ABSTRACT - The effect of phosphorus (P) on formation of the vesicular-arbuscular mycorrhizal (VAM) symbiosis was examined. Using a sand-hydroponic culture chamber, increasing P levels were supplied to soybean (Glycine max L. Merrill) seedlings. It was found that indirect supply of P up to 600 mM did not affect the germination of Glomus mosseae chlamydospores in the rhizosphere. Similar results were found for germination of either G. mosseae or Gigaspora margarita spores in the rhizosphere of soybean growing in soil amended with increasing P levels. These data suggest that P controls VAM formation at some stage after spore germination in the rhizosphere has occurred. Increasing P supply to the soil enhanced carbon exchange rate, sugar content of the roots and decreased sugars in the root exudates. Bioassays using root extracts from plants supplied with different P levels showed differential effects on germination of G. mosseae and G. margarita spores in vitro. Extracts from plants well supplied with P had high levels of sugars and inhibited spore germination. It was proposed that P controls root colonization through its effect on host carbon metabolism. In addition, the relationship between P availability and plant growth response to mycorrhizal formation is discussed.

Index terms: VAM fungi, mycorrhizal formation, spore germination

INTRODUCTION

The factors which may influence the establishment and the degree of colonization by vesicular-arbuscular mycorrhizal (VAM) fungi in developing root systems are very complex. Colonization takes place when roots encounter suitable propagules in the soil. The chance of this happening is a function of both root and inoculum density. Spores usually are not stimulated to germinate by host roots (Daniels & Trappe 1980), but their germ tubes (GT) may show tropism and colonize roots several cm away from the inoculum source (Powell 1976, Hepper & Mosse 1975). Once root and propagules are juxtaposed the process of penetration and cortex colonization will be influenced by the soil environment and by the properties of the host root (Mosse et al. 1981). Among soil factors, phosphorus (P) availability is the most important factor controlling root colonization, host depend-
ence on mycotrophy, and sporulation by the VAM fungi (Menge et al. 1978, 1982; Pugh et al. 1981).

Based on circumstantial evidence, it has been suggested that the effect of P on mycorrhizal formation, as measured by the degree of root colonization, results from its effect on the host plant rather than on the fungus (Sanders 1975, Menge et al. 1978, Hepper 1983, Siqueira et al. 1982). Using information from other biological systems, Woolhouse (1975) proposed the involvement of plant lectins and phosphatase enzymes on the formation of VAM association as related to P nutrition of the host plant. His hypothesis predicts that P-deficient plants are induced to produce phosphatases (5-50X) and these enzymes would combine with root lectins, forming inactive dimers.

Since lectins can bind specifically to N-acetylgalactosamine of fungal cell wall, preventing spore germination and GT growth (Mirelman et al. 1975, Callow, unpublished; cited by Woolhouse 1975), they would act as a controlling factor. In addition, they may prevent the dissolution of host cell wall by inhibiting the activity of fungal polygalacturonases (Albersheim & Anderson 1971), which play an important role in host wall penetration. The lectin hypothesis has been recently tested by the senior author (Siqueira 1983). His results suggest that the involvement of these molecules as a controlling factor for mycorrhizal formation must be regarded as hypothetical.

More recently, a decrease in host cell membrane permeability as the result of improved host P nutrition has been suggested as a possible mechanism to control the degree of root colonization (Ratnayake et al. 1978, Graham et al. 1981). It predicts that plants well supplied with P would have reduced root exudation, consequently reduced fungal spore germination and GT growth in the rhizosphere, and hence a lower root colonization rate. However, clear relationship between spore germination in the rhizosphere and final root colonization rates has not been found.

A third possible mechanism can be hypothesized. Our data (Siqueira et al. 1982, Siqueira 1983) and that of others (Mosse 1959, Hepper 1979, Daniels & Trappe 1980, Koske 1981, Hep- per 1983) showed that soluble P did not inhibit spore germination as did organic acids and sugars. This supports early suggestions that P controls mycorrhizal formation through its indirect effect on the host (Sanders 1975, Menge et al. 1978), and that host carbon metabolism may play a key role in mycorrhizal formation (Siqueira et al. 1982).

The objective of this study is to investigate the effect of P on formation of the VAM symbiosis.

MATERIAL AND METHODS

To investigate the relationships between P availability and mycorrhizal formation, several experiments were performed. The first experiment, designed to examine the effect of host P nutrition on spore germination in the rhizosphere, was performed as follows.

