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Production of Vesicular-Arbuscular Mycorrhizal Fungus Inoculum in Aeroponic Culture†

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Bahia grass (*Paspalum notatum*) and industrial sweet potato (*Ipomoea batatas*) colonized by *Glomus deserticola*, *G. etunicatum*, and *G. intraradices* were grown in aeroponic cultures. After 12 to 14 weeks, all roots were colonized by the inoculated vesicular-arbuscular mycorrhizal fungi. Abundant vesicles and arbuscules formed in the roots, and profuse sporulation was detected intra- and extraradically. Within each fungal species, industrial sweet potato contained significantly more roots and spores per plant than bahia grass did, although the percent root colonization was similar for both hosts. Mean percent root colonization and sporulation per centimeter of colonized root generally increased with time, although with some treatments colonization declined by week 14. Spore production ranged from 4 spores per cm of colonized root for *G. etunicatum* to 51 spores per cm for *G. intraradices*. Infectivity trials with root inocula resulted in a mean of 38, 45, and 28% of bahia grass roots colonized by *G. deserticola*, *G. etunicatum*, and *G. intraradices*, respectively. The germination rate of *G. etunicatum* spores produced in soil was significantly higher than that produced in aeroponic cultures (64% versus 46%) after a 2-week incubation at 28°C. However, infectivity studies comparing *G. etunicatum* spores from soil and aeroponic culture indicated no biological differences between the spore sources. Aeroponically produced *G. deserticola* and *G. etunicatum* inocula retained their infectivity after cold storage (4°C) in either sterile water or moist vermiculite for at least 4 and 9 months, respectively.

Soil-based pot culture is a common method for production of vesicular-arbuscular mycorrhizal (VAM) fungal inoculum (10). Recently, solution culture techniques such as nutrient film (5, 11) and aeroponics (15) have been adapted for the production of inocula of VAM fungi. These methods provide an alternative to soil-based pot culture for mass production of clean, soilless VAM inoculum. Clean propagules, especially spores, are not only useful for inoculation, but are also essential for critical physiological and genetic studies. Both solution culture techniques provide well-colonized root inocula (5, 11, 15), but results of sporulation experiments were quite different. In the nutrient film technique, sporulation was sparse, except with full-strength Hoagland nutrient solution, when plants suffered manganese toxicity (11). In contrast, the highly aerated rooting environment of aeroponic culture stimulates rapid and abundant sporulation of the VAM fungi. In an aeroponic culture, inoculation of bahia grass (*Paspalum notatum* Flugge) with *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Glomus intraradices* Schenck & Smith resulted in root colonization and sporulation superior to that previously reported for a soil-based pot culture (15, 15a).

For effective use of the aeroponic culture technique for inoculum production, the following questions must be addressed. (i) Can this technique be applied to other host plants and VAM fungi? (ii) Are aeroponically produced inocula infective? (iii) Is it possible to store inoculum in a viable state for an extended period?

The objectives of this study were to investigate host-fungus interactions in aeroponic culture, to test the infectivity of aeroponically produced root and spore inocula, and to determine the viability of these inocula after cold (4°C) storage for different periods.

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MATERIALS AND METHODS

In these studies we compared the establishment of three VAM fungi: *Glomus deserticola* Trappe, Bloss, & Menge, *G. etunicatum* Becker & Gerdemann, and *G. intraradices* on two host plants: bahia grass and industrial sweet potato (*Ipomoea batatas* (L.) Lam. cv. White Star).

Precolonization. Aeroponic cultures were initiated with precolonized seedlings. Spores of *G. deserticola* (isolate S306), *G. etunicatum* (isolate S329), and *G. intraradices* (isolate S303) were wet sieved from 400 g of cold-stored (4°C) soil inoculum and mixed with pasteurized (75°C for 4 h) loamy sand (siliceous hyperthermic Grossarenic Paleudult). Surface-disinfected (30% H_2O_2, 10 min) bahia grass seeds and slips of industrial sweet potato were then placed in the inoculum in shallow (6-cm-deep) trays. Seedlings were grown in a nonshaded greenhouse for 6 to 8 weeks (see below for details of greenhouse environmental conditions), after which roots were washed and trimmed to a length of 6 cm for bahia grass and 8 cm for industrial sweet potato. Root colonization by VAM fungi was confirmed by the nondestructive autofluorescence method of Ames et al. (1).

