Journal of General Microbiology (1971), **69**, 157–166
Printed in Great Britain

The Influence of Phosphate and Other Nutrients on the Development of Vesicular-arbuscular Mycorrhiza in Culture

By BARBARA MOSSE AND J. M. PHILLIPS
Rothamsted Experimental Station, Harpenden, Hertfordshire

(*Accepted for publication* 18 *August* 1971)

SUMMARY

Vesicular-arbuscular mycorrhiza were established in *Trifolium parviflorum* in culture by inoculation with Endogone spores. In a medium containing 265 mg. phosphorus (P)/l., as $CaHPO_4$ and K_2HPO_4, infection occurred only when the medium lacked nitrogen (N). In a medium containing only 100 mg. P/l., infection occurred readily in the presence of 0·5 g. KNO_3/l.

Calcium monohydrogen phosphate, Ca phytate, Na phytate, Fe phytate, phytin, lecithin and DNA were adequate sources of phosphate for both plant and fungus. Ca phytate and DNA greatly stimulated fungal growth, and DNA also stimulated spore formation, in the agar medium. With Na in the medium infections in the root were sparse. Inositol may serve as a carbon source for Endogone.

Mycorrhizal infection occurred with either $FeCl_3$ or Fe-EDTA in the medium; when so little iron was present that plants grew poorly, there was also little mycorrhizal infection.

INTRODUCTION

Typical vesicular-arbuscular (VA) mycorrhiza were established for the first time, in monoxenic culture in clover seedlings in an inorganic salt medium commonly used in studies on nodulation (Jensen, 1942); this medium lacked nitrogen (N) and contained 265 mg. phosphorus (P) (as $CaHPO_4$ and K_2HPO_4)/l. Germinated, surface-sterilized spores of an Endogone sp. were used as inoculum. Infection occurred only with spores already germinated and when entry into the root was assisted by bacteria, by an extract from a bacterial culture, by EDTA or by pectinase (Mosse, 1962). If N was added infections occurred only after the host plant had removed this N from the medium. We have now obtained typical VA infections in a medium containing N with surface-sterilized Endogone spores not previously germinated. The two media differ in the source and amount of phosphate, the presence or absence of charcoal, the use of Fe-EDTA instead of $FeCl_3$, and the use of $CaCl_2$ instead of NaCl. This paper examines which of these differences is responsible for the better results in the second medium.

We also tested various other organic P compounds, viz. phytin, sodium phytate, iron phytate, glucose-6-phosphate, lecithin and DNA, and inositol (the carbon framework of the phytates). The results allow some deductions about the physiology of the *Endogone* sp., which is an obligate symbiont not so far cultured without a host plant.

METHODS

Test plants. Seeds of *Trifolium parviflorum* were sterilized in concentrated H_2SO_4, chilled and germinated on agar plates (Nutman, 1949). The germinated seeds were placed singly on agar slopes in test tubes kept in a glass house with a day temperature of 20° and a night

temperature of 15°. During the winter a 12 h. day was maintained by supplementary fluorescent lighting.

Inoculum. Resting spores of *Endogone mosseae* (Nicolson & Gerdemann, 1968) were excized from sporocarps and surface sterilized in 2 % (w/v) Chloramine T containing 200 mg. streptomycin/l. and a trace of detergent (Mosse, 1962). Spores were either transferred by capillary pipettes to watch glasses containing the sterilant and then rinsed in three changes

