSTEMS IN PHOSPHATE-FIXING AGRICULTURAL SOILS

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Abstract

The scarcity of available phosphate in many soils is a critical limiting factor in legume-Rhizobium-systems because it affects not only plant growth but nodulation and N_2-fixation by the micro-symbiont. Hence, VA mycorrhizas, which are widespread in legumes, play an important role in the development of such crops and are thus of great interest for food production in the biosphere.

This paper discusses the work developed in this laboratory in relation to the significance of VA mycorrhiza in N_2-fixation within two legume-Rhizobium-systems: Medicago sativa (alfalfa)-Rhizobium meliloti and Hedysarum coronarium (sulla)-Rhizobium sp.. Several experiments have been carried out to study the interactions between natural and introduced VA endophytes and Rhizobium, and soluble phosphate fertilizer on growth, nodulation and N-uptake of the two test legumes in natural (unsterilized) agricultural soils. The tests were conducted under both pot and field conditions

In studying the interactions between introduced and indigenous endophytes, the situations were defined where legume inoculation with VA mycorrhizal fungi in phosphate-fixing soils was successful, since improved plant growth and nutrition also enhanced the activity of Rhizobium applied as inoculant. In such responsive soils field inoculation with VA mycorrhiza may, therefore, be worth trying.

The effectiveness of mycorrhizal fungi was established in field trials, where the fungi swiftly became established, survived and infected plants growing in the field. This can be deduced from the increase in shoot growth and nutrition caused by the introduced fungus.

Although our results further indicate that mycorrhizal inoculation improve the N content in leguminous plants, there is no evidence to show that N is due to increased N_2-fixation or to increased N uptake from soil. The use of ^{15}N to label soil assimilable N is proposed as a suitable way to quantitatively estimate the amount of N in plant which is coming from symbiotic dinitrogen fixation by legumes growing under field conditions.

INTRODUCTION

Most soils do not contain sufficient available phosphate for maintaining suitable plant growth. In the case of legumes, the scarcity of soluble phosphorus is a critical limiting factor because it affects not only plant growth but also nodulation and nitrogen fixation. It has been shown that the infection mechanism of a legume root by its specific Rhizobium and the process of nitrogen fixation have a high energy requirement, because nitrogenase is dependent on ATP to reduce dinitrogen to ammonia. Approximately, twenty one moles ATP are converted to ADP per mole dinitrogen reduced. In fact, nodules often contain 2-3 times more phosphorus per unit dry matter than the root on which they are formed (8).

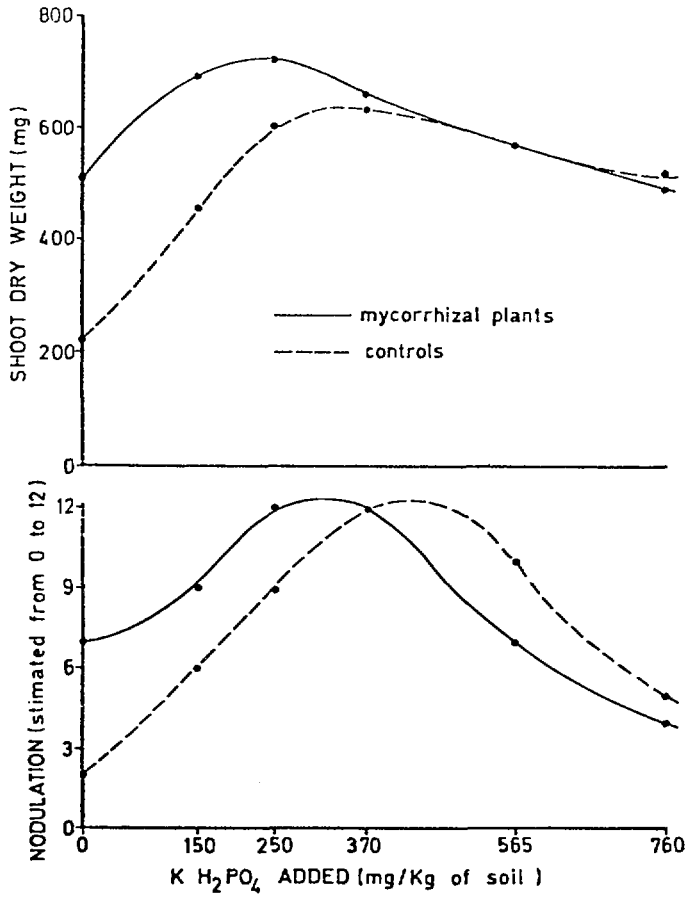
Vesicular-arbuscular mycorrhizas by means of their network of hyphae ramify the surrounding soil, beyond the root hair zone, and are therefore capable of absorbing more than the root alone. This is particularly important for ions which diffuse slowly in soil, such as phosphate and molybdate. Hence, vesicular-arbuscular mycorrhiza can improve growth, nodulation and nitrogen fixation in legumes, crops of great interest for food production in the biosphere (6,9).

The VA mycorrhizal symbiosis is widespread in legumes. Among the three sub-families included in the Leguminoseae, two of them, i.e., Papilionoideae and Minosoideae usually have VA mycorrhiza. However, legume species differ in their growth responses to mycorrhizal infection. Putting it in another way, many legumes are rather dependent on mycorrhiza for P uptake, and, therefore, for nodulation in P deficient soils. However, the mycorrhizal dependence in Lupinus, for example, is quite small. Thus, the degree of dependence is clearly dictated by species (8).

In contrast with Rhizobium VA mycorrhizal fungi can infect a very wide range of plants. There is then a lack of specificity ("sesum latum") in VA infections. However, certain strains are much better than others in providing nutrients to their host.

THE ROLE OF VA MYCORRHIZA IN N_2-FIXATION IN TWO LEGUME-RHIZOBIUM SYSTEMS: MEDICAGO SATIVA (ALFALFA) - RHIZOBIUM MELILOTI AND HEDYSARUM CORONARIUM (SULLA) - RHIZOBIUM SP.

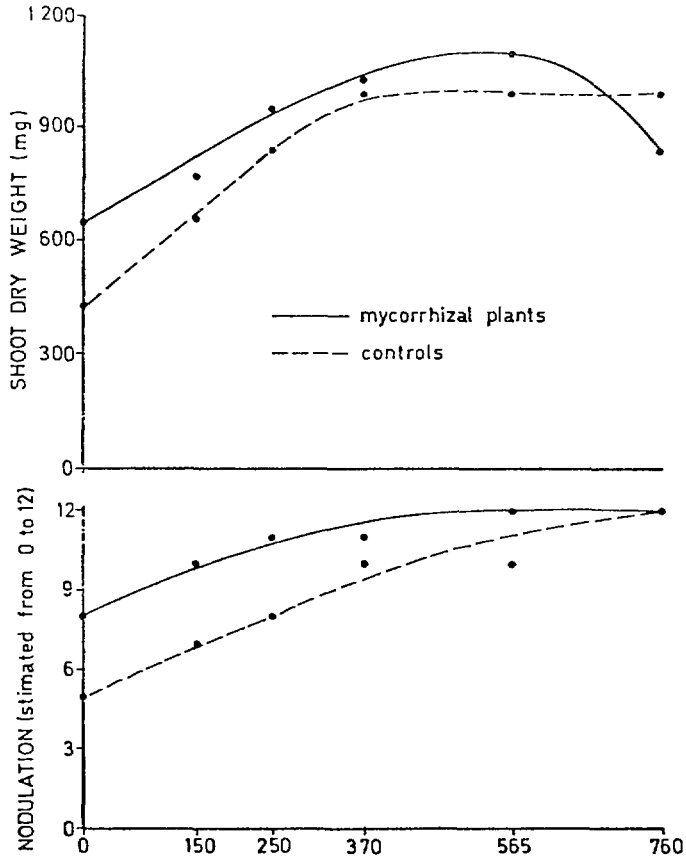
In both systems, we first studied briefly the interactions between increasing soil phosphorus level and VA mycorrhiza on plant growth and nodulation. In the case of Medicago (Figure 1), mycorrhizal plants grew and nodulated significantly better than non-mycorrhizal ones in unamended soil, and also when less than 370 mg KH_2PO_4/kg soil was added. Additions of 370 mg KH_2PO_4/kg or higher to the soil eliminated the mycorrhizal effect on plant growth and nodulation.



Medicago sativa - R. meliloti system

Figure 1

In case of Hedysarum (Figure 2), mycorrhizal inoculation also improved significantly plant growth and nodulation at low phosphate additions (0, 150 and 250 mg/kg). However, the response differed from that of Medicago, in that high additions of phosphate did not show significant detrimental effects on nodulation, and only the higher doses were "supra-optimal" for plant growth in mycorrhizal plants.



Hedysarum coronarium-Rhizobium sp. system

Figure 2

In this context, Asimi et al. (1) carried out some experiments aimed at studying the influence of increasing soil phosphorus levels on interactions between vesicular-arbuscular mycorrhiza and Rhizobium in soybeans. They found that increasing doses of phosphate fertilizers, first of all eliminated the positive effect of mycorrhiza on plant growth and then progressively eliminated mycorrhizal effects on nodulation, and finally on nitrogenase activity. This agrees with the results obtained by Islam (7) showing that nitrogenase activity was doubled by mycorrhizal inoculation of soybean, while growth was only slightly increased.

Indeed, several authors (1,10) have also confirmed that enhancement of nitrogenase activity by mycorrhiza preceeded plant growth responses to mycorrhiza.

The above statements give us a general view on the role of VA mycorrhiza in supplying phosphate for nodulation and nitrogen fixation in legumes.

Although it is known that legume nodules are usually not invaded by the VA fungus, which remains confined to the root cortex, the above results suggest that phosphate is, in some way, made directly available to the nodules. A time-course experiment using ^{32}P would be of great interest to clarify this.

On the basis of the evidence just summarized, several experiments were carried out to study the results of the interactions between natural and introduced VA endophytes and Rhizobium, and soluble phosphate fertilizer on growth, nodulation and N uptake of our two test legumes in natural (unsterilized) agricultural soils. The experiments were conducted under both pot and field conditions.

STUDIES ON THE MEDICAGO SATIVA-R. MELILOTI SYSTEM

Pot Experiments

Two types of assays were designed with the aim of establishing first the different role of soluble phosphate applied at an agronomical dose (equivalent to 1g KH_2PO_4/kg of soil) and the inoculation with a selected VA mycorrhizal fungus on the growth, nodulation and N uptake of Medicago sativa. Second, we tried to define the type of interaction, whether synergestic or antagonistic, between native and introduced endophytes (5).

Two different soils were used (soil No. 8 and No. 9). Both have many common characteristics, such as high clay content, pH, calcium content, which make these soils phosphate retentive, thus requiring large dressings of P fertilizers in order to obtain adequate crop yields. The phosphate-fixing capacity of these soils is such that about 70 and 61% of the soluble phosphate given was fixed by soils Nos. 8 and 9, respectively, during 3 weeks under our glass-house conditions.

Table 1 summarizes the effect of VA fungus (Glomus mosseae) and soluble P on shoot dry weight, nodulation and N uptake of alfalfa grown for 7 weeks in unsterilised soil No. 8. All plants received a standard inoculum of R. meliloti. The data show that the effects of the inoculation of VA endophytes on plant growth were similar to those achieved by the dose of soluble P fertilizer used (M versus SP treatments). Inspite of the number of nodules formed on phosphate treated plants being higher than that developed on mycorrhizal plants, the N content (% of dry matter) was higher in mycorrhizal plants, and the total N uptake was similar. This shows that nodules developed on mycorrhizal roots were more efficient in nitrogen fixation.

TABLE 1

Effects of VAM (*Glomus mosseae*) and soluble P on growth and nutrition of *Medicago sativa* grown in unsterilized soil No 8. After Barea et al., 1980.

Treatment(*)	Shoot Dry weight (mg)	P %	Number of nodules	Nitrogen %	Nitrogen total
Control	145[a]	.16	58[a]	3.28	4.8[a]
Mycorrhiza (M)	432[b]	.19	90[b]	4.09	17.7[b]
Soluble P (SP)	483[b]	.28	169[c]	3.46	16.7[b]
M + SP	567[c]	.34	143[c]	3.57	20.2[c]

(*) All plants received a *Rhizobium meliloti* inoculum

P = 0.05

Table 2 shows the results obtained in soil No. 9. In general, the response is similar to that found in soil No. 8. Inoculation of VA endophytes had the same effect as the soluble P fertilizer. Mycorrhizal inoculation tends to improve N uptake by plants if it is compared with the corresponding non-inoculated control.

TABLE 2

Effects of VAM (*Glomus mosseae*) and soluble P on growth and nutrition of *Medicago sativa* grown in unsterilized soil No 9. After Barea et al., 1930.

