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The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi

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Abstract. A low-cost, low-maintenance system for soilless production of vesicular-arbuscular mycorrhizal (VAM) fungus spores and inoculum was developed and adapted for production of acidophilic and basophilic isolates. Corn (*Zea mays*) plants were grown with *Glomus etunicatum*, *G. mosseae* or *Gigaspora margarita* in sand automatically irrigated with modified Hoagland's solution. Sand particle size, irrigation frequency, P concentration, and buffer constituents were adjusted to maximize spore production. Modified half-strength Hoagland's solution buffered with 4-morpholine ethanesulfonic acid (MES) automatically applied 5 times/day resulted in production of 235 *G. etunicatum* spores/g dry wt. of medium (341000 spores/pot) and 44 *G. margarita* spores/g dry wt. of medium (64800 spores/pot). For six basophilic isolates of *G. mosseae*, $CaCO_3$ was incorporated into the sand and pots were supplied with the same nutrient solution as for acidophilic isolates. The increased pH from 6.1 ± 0.2 to 7.2 ± 0.2 resulted in spore production ranging from 70 to 145 spores/g dry wt. (102000–210000 spores/pot). Spore production by all isolates grown in the soilless sand system at Beltsville has exceeded that of traditional soil mixtures by 32–362% in 8–12 weeks.

Key words: Nutrient solution – Spore production – *Glomus etunicatum* – *Glomus mosseae* – *Gigaspora margarita*

Introduction

Traditionally, soil has been included in potting media used for the production of vesicular-arbuscular mycorrhizal (VAM) fungi (Ferguson and Woodhead 1982) because of its availability, low cost, and general suitability. Residual soil particles remaining on the ultimate product have not interfered with its use as pot and field inocula. However, recent research on biochemical (Hepper et al. 1988), genetic (Millner and Meyer 1990), immunologic (Wright et al. 1987) and ontogenetic (Franke and Morton 1990; Morton and Benny 1990) aspects of VAM fungi required spores free from residual soil particles and organic matter commonly present in soil-media cultures after harvest.

Other approaches to soilless culture of VAM fungi have included static hydroponics (Parvathi et al. 1984), flowing hydroponics (Howeler et al. 1982; Mosse and Thompson 1984), aeroponics (Sylvia and Hubbell 1986), and in vitro with transformed roots (Mugnier and Mosse 1987; Becard and Piche 1989). For these methods, spore production was reported only for the aeroponics and transformed roots (Becard and Piche 1989). In addition, all these methods involved considerable cost in terms of set-up or maintenance. We were interested in having a system that could be set up with standard equipment available in the horticultural industry at low cost, e.g. a hydroponic sand system (Ojala and Jarrell 1980). Current systems which recirculate nutrient solution preclude the culture of many different isolates from diverse geographic areas on the same nutrient supply because of risk of contamination. Acceleration of the time course for spore production over the usual 3–4 months required in soil-based pot cultures would decrease the opportunity for contamination. Ultimately, culture in a soilless system would eliminate much of the time-intensive purification usually needed to rid spores of soil and organic debris.

This report describes the development of a low-cost, low-maintenance, soilless system for the fast, efficient production and harvest of clean VAM fungal spores. It was adapted so that VAM fungal isolates requiring acidic or alkaline conditions could be grown using the same nutrient supply source.

Materials and methods

Four experiments were conducted in succession to establish optimal conditions for production of VAM fungal spores in industrial sand medium using a drip irrigation system to add essential nutrients and water.