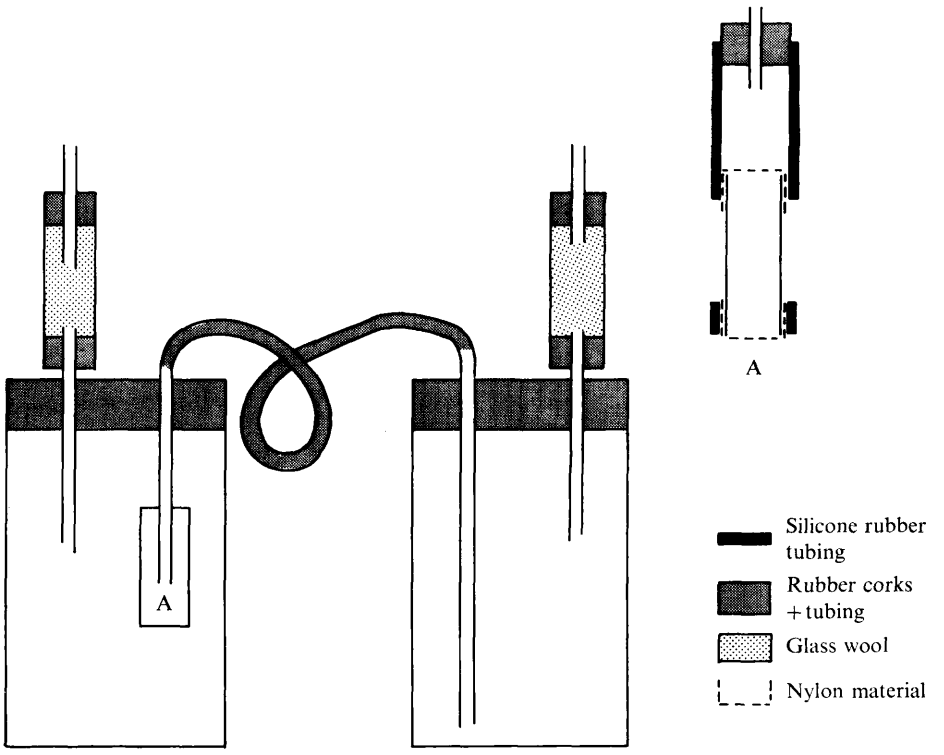

Fig. 1. Apparatus used for sterilizing Endogone spores.

of water, or they were placed in a small glass tube (A) closed at the ends by nylon gauze held in place by a rubber ring, and connected by flexible tubing to a reservoir containing sterilant or water (Fig. 1). Spores were sterilized and rinsed in this container by raising and lowering the reservoir several times. Both methods produced surface-sterilized spores capable of germination. When seedlings had formed some first-order laterals, usually two weeks after sowing, they were inoculated by placing 10 to 15 sterilized spores near the roots growing on the agar surface.

Media. The composition of media tested is given in Table 1.

Phytin was extracted from wheat bran as described by Clark (1914). Iron phytate was prepared by adding excess $FeCl_3$ to an acid solution of sodium phytate. All media were adjusted to pH 6·3 before autoclaving at 121° for 15 min. There were eight separate experiments comparing treatments, and replication ranged from 12 to 24 tubes in each. Results from different experiments are not directly comparable because of seasonal differences in plant growth and infection, attributable to differences in light intensity.

Records. Ten to twelve weeks after inoculation, plants were tested for contamination and

plant size and infection were recorded. Sterility was tested by adding some supernatant liquid from each tube to sterile nutrient broth (Oxoid), in which Endogone does not grow: broth with liquid from monoxenically infected, uncontaminated seedlings remained clear. The seedlings were then carefully removed from the melted agar, rinsed in warm water, dried between filter papers and weighed. Roots and attached external mycelium were stained by plunging into boiling lactophenol containing cotton blue (0·01 %, w/v), leaving to simmer for 10 min. and destaining in several changes of lactophenol. This treatment stained the fungus and cleared the tissue sufficiently for microscopic examination of whole, unsectioned roots.