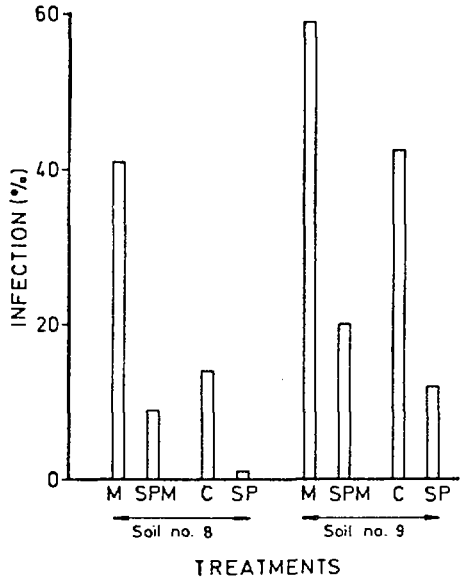
Treatment(*)	Dry weight (mg)	P %	Number of nodules	Nitrogen %	Nitrogen total
Control	398[a]	.29	112[a]	3.53	14.1[a]
Mycorrhiza (M)	667[b]	.31	131[a]	3.31	22.1[bc]
Soluble P (SP)	659[b]	.31	122[a]	2.86	18.9[b]
M + SP	669[b]	.39	123[a]	3.72	24.9[c]

(*) All plants received a *Rhizobium meliloti* inoculum

P = 0.05

Figure 3 records the development of mycorrhizal infection in soils 8 and 9 as affected by P fertilizer.

Soluble phosphate significantly depressed the degree of infection by native or introduced VA endophytes. Although the intensity of the VA infection by indigenous fungi in soil no. 9 is remarkable, the introduced VA fungi improved the degree of infection over the level achieved by native ones. The inoculated endophytes became, therefore, established in unsterile conditions.



M = mycorrhiza inoculated
SP = soluble phosphate
C = uninoculated control (native mycorrhiza)

Figure 3

To study the interactions between introduced and indigenous endophytes, we compared the effect of inoculation with VA endophytes on plant growth in unsterile soil and in soil sterilized by autoclaving to kill the indigenous mycorrhizal fungi. The results are observed in Table 3. In the four experimental conditions tested, mycorrhizal inoculation significantly increased plant dry weight. The growth increases produced by native and introduced endophytes were calculated from these data and summarized in Table 4. The effect of native endophytes is revealed by comparing unsterile and sterile controls. The effect of inoculated endophytes is determined by plant response to mycorrhizal inoculation in sterile soil. To estimate the response of the plants to the interaction between native and inoculated fungi it is necessary to compare the growth of mycorrhizal inoculated plants in unsterile conditions versus that of uninoculated plants grown in sterile soil. As shown by the results presented on Table 4, for both soils the experimental data obtained for the inoculated treatment is similar to that obtained by adding up the individual effects of native and inoculated endophytes. These results suggest an additive effect of indigenous and introduced endophytes.

TABLE 3

Effects of native and introduced VA endophytes on plant growth. After Barea et al., 1980.

Treatment(*)	Dry weight (mg)	
	Soil No 8	Soil No 9
Unsterile Soil		
Control	145	398
Mycorrhiza	432	667
Sterile soil		
Control	109	248
Mycorrhiza	385	493

(*) All plants received a *Rhizobium meliloti* inoculum

TABLE 4

Growth increases in *Medicago sativa* produced by native or inoculated VA endophytes. After Barea et al., 1980.

Effect of VA endophytes	Percent growth (shoot dry weight) increase	
	Soil No 8	Soil No 9
1) Native (C unst. vs. C st.)	33.0	60.5
2) Inoculated (M st. vs. C st.)	253.2	98.8
Native + inoculated		
Calculated (1 + 2)	286.2	159.3
Experimental (M unst. vs. C st.)	296.3	168.9

C = uninoculated control; M = Mycorrhiza inoculated;
unst. = unsterile soil; st. = sterile soil

Hence, it can be concluded that legume inoculation with VA mycorrhizal fungi in phosphate-fixing soils was successful, since it not only improved plant growth and nutrition, but also enhanced the activity of Rhizobium applied as inoculant. Field inoculation of legumes with VA mycorrhiza may, therefore, be worth trying.

Field Experiments

Production of high quality inocula is the main factor which limits the performance of large scale field experiments. This could however be solved when pure fungal cultures are obtained. Field experiments were in the meantime developed with available inocula, using microplots.

Field inoculation experiments were carried out on Medicago sativa inoculated with its symbiotic partners Rhizobium meliloti and the endomycorrhizal fungus Glomus mosseae in untreated arable soils (2, 3).

These experiments were executed in soil no. 9, but in plots that had been subjected to different agronomic practices by farmers and which therefore

differed from each other in some respects. These differences are shown in Table 5. The most important of these are the amount of soluble Olsen P, and the number of Endogonaceae spores. Plot "9b" possessed twice more soluble phosphate and three times more VA propagules than plot "9a".

TABLE 5

Some analytical characteristic of the test soils. After Azcón-Aguilar and Barea, 1981.

Soils	pH (water)	Organic matter (%)	Total P (ppm)	Soluble P (ppm)	No. *Endogonaceae* spores/100 g
9[a]	7.8	1.74	611	9.2	24
9[b]	7.4	1.94	1164	17.6	75

The results of the experiment carried out in plot "9a" are shown in Figure 4. Inoculation with Glomus was always effective in promoting plant growth, but Rhizobium was only able to enhance growth of Glomus-inoculated plants. Probably, phosphorus was the limiting factor for Rhizobium activity, since available phosphate as well as the number of native Endogonaceae spores in this test soil were low. Hence, plants did not respond to Rhizobium inoculation unless they were also inoculated with VA fungi.

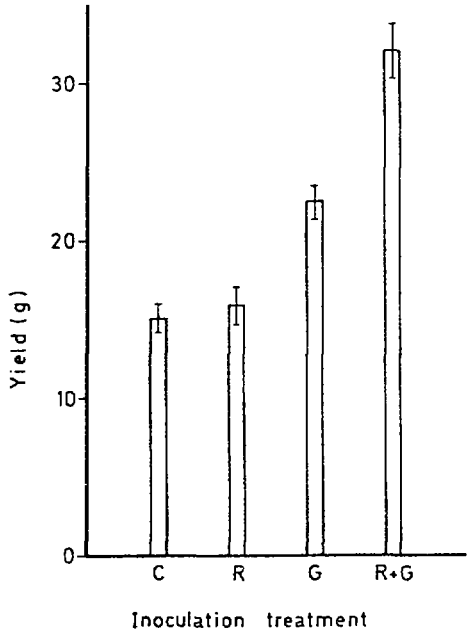


Figure 4

C = control
R = Rhizobium - inoculated
G = Glomus mosseae - inoculated

In contrast, Rhizobium meliloti was effective when inoculated alone in plants growing in plot "9b" (Figure 5). The efficiency of indigenous VA fungi, together with the higher concentration of available P in the soil, could cause plants to respond to Rhizobium inoculated alone.

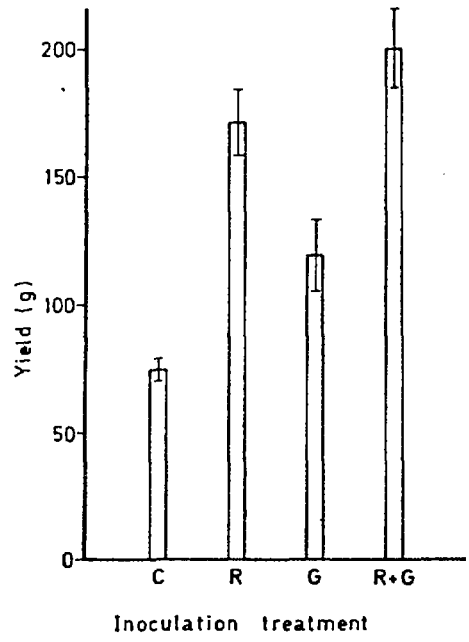


Figure 5

C = control
R = Rhizobium - inoculated
G = Glomus mosseae - inoculated

In both instances, inoculation of Rhizobium plus Glomus more than doubled the yield, compared to the uninoculated control.

As alfalfa plants are usually harvested by serial cutting, and because they regrow after cutting, the persistence of the effect of endosymbionts in a second harvest was also studied.

Figure 6 illustrates the effects of microbial inoculation on the yield of Medicago sativa plants in the second harvest. It is obvious that the effect of the introduced endophytes persisted. The dual inoculum (R+G) was significantly more effective than any other treatment.

It is noteworthy that plants inoculated with Glomus (G and R+G treatments) possessed higher N and P content than uninoculated control or R treatments, either at the 1st (Table 6) or at the 2nd harvest (Table 7).

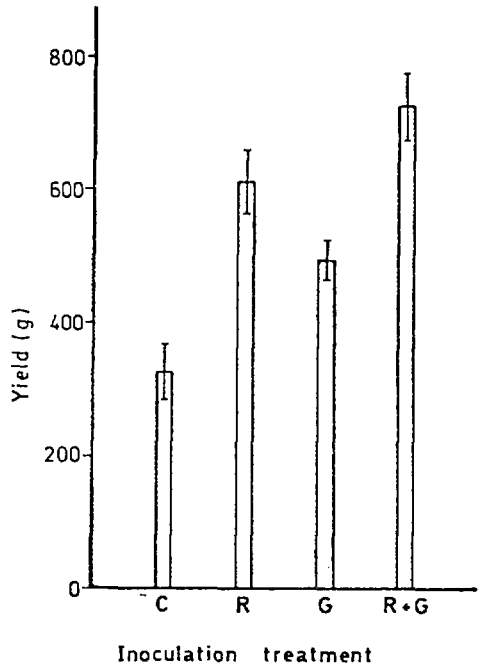


Figure 6

C = control
R = Rhizobium - inoculated
G = Glomus mosseae - inoculated

TABLE 6

Effect of *Rhizobium* and *Glomus* on N and P uptake by *Medicago sativa*. Field experiment, 1st harvest. After Azcón-Aguilar and Barea, 1981.

Treatments	Nitrogen		Phosphorus	
	Control (% Dry matter)	Total (g/replicate)	Content (% Dry matter)	Total (g/replicate)
Control (C)	3.88^a	2.91^a	$.32^a$	$.24^a$
Mycorrhiza (G)	4.63^b	5.51^b	$.35^a$	$.42^b$
Rhizobium (R)	3.54^a	6.07^b	$.32^a$	$.55^b$
R + G	4.57^b	9.13^c	$.36^a$	$.72^c$

Each replicate consisted of 400 plants

The effectiveness of G. mosseae was confirmed in these field trials, where the fungi swiftly became established, survived and infected the alfalfa plants growing in the field. This can be deduced from the increase in shoot growth, N and P content that resulted from inoculation of the fungus below oversown seeds.

TABLE 7

Effect of *Rhizobium* and *Glomus* on N and P uptake by *Medicago sativa*. Field experiment, 2nd harvest. After Azcón-Aguilar and Barea, 1981.

Treatment	Nitrogen		Phosphorus	
	Content (% Dry matter)	Total (g/replicate)	Content (% Dry matter)	Total (g/replicate)
Control (C)	2.52[a]	8.21[a]	.16[a]	0.52[a]
Mycorrhiza (M)	2.88[bd]	14.28[b]	.22[b]	1.09[b]
Rhizobium (R)	2.74[ab]	16.76[b]	.18[a]	1.10[b]
R + G	3.13[d]	22.71[c]	.21[b]	1.52[c]

Each replicate consisted of 400 plants

P = 0.05

In conclusion, the results of the present field trials show that G. mosseae is an efficient organism that helps legume-rhizobia systems to obtain their nutrients when they grow in phosphate-fixing soils.

Hedysarum coronarium-Rhizobium sp.

With respect to the other legume-Rhizobium system, we have conducted some experiments aimed to help the introduction of Hedysarum coronarium L. into new habitats. A survey was first undertaken to examine whether H. coronarium is nodulated and mycorrhizal in its natural growing area. Pot experiments were then set up to assess the feasibility of inoculation in soils (new habitats) previously studied for some of their physical, chemical and biological (microbial) characteristics. Finally, a field experiment was carried out in a selected soil (4).

The survey of the natural growing area of Hedysarum coronarium showed that all the root samples were mycorrhizal and possessed nodules. The indigenous VA mycorrhizal infection occupied about 50-75% of the feeder roots.

The soil used in the present study involving Hedysarum coronarium had been under intensive cultivation and had received large supplies of fertilizers. This is reflected in the data of soil analysis (Table 8). In spite of the total P being high, the available P is quite low in soil no. 10, probably because the P fertilizer added became fixed as a consequence of the high pH, Ca and clay content of this soil. Soils also differ in their native VA endophyte population. Soil no. 10 had a higher number of spores (80 vs. 35 per 100 g soil) and also greater mycorrhizal infection (50% vs. 17%) than soil no. 11. This could be related to the lower available phosphate content in soil no. 10. However, both soils possess a fairly low endophyte population and this is probably due to the fertilizer application these soils received in the past, treatments that can depress the numbers of spores in soil. Glomus mosseae was the dominant species and it was chosen for mycorrhizal inoculation.

TABLE 8

Analysis of the test soils (new habitats). After Azcón-Aguilar et al., 1982.

Soil No.	Clay (%)	pH (water)	Organic matter (%)	$CaCO_3$ activ. (%)	Total P	Soluble P [a] (ppm)
10	44.1	7.4	1.39	12.5	618	5.6
11	25.2	7.2	2.17	2.1	1557	33.0

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

The results of the glasshouse experiments to assess the feasibility of G. mosseae inoculation are summarized in Tables 9 and 10. It is obvious that G. mosseae was successfully introduced into Hedysarum coronarium rhizosphere in soil no. 10, and, through its activity, increased plant growth and nodulation under both sterile and unsterile conditions. These results therefore show that field inoculation with the selected endophytes may be worth trying in soil no. 10.