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Effect of 4-morpholine ethanesulfonic acid (MES) buffer in sand culture on spore production as compared with soil-mix culture (experiment I)

Plastic pots (15 cm in diameter, 12 cm high) were filled with either sand:soil:turface 1:1:1 (soil-mix), which had cation exchange capacity (CEC) of 6.0 meq/100 g, 3 ppm NO_3^- N, 24 ppm plant-available P (Bray's), and 3 ppm K, or with sand having a particle size distribution of 4% >850 µm, 80% 280–850 µm, 16% <280 µm (2Q-Rok, Pennsylvania Glass Sand Corp., Pittsburgh, Pa.). The soil was a loamy sand which was acquired at a site in Beltsville, Md. mapped as Galestown-Evesboro complex. Turface (Terra Green-MS, Oil Dri Corp. of America, Chicago, Ill.) is a heat-treated calcined montmorillonite. One-half of the pots was inoculated with 1800 spores/pot *Glomus etunicatum* Becker and Gerdemann isolate B1 and the remaining pots with 350 spores/pot *Gigaspora margarita* Becker and Hall isolate INVAM 105. Inoculum was thoroughly mixed with the potting media (ca. 1150 g soil mix/pot and 1070 g sand mix/pot). In this experiment and subsequent ones reported here, 1-week-old, fungicide-free sweetcorn (*Zea mays*) seedlings cv. "Iochief" grown in standard seed-towels with water only were transplanted into each pot (2 per pot). Soil-mix treatments were hand-watered and were fertilized with (25-0-25) Peter's No-Phos special fertilizer (Peter's Fertilizer Products, Fogelsville, Pa.) according to our standard culturing practice, which was when leaf appearance indicated need for supplemental nitrogen. Sand pots were automatically drip-irrigated 3 times/day with 30 ml of modified full-strength Hoagland's solution (Table 1) containing 2 µM P either with (pH 6.1) or without (pH 6.7) MES, Sigma, St. Louis, Mo.) buffer. Hoagland's solution was chosen as a standard complete nutrient solution because it lacks ammonium salts, which often result in an altered solution pH during plant growth (Asher and Edwards 1983), and it contains necessary micronutrients. Each treatment was replicated three times. Nutrient-solution pH was measured before use from the reservoir and again as fresh effluent draining from the pots immediately after irrigation. After 10 weeks, plant heights were recorded, and shoots and roots were harvested, dried for 7 days at 100° C and weighed. Spore production was evaluated.

In subsequent experiments, larger particle-size sand (no. 4 coarse sand, Morie Co., Millville, N. J.) was used to promote better drainage. This sand was mixed 1:1 with the fine sand used previously. The resulting particle size was 60% >850 µm, 27% 550–850 µm, and 13% <550 µm.

Table 1. Modified Hoagland's solution

Salt	Final concentration	
	Full strength[a]	Half strength[b]
$Ca(NO_3)_2 \cdot 4H_2O$	5.0 mM	2.5 mM
KNO_3	5.0 mM	2.5 mM
$MgSO_4 \cdot 7H_2O$	2.0 mM	1.0 mM
NaFe EDTA	0.1 mM	50.0 µM
KH_2PO_4	2.0 µM[c]	20.0 µM
H_3BO_3	10.0 µM	10.0 µM
$Na_2MoO_4 \cdot 2H_2O$	0.2 µM	0.2 µM
$ZnSO_4 \cdot 7H_2O$	1.0 µM	1.0 µM
$MnCl_2 \cdot 4H_2O$	2.0 µM	2.0 µM
$CuSO_4 \cdot 5H_2O$	0.5 µM	0.5 µM
$CoCl_2 \cdot 6H_2O$	0.2 µM	0.2 µM
$NiSO_4 \cdot 6H_2O$	0.2 µM	0.2 µM
HCL 3N	25.0 µM	25.0 µM
MES buffer	0.5 mM	0.5 mM

[a] Used in experiments I–III
[b] Used in experiment IV
[c] Concentration of KH_2PO_4 for experiment I only

Effect of rock phosphate and a gradual increase in solution P from 5 to 20 µM on spore production in sand culture (experiment II)

Inocula of *G. margarita* (1800 spores/pot) or *G. etunicatum* (2000 spores/pot) were thoroughly mixed into six replicate pots (ca. 1450 g sand/pot) and planted with corn. One-half was amended with 22 µg/g P as rock phosphate (0.87% available P; 2% available $P_2O_5^-$) and the remaining pots were left unamended. Rock phosphate was used to supplement phosphorus in a slow-release, not readily available form. Each treatment had three replications. Pots were drip irrigated 4 times/day with 40 ml modified full-strength Hoagland's solution containing MES buffer and increasing levels of solution P (see Tables 3 and 4 for details). *G. margarita* cultures were harvested at 9 weeks. *G. etunicatum* cultures were harvested at 12 weeks.