Aeroponic culture. Colonized seedlings (30 for bahia grass and 20 for industrial sweet potato) were placed into aeroponic chambers in a nonshaded greenhouse with a ca. 10-cm spacing between plants for bahia grass and a 12-cm spacing for industrial sweet potato. The apparatus of aeroponic chambers was adapted from Zobel et al. (17). *G. deserticola* was cultured from April to September 1986 with mean maximum and minimum temperatures of 37 and 25°C and a maximum photosynthetic photon flux density (PPFD) of 1,725 μmol s^{-1} m^{-2}. *G. etunicatum* was cultured from June to November 1986 with mean maximum and minimum temperatures of 35 and 24°C and a maximum PPFD of 1,850 μmol s^{-1} m^{-2}. *G. intraradices* was cultured from August 1986 to January 1987 with mean maximum and minimum temperatures of 31 and 22°C and a maximum PPFD of 1,850 μmol s^{-1} m^{-2}. Low-P_i (0.03 μg g^{-1}) dilute Hoagland nutri-

ent solution was used in each chamber as described by Sylvia and Hubbell (15), with the solution pH initially adjusted to 6.50 ± 0.05 with 1 N NaOH. Roots of four randomly selected bahia grass and three industrial sweet potato plants from each chamber were harvested 14 days after seedlings had been placed in the aeroponic chambers. Successive harvests were continued thereafter at 2- or 3-week intervals up to 14 weeks. At each harvest, only roots grown out of the original roots (8 cm for industrial sweet potato and 6 cm for bahia grass) were collected; they were cut into 5-cm segments. These root segments were checked for sporulation under a dissecting microscope and then cleared in 10% KOH and stained with 0.05% trypan blue. The total root length and root length colonized by VAM fungi were estimated by a gridline intersect method (6), and the total number of spores produced in each segment was recorded. After each harvest, the final pH of the nutrient solution in the chambers was recorded and the solution was changed. Roots of the remaining seedlings were trimmed to a length of 12 to 15 cm to prevent growth into the nutrient solution.

Infectivity of root inocula. A completely randomized experiment was conducted with fresh root inocula of *G. etunicatum* and *G. deserticola*. There were 7 treatments (2 × 3 factorial and 1 noninoculated control) and 6 replicates. The two inoculation rates were 1:250 and 1:1,250 (vol/vol, root inoculum/soil). The three sources of inocula were colonized bahia grass and industrial sweet potato roots produced aeroponically and bahia grass roots colonized in soil. Roots from each host-fungus combination were harvested from aeroponic chambers after 14 to 17 weeks of growth, cut into 1-cm pieces, and mixed thoroughly. Root inocula from soil were collected by washing fresh soil-based pot cultures, and the roots collected were cut into 1-cm pieces. White leach tubes (capacity, 165 ml; Ray Leach Cone-Tainer Nursery, Canby, Oreg.) were filled with pasteurized loamy sand, and root inocula (0.14 and 0.68 g) were placed 2 cm below the surface. Tubes were seeded with bahia grass and placed in a greenhouse. The mean maximum and minimum temperatures were 29 and 20°C, and the maximum PPFD at the seedling level was 1,650 μmol s^{-1} m^{-2}. After 8 weeks roots were harvested, cleared, stained, and assessed for mycorrhizal colonization as described above. A similar experiment with five treatments (2 × 2 factorial plus 1 noninoculated control) was established for *G. intraradices* dry root inocula (air dried for 2 days) at two inoculation rates (same as above) and two sources of inocula (colonized bahia grass and industrial sweet potato roots produced aeroponically).

Infectivity of *G. etunicatum* spores. Spores of *G. etunicatum* were collected on a sieve (pore size, 90 μm) by washing colonized roots of industrial sweet potato from aeroponic culture with a strong stream of tap water. Spores of *G. etunicatum* were also obtained from fresh soil-based pot cultures by wet sieving and decanting, followed by centrifugation in H_2O and 40% sucrose (4).