TABLE 9

Mycorrhizal infectivity of the test soil No. 10 and additional effects of inoculation with *Glomus mosseae* in the formation of and responses to microbial symbiosis with *Hedysarum coronarium* plants grown in the glasshouse. After Azcón-Aguilar et al., 1982.

Parameter	Sterile soil		Unsterile soil	
	Control	Mycorrhiza	Control	Mycorrhiza
% VA infection[a]	0	57 ±7	20 ±5	74 ±11
Nodulation[b]	2	4	2	4
Shoot weight(g)[a]	2.1 ±0.2	3.8±0.1	2.5±0.2	4.1±0.3
Root weight(g)[a]	2.7 ±0.2	3.5±0.7	2.4±0.5	3.3±0.4

[a] Mean value ± Confidence Limit at 5% level of significance

[b] Estimated on a scale from 0 (no nodules) to 4 (abundant nodulation)

TABLE 10

Mycorrhizal infectivity of the test soil No. 11 and additional effects of inoculation with *Glomus mosseae* in the formation of and responses to microbial symbiosis with *Hedysarum coronarium* plants grown in the glasshouse. After Azcón-Aguilar et al., 1982.

Parameter	Sterile soil		Unsterile soil	
	Control	Mycorrhiza	Control	Mycorrhiza
% VA infection[a]	0	3 ±2	52 ±10	60 ±6
Nodulation[b]	3	4	3	4
Shoot weight(g)[a]	4.1±0.3	4.2±0.7	5.0±1.0	4.9±1.0
Root weight(g)[a]	2.1±0.2	2.1±0.2	2.3±0.4	2.3±0.8

[a] Mean value ± Confidence Limit at 5% level of significance

[b] Estimated on a scale from 0 (no nodules) to 4 (abundant nodulation)

In contrast, G. mosseae was not successfully introduced in soil no. 11, probably because of the high level of available phosphate in this soil.

TABLE 11

Growth increases in *Hedysarum coronarium* produced by native or inoculated VA endophytes. After Azcón-Aguilar et al., 1982.

Effect of VA endophytes	Percent growth (shoot dry weight) increase	
	Soil No. 10	Soil No. 11
1) Native (C unst. vs C st.)[a]	19.2	23.1
2) Inoculated (M st. vs C st.)[a]	82.7	3.7
Native + Inoculated:		
Calculated (1 + 2)	101.9	26.8
Experimental (M unst. vs C st.)[a]	93.7	21.2

[a] C = Control, M = Mycorrhiza inoculated, unst. = unsterile soil, st. = sterile soil

The growth increases produced by native and introduced endophytes were calculated from the data in the above two Tables and are summarized in Table 11. The effect of native or introduced endophytes and the type of their interactions on Medicago were studied in a fashion similar to that described above. The results suggest that in soil no. 10, the indigenous and introduced endophytes together enhanced plant growth, although the indigenous strains were of low infectivity and effectiveness. This agrees with several reports suggesting that naturally occurring symbionts are not the most effective. In soil no. 11 no growth response to G. mosseae inoculation was realized. However, the indigenous endophytes were highly infective and seemed more adapted to the environmental conditions of the soil, mainly to its high P content, but they did not produce significant growth increases. Hence, soil no. 11 was not selected for field inoculation trials.

Tables 12 and 13 show the effect of chemical (NPK) and biological treatment (Glomus mosseae + Rhizobium sp. specific for Hedysarum coronarium) on the growth and nutrition of this legume in the field.

TABLE 12

Effect of chemical and biological fertilizers on mineral composition of leaves of *Hedysarum coronarium* growing in the field soil No. 10 After Azcón-Aguilar et al., 1982.

Nutrient	Leaf content (% Dry Matter)[a]		
	Control (Nil)	Chemical (NPK)	Biological (RM)
N	1.81 ± 0.32	2.67 ± 0.29	3.30 ± 0.16
P	0.20 ± 0.03	0.25 ± 0.02	0.26 ± 0.01
K	1.00 ± 0.07	1.27 ± 0.17	1.37 ± 0.16
Ca	2.28 ± 0.22	2.35 ± 0.25	2.23 ± 0.16
Mg	0.70 ± 0.05	0.64 ± 0.05	0.68 ± 0.07

[a] Mean value ± Confidence Limit at 5% level of significance

TABLE 13

Effect of chemical and biological fertilizers on the yield and nutrition of *Hedysarum coronarium* grown in the field soil No. 10. After Azcón-Aguilar et al., 1982.

Treatments (fertilizers)	Shoot dry weight[a] (g)	Shoot content (% Dry Matter)[a] N	P
Control (Nil)	805 ± 30	2.40 ± 0.26	0.23 ± 0.03
Chemical (NPK)	903 ± 44	2.58 ± 0.12	0.23 ± 0.02
Biological (RM)	1151 ± 102	3.64 ± 0.13	0.30 ± 0.01

[a] Mean value ± Confidence Limit at 5% level of significance

Table 12 shows the results of the leaf analysis carried out for evaluating the nutritional status of growing plants. These results indicate that the foliar content of N, P and K was highest in plants inoculated with Rhizobium-mycorrhizal fungus G. mosseae. A growth response to microbial inoculation was also evident at harvest (Table 13).

The results of the field trial show that the mycorrhizal inocula used were quite efficient at helping the legume Hedysarum coronarium to obtain its nutrients from phosphate-fixing agricultural soils.

Our results indicate that mycorrhizal inoculation improves the N content in leguminous plants, but they do not indicate whether the extra N is due to increased N_2-fixation or increase N uptake from soil. The use of ^{15}N to label soil assimilable nitrogen is a suitable way to quantitatively estimate the amount of N in the legume which is coming from symbiotic dinitrogen fixation under field conditions. This will be the subject of our future research.

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CARBON AND NITROGEN TRANSFER IN MYCORRHIZAL LEGUMINOUS PLANT SYSTEM

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Abstract

A suitable double compartment growth chamber, which allowed plants to be labelled with ^{14}C and ^{15}N was used to measure belowground respiration, distribution of photosynthate within different plant parts as well as the extent of dinitrogen fixation in mycorrhizal and non-mycorrhizal Vicia faba. The results showed that mycorrhizal infection did not stimulate dinitrogen fixation in Vicia faba. Also, mycorrhizal infection resulted in a higher photosynthate demand for growth and nitrogen fixation than in non-mycorrhizal plants. The extra drain exerted on the photosynthate supply to plant roots by the mycorrhizal fungus did not however, result in lowered dry matter yield. It is therefore likely that mycorrhizal plants have a mechanism for compensating for this assimilate drain, and this needs to be investigated further.

INTRODUCTION

Carbon transfer within nodulated leguminous plants, such as Vicia sp. (Lawrie and Wheeler 1975) and Pisum sp. (Minchin and Pate 1973) has been well documented. Nitrogen transfer in nodulated leguminous plants has also been well documented (Oghoghorie and Pate 1972, Pate 1975). The association between Rhizobium sp. and nodulated leguminous plants is symbiotic and therefore, in addition to adequate P-nutrition, nodule formation and N_2 fixation are dependent upon the availability of photosynthate to the Rhizobium bacteriods within the nodules.

Mycorrhizal plants, either ectomycorrhizal or endomycorrhizal, are more efficient than non-mycorrhizal plants in exploring phosphate from soil (Mosse 1973, Bowen et al 1975, Azcon et al 1976). Therefore, mycorrhiza are able to provide more phosphate to mycorrhizal plants compared to non-mycorrhizal ones. VA mycorrhizal plants grown in infertile soils have also been found to be more efficient in the uptake

of other nutrients, e.g., Zn, Ca, K, Mn and Cu (see reviews by Smith 1974, Bowen 1980). Furthermore, there are claims that VA mycorrhiza stimulate nodulation of legumes and hence increase rate of N_2-fixation by legumes (Crush 1974, 1976, Daft and El-Giahmi 1974, 1976; Mosse et al. 1976; Azcon-G. de Aguilar and Barea 1978). The stimulation of nodulation may probably be due to the ability of mycorrhizal fungal hyphae to provide adequate phosphorus supply to the plant. Spores of vesicular arbuscular (VA) mycorrhiza, an endomycorrhiza, are abundant in soils and most agricultural crops are invariably infected by one or more species of VA mycorrhiza. VA mycorrhiza, unlike ectomycorrhiza which infect root cortical cells intercellularly and form fungal mantle on root surface (Harley 1969), infect cortical cells of host plant roots both intercellularly and intracellularly with the formation of vesicles and arbuscules at the ends of fungal hyphae (Harley, 1969).

Since VA mycorrhizal fungi also live within the plant roots and utilise plant photosynthate, questions have been raised as to whether VA mycorrhiza influences the rate of phtosynthesis by legumes and also the dinitrogen fixation from the atmosphere. Pang and Paul (1980) showed that VA mycorrhiza indeed affected the rate of photosynthesis of 6 1/2 week old Vicia faba, but had no effect on the rate of N_2-fixation under controlled environment. Paul and Kucey (1981) confirmed these earlier conclusions by Pang and Paul (1980) with 5 week old Vicia faba. Other than these two publications, no other literature is available on the subject of carbon and nitrogen transfer in this three component system: the leguminous plant, the VA mycorrhizal fungus and the dinitrogen fixing bacterium. A brief review of the publication by Pang and Paul (1980) at this meeting will show the importance of stable and radioactive isotopes in studying carbon and nitrogen transfer between legumes, Rhizobium, and VA mycorrhizal fungi.

MATERIALS AND METHODS

A soil of low phosphate level (6 ppm-P) was chosen for the experiment and the test plant was Vicia faba. The plants were inoculated with (a) Rhizobium sp. (Nitragen Co., Milwaukee, Wisconsin) and (b) a combination of Rhizobium sp. and Glomus mosseae spores (propagated in sand culture from isolates collected in a Vicia faba field; host plant was Vicia faba).

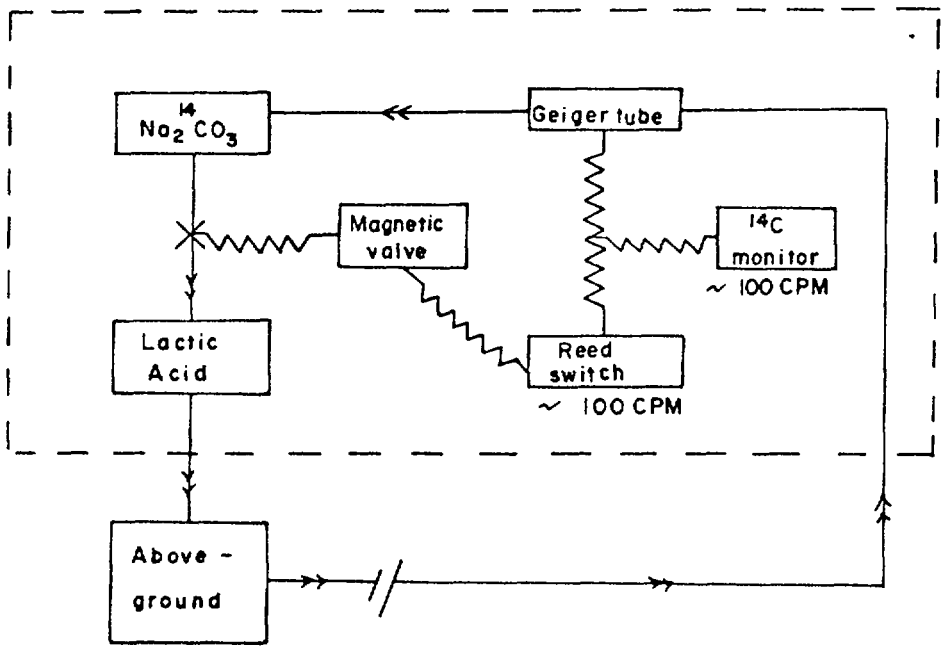

Fig. 1. $^{14}CO_2$ source

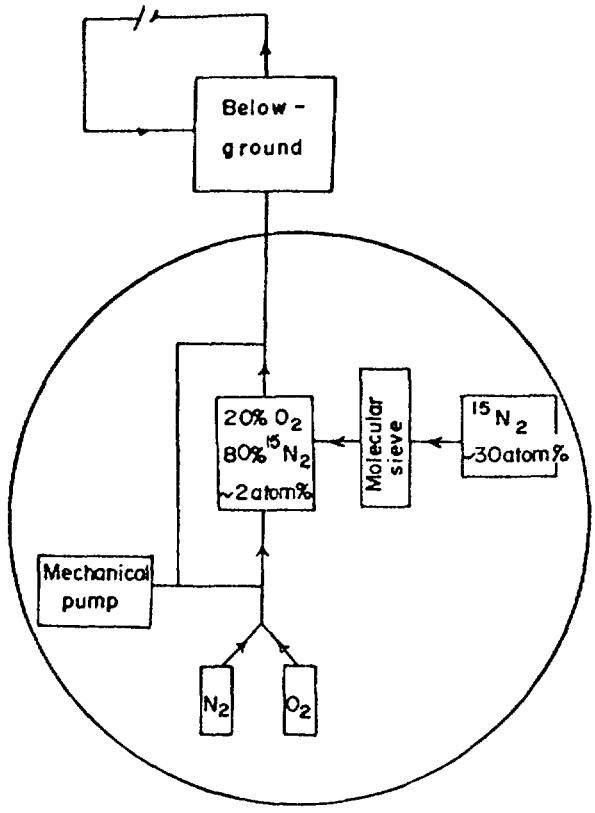

Fig. 2. $^{15}N_2$ source

A two-compartment system, consisting of a carbon-14 compartment (Fig. 1) and a nitrogen-15 compartment (Fig. 2), as well as a growth chamber system (Fig. 3) were designed so that plants could be labelled with ^{15}N during N_2-fixation by belowground plant parts and with ^{14}C during photosynthesis by aboveground plant materials. Cross-over of the