Effect of 100 µM P on spore production (experiment III)

Glomus mosseae Nicolson and Gerdemann INVAM 156 (460 spores/pot) was cultured in sand amended with $CaCO_3$ to adjust the pH to 7.2±0.2. One-half of the pots was amended with 100 µg/g P as rock phosphate (as above) and one-half was left unamended. Each treatment had three replications. All pots were drip irrigated 3 times/day with 40 ml of full-strength Hoagland's solution containing MES buffer with 20 µM P for 4 weeks and 100 µM P for 8 weeks, or with 100 µM P for 12 weeks. *G. etunicatum* was cultured in sand without $CaCO_3$ and rock phosphate, but inoculated with sievings containing hyphae, root pieces and ca. 2000 spores. Pots were irrigated with the same nutrient solution as *G. mosseae*. Nutrient solution pH was measured from the reservoir and the fresh effluent from the pots immediately after irrigation. *G. etunicatum* and *G. mosseae* cultures were harvested at 11 and 12 weeks, respectively.

Effect of modified half-strength Hoagland's solution and 20 µM P on spore production (experiment IV)

Half-strength Hoagland's solution with 20 µM P (Table 1) supplied as 60 ml, 5 times/day was tested on spore production in sand culture. Inoculants were *G. margarita* (5000 spores/pot or sievings, three replicates each), *G. etunicatum* (sievings, three replicates). To determine the effect of these sand nutrient-solution conditions on sporulation of basophilic isolates, we obtained observations on six isolates of *G. mosseae* (Table 5). From previous culturing in sand (unpublished), we knew that these isolates required a pH higher than 7.0 to achieve spore production averaging 3–10 spores/g soil. *G. margarita* cultures were harvested at 9 weeks and *G. etunicatum* and *G. mosseae* cultures were harvested at 11 weeks.

All plants were grown in a greenhouse at 30° C under sodium vapor lights [675 µE of photosynthetically active radiation (PAR)]. Plant shoots and roots were harvested, dried and weighed as in the first study, and spore production was evaluated as described below. A simple analysis of variance (alpha = 0.05), using log transformations to normalize data when necessary, was performed on data from experiments I, II (*G. etunicatum*), and III (*G. mosseae*). Duncan's multiple range test was used for means separation (SAS 1985) where appropriate. Means and standard error of the mean were calculated for root weights and spore counts for *G. etunicatum* and *G. margarita* data (see Tables 3, 4).

Evaluation of spore production

After harvesting the shoots, the potting media with roots were air dried and weighed. Potting media with roots were then soaked in water, agitated gently by hand, and wet-sieved. The roots were sprayed clean, collected from the sieves, dried for 7 days at 100° C and weighed.

Pots containing *G. margarita* were wet-sieved with a 850-µm and 150-µm sieve series, *G. etunicatum* pots were sieved with a 850-µm and 45-µm series and *G. mosseae* with 850-µm and 106-µm series. For routine retrieval of spores, intermediate sieves can be used to reduce the amount of sievings at the expense of nominal spore loss. However, for these studies the retrieval of all spores was considered important. Total time needed to wet-sieve each pot was recorded for the first study.

Spores were separated from root pieces in sievings by centrifugation (3 min, 3000 rpm) through a 20%, 40%, and 60% sucrose gradient in a 50-ml centrifuge tube. Spores from the upper layers were washed on a 45-µm sieve, then resuspended in 40 ml distilled water. Aliquots of the spore suspensions containing 1000–2000 spores (0.5, 0.25, or 0.2 ml) were distributed onto a filter paper for counting with a binocular microscope. Counts from three replicate aliquots were averaged for each sample; averages were multiplied by 2, 4, or 5 to determine spores/ml, and then multiplied by the total volume of the spore suspension to determine total spores/pot. Sediment at the bottom of the tube after sucrose centrifugation was treated in the same manner as the spore suspensions. Spore numbers from sediment were included in the total spore count for the pot.