Table 1. *Composition of media* (*g./l.*)

No.			mg. P/l.
I	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)		30
II	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100 + 30
III	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 1·43 $CaHPO_4.2H_2O$	260 + 30
IV	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·63 Ca phytate[b]	100 + 30
V	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·63 Na phytate[c]	100 + 30
VI	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·73 Fe phytate	100 approx. + 30
VII	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·73 phytin	100 approx. + 30
VIII	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·76 DNA[cd]	60 approx. + 30
IX	10 charcoal[a] + 0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 1·06 lecithin	60 approx. + 30
X	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)		0
XI	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100
XII	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 1·43 $CaHPO_4.2H_2O$	260
XIII	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·63 Ca phytate	100
XIV	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·76 DNA	60 approx.
XV	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 1·0 glucose-6-phosphate	100
XVI	0·2 $CaCl_2$ + FeEDTA (7 mg. Fe)	+ 0·12 inositol[e]	0
XVII	0·2 NaCl + $FeCl_3$ (3 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100
XVIII	0·2 $CaCl_2$ + $FeCl_3$ (35 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100
XIX	0·2 $CaCl_2$ + FeEDTA (1 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100
XX	0·2 $CaCl_2$ + FeEDTA (1 mg. Fe)	+ 0·63 Ca phytate	100
XXI	0·2 $CaCl_2$ + $FeCl_3$ (1 mg. Fe)	+ 0·55 $CaHPO_4.2H_2O$	100
XXII	0·2 $CaCl_2$ + $FeCl_3$ (1 mg. Fe)	+ 0·63 Ca phytate	100

All media contained in addition 15 g. Difco agar, 0·5 g. KNO_3 and 0·2 g. $MgSO_4.7H_2O$.

[a] Total P content 0·3 % (w/w).
[b] Koch-Light Ltd, Colnbrook, Buckinghamshire.
[c] British Drug Houses Ltd, Poole, Dorset.
[d] From calf thymus gland.
[e] Amount of inositol in 0·63 g. Ca phytate.

Presence or absence of VA infection was recorded and the most heavily infected part of the root system, usually the top quarter, was used for more detailed observations on the spread of infection, vesicle production in the root, the amount of external mycelium and numbers of extra-matrical spores. The complete root system was laid out in a Petri dish and 1·5 cm. was cut from within the most heavily infected part. The segments were placed on a microscope slide and individual roots were eased apart and covered with a coverslip. With the aid of an eye-piece graticule with crossed wires, three traverses were made across the root segments (at both ends and in the middle of the coverslip). Root infection was recorded in three categories; without infection (rating 0), roots with about one-third of the cortex infected (rating 1), and those with about two-thirds of the cortex infected (rating 2). The total infection rating was then expressed per 100 roots traversed giving a maximum rating of 200 (all roots with about two-thirds of the cortex infected). Roots with vesicles and the number of external spores met in the traverse were recorded separately. The numbers of external hyphae traversed were recorded as single hyphae or, when in strands, as two

sizes containing approximately 25 (rating 25) or approximately 50 (rating 50) individual hyphae. These ratings were added to the number of single hyphae recorded. Four separate criteria of infection were therefore measured: (i) the intensity of VA infection in the root; (ii) the number of roots with vesicles; (iii) the estimated number of external hyphae; (iv) the number of extra-matrical (external) spores. In one experiment the total length of infected root system was measured.

RESULTS

Effects of different sources and amounts of P on the incidence and intensity of infection.

Ungerminated Endogone spores produced typical VA infections in plants grown on a basic medium containing N and up to 100 mg. P/l. (Table 2). All the media contained

Table 2. *Effects of different phosphate sources on the incidence of infection by Endogone sp.*

Medium	Main phosphate source	No. of plants examined	No. of plants without contamination	No. of plants with VA infection	No. of VA plants without contamination
I	* None	21	14	8	6
II	$CaHPO_4$	23	16	5	4
IV	Ca phytate	18	13	13	8
V	Na phytate	24	15	6	2
VI	Fe phytate	24	20	9	7
VII	Phytin	24	15	3	3
VIII	DNA	24	15	6	3
IX	Lecithin	22	18	5	5