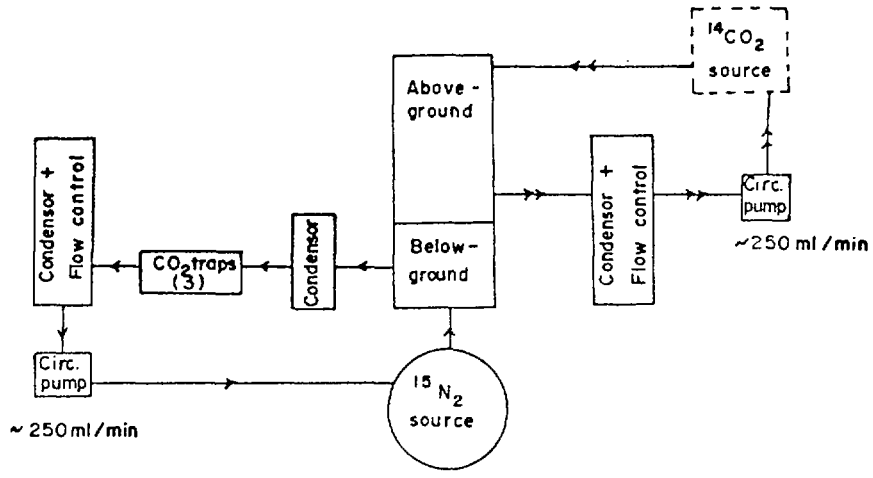

Fig. 3 Flow diagram of the two-compartment labelling growth chamber

gaseous atmosphere between the aboveground and belowground compartments did not occur with the use of Terostat VII sealant (Teroson GmbH, Germany) which is not toxic to plants. The 6 1/2 week old Vicia faba plants were labelled for 48 hours, after which $^{15}N_2$ and $^{14}CO_2$ were replaced by non-labelled N_2 and CO_2. Plants were harvested after a further 4 1/2 days growth in the chamber. $^{14}CO_2$ from belowground was trapped continuously (Fig. 3) and analysed for concentration and radioactivity. At harvest, plants were separated into different plant parts, and roots were examined for percentage mycorrhiza infection after staining with 0.01% acid fuchsin. Results were reported along with the standard errors and the average of six replicates.

RESULTS AND DISCUSSION

Different species of wild legumes were collected from a Saskatchewan virgin range land and examined for VA mycorrhiza infection. Their percentage infection of roots ranged from 13 to 83% (Table 1). The N_2

Table 1 Mean percentage of vesicular-arbuscular mycorrhizal infection in various prairie legumes collected from Cranberry Flats, Saskatchewan in May, 1976

Species	% VA infection
Astragalus tenellus	26 ± 3
Oxytropis sevicea	43 ± 10
Psoralea lanceolata	50 ± 14
Thermopsis rhombifolia	83 ± 6
Vicia americana	13 ± 6

fixation potential of these plants were also assayed by the acetylene reduction technique. Since all wild legumes collected were VA mycorrhizal, it was not possible to compare the N_2-fixation rate of the same legume species as affected by VA mycorrhizal infection.

At the early stage of our laboratory experiment with Vicia faba, no significant difference in $^{14}CO_2$ specifc activity of the mycorrhizal and non-mycorrhizal Vicia faba was detected (Fig. 4). However, the differences were quite significant at the latter part of the experiment (Fig. 4). This may be due to higher respiration of the VA mycorrhiza infected plant. This hypothesis was confirmed when $^{14}CO_2$ respired from mycorrhizal and non-mycorrhizal plants were calculated (Fig. 5).

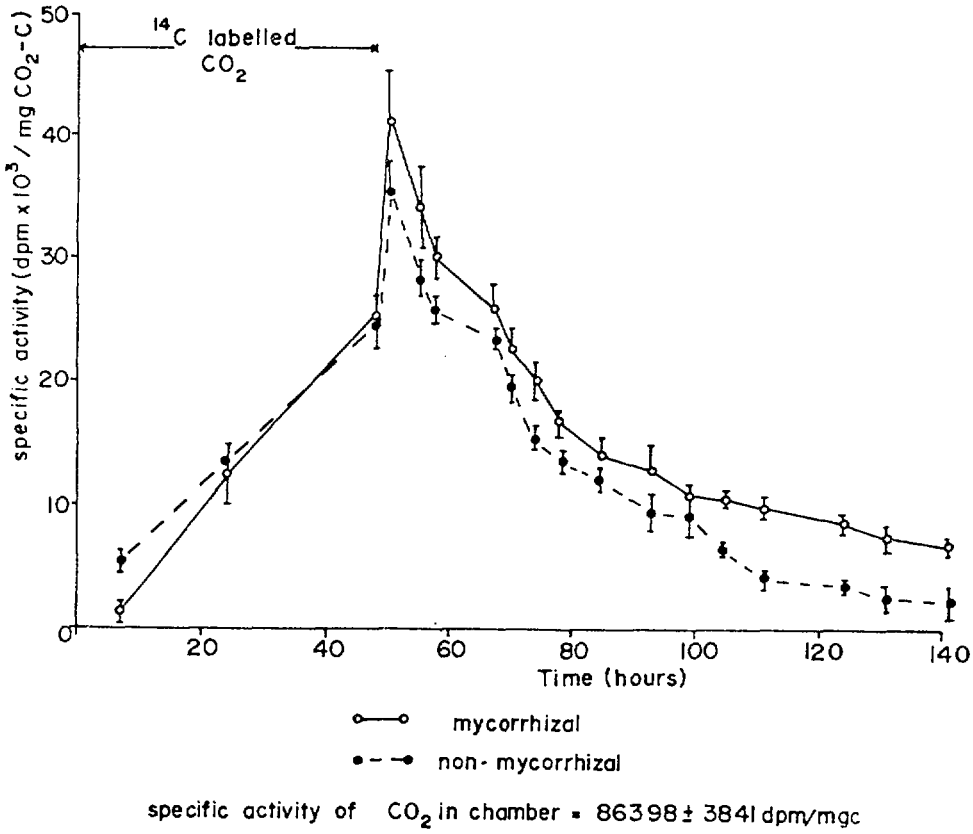

Fig.4. Relative specific activity of belowground respiration of mycorrhizal and non-mycorrhizal fababean (6½ weeks)

The cumulative quantity of $^{14}CO_2$ respired from belowground of mycorrhizal plants at the termination of the experiment was approximately twice that of the non-mycorrhizal one. With such a large difference in belowground respiration, differences in the yield of plant dry matter

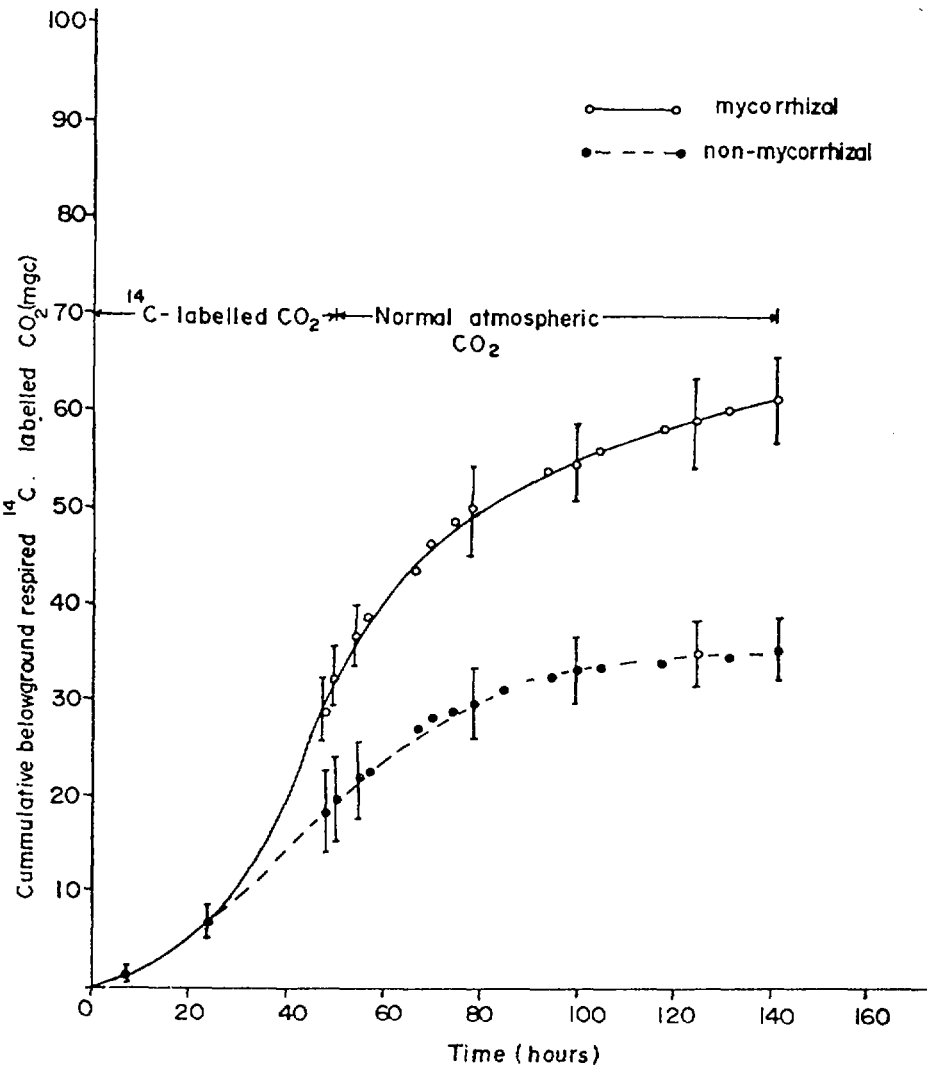

Fig. 5. Cummulative belowground ^{14}C labelled CO_2 of mycorrhizal and non-mycorrhizal fababean

between the two should be expected. However, there was no significant yield difference between the VA mycorrhiza infected and non-infected plants (Table 2). The yield data therefore indicated that although the presence of fungi increased root respiration, growth of Vicia faba was not affected under our experimental conditions with light intensity of 8000-10500 lx during the 18 hours photoperiod at 20C. Percent distributuion of ^{14}C (Fig. 6) and ^{15}N (Fig. 7) in plant parts showed that there were no significant differences between the mycorrhizal and the non-mycorrhizal plants, with the exception of the belowground (root + fungi + bacteria) respired $^{14}CO_2$ (Fig. 5). In mycorrhizal plants, 30% of the total net photosynthate was accounted for by belowground respiration, and in non-mycorrhizal plants, 18% was accounted for. The mycorrhizal Vicia faba was calculated to fix 2.3 ug N_2-N.mg^{-1} C

photosynthesized during the 48-hour ^{14}C–^{15}N labelling period and the non-mycorrhizal plant fixed 3.2 ug N_2–N . mg^{-1}C photosynthesized during the same period.

Table 2 Yield of fababean plant material (6½ week old).

	Yield (g)	
	Mycorrhizal	Non-mycorrhizal
Leaves	0.73 ± 0.04	0.82 ± 0.07
Stems	0.56 ± 0.07	0.66 ± 0.04
Roots + nodules	1.11 ± 0.07	1.22 ± 0.10
Total	2.40 ± 0.07	2.70 ± 0.19

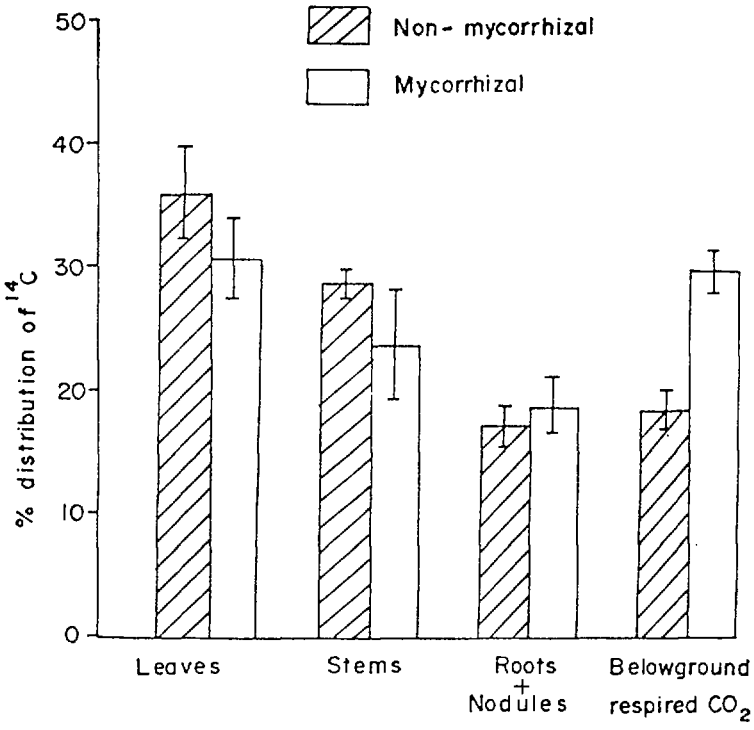

Fig. 6. Percent distribution of ^{14}C in plant parts and belowground respired CO_2 of 6½-week old fababean after 48 hours labelling with $^{14}CO_2$