Irrigation system

The irrigation system consisted of 1.9 cm internal diameter (ID) irrigation polypipe with 1.52 mm ID leader tubes and 15 cm diameter dribble rings. Dribble rings were connected to the leader tubes, placed inside the pot and adjusted to lie flat on the surface of the sand. Each dribble ring had four equidistant holes to provide even distribution of nutrient solution. Polypipe dribble rings were chosen over "drop in" weights to avoid contamination by metals.

An on/off valve was attached to one end of the irrigation system and connected by tygon tubing to a special epoxy-encapsulated, chemically resistant, submersible, centrifugal pump (Grainger, Baltimore, Md.). The pump was placed in a covered 121-l plastic barrel which was used as a reservoir for the nutrient solution (Figs. 1, 2). A programable timer connected to the pump controlled the application of nutrient solution. The irrigation system was ultimately set to deliver 60 ml nutrient solution during a 45-s irrigation period, 5 times/day during daylight hours. Algal growth on the sand surface was controlled by covering the pots with black plastic.

Results

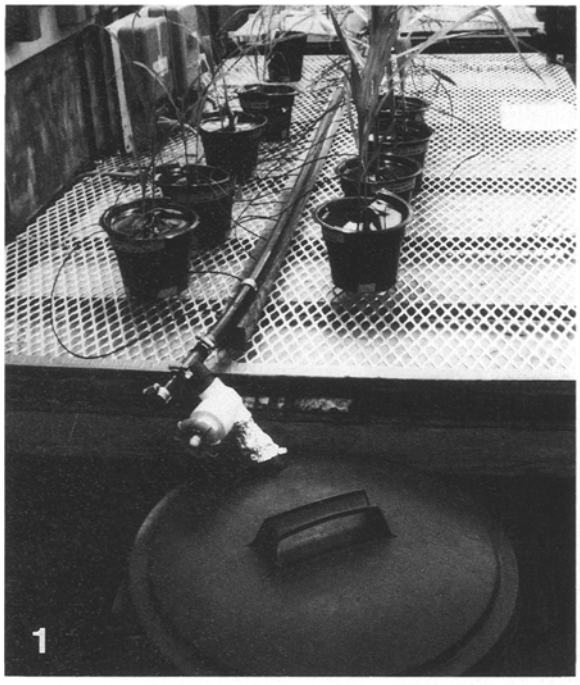
In the experiment comparing sand to soil cultures (I), plants grown in the soil-mix had higher shoot and root dry weights than those grown in the sand nutrient solution with or without MES buffer (Table 2). Sand culture pots were waterlogged at the bottom of the pots and plants were stunted with leaf-tip damage. Hence, a coarser sand medium with better drainage was selected for subsequent experiments. Soil cultures produced more spores/g dry wt. medium than the nutrient solution without MES buffer. When MES buffer was included in the nutrient solution, however, spore production was equivalent to that in the soil-mix.