* All media contained charcoal as an additional source of P.

charcoal which supplied an extra 30 mg. P/l. Although some infected seedlings were also contaminated with other micro-organisms, mostly bacteria, there was no indication that contamination assisted VA infection. Although most plants became infected in the medium containing Ca phytate, many also became infected in media containing inorganic phosphate ($CaHPO_4$) or no phosphate. Infection did not therefore depend on any particular form of phosphate. However, it was sensitive to different concentrations (Table 3). In media with two levels of inorganic phosphate and no charcoal (X, XI, XII), the optimum P concentration for both incidence and intensity of infection was 100 mg/l.; with 260 mg./l. both plant growth and infection were less. In media containing charcoal (I, II, III), intensity of infection was greatest in medium I (30 mg. P/l.), less in II (130 mg. P/l.) and least in III (290 mg. P/l.). The incidence of infection was also least in medium III although the higher P level did not affect spore germination.

There was a significant interaction between charcoal and phosphate. Charcoal improved infection only when it was the sole source of P (cf. media X and I). It diminished the intensity of infection (though not its incidence) when 100 and 260 mg. P/l. were present as $CaHPO_4$, probably by increasing the total P above the optimum. With 290 mg. P/l. (medium III), infection was no better than without P (medium X), although plants were much larger. The optimum P concentration for VA mycorrhiza seemed to be between 30 and 100 mg./l.

Fungal growth outside the root, as measured by external mycelium and spores, was greater with the two organic forms of phosphate (Ca phytate and DNA) than with the inorganic. DNA in particular greatly stimulated the number of external spores (medium XIV, Fig. 2*a*) and they were also significantly larger ($P < 0{\cdot}01$) with a mean diameter of

25·3 μm. compared with 22·5 μm. with Ca phytate (medium XIII) and 21·6 μm. with $CaHPO_4$ (medium XI). Adding charcoal markedly reduced numbers of external spores in the DNA medium.

Table 3. *Development of VA infection and of external mycelium in media containing different amounts and sources of P.*

Mean of 6 replicates.

Medium	Source of P	mg. P/l.	Fresh wt (g.)	Plants infected* (%)	Intensity of infection†	Roots with vesicles†	External mycelium†	External spores†
				No charcoal				
X	None	0	0·06	55	30	0·2	43	5
XI	$CaHPO_4$	100	0·23	100	86	10	152	62
XII	$CaHPO_4$	260	0·16	60	44	6	102	48
XIII	Ca phytate	100	0·30	100	110	28	298	55
XIV	DNA	60	0·24	86	93	6	304	138
				With charcoal				
I	charcoal	30	0·11	100	55	3	115	15
II	$CaHPO_4$	100	0·30	100	30	3	44	10
	charcoal	30						
III	$CaHPO_4$	260	0·29	70	16	0·3	34	5
	charcoal	30						
IV	Ca phytate	100	0·31	100	147	31	448	61
	charcoal	30						
VIII	DNA	60	0·21	94	111	6	214	23
	charcoal	30						

Standard errors: Intensity of infection ± 11 Roots with vesicles ± 1·8. External mycelium ± 38. External spores ± 13.

As there was a significant interaction between charcoal and phosphate treatments, and as the range of variation within treatments differed considerably, the standard errors should be regarded as approximations only. More reliable analyses using logarithmic transformations were also done.

* % plants infected refers to 10 or more plants/treatment. In all treatments there were some contaminated replicates which are included in these figures as there was no indication that contamination had affected either the incidence or character of the VA infection.

† For assessment methods, see text.