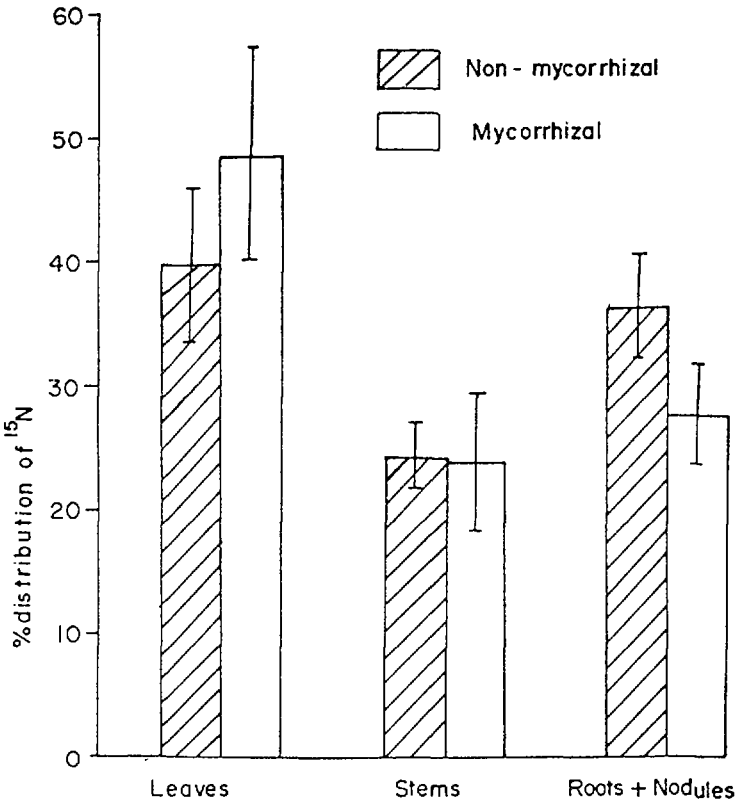

Fig. 7. Percent distribution of ^{15}N in plant parts of 6½-week old fababean after 48 hours labelling with $^{15}N_2$

CONCLUSIONS

(1) A two compartment ^{14}C-^{15}N labelling growth chamber was found to be effective in measuring the effect of VA mycorrhizae on belowground respiration, distribution of photosynthate and symbiotic dinitrogen fixation of a three component system of PLANT-MYCORRHIZA-RHIZOBIUM.

(2) There was no significant contribution of VA mycorrhiza (G. mosseae) to dinitrogen fixation by Vicia faba 'Ackerperle'. Since our data contradict results of other investigators (Crush 1974, 1976,; Daft and El-Gianemi 1974, 1976; Mosse et al 1976; Azcon de Aguilar and Barea 1976) who had demonstrated that VA mycorrhiza stimulated nodule formation and N_2-fixation of legumes, our results may serve as an indication that host plant-Rhizobium-VA mycorrhiza specificity exists amongst the three components to provide positive stimulation.

(3) Under some conditions, certainly of ours, VA mycorrhizal infection might have harmful effects on plant growth in the long run. The reason is because of the inefficient metabolism of photosynthate by VA mycorrhiza. The VA mycorrhiza continuously obtained carbon source from plant and consequently induced the plant to do more work during photosynthesis to allow the plant to provide enough energy for the growth of the VA mycorrhizal fungi and also the energy consuming process of nitrogen fixation by Rhizobium bacteriods.

(4) Leguminous plants were able to compensate for the increased photosynthate needs of its microbial partners because the mycorrhizal plants evolved considerably more CO_2 compared to non-mycorrhizal plants without a deleterious effect on plant growth.

(5) Further experiments are required to gain a better understanding of how these partners react under natural conditions.

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THE FATE OF ASSIMILATES AND THE COST OF THE VESICULAR-ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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Abstract

A susccessful vesicular-arbuscular mycorrhizal (VAM) infection results in an assimilate drain from the plant to the fungus for its growth and maintenance. This drain could therefore affect the growth of mycorrhizal plants. Under many situations (especially in nutrient-poor soils) however, the benefits obviously far outweigh the assimilate cost of the symbiosis, and growth responses have been reported in such cases. The evidence available suggests that mycorrhizal plants may compensate for the larger sink in the root through greater sustained photosynthesis during the day than non-mycorrhizal plants. On the other hand, there are data which show that depression of plant growth can result from VAM formation in some cases. Such depressions, however, appear to be the exception rather than the rule even for high fertility situations and may be affected considerably by the host-fungus combination and the extent of the infection in the root.

INTRODUCTION

It is a common saying that there is no such thing as a free meal and it is therefore appropriate to ask such questions as 'What is the cost of the v.a.m.?' and "Can situations occur in which the association is to the detriment of plants?" In this contribution I indicate some of the aspects of such questions. It is an area which until now has attracted only spasmodic attention although it is now an area of increasing study.

First, it has to be said that under nutrient deficiency conditions (or other conditions in which a mycorrhizal response is obtained) obviously the benefits far outweigh the assimilate cost of the symbiosis.

The question needs to be redefined in several ways. The types of pertinent questions are:

1. Does a plant which is mycorrhizal grow as well as a non-mycorrhizal plant with the same nutrient (e.g. phosphate) content. That is, is the assimilate going to the fungus, both for growth and maintenance, an appreciable drain?

2. Does a fertility situation arise in which the mycorrhizas may be a liability?

and 3. Are there some v.a.m. - plant combinations in which the fungus is detrimental to growth under most conditions?

Growth Depressions

Two general types of growth depressions may be recognized, transitory and prolonged.

(1) Transitory growth depressions These are usually small and last for a few weeks after plant emergence, i.e. until fungus growth into soil occurs and the plant starts to benefit from the increased supply of nutrients. This may take as long as 10 weeks (Smith and Daft, 1977) but is often only three to four weeks. It represents an assimilate drain to the fungus for its growth and maintenance and is likely to vary with the extent of the fungus development within the root. This will be quite variable between v.a.e. (see Bowen, pp. 85-102).

(2) Prolonged (? permanent) depressions of growth In laboratory studies, substantial growth depressions following inoculation with v.a.e. have sometimes been recorded. For example Mosse (1973) found 40 percent depression of onion growth when high levels of soluble phosphate were applied; Sparling and Tinker (1978) found reduced growth of grasses after 43 weeks after inoculation on soils which had been γ-irradiated and they attributed the depression to release of nutrients by the irradiation so that the mycorrhizas were superfluous;

Hall (1978) found v.a.m. increased clover growth at 12 kg P ha^{-1} but decreased it at 36kg P ha^{-1}, and Crush (1976) found decreases in growth of mycorrhizal *Medicago sativa* and *Trifolium hybridum* of 3.5 - 16.2 per cent, the effect being marked at high fertilities.

Possibilities for decreased growth due to v.a.m.

The data above suggest reduction of growth can take place in some circumstances but these seem (so far) to be related to high fertility levels in soil.

There appear to be four general possibilities for growth depressions by v.a.m.

(1) Direct pathogenic effects. This raises the question of definition of a pathogen in physiological terms. It may be due to the production of pathogenic substance (so far undetected in v.a.m.), the killing of roots (so far undetected), blocking xylem vessels as in some plant pathogens (so far undetected in v.a.m.) or the direction of an inordinate amount of assimilate to the fungus at the expense of the plant, i.e. an assimilate drain (see below). Hendrix and Modjo (1981) have claimed a 60-70 percent reduction in tobacco following inoculation with a strain of *Glomus macrocarpus*.

(2) A disease vector. Growth depressions could be due to the fungus acting as a vector of plant disease. So far this possibility has not been adequately explored with v.a.m. It does pose problems of transmission of the presumed disease, e.g. virus, to the higher plant. While the arbuscule of the v.a.m. is still intact, there is no mixing of the cytoplasm of the fungus and the plant; however on arbuscule degeneration (4-11 days after formation) release by the fungus could occur, bringing the presumed virus in close proximity to the plant plasmalemma. So far, there is no electron microscope evidence of virus-like inclusions in v.a.e. but they have rarely been looked for.

(3) Phosphate toxicity Mosse (1973) considered the depression of growth recorded above, was phosphate toxicity due to the highly efficient absorption of phosphate from soil by v.a.m., in this case soils with a high phosphate addition. This may occur on occasions but as Tinker (1975) pointed out, growth depressions can occur in situations in which phosphorus toxicity was not a possibility.

(4) Assimilate drain by the fungus. - Note that most of the depression in yield recorded so far are in relatively fertile soil. While the 'conventional wisdom' is that high phosphate first restricts infection and then prevents it, often unrealistically high phosphate levels are needed to achieve this. Further, as Table 1 of my 'epidemiological' paper in this meeting shows, there is considerable variation in fungus sensitivity to this. It is highly likely that many fertilized situations could arise in which the plant is heavily infected by mycorrhizal fungi but derives no benefit from it. This would give the possibility for yield reduction by an assimilate drain.

The size of the fungal sink

There is little data on the variation in size of the fungal sink. Pang and Paul (1980) found with matched mycorrhizal and non-mycorrhizal *Vicia faba*, that non-mycorrhizal plants transferred 37 percent of fixed carbon below ground and the mycorrhizal ones transferred 47 percent, the extra ten percent being lost mainly in respiration.

There is little doubt that the fungal component accumulates a higher average level of assimilate than root cells for Bevege *et al.* (1975) found ^{14}C assimilate levels in external hyphae of mycorrhizal clover and *Araucaria* to be four times that of the roots (plant plus internal hyphae) they were taken from. However it must be remembered that most root data is the average of many types of cells, e.g. young growing apices, mature cells and non-accumulating vascular tissues.

Bowen (1978) has suggested mycorrhizas and growing tips of roots are potentially competitive sinks which, in perennials, may be out of phase in their development, and thus rarely compete.

The fates of assimilates going to the fungal hyphae are discussed by Bevege *et al.* (1975), but briefly they are used in growth, in storage pools (organic acids, lipids, glycogen), and in respiration. Estimates of hyphae external to the root are few and vary from 1 percent to 6 percent of the weight of the plant (usually 1-2 percent). There is great variation in weight of the fungus within the root but again there is little good data on this. Tinker (1975) considered the volume of the fungus in well infected onion roots was only 1-2 percent of the root volume. However, much heavier infections than this are possible. For example, calculations from the data of Hepper (1977) shows some 17 percent of the root weight can be fungal material.

Respiration from mycorrhizal roots is high. Thus Paul and Kucey (1981) calculated that of all assimilate going to the uninfected root of faba bean, 63 percent was respired but the corresponding figure for mycorrhizal roots was 75 percent. Sanders, Martin and Bowen (unpublished) found respiration of mycorrhizal onion roots was 0.32 mg. C g^{-1} f.w. day^{-1}, i.e. six times that of corresponding non-mycorrhizal roots in which respiration was 0.05 mg C g^{-1} f.w. day^{-1}.

Therefore, the fungus could be an appreciable assimilate drain under certain conditions. I suggest there may be four general situations in which growth reduction <u>via</u> the fungus being an assimilate sink would be most likely.

(1) 'Excessive' development of the fungus in the host - This may be from 1 percent to 17 percent of the root dry weight and is a function of the fungus-plant combination.

(2) Mycorrhizas developing in situations in which they are superfluous, i.e. in highly fertile soils. Most cases so far of plant growth

TABLE 6

Variation in spore germination between *G. mosseae* spores of different origin (Daniels and Duff. 1978)

Source	Spore Germination Percent	
	Water Agar	Soil
Washington	76	64
Bend	0	35
Corvallis	0	38
Illinois	0	40

Fungal growth through soil to the root There has been no good experimental study of the distance germinated spores of v.a.e. may grow through soil to the root. There appears to be no strong root stimulus in this (Powell 1976) and it has been suggested that all other factors being equal, the distance is proportional to the size of the spore (which represents the reserves it has to draw on for growth) (Bowen, 1979). However, the size of the spore is not the only consideration for if the germinated spore does not encounter a root after a period of growth it can become dormant again.

The distance a spore 'needs' to grow through, to encounter a root is affected by the rooting intensity of the species; and there is almost certainly a relationship between the number of propagules, propagule size, rooting intensity and infection level (by Bowen 1979, 1980,). Daniels and Menge (1978) state that J. Ferguson (per. comm.) has demonstrated that larger spore densities are required of small-spored v.a. mycorrhizal species for good infection.

It is probably during growth through a soil, a non-selective environment harbouring a wide range of soil organisms, that the fungus is most vulnerable.

depression indicated above fall into this category. Excessive development of the fungus will exacerbate this effect.

(3) Where photosynthesis is low, e.g. low light intensity, but where infection has occurred (perhaps previously). This is probably of little agricultural moment but may be, in understorey plants in a mixed ecosystem.

and

(4) Where infection develops well but where fungus strain or soil conditions are such that little mycelial growth occurs in soil. I believe this possibility warrants much more study. It is a situation where the plant pays the cost and gets no benefit. That is, in the terms mentioned earlier, the price of the 'dinner' has been paid but it did not materialize.

Can the plant compensate?