Surface sterilized (0.2% HgCl for 3 min.) soybean (Glycine max (L) Merrill) seeds were germinated on 1% agar medium plates. One four-day-old uncontaminated seedling was transferred to a sand-hydroponic plastic culture chamber (Fig. 1). Seedlings were planted in sand in the upper compartment of the chamber and some roots were directed to grow through small holes into the lower compartment of the chamber. Simultaneously, 25 Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe chlamydospores placed between 2 gelman membrane filters (MF) were placed to germinate in the rhizosphere in the sand compartment of the chamber. The lower compartment of the chamber was filled with 100 ml of Hoagland's nutrient solution (Hoagland & Arnon 1950) containing 0, 300, 600, and 1200 mM P as CaH2PO4. Additional water was added to the sand compartment to keep moisture at optimum level for spore germination. An identical culture chamber, lacking the host plant, was assembled and nutrient solution was applied to the top of the sand compartment and allowed to drain out to the lower compartment. The experiment was carried out under growth chamber conditions (as defined later) for 23 days and nutrient solution was replaced weekly. At harvesting, MF containing spores were recovered from the chambers and stained with 0.01% acid fuchsin for 5 min. The excess staining solution was washed out with deionized water and spores were observed under a dissecting microscope. Spore germination and germ tube (GT) growth were assessed as described by Siqueira et al. (1982). Each treatment was replicated 3 times. This experiment was repeated with Gigaspora margarita Becker & Hall and produced similar results.

A second experiment was performed under growth chamber conditions using soil as the growth substrate for soybean. P levels of 0, 30, 60, 120, and 240 μg/g of soil as CaH4(PO4)2 were added to autoclaved soil. Soil samples were collected from a sandy-soil (Spodosol) with
pH = 5.5, containing P = 70; K = 28; Ca = 320; Mg = 60; Zn = 8; Cu = 7; Mn = 6, nutrients in ppm, determined by double-acid extraction. Additionally, 500 µg/g soil of CaCO$_3$ was added to the soil before application of P treatments. The soil samples were brought to 9% (V/M) moisture and incubated for 10 days. Following the incubation, 200 g of soil (dry weight basis) were packed into styrofoam cups, half of which were sown with 4 soybean seeds per cup. At the same time, 15 azygospores of G. margarita sandwiched between two MF were placed in each cup 1.5 cm below the seeds or 2.5 below the surface for the cups with no seeds. Each treatment was repeated six times. The soil moisture content was readjusted to 9% with deionized water; 5 ml of 1/3 strength Hoagland nutrient solution lacking P was applied every other week.

The experiment was kept in a growth chamber equipped with incandescent and fluorescent light with 13h photoperiod, and 18.5 lux light intensity. Temperature was controlled to 28 and 25°C during the day and night, respectively. After four weeks, the MF with the spores were recovered from the soil and treated as described earlier. At harvesting, root exudates and alcohol extracts were collected and treated according to Graham et al. (1981). The exudates and extracts were analyzed for their carbohydrate content by the anthrone method (Ashwell 1957) and by thin layer chromatography (TLC). For TLC analysis, 20 µl of sample were applied on silica coated plates, developed in isopropanol-water and detected by the silver-alkali reagent (Menzies & Seakins 1969). Root exude samples from the same P level were pooled together and used as solvent to prepare 1% agar media. The root extracts were evaporated to dryness and brought to the same volume as the exudates with deionized water. One percent agar media were also made using root extract solution as solvent. Five plates per treatment were then assayed for their effect on spore germination and GT growth in vitro using G. margarita azygospores. This experiment was repeated with only 2 P (0 and 40 µg/g soil) levels and showed similar results.