Comparison of Ca phytate, inositol and glucose-6-phosphate

Seedlings in medium XV with glucose-6-phosphate were not appreciably larger than those in medium XVI, which contained no added phosphate (Table 4). Apparently neither the plant nor the fungus used P supplied as glucose-6-phosphate. Only half the seedlings became infected in this medium, and the intensity of infection was less than with either inositol or Ca phytate. In other experiments, in which glucose-6-phosphate was sterilized by filtration, plants grew very well but incidence (50 %) and intensity of infection were again much less than in comparable plants given Ca phytate. Spore germination was also less. This result agrees with an earlier observation (Mosse, 1959) that glucose depresses growth of the germ tubes of the fungus.

The effects of inositol (medium XVI) were very striking. Not only was the infection nearly as well developed as in medium XIII containing phytate, but the fungus was also distributed much more evenly through the medium, including the bottom half of the tube. This was reflected in the significantly greater infection of roots growing near the bottom of the tube in the inositol medium, in spite of the obvious phosphate starvation that kept the plants small and presumably also affected fungal growth. When 0·005 g./l. was given, instead of

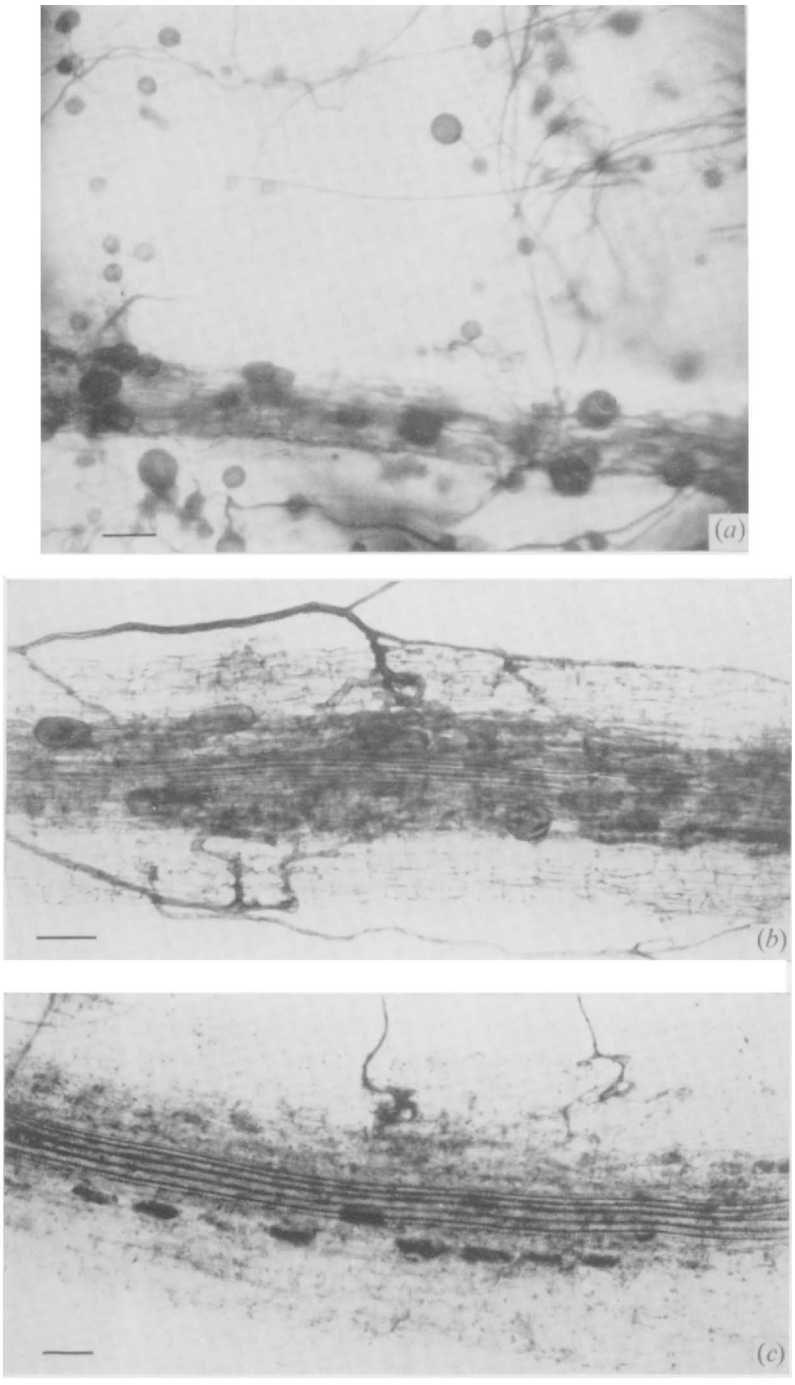

Fig. 2. (*a*) External mycelium and spores in medium XIV containing DNA; (*b*) Clover root with typical VA infection in medium XVIII containing $CaCl_2$; (*c*) Clover root with typical VA infection in medium XVII containing NaCl. Scales represent 100 μm.

Table 4. *Development of VA infection and external mycelium in media containing Ca phytate, inositol and glucose-6-phosphate*

Mean of 14 replicates.

		Roots in region of maximum infection						Roots near bottom of test tube			
Medium	Source of P (mg./l.)	Fresh wt (g.)	Plants infected (%)	Intensity of infection	Roots with vesicles	External mycelium	External spores	Intensity of infection	Roots with vesicles	External mycelium	External spores
XV	glucose-6-phosphate 100	0·15	57	16±4·1*	0·3	93	20±7	0	0	0	0