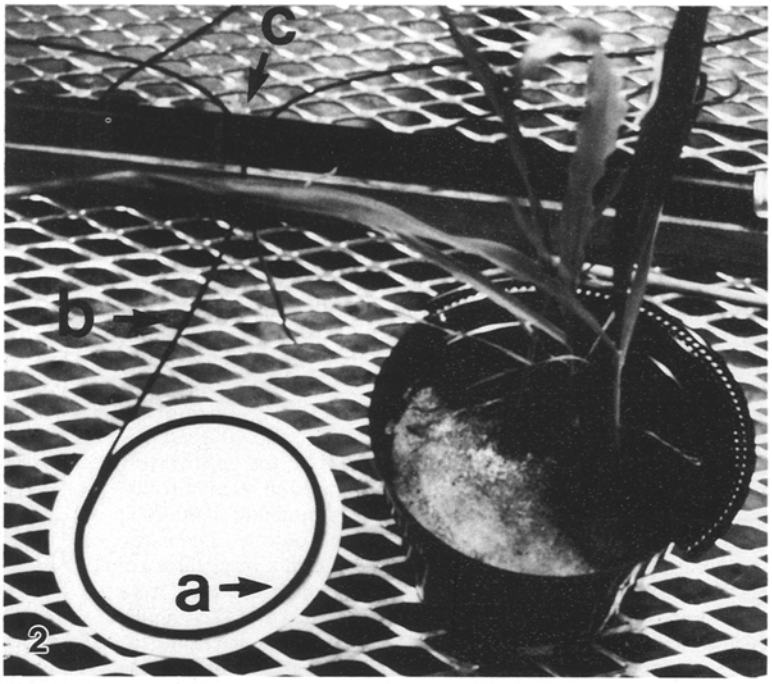
The absence of clay particles and soil organic matter in sand cultures resulted in cleaner spore preparations (Fig. 3a, b). The time involved in sieving and centrifugation was half that needed to process the soil-mix cultures (Table 2). The pH of unbuffered nutrient solution in the reservoir fluctuated between 6.5 and 7.0 during the course of the experiment; fresh effluent ranged from pH 6.0 to 6.7. Fresh effluent from the soil-mix pots was pH 5.8. When buffered, the pH of the nutrient solution in the reservoir was the same as that of the fresh effluent (ranging from 5.9 to 6.3). This stability in pH, together with increased spore production, warranted the use of MES to buffer Hoagland's solution for all subsequent studies.

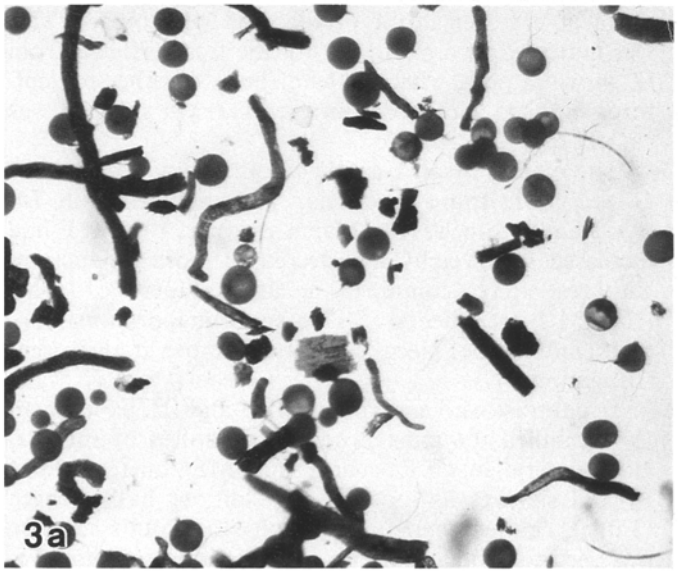
In tests of the effects of rock phosphate and solution P concentration (II and III), addition of up to 100 µg/g P as rock phosphate did not significantly increase ($P = 0.05$) plant growth or spore production of *G. etunicatum* (Table 3), *G. mosseae* (data not shown) or spore production of *G. margarita* (Table 4). The addition of $CaCO_3$ to *G. mosseae* cultures increased the host environment pH even in the presence of MES buffer. This was indicated by a pH of 7.2 for the fresh effluent from *G. mosseae* pots, whereas the effluent of other pot cultures supplied with MES-buffered nutrient solution was ca. pH 6.1.

The effects of all treatments on *G. etunicatum* and *G. margarita* spore production are summarized in Tables 3 and 4 respectively. Plants grown at 100 µM P had increased root weight, and decreased spore production. Cultures which contained a large volume of roots (100 µM P treatments) yielded spore preparations containing many root pieces even after sucrose gradient centrifugation.