Plants from the repeated experiment were used for additional studies. Using two replicates per treatment, plants with no P and 40 µg P/g soil, inoculated with either G. mosseae or G. margarita and uninoculated control, were used for determination of CO$_2$ exchange rate (CER) and other physiological parameters. These studies were performed with a semi-mobile leaf gas exchange system, located at Irrigation, Research and Education Park, University of Florida, Gainesville. The plants were grown in pots containing 1,000 g of soil under growth chamber conditions for 8 weeks. Following the growing period plants were transferred to a plexiglass chamber and equilibrated for four days. The first fully developed leaf from each plant was mounted in a small leaf chamber similar to those used by Harris (1982). The environment in individual leaf chambers was monitored by a computer programmed to record basic sensor signals: quantum flux density, air temperature, dew point temper-
and plants were thinned to one, five days after emergence. Plants were watered every other week with 10 ml per cup of 1/4 strength Hoagland's nutrient solution lacking P (Hoagland & Arnon 1950). The experiment was carried out for ten weeks in an open light greenhouse with temperature set at 26°C. At the end of the growing period, the entire cup contents were wet sieved on nested sieves (850, 425, and 106 μm). The entire plant and the MF with spores were recovered from the first sieve. Root samples were taken from the top sieve for root colonization assessment and total spore numbers were determined in the fraction collected on sieve 106. The MF with the spores were treated as described earlier. Plant height, root fresh weight, shoot dry weight, and number of nodules were recorded. Root colonization was estimated according to Giovannetti & Mosse (1980) on 10% KOH cleared roots stained with 0.1% lactophenol trypan blue (Phillips & Hayman 1970). Root segments were considered colonized if either hyphae, arbuscules or vesicles, or spores were visible. Root extracts were prepared and treated according to Graham et al. (1981). Total sugar analysis was performed colorimetrically (Ashwell 1957).

The alcohol extracts of roots were evaporated to dryness at 40°C using a rotovaporator and returned to original volume with deionized water. Individual replicates were diluted 15 times with deionized water and assayed for their effect on spore germination and GT growth using sand plate bioassay as follows. Fifteen G. mosseae chlamydospores were placed between two MF and incubated on sand plates containing 100 g of washed river sand moistened with 10 ml of the diluted root extract from the different treatments. After 14 days of incubation, MF were recovered, stained and the spores assessed under a dissecting microscope at 25 x for spore germination and GT growth rate (Siqueira et al. 1982).

Data were statistically analyzed utilizing the statistical analysis system package (SAS) at the Northeast Regional Data Center of the University of Florida, USA.

RESULTS

Sand-Hydroponic Culture Experiment.

The system was effective for spore germination studies. G. mosseae chlamydospores showed high germination rates under the experimental conditions. Germination and GT growth were not significantly affected by the presence of roots (Fig. 2). Spores germinated in the rhizosphere showed extensive hyphal growth and produced abnormal, highly ramified, lobated hyphae, along with a number of auxiliary cells and vegetative spores (Siqueira 1983). These tropic responses, are apparently induced by the host and were not induced by the in vitro studies with either root exudates or extracts. As seen in Fig. 2, the presence of host roots did not significantly affect spore germination and GT growth, but induced tropic responses in the free living mycelium growing on membrane filters in the rhizosphere. An indirect supply of P up to 600 mM did not reduce spore germination and GT growth (Fig. 2).

Growth Chamber Experiments.

The effects of P supply on the sugar content of root exudates and extracts, and spore germination under growth chamber conditions are presented on Fig. 3. Addition of small amounts of P (up to 60 μg/g soil) increased the sugar content of the root extracts and decreased it in the exudates of soybean plants. Root extracts with high sugar content were highly inhibitory to spore germination in vitro,
sporulation efficiency did not follow the same trend (Table 1). The decrease in spore number per plant was closely related to PRC and ARC with R values (P < 0.05) of 0.72 and 0.77, respectively. The reduction on sporulation is due to a decrease in root colonization, rather than its direct effect on the fungus. P application had little effect on spore germination in the rhizosphere (Table 1 and Fig. 5) and no significant relationship was found between spore germination in the rhizosphere with either PRC or ACR. Addition of P up to 80 μg/g soil did not reduce spore germination in the rhizosphere but did reduce root colonization by 42% and 62% over the control (with no P added) for G. mosseae and G. margarita, respectively. Root extracts from plants grown under high P supply were highly inhibitory to spore germination in vitro (Table 1). Such an inhibitory effect was greater for G. mosseae than for G. margarita (Fig. 5).

**DISCUSSION**

If conditions are suitable, spore germination is not affected by the presence of roots. However, germinating spores in the rhizosphere showed extensive hyphal growth and exhibited tropism. Similar results have been reported by Daniels & Trappe (1980) and Powell (1976). The fact that these tropic responses were not induced by either root exudates or extracts suggests the involvement of volatile compounds active in the rhizosphere as demonstrated by Koske (1982) and St. John et al. (1983).