XVI	inositol 0	0·12	93	70±8·3	1·5	134	28±4·5	53±9·8	1	45	6
XIII	Ca phytate 100	0·42	100	79±9·3	15	193	54±6·8	20±6	1·4	8	0·3

* Standard error: intensity of infection, maximum score = 200. External mycelium is expressed as number of hyphae (see text). In all treatments there were some contaminated tubes. These are included in the figures as there was no indication that contamination had affected the incidence or character of the VA infection.

0·12 g./l. as in medium XVI, the intensity of infection was halved, showing that the response to inositol was to some extent dependent on concentration.

Some beneficial effects of Ca phytate noted earlier may therefore have been due to its inositol component, which could have acted as a carbon source for the fungus. In the purely inorganic medium the only carbon sources available to the fungus would have come either from the plant or, less probably, from atmospheric CO_2.

To investigate the relationship between fungal development inside and outside the root, correlations were calculated between intensity of infection and external mycelium. In media containing $CaHPO_4$ the correlation coefficient was $0{\cdot}85 \pm 0{\cdot}05$, and for media containing Ca phytate it was $0{\cdot}86 \pm 0{\cdot}05$, but for media with inositol it was $0{\cdot}40 \pm 0{\cdot}15$. Thus, with Ca phytate or $CaHPO_4$, the external development of the fungus was closely related to its spread within the root, whereas with inositol it was less dependent on internal infection, again suggesting that inositol may act as an alternative carbon source.

Comparison of Fe-EDTA and $FeCl_3$

When, as in Jensen's medium, $FeCl_3$ (0·1 g./l.) was given (medium XVIII) all plants became infected. Therefore Fe-EDTA had no specific effect on VA infection. However, when very small amounts of Fe-EDTA and $FeCl_3$ were supplied (1 mg. Fe/l., Table 5) the Fe-EDTA was insufficient for normal growth, and infection was also decreased to 30% when $CaHPO_4$ was the phosphate source (medium XIX). Analysis of the Ca phytate showed it to contain a small amount of iron as impurity (less than 5 μg./g.), and this was enough to give normal growth and 100% infection (medium XX).