In contrast with experiments I, II and III, experiment IV combined the most productive features of the previous experiments, i.e., coarse sand, MES buffer, 20 µM P, and sievings as inocula. In addition, half-strength (Table 1) rather than full-strength Hoagland's solution was used to supply nutrients. Production of *G. etunicatum* averaged 235 spores/g dry wt. medium, which was 362% more than the highest previous average of 50.8 spores/g dry wt. medium (Table 3). Production of *G. margarita* averaged 25.8 and 44.7 spores/g dry wt. medium for spore- and sievings-inoculated treatments, respectively. This was 32 and 128% greater than the highest previous average of 19.6 spores/g dry wt. medium (Table 4). Spore production by the six *G. mosseae* isolates ranged from 70–145 spores/g dry wt. medium (Table 5) which was seven-fold greater than that in our soil pots, which typically ranged 3–10 spores/pot (unpublished data). The highest previous spore count with our sand nutrient solution system yielded 6.4 spores/g dry wt. medium (data not shown, experiment III). This poor sporulation in experiment III was attributed to the full-strength Hoagland's solution and the high P (100 µM) used. The highest spore yields from the three species of VAM fungi used in this study are in part attributable to the inclusion of colonized root pieces and hyphae in the







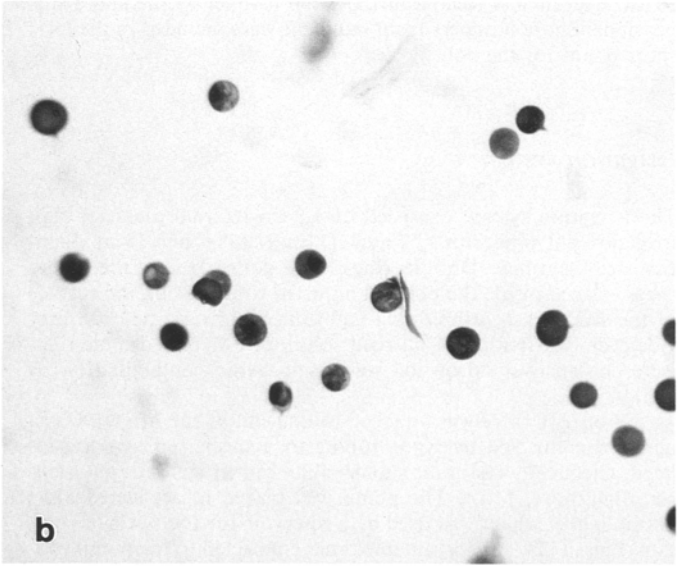


Fig. 1. Nutrient solution drip-irrigation system

Fig. 2. *a*, Dribble ring; *b*, leader tube; *c*, 1.9-cm main line

Fig. 3a, b. Spore preparations after sieving and sucrose centrifugation. **a** Traditional soil-mix pots. **b** Sand nutrient solution pots

inocula (sievings), although spore carry-over from the inocula accounted for less than 10% of the final spore yield/pot.

Discussion

Compared with soil : sand : turface cultures of VAM fungi, the soilless method successfully reduced the handling time for spore retrival by 50%. High density spore populations with low organic debris and no soil particles were harvested in 8–12 weeks.

Through this series of experiments, we have determined that a selective combination of culturing factors, i.e. baseline nutrient solution content, solution P concentration, MES buffer, sand particle size, and inoculum composition, can be used to significantly enhance spore production of three different VAM fungi. The precise conditions that we found for efficient mass production of soil-free VAM fungus spores are summarized below.

1. Half-strength Hoagland's nutrient solution with 20 μM P and 0.5 mM MES buffer provided adequate

Table 2. Experiment I: effect of media/fertilizer treatment on plant growth, spore sieving time and spore production of *Glomus etunicatum* and *Gigaspora margarita*[a]

Media/Fertilizer treatment	Top wt. (g)	Root wt. (g)	Sieving time (min)	Spores/g dry wt. media[b]	(spores/pot)
Soil mix +25-0-25 (no phos)	16.76[a]	2.82[a]	26.7[a]	29.8[a]	(34400)
Sand +2 μM P Hoagland's	1.42[b]	0.43[b]	10.3[b]	19.7[b]	(21300)
Sand +2 μM P Hoagland's +MES buffer	1.93[b]	0.40[b]	10.3[b]	30.0[a]	(32200)