(i) Germination test. The germination test experiment had a randomized complete block design with two sources of spores (soil and aeroponic culture) and three blocks, each with three replicates. Pasteurized sandy loam (3 kg) was mixed with 200 ml of deionized water (final soil moisture content, 5.9%) and placed in a plastic tray (4.5 by 19 by 30 cm). Approximately 50 spores were placed on each of 18 Metricel membrane filters (diameter, 25 mm; pore size, 0.2 μm; Gelman Sciences, Inc., Ann Arbor, Mich.), which were then folded twice and inserted into the soil to a depth of 1 cm. The soil tray was sealed with Saran wrap and incubated in the dark at 28°C. To permit some aeration, holes were punched with a needle at the corners of the plastic cover. After a 2-week incubation, filters were removed, unfolded, stained with 0.05% trypan blue, and checked under a compound microscope (magnification, ×100 to 200) for percent spore germination.

(ii) Infectivity test. The infectivity test experiment had a completely randomized design with 9 treatments (2 × 4 factorial plus 1 noninoculated control) and 18 replicates. The two sources of inocula used were spores collected from soil and aeroponic culture, and four rates were applied: 25, 50, 75, and 100 spores per 165 ml of soil. The experimental setup was the same as that described above for infectivity of root inocula. After 8 weeks, six plants were randomly selected to assess VAM development. The remainder were harvested after 12 weeks. Roots were cleared, stained, and assessed for VAM development as described above. Data from different harvests were analyzed separately.

Storage study. Aeroponically produced *G. etunicatum* and *G. deserticola* inocula containing colonized roots with external hyphae and spores were cut to 1-cm pieces and stored in Mason jars with sterilized distilled water or with sterilized moist vermiculite (50 cm^3 of vermiculite and 30 ml of distilled water) at 4°C. Inocula from different fungal and plant species were stored separately. Inocula associated with industrial sweet potato were stored either in sterile water or in moist vermiculite, whereas inocula associated with bahia grass were stored in sterile water only. Infectivity tests were carried out after cold storage for 2, 4, 6, and 9 months, when a completely randomized experiment was conducted for each fungal species grown in a greenhouse (2 and 4 months) or a growth chamber (6 and 9 months). For 2-month storage, the mean maximum and minimum temperatures were 27 and 24°C and the PPFD at the seedling level was 1,650 μmol s^{-1} m^{-2}. For 4-month storage, the mean maximum and minimum temperatures were 28 and 21°C and the maximum PPFD at the seedling level was 1,740 μmol s^{-1} m^{-2}. For 6- and 9-month storage, the mean maximum and minimum temperatures were 30 and 22°C and the maximum PPFD at the seedling level was 1,850 μmol s^{-1} m^{-2}. The four treatments were noninoculated control, industrial sweet potato-associated inocula stored in sterile water, industrial sweet potato-associated inocula stored in moist vermiculite, and bahia-grass-associated inocula stored in sterile water. The experimental setup was the same as described above for infectivity of root inocula. Bahia grass was used as the test plant, white leach tubes (capacity, 165 ml) were used as the containers, and the inoculation rate was 1:1,250 (vol/vol, inoculum/soil). Roots were harvested after 8 weeks, and root colonization was assessed as described above.

Data analysis. All data were subjected to analysis of variance, and data from different fungal species were analyzed separately. When significant main effects occurred, treatment means were separated by Tukey's (equal sample size) or Scheffe's (unequal sample size) multiple pairwise comparison at $P \leq 0.05$ (13). For storage study, treatment means were separated by orthogonal contrast.

RESULTS AND DISCUSSION

Aeroponic culture. By the final harvest, all roots had been colonized by inoculated VAM fungi and contained abundant vesicles and arbuscules; spores were produced both intra- and extraradically. The pH of the nutrient solution remained within 0.1 unit of the initial level throughout the experiment. Analysis of variance indicated that no time × position