Table 5. *Effects of low levels of Fe-EDTA and $FeCl_3$ on the incidence of VA infection in, and growth of Trifolium parviflorum*

Medium	Iron source (1 mg. Fe/l.)	P source	Mean fresh wt (g.)	Plants infected (%)
XIX	Fe-EDTA	$CaHPO_4$	0·13	30
XX	Fe-EDTA	Ca-phytate	0·42	100
XXI	$FeCl_3$	$CaHPO_4$	0·34	100
XXII	$FeCl_3$	Ca-phytate	0·36	100

Effect of sodium

In two experiments, sodium, present in the medium either as NaCl (80 mg. Na/l., medium XVII), or as Na phytate (70 mg. Na/l., medium V) affected the internal development of the fungus, restricted its longitudinal spread and often confined infection to a single layer of cells next to the endodermis. Fresh weight of plants, number of infections per plant and

Table 6. *Effects of NaCl and $CaCl_2$ on VA infection in Trifolium parviflorum*

Mean of 14 replicates.

Medium	Fresh wt (g.)	Plants infected (%)	No. infections/plant	Total length infected tissue/plant (cm.)
XVII (NaCl)	0·28	100	16	27
XVIII ($CaCl_2$)	0·42	100	40	63

total length of infected tissue were significantly less in medium XVII containing NaCl, than in medium XVIII with $CaCl_2$ (Table 6). Instead of 78% of roots with two-thirds of the cortex infected, there were only 32% in the NaCl medium. Fig. 2, (*b*) and (*c*) show

typical infections. Although the two media (XVII and XVIII) also contained different amounts of Fe, 3 and 35 mg./l. respectively, it is improbable that this would have accounted for the result. Even 1 mg. Fe/l. given as $FeCl_3$ (media XXI and XXII, Table 5) was sufficient for normal growth and infection. Infections were also sparser and more restricted with Na phytate than with $CaHPO_4$ as a source of P. The large sodium content of Jensen's medium may therefore have contributed to the difficulties of obtaining VA infections in it.

DISCUSSION

Clearly the synthesis of VA mycorrhiza in culture is much affected by amounts of phosphate in the medium. Optimum amounts of $CaHPO_4$ were of the order of 100 mg. P/l. (medium XI, Table 3). The intensity but not the incidence of infection was diminished when an extra 30 mg. P was given in the form of charcoal (medium II, Table 3). More (260 mg./l.) reduced both incidence and intensity of infection (medium XII, Table 3) and additional charcoal still further decreased the intensity (medium III, Table 3). Although there were significant interactions with charcoal, there was no evidence that it was harmful provided P concentration did not exceed the optimum. Charcoal had originally been added as an adsorbent of possible toxic substances that might interfere with mycorrhizal development, but infection clearly did not depend on it (Table 3). It is concluded that the difficulties experienced in obtaining VA mycorrhiza in Jensen's medium were connected with its high P content (265 mg./l.), especially as some of this P was in the form of K_2HPO_4 with a solubility of 167 g./l. compared with 0·32 g./l. for $CaHPO_4$, equivalent to 60 mg. P/l. If, as is improbable, all the P in 10 ml. of medium XI were absorbed into a plant of 40 mg. dry weight, the concentration of P would reach 2·5 % (w/w) and with more than twice as much P it could exceed this because more phosphate dissolves and is gradually taken up. Greenwood & Hallsworth (1960) and Asher & Loneragan (1967) found a decrease in growth and other adverse effects of supra-optimal phosphate uptake in subterranean clover containing 1·3 % P. Therefore the phosphate concentrations in Jensen's medium may affect metabolic processes in the host plant that might alter its liability to infection. However, high P concentrations in the medium could also affect the fungus directly, although customary media for growing fungi often contain up to 1 g. K_2HPO_4/l. equivalent to 228 mg. P/l. However *Endogone* may well be an unusual fungus.

It is known that in soil also high P levels decrease and finally eliminate mycorrhizal infection (Baylis, 1967; Mosse, 1971), and Daft & Nicolson (1969) obtained decreasing infection with increasing additions of KH_2PO_4 in sand culture. How N starvation counteracts the effects of too much P is not understood, but both in Jensen's medium (Mosse, 1962) and in pot experiments with soil (Baylis, 1967; Mosse, 1967) this effect was observed. According to Cochrane (1958) lack of N in a fungal medium may be expected to act directly on P absorption by restricting the energy required for its uptake. On the other hand symptoms of P toxicity in plants can be reduced by adding N (Greenwood & Hallsworth, 1960; Bhatti & Loneragan, 1970).