[a] Treatment means are averages of both fungi because there was no species by fertilizer interaction. Means in a column followed by the same letter are not significantly different ($P=0.05$)

[b] Log transformation was used for mean separation by Duncan's multiple range test

Table 3. Summary comparison of root weight and spore yield for all the *G. etunicatum* experiments using 25-0-25 (no-phos) fertilizer or drip irrigated Hoagland's solution

Fertilizer treatment	Inoculum	Root wt. (g)	Spores/g dry wt. media	(spores/pot)
Experiment I		$\bar{X}\pm$SEM[a]	$\bar{X}\pm$SEM[a]	
Soil mix +25-0-25 (no phos)	1800 spores/pot	2.71±0.24	40.6± 7.0	(46400)
Full-strength Hoagland's +2 μM P	1800 spores/pot	0.44±0.01	35.0±19.0	(37500)
Full-strength Hoagland's +2 μM P +MES buffer	1800 spores/pot	0.34±0.22	50.8± 2.3	(54400)
Experiment II[b]				
Full-strength Hoagland's +5–20 μM P +MES buffer	2000 spores/pot	1.01±0.06	35.9± 7.1	(52100)
Full-strength Hoagland's +5–20 μM P +MES buffer +22 ppm rock phosphate	2000 spores/pot	1.03±0.12	29.5± 6.7	(42800)
Experiment III				
Full-strength Hoagland's +20–100 μM P +MES buffer[c]	Sievings[d]	2.20±0.18	12.0± 2.9[e]	(17400)
Full-strength Hoagland's +100 μM P +MES buffer	Sievings	2.49±0.08	7.4± 1.4[e]	(10700)
Experiment IV				
Half-strength Hoagland's +20 μM P +MES buffer	Sievings	3.17±0.14	235.0±10.0	(341000)

[a] Mean and standard error of the mean, $n=3$

[b] Corn plants were grown for 1 week at 5 μM P, followed by 4 weeks at 10 μM P and then 7 weeks at 20 μM P

[c] Corn plants were grown for 3 weeks at 20 μM and 8 weeks at 100 μM P

[d] Sievings contained hyphae, root pieces and ca. 2000 spores from experiment I

[e] Mean and standard error of the mean, $n=2$

nutrients for growth at lower expense than full-strength Hoagland's solution. Other concentrations of P tested were suboptimal relative to 20 μM because they yielded poor shoot and root growth (2.0 μM P) or poor spore production (100 μM). During the 8- to 12-week phase of spore production culture, solution P concentrations greater than 2.0 μM are needed to avoid stunting and to sustain new root growth.

2. Sand containing 60% >850 μm, 27% 550–850 μm, and 13% <550 μm particles provided good drainage as well as adequate moisture retention.

3. Inocula as 10 ml of sievings (from 12-week sand or soil cultures) containing hyphae, colonized root pieces, and ca. 2000 spores enhanced spore production within 8–12 weeks.

Table 4. Summary comparison of root weight and spore yield for all the *G. margarita* experiments using 25-0-25 (no phos) fertilizer or drip-irrigated Hoagland's solution. ND, No data available

Fertilizer treatment	Inoculum	Root wt. (g)	Spores/g dry wt. media	(spores/pot)
Experiment I		$\bar{X} \pm$ SEM[a]	$\bar{X} \pm$ SEM[a]	
Soil mix +25-0-25 (no phos)	350 spores/pot	2.90±0.09	19.6±5.25	(22500)
Full-strength Hoagland's +2 μM P	350 spores/pot	0.42±0.08	4.2±0.46	(4490)
Full-strength Hoagland's +2 μM P+MES buffer	350 spores/pot	0.46±0.03	8.9±1.65	(9520)
Experiment II[b]				
Full-strength Hoagland's +5–20 μM P+MES buffer	1800 spores/pot	ND	6.0±0.72[c]	(8700)
Full-strength Hoagland's +5–20 μM P+MES buffer +22 ppm rock phosphate	1800 spores/pot	0.51±0.03	3.7±0.39	(5370)
Experiment IV				
Half-strength Hoagland's +20 μM P+MES buffer	5000 spores/pot	2.68±0.13	25.8±3.5	(37400)
Half-strength Hoagland's +20 μM P+MES buffer	Sievings[d]	3.00±0.09	44.7±5.5	(64800)