Most of the situations I have discussed where depressions have been recorded have been in high fertility situations. However, even in the high fertility soils, do such depressions represent the 'norm' or do they represent very special situations? Certainly much more testing of this point is necessary. The existence of a larger sink in the root does not necessarily mean this is at the expense of top growth (Bowen, 1978): if feedback from various sinks controls photosynthesis, a larger sink in the root could lead to compensation by the plant by more sustained photosynthesis during the day. Indeed, Table 1 indicates that this may happen.

TABLE 1

Daily photosynthesis of matched mycorrhizal and Non-mycorrhizal faba beans

Authority	Photosynthesis ($mg\ C\ g^{-1}\ day^{-1}$) Non Mycorrhizal	Mycorrhizal
Pang and Paul (1980)	36	46
Paul and Kucey (1980)	18.3	20.2

Conclusion

In summing up the evidence, drawn mainly (if not entirely) from pot studies, one must conclude that depressions of growth can result from v.a.m. formation in some cases. These appear to occur with relatively high fertilizer applications and thus represent a special situation. Furthermore, what has been reported under these circumstances may be the exception rather than the rule even for high fertility situations and may be affected considerably by the host-fungus combination and the extent of the infection in the root. It is important to clarify this and to recognize the plant-fungus combinations which are most prone to such depression of growth. It may be that application of high levels of fertilizer to maximize production can be counter-productive.

The energy 'cost' of the symbiosis is still open to question. There is evidence that the plant may compensate for assimilate drain to the fungus by increasing photosynthesis. If certain mycorrhizal fungi can stimulate cytokinin production as suggested by Allen *et al.* (1980), it may well affect photosynthesis. However, it is possible that only certain fungus-plant combinations can compensate in this way for an assimilate drain. These are areas requiring more detailed

study, not only under controlled laboratory conditions but also in field studies. The methods developed by Martin (1977) to investigate carbon balance in plants are eminently suitable for such studies, such methods depend on the assimilation of $^{14}C\ O_2$ by the plant and are the best way to approach the problem.

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FIELD INOCULATION PROCEDURES WITH VASICULAR-ARBUSCULAR MYCORRHIZA

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Introduction

The goal of field studies with vesicular-arbuscular mycorrhiza (VAM) is to improve crop yield by establishing vigorous VAM infections that will substantially enhance the uptake of phosphate. This objective is usually approached by introducing efficient inoculum into a field crop early enough and at a high enough rate to affect plant growth. Some consideration should also be given to ways of manipulating the native mycorrhizas because satisfactory field inoculation procedures for large-scale application are not yet well developed and the indigenous soil populations of VAM fungi are sometimes symbiotically efficient but too sparse to be effective. Thus mixed cropping systems and crop rotations that include strongly mycorrhizal plant species may well prove to be useful for building up the native VAM population. However, in this article, we are concerned primarily with the methodology of field inoculation, using VAM endophytes that have already been cultured on stock plants and tested for their symbiotic potential and soil/plant preferences.

Before the numerous steps that should be followed in field inoculation studies are outlined, a few of the 30 or so published field experiments (11) will be mentioned to illustrate some of the inoculation procedures already used. These experiments fall fairly distinctly into two categories: those done in fumigated soil and those done in unsterile soil. Considering the vast number of

investigations made on VA mycorrhizal plants grown in pots in the glasshouse, surprisingly few field experiments have been done, particularly under realistic agricultural conditions. Probably some field experiments have not been reported due to a lack of growth response to VA mycorrhiza under the conditions studied.

Field experiments in fumigated soils

Most of these experiments have been done in areas treated with fumigants, especially methyl bromide and chloropicrin, to control root diseases. The fumigation treatments eliminate not only pathogens but also the indigenous mycorrhizal fungi. Generally fumigation can lead to poorer crop growth unless suitable VAM endophytes or phosphate fertilizer are introduced in instances where the crop is one that is especially dependent on mycorrhiza. Nearly all VAM studies in fumigated soils have been done in the United States, largely with woody species in plant nurseries.

Citrus is probably the crop most studied in VAM inoculation experiments in fumigated soils. The reversal of citrus stunting by mycorrhizal infection in nursery soils treated with methyl bromide in California and Illinois was shown some ten years ago (8). Inoculation of field plots was achieved by placing soil and roots from a pot culture of sudangrass infected with Glomus mosseae beneath the seeds sown in furrows. In later experiments (3) field inoculation was done by coating seeds with inoculum of Glomus fasciculatus. The inoculum was extracted from stock plant cultures by wet-sieving and stuck to the seeds with methyl cellulose. In both studies mycorrhizal inoculation improved plant growth considerably. This reflected the occasional non-stunted seedlings seen in fumigated nurseries which were found to have developed mycorrhizal infection in situ. In Florida, citrus seedlings in

fumigated soil showed marked growth responses to inoculation with Glomus etunicatus but not with G.mosseae (Nemec, pers.comm.), even though both inocula had been standardised in terms of numbers of spores added. In earlier tests both Glomus species had stimulated growth at some locations when seedlings were pre-inoculated or inoculum was added to trenches beneath the seeds (13). Pre-inoculation of citrus seedlings in bulk containers before transplanting to the nursery now seems the best method for achieving the required infection.

Peach was responsive to VAM inoculation in methyl bromide-treated zinc-deficient soil in a commercial nursery in California (9). Placing inoculum of Glomus fasciculatus in the form of soil and roots from sudangrass pot cultures below field-sown seeds (5g/seed) increased seedling vigour and decreased nutrient deficiency symptoms, especially for zinc.

Soybeans can also benefit from VAM inoculation in the field where the soil is fumigated, although growth responses may be less than with citrus and peach. Very clearcut results were obtained in North Carolina (15) for plants grown in large fibre-glass bins buried in soil. The soil in the bins was treated with methyl bromide and chloropicrin, then half were re-infected with VA mycorrhiza by adding a portion of soil containing many VAM spores. Adding phosphate fertilizer nearly doubled plant growth in the non-mycorrhizal set, whereas the mycorrhizal plants grew just as well irrespective of fertilizer. Soybeans were also tested in small field plots fumigated with methyl bromide in Florida (16). Mycorrhizal inoculum was placed in peat pellets in which the seeds were germinated and the young seedlings transplanted to the field. Mycorrhiza did not increase growth of a non-nodulating isoline but increased the yield of a nodulating isoline. Mycorrhiza-

enhanced nitrogen nutrition from increased nitrogen fixation rather than nitrogen uptake was implied.

Peas responded to mycorrhizal inoculation in a field in south-east England where treatment with telone and dazomet to kill virus-transmitting nematodes also killed the indigenous mycorrhizal fungi. Inoculum from stock cultures was placed below seeds of semi-leafless peas sown in rows and increased growth over the uninfected controls about 2½-fold (12). This paralleled earlier observations of plants in unsterile soil which were heavily infected by the native VAM fungi and approximately twice as tall as uninfected plants in sterilized soil. However, in a subsequent experiment where leafless pea seedlings were transplanted in balls of inoculum at a much denser planting rate than before, responses to VAM or phosphate were much smaller.

Field experiments in unsterile soils

In soils that have not been treated with sterilants, potential crop responses to inoculation will be superimposed on any growth effects produced by the indigenous mycorrhizal population. Therefore field experiments in this category have tended to involve soils where the native VAM endophytes appear sparse or not very effective. There is also an emphasis on low-phosphate, agriculturally marginal soils.

Maize and wheat grew better when inoculated with VAM fungi in very infertile soil in Pakistan (7). Seedlings were pre-inoculated or uninoculated (controls) and transplanted to the field by hand. Although it is difficult to relate this procedure to standard sowing densities and methods, it does show that benefits from inoculation are possible in specific circumstances.

Pre-inoculated plants built up mycorrhizal infection much faster and to a much higher level than the controls which relied on the indigenous VAM fungi.

Mycorrhizal inoculation increased potato yields in field plots, but only in the absence of phosphate fertilizer (1). The experimental site was a field which had been fallow for two years and contained a small indigenous mycorrhizal population such that VAM infection developed only slowly in the uninoculated plants. Inoculum was supplied to the seed furrows as crude field soil from adjacent barley plots which contained many VAM spores.

White clover has attracted much attention in upland agriculture because it contributes nitrogen and good quality herbage to hill grasslands. Mycorrhizal inoculation has been employed to try and improve clover establishment in these areas, especially in New Zealand (^{14}) and the U.K. (4). In the mountains of mid-Wales, inoculation with Glomus mosseae and G.fasciculatus doubled clover growth and greatly increased its nodulation and survival (6). The clover seedlings had been pre-inoculated before transplanting to field plots containing inefficient native endophytes and given standard dressings of phosphate fertilizer. Results from furrow inoculation of seeds were more variable. In other infertile sites plants grew well irrespective of inoculation because of a very effective native endophyte population.

Methodology

A more efficient methodology for mycorrhizal studies under field conditions needs to be developed if VA mycorrhiza is to be harnessed in large-scale crop production. Furthermore, no mycorrhizal programme can function effectively if the basic methods are not properly understood or carried out. According

to what we know from fundamental studies on VA mycorrhiza and from the relatively few applied experiments, the procedures for a field inoculation programme can be outlined as a series of seven steps.

1. Initial considerations

There are four major factors that determine the potential benefits of mycorrhizal inoculation in the field and which must be considered in the initial planning stages. They are :

(i) The native endophytes

(ii) Soil fertility

(iii) Crop species

(iv) Cultivation practices

These aspects are discussed further in the paper "Effects of plant species, soil and environmental factors on VA mycorrhizal infection and nutrient uptake." (See pp. 29-47).

2. Establishment of a culture collection

The best possible strains of VAM fungi should be obtained and maintained on stock plants as follows :

(i) Isolate local endophytes by wet-sieving, centrifugation, baiting, etc. Different morphological species and different physiological strains should be recovered from as many sites as possible.

(ii) Obtain two or three internationally known isolates considered likely to be effective under the specific soil and environmental conditions prevailing locally.

(iii) Screen endophytes on important, mycorrhiza-responsive crops grown in representative local soils in pots.

(iv) Select the most promising ones for the culture collection.

(v) Maintain on suitable stock plants.

3. Choice of endophyte(s) for field inoculum

This decision must take into account the soil type and crop species to be tested. A good screening programme is vital to provide information on differences between VAM endophytes in relation to range of tolerance and suitability in diverse situations. Potentially successful endophytes may then be selected from the stock culture collection for use in the field. Additional endophytes could be obtained from the individual test sites to which they should be well adapted and hence potentially effective. It might be possible to speed up strain trials to less than half the customary 10 weeks or so needed to measure growth responses by using isotopes like ^{32}P. These could indicate which strains begin to function quickly in taking up P and in stimulating most P uptake within a defined, brief period.

An alternative approach is to use a mixed inoculum and let the crop and soil select the most appropriate endophyte(s) *in situ*. It may be difficult to determine whether mixed infections are less or more effective than single infections, but such an approach avoids "putting all your eggs in one basket" if there is no obviously superior single strain for the particular situation.

4. Production of inoculum

For this purpose pot cultures of VAM endophytes are needed on stock plants which are :

(i) readily maintained under local glasshouse conditions,

(ii) suitable hosts for the endophytes selected,

(iii) unrelated to the test crop, to reduce the risk of contamination by host-specific pathogens

(iv) able to be grown in large numbers to provide enough inoculum for use on a field scale.

Menge, Lembright and Johnson (10) described a useful procedure for producing VAM inoculum to infect nursery-grown citrus. Endophytes are first isolated from citrus soils by extracting spores or infected roots and inoculating these onto sudangrass seedlings. Other hosts could be substituted, e.g. tomato, soybean, maize or safflower. The resulting pot cultures are checked for purity by seeing whether only one endophyte species becomes established, as determined from the uniformity of spores produced. These spores are extracted by wet-sieving or other techniques and aliquots of say 10 to 20 morphologically identical spores are then surface-sterilized to kill possible contaminating pathogens before placing on the roots of aseptically grown plants. Root pieces are removed once these infections are established monoxenically and added to fresh seedlings in pots or seed trays in the glasshouse on a scale large enough to produce inoculum in bulk. These cultures can be treated with appropriate nematicides and fungicides to keep out specific citrus pathogens but without killing the mycorrhiza. Inoculum from these cultures is then used for inoculating citrus seedlings in the nursery. These procedures can be modified for one's own requirements, but the basic principles must be followed and special care given to quality control.

5. Choice of inoculation methods

Some idea of the variety of methods to choose from is evident from the field experiments cited earlier. Essentially inoculum

must be concentrated near the seed, not broadcast thinly over a wide area, to achieve good infection (5). There are at least six methods:

(i) Pre-inoculated transplants. Seedlings of various crops are infected with VAM fungi before planting out in order to gauge the mycorrhizal potential of a particular site in terms of plant yield without risking failure resulting from unsuccessful inoculation. However, in practice, this technique can only be considered for crops that are normally transplanted to the field — primarily container-grown plants.

It is particularly easy to add inoculum to tree seedlings in containers to establish good VAM infections before they are transplanted, e.g. citrus, cacao, oil palm. The mycorrhizal technology for other tree crops, and perhaps transplanted vegetables such as onion, could become equally well developed.

(ii) Seed coating. Adhesives like methyl cellulose are used to stick inoculum particles onto single seeds. This technique has been shown to work, but there are at least two difficulties. Firstly, the particles of inoculum (i.e. infective propagules) are too big to be easily stuck onto small seeds in the way rhizobia, for example, can be pelleted onto seeds. Secondly, when the seed germinates, the seed-coat may not remain in the region of the growing root so that the root misses the mycorrhizal inoculum. Possibly inoculum could be incorporated into a carrier to produce a large pellet with the seed in the middle.