As widely reported (Gerdemann 1968, Mosse 1981, Mosse et al. 1981) it was found that additions of soluble P decreased root colonization by the VAM fungi. However, the mechanism by which P inhibits mycorrhizal formation remains unknown. Results from our sand-hydroponic culture experiment do not support the membrane permeability hypothesis as proposed by Ratnayake et al. (1978) and indicate that the mechanism by which P controls PRC acts at the penetration or post-penetration phase rather than on spore germination in the rhizosphere as suggested by Graham et al. (1981). The amount of sugars in the root exudates and extracts was affected by P supply. Increasing

FIG. 4. Effect of P supply and inoculation with VAM fungi on carbon exchange rate (CER) of soybean plants. P = 40 µg P/g soil, Mar = G. margarita, Mos = G. mossaeae, PAR = photosynthetically active radiation. Means followed by the same letters are not statistically different by The Least Square means procedure range test at P = 0.05.

the level of available P in the soil resulted in a decrease in the amount of sugar in the root exudates and an increase in the sugar content of root extracts. Similar results are reported by Graham et al. (1981). Nevertheless, at high P levels sugar content of root extracts decreased. This seems to result from an unbalanced nutrition caused by high P levels which are approaching toxicity level. Soybean plants with P levels higher than 30-40 µg P/g soil were stunted, chlorotic and had lower dry weight (Table 1). Plants grown at P levels equal to or lower than 60-80 µg P/g soil showed no symptoms of unbalanced nutrition and their PRC was less than half that of control to which no P was added. Hence, our discussion will be concentrated on those plants.

Host P nutrition partially controls carbon metabolism and translocation of photosynthates to other parts of the plant (Herold & Walker 1979, Heber & Heldt 1981). Either P application or mycorrhizal colonization increased CER by soybean plants. The cytoplasmic P supply may not only increase host photosynthetic rate, but also can increase the exportation of triose phosphate (TP) out of the chloroplast envelope (Herold & Walker 1979, Heber & Heldt 1981). As a result of the enhanced TP exportation, sucrose synthesis in the cytosol is also enhanced because TP is the precursor for sucrose synthesis (Fig. 6). Depending upon the source-sink relationships, the more sucrose is synthesized the more it is translocated to other parts of the plant, including the roots. As already suggested, VAM fungi in the roots may constitute an important sink for carbohydrate
TABLE 1. Overall effect of P levels and inoculation with VAM fungi on several parameters, for the soybean experiment under greenhouse conditions. (Data are LS means given by the general linear model procedure).

<table>
<thead>
<tr>
<th>Variable</th>
<th>P added (µg/g soil)</th>
<th>STD error (P = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>34.3</td>
<td>35.1</td>
</tr>
<tr>
<td>Shoot DW (g)</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Root FW (g)</td>
<td>9.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Nodules (number/plant)</td>
<td>15.7</td>
<td>17.7</td>
</tr>
<tr>
<td>Sugar (mg/plant)</td>
<td>20.4</td>
<td>24.2</td>
</tr>
<tr>
<td>Colonization (%)</td>
<td>60.9</td>
<td>28.9</td>
</tr>
<tr>
<td>ACR</td>
<td>6.8</td>
<td>2.9</td>
</tr>
<tr>
<td>G - rhizosphere (%)</td>
<td>33.1</td>
<td>38.2</td>
</tr>
<tr>
<td>G - bioassay (%)</td>
<td>70.1</td>
<td>62.0</td>
</tr>
<tr>
<td>Sporul. efficiency</td>
<td>19.4</td>
<td>21.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>G. mosseae</th>
<th>G. margarita</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>36.2</td>
<td>34.6</td>
<td>32.5</td>
</tr>
<tr>
<td>Shoot DW (g)</td>
<td>1.6</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Root FW (g)</td>
<td>11.1</td>
<td>8.1</td>
<td>6.8</td>
</tr>
<tr>
<td>Nodules (number/plant)</td>
<td>20.0</td>
<td>14.2</td>
<td>18.6</td>
</tr>
<tr>
<td>Sugar (mg/plant)</td>
<td>18.8</td>
<td>28.9</td>
<td>14.6</td>
</tr>
<tr>
<td>Colonization (%)</td>
<td>31.9</td>
<td>27.0</td>
<td>-</td>
</tr>
<tr>
<td>ACR</td>
<td>3.8</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>G - rhizosphere (%)</td>
<td>43</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>G - bioassay (%)</td>
<td>34.2</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>Sporul. efficiency</td>
<td>11.9</td>
<td>25.8</td>
<td>-</td>
</tr>
</tbody>
</table>

DW - dry weight; FW - fresh weight; ACR = percentage colonization x root FW (g); G - germination; sporul. efficiency = spore/g colonized root.