[a] Mean and standard error of the mean, $n=3$
[b] Corn plants were grown for 4 weeks at 5 μM P, followed by 4 weeks at 10 μM P and then 1 week at 20 μM P
[c] Mean and standard error of the mean, $n=2$
[d] Sievings contained hyphae, root pieces and ca. 2000 spores from experiment I

Table 5. Spore production of *G. mosseae* isolates with modified half-strength Hoagland's solution, 20 μM P[a]

Isolate[b]	Geographic origin	Spores/g dry wt. medium	(spores/pot)
G1	UK	142	(206000)
G2	UK	98	(142000)
G3	UK	117	(170000)
JII 1712	UK	70	(102000)
Granada	Spain	122	(177000)
INVAM 365	USA (Fla.)	145	(210000)
		116 Average	(167000)

[a] $CaCO_3$ increased culture pH to 7.2±0.2
[b] Inocula consisted of sievings containing hyphae, root pieces, and ca. 2000 spores from soil cultures

4. The drip irrigation system delivered 60 ml evenly on the sand surface, 5 times/day (during daylight hours only). This schedule was optimal for our growing conditions, but can be adjusted.

5. Sweetcorn was the host. In general, our past experience led to the selection of sweetcorn as a host for spore production because of its (a) short growth/maturation cycle, (b) white uncolonized versus yellow colonized roots, (c) wide range of temperature tolerance, (d) easily recognizable P deficiency symptoms, and (e) its high photosynthetic efficiency relative to other common hosts, i.e. onions (*Allium* spp.), clovers (*Trifolium* spp.), and sudan grass (*Sorghum sudanense*).

6. Sodium vapor lights provided a consistent high intensity (675 μE PAR) light source throughout the growth period.

7. MES buffer stabilized the root environment pH at 6.1 and enhanced spore production of acidophilic isolates. Addition of $CaCO_3$ to the sand supported the growth and spore production of basophilic isolates.

The successful sporulation of acidophilic isolates (*G. margarita* and *G. etunicatum*) with the half-strength solution and the six basophilic isolates of *G. mosseae* by incorporation of $CaCO_3$ demonstrates the adaptability of this system for clean spore production by VAM fungi obtained from and conditioned to a range in pH. In addition to the species and isolates for which data are presented, we have successfully cultured and enhanced spore production of six other isolates of VAM fungi including *Acaulospora longula* ex Columbia, South America, *Gigaspora gigantea* ex Pennsylvania, *Glomus deserticola* ex Utah, *G. intraradices* ex Florida, *G. monosporum* ex France and *Scutellospora coralloidea* ex California (data not shown). This supports our finding that this method is applicable to a variety of VAM fungal isolates from diverse geographic locations.

Previous reports of hydroponic culture of VAM fungi have emphasized the plant response to VAM, primarily in terms of yield (Ojala and Jarrell 1980; Howeler et al. 1982; Mosse and Thompson 1984; Parvathi et al. 1984). In contrast, the system described here emphasizes the fungal growth and sporulation response. Other systems recirculate the source nutrient solution, whereas our system does not. Without recirculation of nutrient

solution, our system can accommodate different isolates simultaneously because the nutrient supply is protected from possible contamination by spores and hyphae that may elute from culture pots.

We have used water-washed spores obtained from this system for amplification of rDNA by the polymerase chain reaction with good success and minimal spore pre-treatment. The overall cleanliness of the spores obtained from this system makes quality control checks for contaminants very easy. Spore development (Franke and Morton 1990) and extraradical hyphae (Sylvia 1988) can be observed much more easily when cultures are produced by this soilless method.

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