(iii) Multi-seeded pellets. - virtually the reverse of seed coating. Inoculum is made into spheres of about 1cm and seeds stuck to the outside to form multi-seeded spheres or pellets. Which-

ever way up the pellet lands during sowing, at least some roots from the germinating seeds will grow geotropically through the inoculum.

(iv) Furrow inoculation. Placing inoculum directly in the furrow and sowing the seeds on top is probably the most successful and commonly used method in field inoculation studies. Soil-root inoculum from stock cultures is spread loosely along the furrow or applied at intervals as spheres or cubes of about 10g. Then a layer of soil is added between the inoculum and seeds and over the seeds. So far this has been done by hand on a small scale. On a larger scale seed drilling machinery might be adapted to incorporate both seeds and inoculum together in the furrows, but the amount of inoculum required may be impractical - calculated to be around 2 to 3t/ha (5).

(v) Fluid drilling and slurry inoculation. This method is adapted from the technique developed to achieve earlier emergence of vegetables in cold field soils in the spring. Seeds are pre-germinated so that their radicles are just visible and suspended in a viscous fluid such as 4% methyl cellulose. This fluid is added to furrows as a slurry. For mycorrhizal purposes wet-sieved inoculum is suspended in the slurry along with the seeds. If legumes are used, rhizobia can also be incorporated in the slurry. Thus the inocula are placed in close contact with the germinating seeds and emerging radicles. Infection as good as in method (iv) can be achieved but with only a fraction of the original crude soil-root inoculum (5).

(vi) Highly infective soil. The indigenous VA mycorrhizal population of a field plot is increased by growing a strongly

mycorrhizal crop there and using the top soil as crude inoculum (1). Such inoculum will include other soil micro-organisms, both beneficial and harmful.

An alternative is to raise the level of mycorrhiza in the test site by pre-cropping with a highly infected host species. In practice this approach could be considered in some crop rotations.

6. Form of inoculum

A decision on what form of inoculum to use is closely linked to the inoculation method selected. The main forms are :

(i) Soil from stock plant cultures containing mycorrhizal spores, infected root fragments and mycelium.

(ii) Wet-sievings from stock plant cultures, primarily the organic fraction from (i) in which spores, roots and mycelium are concentrated.

(iii) Infected roots. These can be washed from soil, but large quantities can be produced cleanly in NFT (nutrient film technique) cultures (2). Storage without loss of viability is a problem, but infected roots from some soil-grown plants can survive at least 6 months in dried soil (17). Infections can be produced faster from infected roots than from spores.

(iv) Pure cultures of the endophyte. Success in growing VA mycorrhizal endophytes axenically would change our whole attitude towards inoculum production and inoculation techniques.

(v) Inoculum in situ (indigenous endophyte population), its level raised in the field by judicious pre-cropping.

Conclusions

1. More VAM endophyte screening programmes are needed. We have little information on the genetic stability of different strains and species, or on the possibility of selecting and breeding for particular conditions.

2. Inoculum must be producible in bulk, readily transported to the field, and easily introduced on a large scale in the seedling rooting zone.

3. The native mycorrhizal populations are important in some soils and more information is required on ways of manipulating these populations.

4. The crops that depend most on mycorrhiza should be identified.

5. With perennial crops it needs to be clarified whether there is most benefit from inoculation in the first year, whether benefits persist in subsequent years, and how fast infection spreads from the points of inoculation.

6. Fumigated soils with the native mycorrhizal population eliminated are a special case particularly favourable to the concept of introducing selected endophytes by inoculation.

7. Large growth responses can be achieved where the field conditions are well understood and sowing and inoculation carefully controlled. However, growth responses under commercially acceptable

conditions are proving more tenuous. Therefore in temperate regions it may be more realistic to look for small but significant growth effects in fairly fertile soils. Large growth effects are most likely in nutrient-stressed sites, e.g. upland grasslands and eroded or reclaimed land such as mine spoils.

8. Field inoculation with VA mycorrhiza will probably have most impact in the less intensive agricultural systems of the tropics where the soils are often phosphate-fixing, phosphate fertilizers are in short supply, and the temperatures favour high microbial activity.

9. Some phosphate input is needed to prevent mycorrhiza exhausting the available soil P too quickly since natural replenishment from the bulk of insoluble soil P takes place slowly.

10. The costs of mycorrhizal inoculation need to be assessed in specific situations in order to determine the extent to which it can yield benefits which are economically practical.

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EXPERIMENTAL PLANS

1. INOCULUM PRODUCTION

Introduction:

A more efficient methodology for mycorrhizal studies under field conditions needs to be developed if the benefit of mycorrhizal symbiosis is to be utilised in large-scale crop production. Furthermore, no mycorrhizal programme can function effectively if the basic methods are not properly carried out.

Research objectives:

To obtain the best possible strains of VA mycorrhizal fungi and develop practical inoculation methods to achieve benefits from mycorrhiza in various cropping systems.

Research approach:

(i) Isolate local endophytes by wet-sieving, baiting, etc. Not only different species, but also different strains of the same species should be obtained from as large a number of sites as possible

(ii) Obtain 2 or 3 internationally recognised isolates considered likely to be effective under the specific environmental conditions prevailing locally.

(iii) Screen many endophytes in pot trials using representative local soils.

(iv) Select the most promising species and strains to form a culture collection which is maintained on stock plants.

(v) Build up inoculum for large-scale field inoculation using the methods outlined separately and paying special attention to quality control.

(vi) Develop suitable inoculation techniques

(vii) Evaluate the benefits from introducing the selected strains into a field situation, taking into account the contribution made by indigenous endophytes.

Isotopes, e.g., ^{32}P, could be useful in speeding up strain trials by giving a rapid indication of which ones begin to function in P uptake most rapidly and which stimulate most P uptake within a fairly short period (compared to the 2 months or so needed to measure growth responses in the normal way). Efficient screening of endophytes is vital.

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2. NUTRIENT AVAILABILITY AND EFFICIENCY OF FERTILIZER USE

Introduction:

Many glasshouse and field trials have shown a substantial increase in nutrient uptake and plant growth in nutrient deficient soils, following inoculation with mycorrhizal fungi. The increased nutrient absorption is achieved by fungus growth from the root into soil, absorption of nutrients by the fungi and nutrient translocation to the plant. It follows that mycorrhizas could have a significant impact on IAEA/FAO programmes involving the study of roots and plant nutrition. They may be an important complementary approach to genetic selection of plants for high production in nutrient poor soils.

Most studies involving mycorrhizal nutrition have emphasized phosphate uptake. However, experimental evidence indicates increased absorption of other poorly mobile ions such as zinc and copper by VA mycorrhizas and also of ions such as ammonium, potassium, and, in a few cases, sulphate. There are suggestions that mycorrhizas may play an important role in the uptake of nitrate and other highly mobile ions in mixed ecosystems (see Section 4 on "Mixed Cropping").

Research Approaches:

(i) Inorganic ions - There is need for more study of phosphate uptake and also the uptake of other ions from soil by mycorrhizas. These include Zn and Mo (especially in relation to legume nitrogen fixation), ammonium ions, sulphate, potassium and in mixed ecosystems, nitrate. These can be investigated most effectively by laboratory studies in which isotopes are injected into soil containing mycorrhizal fungi growing from roots to assess the efficiency of uptake compared with non-mycorrhizal plants, and confirming this by plant growth studies in the field following appropriate mycorrhizal inoculation (see Section 1 on inoculation methods).

There is a need to examine whether fungi can absorb phosphate ions from lower soil solution concentration than plant roots alone. This is approached best by using isotopic methods.

(ii) Organic sources of nutrients - Almost all studies have focussed on inorganic ions and inorganic sources of plant nutrients. However, there is a need to examine the penetration of mycorrhizal fungi into senescent roots, into litter and animal dung (an important nutrient source in some agricultural systems) and the absorption of inorganic and organic nutrients from these sources in competition with other soil microorganisms. Such studies would be performed most effectively in field and laboratory studies by using either isotopically labelled organic materials or the indirect labelling (A value) approach.

(iii) Forms of phosphate. Although mycorrhizal plants apparently use the same forms of inorganic phosphate as do uninfected plants, the former do so more effectively. Additions of phosphate to soil to balance phosphate removed should be in a form compatible with mycorrhizal growth and functioning. The penetration of fertilizer bands and granules of poorly soluble phosphates (such as rock phosphate), and the

absorption of P from such sources should be examined, using labelled phosphate fertilizer. In this way mycorrhizal uptake of phosphate from such sources can be distinguished from phosphate uptake from soil resources. It is likely that some fungi will be able to penetrate these sites more effectively than others.

(iv) Mycelial penetration of soil. The main basis of mycorrhizal stimulation of nutrient uptake is considered to be the growth of fungi from the roots into soil. This will be affected considerably by the fungus-plant combination used and by soil conditions. However, little is known about fungus growth into soil (or organic matter and fertilizer granules) although an understanding of this is critical to prediction of the most appropriate fungus to use in particular conditions. Such studies would be performed best by examining the uptake of isotopes injected into soil (at various depths and distances) containing mycorrhiza-plant combinations maintained under controlled conditions (initially in the laboratory).

3. THE ROLE OF MYCORRHIZA IN SYMBIOTIC N_2 FIXATION

Introduction:

Nitrogen and phosphorus are major nutrient elements which often limit plant growth. Although inorganic N fertilizer application is usually used to correct any deficiency of this element, the high cost of N fertilizer and the logistics of transport and distribution limit its use. A successful legume-*Rhizobium* association, which results in the conversion of substantial amounts of N_2 gas directly into forms utilizable by the plant is therefore an alternative to inorganic N fertilizer.

The successful establishment and efficient functioning which are necessary for deriving the maximum benefit of this association are, however, limited by several factors, of which soil nutrient deficiencies, in particular P, play a major role. That the addition of P in a P-deficient soil increases the amount and proportion of N a legume derives from fixation has been established by the IAEA/FAO

Co-ordinated Research Programme on nitrogen fixation, using ^{15}N tracer techniques. There is also evidence in the literature to show that mycorrhizas are able to improve the P and N content of leguminous plants grown in P-deficient soils, but the evidence that the extra N in mycorrhizal plants is due to increased N_2 fixation and not to increased N uptake from soil is weak. Mycorrhizal plants have also been shown to be affected to varying degrees by different forms of P fertilizer, this effect being in itself dependent on strains of mycorrhiza used.

These factors will therefore affect the effectiveness of mycorrhizal establishment and functioning, and will determine the ability of the fungus to increase P uptake in the plant, and need to be studied. Thus, there is good reason to believe that mycorrhizal infection can result in increased N_2 fixation by legumes, by improving the P status of plants and this can be proved by the use of ^{15}N.

Research Approach:

To study the role of mycorrhizas in enhancing N_2 fixation in the legume-*Rhizobium* association through improved P uptake, it will be necessary to determine the amounts and proportions of N derived from various sources, so as to assess the efficiency of the N_2 fixing system. The use of ^{15}N is the only direct method for partitioning the various sources of N in a legume plant. The method used will be based on that by either Fried and Middelboe (1977) or Fried and Broeshart (1975). Since maximum benefits of mycorrhizal infection are often attained under conditions of low P availability, and, also, since it is under such conditions that supplemental P is needed, particular emphasis will have to be placed on selecting sites with low soil P, or conditions under which P response is expected. The study could be performed with either food or forage legumes, and at a later stage, N_2 fixing trees. It will be essential to apply an efficient *Rhizobium* strain to all plots, and these studies should be performed in the field. Essential treatments may include:

(i) *Rhizobium* alone

(ii) *Rhizobium* + Mycorrhiza

(iii) Rhizobium + optimum P level

(iv) Fumigated plot, inoculated with Rhizobium, but no mycorrhiza.

Chemical analysis will have to be performed on plant samples to determine the effect of mycorrhiza on uptake of other elements like Cu, Zn, Mo, which can affect plant growth and/or nitrogen fixation.

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4. THE ROLE OF MYCORRHIZAS IN MIXED CROPPING SYSTEMS:

Mixtures of two or more species are extremely important, economically, especially in the developing world. Examples include (1) food crops, e.g., cereal plus legume, (2) non-food crops, e.g., rubber, oil palm, (3) crops grown with a shade species, e.g., tea, (4) mixtures grown for forage, e.g., cereals plus forage legume, (5) grazing lands, including savanna, and (6) forest timber production. Most of the ways in which mycorrhizas can influence monoculture crops (see above) can apply to mixed crops also, but here we concentrate on additional topics specific to mixed crops.

In mixed crops the correct balance betwen species is very important. Mycorrhizas have been shown to affect the balance between grasses and clover (e.g., Crush 1974), markedly increasing the ability of clover to compete against grass. Such experiments should be carried out with different species combinations, especially legume plus non-legume, preferably in field plots. The species will compete for nutrients, and mycorrhizas may influence how they share the available nutrients. The uptake of not only immobile ions but highly mobile ones

whose uptake is probably not influenced by mycorrhizas in monoculture could be affected. Mycorrhizas may thus influence uptake of a greater range of nutrient elements in mixtures than in monoculture. This should be investigated, using isotopic methods in pot culture, and, also in the field. The placement of fertilizers and timing of their application must aim at allowing the best balance of uptake by the species. Experiments involving isotopes should be carried out to investigate the best fertilizer practice for major mixed-crop combinations when mycorrhizal. For further details on relevant techniques of isotope use see section on Nutrient Availability (Section 2).