The agreement between results with plants grown in soil and in agar culture is encouraging, because the latter offer considerable advantages for studying the physiology of mycorrhizal associations. The responses and development of the external (extra-matrical) mycelium can be more accurately assessed and the method described in this paper showed that Ca phytate, DNA and inositol greatly stimulated the external mycelium. Inositol in particular seemed to make hyphal growth in the agar independent of its spread in the root. Inositol can function as a growth factor for some micro-organisms but, since its effect was to some extent de-

pendent on concentration it may well have acted as a carbon source. With inositol in the medium even small, phosphate-starved plants were heavily infected (medium XVI, Table 4) whereas generally, small and nutritionally deficient plants like those in media X (Table 3), XV (Table 4), XIX (Table 5) and XVII (Table 6), were only slightly or not at all infected. That inositol and phytates may be beneficial for independent growth of Endogone is interesting and possibly relevant to its ecological situation. In soils glucose, which does not appear to be utilized by Endogone, is present in small amounts and many micro-organisms compete for it. On the other hand phytates form the greater part of the organic phosphate fraction in soils (Anderson, 1956) and are clearly relatively resistant to microbial attack, so that Endogone might have a readily available carbon source in many soils.

We thank Miss G. A. Oakey for much painstaking technical help and for the diagram in Fig. 1.

REFERENCES

ANDERSON, G. (1956). Identification and estimation of soil inositol phosphates. *Journal of the Science of Food & Agriculture* **7**, 437–44.

ASHER, C. J. & LONERAGAN, J. F. (1967). Response of plants to phosphate concentration in solution culture. I. Growth and phosphorus content. *Soil Science* **103**, 225–33.

BAYLIS, G. T. S. (1967). Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytologist* **66**, 231–43.

BHATTI, A. S. & LONERAGAN, J. F. (1970). The effect of early superphosphate toxicity on the subsequent growth of wheat. *Australian Journal of Agricultural Research* **21**, 881–92.

CLARK, G. (1914). Phytin and phytic acid. *Journal of the Chemical Society* **105**, 535–45.

COCHRANE, V. W. (1958). *Physiology of fungi.* New York: John Wiley.

DAFT, M. J. & NICOLSON, T. H. (1969). Effect of *Endogone* mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. *New Phytologist* **68**, 945–52.

GREENWOOD, E. A. N. & HALLSWORTH, E. G. (1960). Studies on the nutrition of forage legumes. II. Some interactions of calcium, phosphorus, copper and molybdenum on the growth and chemical composition of *Trifolium subterraneum* L. *Plant and Soil* **12**, 97–127.

JENSEN, H. L. (1942). Nitrogen fixation in leguminous plants. II. Is symbiotic nitrogen fixation influenced by Azotobacter? *Proceedings of the Linnean Society of N.S.W.* **67**, 205–12.

MOSSE, B. (1959). The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. *Transactions of the British Mycological Society* **42**, 273–86.

MOSSE, B. (1962). The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *Journal of General Microbiology* **27**, 509–20.

MOSSE, B. (1967). *Annual Report Rothamsted Experimental Station for* 1966, p. 88.

MOSSE, B. (1971). *Annual Report Rothamsted Experimental Station for* 1970, p. 89.

NICOLSON, T. H. & GERDEMANN, J. W. (1968). Mycorrhizal Endogone species. *Mycologia* **60**, 313–25.

NUTMAN, P. S. (1949). Nuclear and cytoplasmic inheritance of resistance to infection by nodule bacteria in red clover. *Heredity* **3**, 263–91.