(Bethlenfalvay et al. 1982a, b e 1983) probably through their effects on P uptake, CER rates and other physiological processes caused by fungal invasion of root cells.

Since inorganic P has no direct fungistatic effect on VAM fungi and sugars and organic acids are inhibitory to them in vitro (Mosse 1959, Hepper 1979, Siqueira 1983) the results herein presented strongly support the hypothesis that host carbon metabolism is one of the controlling factors for mycorrhizal formation mediated by soil P availability as summarized in Fig. 6. The amount of sugars and probably other related compounds in the cortical cells appears to be the operative factor. Evidence indicates that these factors act at some stage after spore germination has occurred.

This hypothesis, however, predicts that infective hyphae are deterred from penetrating epidermal cells or, if they do penetrate, they are deterred from spreading out from the penetration point by the high carbohydrate (and/or related metabolites) content of the cortical cells which results from improved P nutrition (Fig. 6).

The proposed mechanism is supported by data from Azcon & Ocampo (1981), Graham et al. (1981) and it is consistent with Harley's concept (Harley 1969) that growth response due to root colonization by mycorrhizal fungi is determined by two opposing processes; a stimulating effect due enhanced nutrient uptake, mainly P, and a detrimental effect caused by a fungal drain on host photosynthate. P is the main nutrient taken up via fungal hyphae. If the plant is well supplied with this nutrient mycorrhizal formation would represent a nonreturnable investment by the host plant. If the plant has no control over the fungal invasion, mycorrhiza may develop as a parasitic...
relationship. In this way, the degree of root colonization is a function of P uptake by the host plant, at least for mycorrhizal soybean plants. If the fungus is not effective for P uptake, the host may have no control over the root colonization, and formation of mycorrhiza may be deleterious to host growth. Association between soybean roots and *Glomus fasciculatum*, well studied by Bethlenfalvay and co-workers (Bethlenfalvay & Yoder 1981, Bethlenfalvay et al. 1982a, b e 1983), illustrates this fact.

In VAM associations a delicate balance exists between the level of P availability, fungal development in the roots and plant growth response. Growth responses may range from mycotrophic growth enhancement at low P levels, through growth inhibition induced by parasitic relationship at intermediate levels of P, to non-mycotrophic growth enhancement of the host at high levels of P (Bethlenfalvay et al. 1982c, 1983). According to the hypothesis proposed herein, the more efficient the mycorrhizal symbiosis is, in terms of P uptake from the growing medium and its translocation to the host, the more delicate is the host-fungus relationship and the more precise is the root colonization control mechanism. The fact that in some host-fungus combinations high colonization rates can be found even at high levels of available

![Diagram](image-url)

**FIG. 5.** Effect of P supply on root sugar content, spore germination, root colonization rate and fungal sporulation by *G. mosseae* (—) and *G. margarita* (——). The data are means for 4 replications. Plants were 10 week old under greenhouse conditions. GER = spore germination.

**FIG. 6.** Simplified diagram for the proposed mechanism by which phosphorus supply controls the formation of VAM symbiosis. RDPC = Ribulose diphosphate carboxylase, TP = triose phosphate, PI = inorganic phosphate, F6P = fructose-6-phosphate, UDP = uridine diphosphate, UTP = uridine triphosphate, UDPG = uridine diphosphate glucose, ADP = adenosine diphosphate, ATP = adenosine triphosphate, VAMF = VAM fungi.

P indicates that the control mechanism may differ for different hosts, as already suggested by Buwald et al. (1982).

In conclusion, it is proposed that the level of soybean root colonization by the VAM fungi is controlled by the host carbohydrate metabolism, which is mediated by host P nutrition.

REFERENCES


BETHLENfalvay, G.J.; BROWN, M.S. & PACOVSKY, R.S. Parasitic and mutualistic associations between a mycorrhizal fungus and soybeans: development of the host. Phytopathology, 72: 889-93, 1982a.