Using ^{32}P, it has been shown for a few grassland species that phosphorus can be transferred from one plant to another and that mycorrhizas increase this transfer substantially (Heap and Newman 1980). This may apply to other elements as well. It could be important in speeding up nutrient transfer between growing plants thus reducing the possibility of their being fixed in soil in forms unavailable for plant uptake. It is important to find out more about this phosphorus transfer, especially how much mycorrhizas increase it, and whether and by how much mycorrhizas enhance transfer of other elements between plants. Transfer of nitrogen from legumes to non-legumes deserves special emphasis. Isotopes would be essential for this work. The isotope can be introduced into some plants by foliar feeding or injection, and the activity in associated plants which did not receive isotopes measured. The influence of mycorrhizas on such transfers can then be ascertained by comparing plants growing near mycorrhizal and non-mycorrhizal plants. So far, such experiments have been conducted only in pots but in the future field experiments should also be included.

Nutrient transfer may involve direct hyphal connections between plants. The frequency of such connections in different mixed crops should be determined. Autoradiography would help with this.

Methods used so far for inoculating mycorrhizas into arable crops are unlikely to work for permanent grassland. Methods suitable for grassland should be developed. Also the effects of management practices, e.g., burning, timing of grazing, on mycorrhizas in

grassland should be investigated, so that grassland can be managed for high mycorrhizal abundance.

As in other crops, the ultimate aim is to find out how mycorrhizas can be used to increase the yield of crops in the field.

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5. THE ROLE OF MYCORRHIZA IN THE TOLERANCE OF PLANTS TO STRESS

Introduction

Many plant stress conditions, e.g., soils of high salinity, high soil temperature, high soil aluminium (low pH), and root diseases, are accompanied by loss of roots or reduced root growth. The consequence of this is reduced uptake of nutrients (and possibly water) from soil. However, many mycorrhizal fungi are not as susceptible as higher plants to these deleterious soil factors; for example some mycorrhizal fungi occur profusely in highly saline soils (up to 8000 ppm Cl) and, they are not susceptible to root disease.

Thus, when such mycorrhizal fungi infect the root, their growth into soil can compensate at least partially for the loss of roots caused by deleterious soil factors. A wide range of mycorrhizal fungi exists, and agronomists therefore, have two genetic resources from which to select to manage such situations. Suitable selection of mycorrhizal fungi may significantly complement plant breeding

programmes aimed at producing genotypes tolerant of deleterious soil conditions. The literature indicates significant tolerance of salinity, low pH and high soil temperatures by mycorrhizal plants compared with non-mycorrhizal plants (Bowen, 1980).

Research Approaches:

1. Salinity

Salinity toxicity may be expressed as a direct toxicity, an inability of the plant to absorb water (osmotic stress) or an imbalance of nutrients, e.g., calcium deficiency. The mycorrhizal fungi may take over a significant part of the nutrient and water absorption functions of the root under saline conditions; it is probable that particular mycorrhizal fungi could be selected to ameliorate the effects of marginal salt toxicity on plant growth. The use of labelled ^{23}Na and ^{36}Cl would be invaluable in screening fungus-soil combinations for efficiency in high salt soils. In addition, the uptake of ^{32}P, ^{45}Ca, and $^{15}NO_3$-N or $^{15}NH_4$-N from saline soils in the presence and absence of mycorrhizal fungi, as indicators of plant nutrient absorption functions, would be of interest.2.

2. Soil Acidity

Soil acidity effects on roots may be via acidity per se or by Al and Mn toxicity, induced P deficiencies or Ca deficiency. In some studies, the growth of plants inoculated with mycorrhizal fungi at pH 4.3 was approximately 60-80% of those at pH 6.6, whereas non-mycorrhizal plants made little growth at the low pH (Graw 1979), Again, it appears that the fungus facilitated the uptake of nutrients. The use of mycorrhizas for improved acid tolerance of plants could be investigated by examining ^{32}P uptake from soil by mycorrhizal plants and mycorrhizal fungus growth into problem acid soils, which are very common in the tropics and sub-tropics (see Section 2 on Nutrient Availability for suggested methods).

3. High Soil Temperatures

The relationship between plant growth and soil temperature can be quite different for mycorrhizal and non-mycorrhizal plants. Certain mycorrhizal fungi are able to absorb soil nutrients from soils at 30-35^{o}C (and probably higher) whereas such temperatures often reduce root growth and function (Bowen, 1980). The nutrient absorption-temperature characteristics of a range of plant-mycorrhizal combinations could be studied best in short term experiments examining ^{32}P uptake from soils. Fungus strains from tropical soils would be especially worth studying in this respect. Such studies could lead to selection of fungi for further field testing in soils experiencing high temperatures.

References

1. Bowen, G.D. (1980) - "Mycorrhizal Roles in Tropical Plants and Ecosystems" In "Tropical Mycorrhiza Research" ed. P. Mikola, pp 165-190, Oxford University Press.

2. Graw, D. (1979). The influence of soil pH on the efficiency of vesicular arbuscular mycorrhizas. New Phytol 82, 687 - 695.

6. MYCORRHIZAS AND WATER USE EFFICIENCY BY FOOD CROPS AND PASTURE SYSTEMS

Introduction:

Moisture stress is a major constraint in rain-fed agricultural system. There is some evidence that mycorrhizal infection not only enhances water-use efficiency by crops, but also, makes them more resistant to drought. The productivity of crops and pasture in semi-arid regions of the world is limited to a large extent by water availability. It is important to determine the relationships between plant nutrition, water availability and mycorrhizal infection in these systems. This is especially important since soil water status can dramatically affect nutrient availability (particularly P), and since mycorrhizal infection has been shown to increase the drought tolerance of several crops. Research involving nuclear techniques could

determine the effectiveness of mycorrhizal infection in making major food crops (including many legumes and non-legumes) and pasture plants more tolerant to low levels of available soil moisture.

Research Objectives:

(i) Increase the tolerance of crops and pasture systems to low soil moisture levels through management and incorporation of VA mycorrhizal fungi.

(ii) Increase phosphorus and nitrogen uptake under low moisture conditions, through mycorrhizal infection (increased fertilizer use efficienty).

(iii) Define the soil moisture regimes and the evaporative demand conditions that are suitable for use of mycorrhizal technology.

Experimental Approach:

Crop systems in arid and semi-arid lands should be established under conditions where moisture becomes limiting to plant growth, which is approximately 3-4 weeks after emergence. This time interval is sufficient to allow mycorrhizal infection. Mycorrhizal infection should be established using indigenous and "international strains".

Soil moisture supply could be controlled by irrigation to allow several cycles of drought while under rain-fed field conditions farming practices such as pitting, terracing, the formation of micro-catchment basins, etc., could be used to establish levels of water supply. Labelled N and P fertilizer could be applied to determine if enhanced water uptake interacts with nutrient supply.

To determine the manner in which mycorrhizal infection can affect water use efficiency, the following measurements or observation would be necessary throughout the growing season:

(i) Soil moisture levels using neutron probes.

(ii) Leaf water potential and diffusive resistance in mycorrhizal and non-mycorrhizal plants before and during periods of water stress.

(iii) Crop growth and phenological development should be determined on a weekly basis.

(iv) Evaporative demand conditions and rainfall should be continuously monitored.

(v) Final dry matter and nutrient yield should be determined. Sequential harvesting at early growth stages to determine the pattern of nutrient uptake would also provide valuable information.

In all field studies care would have to be taken to select uniform sites. Adequate physical and chemical characterization of the soils would be necessary.

7. ROLE OF VA MYCORRHIZA IN PLANT RESISTANCE OR SUSCEPTIBILITY TO PATHOGENS

Introduction:

It is widely documented that both pathogenic and mycorrhizal infections can dramatically alter the water and nutrient relations of many crop plants. The extent to which mycorrhizal systems can alleviate pathogen-induced imbalances and the possible mechanisms involved could be clarified by using isotopes and nuclear technology.

Research objectives:

(i) To increase water and nutrient uptake of diseased plants through the use of mycorrhizal technology, and

(ii) study metabolic changes resulting from pathogen-mycorrhiza interactions.

Research approaches:

(i) A similar approach to that used for nutrient availability (Section 2) but incorporating various pathogenic organisms, including root-rotting, wilt-inducing, and foliar pathogens. This could thus be viewed as a stress situation.

(ii) Produce non-mycorrhizal plants of similar nutritional status as mycorrhizal ones and elucidate any non-nutritional effects of mycorrhiza, e.g., production of unusual metabolites, physical exclusion of the pathogen, etc. Isotopes could indicate which parts of the mycorrhizal infection are most active by showing, for example, which cells or tissues are metabolically incorporating ^{32}P, ^{3}H, etc., and hence, whether there is more pathogen-mycorrhiza interaction in active than in inactive mycorrhizas.

8. EFFECT OF PESTICIDES ON MYCORRHIZA

Introduction

In recent years, the use of different pesticides for crop production has become popular in most countries. However, the possible harmful effects of these chemicals on both the introduced and indigenous mycorrhizal population has not been investigated. This calls for investigating the influence of different pesticides on the native or introduced mycorrhizal populations as well as on their infective ability on the host plant, since mycorrhizas can enhance nutrient uptake. Isotopes, such as ^{15}N and ^{32}P would be very useful in such studies.

Research approaches:

(i) The development of mycorrhiza on the roots of several crops should be monitored using different pesticides.

(ii) Cultivar-mycorrhiza associations should be identified, under greenhouse conditions, which will be tolerant to specific pesticides. These would later be tested in the field using isotopes such as ^{32}P and ^{15}N to assess nutrient uptake in the presence and absence of the pesticides.

RECOMMENDATIONS

The Meeting emphasized the important role mycorrhiza play in increasing crop yields in soils of low fertility through increased uptake of plant nutrients. In addition, mycorrhizal infection can assist plants to tolerate drought and other stresses (such as pathogens, salinity and elevated temperature) better than the uninfected plants. Furthermore, evidence available suggests that mycorrhizas may enhance nitrogen fixation by legumes and are important in mixed cropping systems including pastures.

The Meeting recommended that the IAEA through the Joint FAO/IAEA Division should encourage research on mycorrhiza in Member States as a possible means to improve fertilizer use efficiency. Research using isotopes, such as ^{32}P and ^{15}N was strongly recommended. Since soil moisture is essential for plant growth and strongly influences the uptake of nutrients, studies aimed at understanding the role of mycorrhiza in drought conditions, as well as the effect of soil moisture on mycorrhizal establishment and function were recommended. Isotopes, such as ^{14}C, ^{3}H, together with the use of neutron moisture probes would be useful in such research.

Legumes infected by mycorrhiza in soils of low fertility contain greater amounts of nitrogen than their non-mycorrhizal counterparts. The evidence is not clear as to whether the extra N is due to increased symbiotic N_2 fixation or is a result of enhanced soil N uptake. The use of ^{15}N to establish the sources of N in the mycorrhizal as well as non-mycorrhizal legume was therefore strongly recommended. Also, legumes play an important role in mixed cropping situations, in pastures and as cover crops under tree plantations, etc., and transfer of N from a legume to an associated non-legume may be increased by mycorrhizas, thus improving the nutrition of the non-legume. This transfer of nutrients could also occur for other nutrient elements from living as well as senescent roots. The only direct method for assessing whether such transfers occur and their extent involves the use of isotopes.

Some of the implications of the more efficient exploitation of available nutrients in an already infertile soil by mycorrhiza were described. Studies on suitable fertilizers, which can replenish soil nutrients taken up by plants and yet not impair mycorrhizal infection and

function were recommended. These would include the use of low grade fertilizers such as rock phosphates which are abundant in many developing countries. The use of isotopes such as ^{32}P and ^{15}N, to assess the efficiency of utilization of these fertilizers by mycorrhizal growth, was considered to be essential.

The Meeting documented the need to isolate efficient local strains of mycorrhizal fungi, and test these alongside strains already known to be efficient symbionts, so as to assess the need for mycorrhizal inoculation. This, and the subsequent development of methods for production of high quality inocula and suitable application methods for large-scale field inoculation were considered to be first and vital steps in any successful programme on mycorrhiza. Isotopes, such as ^{32}P and ^{15}N can aid in detecting rapid infection and mycorrhizal effectiveness.

The participants recommended that the IAEA, through its Joint FAO/IAEA Division coordinate a research programme on mycorrhizas. Although various aspects of the proposed studies overlap with those in existing programmes coordinated by the Soils Section of the Division, the expertise needed for mycorrhizal research does not presently exist in these programmes. Training such scientists, whose expertise and interest may be in fields unrelated to mycorrhiza mitigate against advocating that such studies be undertaken by participants in existing programmes. A small training component for selected participants in a mycorrhiza programme was recommended, to bring scientists up-to-date in research in this area, as well as on the use of isotopes and nuclear techniques, in studying plant-mycorrhizal interactions. The Seibersdorf Laboratory should become involved in this programme, as well as in subsequent training, especially on the use of isotopes.

As a long-term goal, these studies could be extended to trees, especialy those capable of fixing N_2.

The Meeting recommended that the papers presented and the isotope-aided research for which plans were developed should be compiled into a non-priced document, an thus facilitate its distribution to interested scientists in developing countries.

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