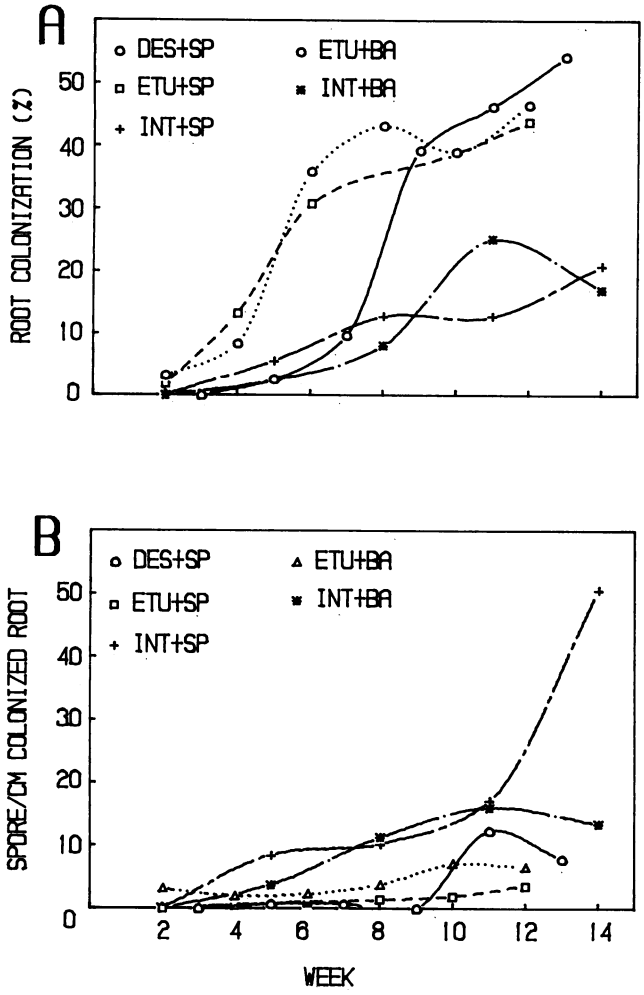

FIG. 1. Mean percent root colonization (A) and sporulation per centimeter of colonized root (B) by *G. deserticola* (DES), *G. etunicatum* (ETU), and *G. intraradices* (INT) on bahia grass (BA) and industrial sweet potato (SP) in aeroponic culture over a 14-week period.

interactions occurred for any variable. At the final harvest (12 to 14 weeks), industrial sweet potato inoculated with *G. deserticola* had at least 50% root colonization, whereas plants inoculated with *G. etunicatum* and *G. intraradices* had 45% and 20% root colonization, respectively (Fig. 1). However, since the experiments for different fungi were set up at different times and plants were grown in a greenhouse rather than an environment-controlled growth chamber, comparison of results among fungi is not possible.

The mean percent root colonization and sporulation per centimeter of colonized root generally increased with time, although with some treatments colonization declined by week 14 (Fig. 1). Percent root colonization decreased toward the apex of the root, except for *G. deserticola* with industrial sweet potato, for which colonization was uniform across the whole root system (Table 1). Spore production ranged from 4 spores per cm of colonized root for *G. etunicatum* to 51 spores per cm for *G. intraradices* (Table 2). Sporulation per centimeter of colonized root decreased acropetally only on roots inoculated with *G. etunicatum* (Table 1). The mean percent root colonization in this study was slightly lower than that reported by Sylvia and Hubbell (15), whereas

TABLE 1. Mean percent root colonization and sporulation per centimeter of colonized root of five host–fungus combinations[a]

Treatment	% Root colonization[b] at following distance from crown (cm):				No. of spores/cm[b] of colonized root at following distance from crown (cm):			
	0–5	5–10	10–15	15–20	0–5	5–10	10–15	15–20
G. deserticola + sweet potato	30A	26A	25A	19A	4A	4A	5A	3A
G. etunicatum + bahia grass	39A	31AB	24BC	19C	8A	4AB	2B	2B
G. etunicatum + sweet potato	34A	27AB	20B	6C	2A	1AB	2A	0B
G. intraradices + bahia grass	21A	17AB	11BC	4CD	16A	15A	7A	6A
G. intraradices + sweet potato	22A	17AB	11BC	3C	23A	21A	24A	19A

[a] The inoculated plants were grown in aeroponic culture for at least 12 weeks.

[b] Means represent at least 15 replicates, and means in the same row not followed by the same letter were significantly different by Tukey's test at $P \leq 0.05$.

sporulation per unit length of colonized root was generally higher. Several factors may have contributed to these differences, such as degree of root colonization of precolonized plants before transfer to aeroponic chambers and environmental conditions. Nevertheless, both reports show that root colonization and abundant sporulation occurred in aeroponic culture. This is in contrast to the nutrient film technique. Mosse and Thompson (11) reported that some spores of *G. fasciculatum* were produced on bean (*Phaseolus vulgaris*) roots by the nutrient film technique, and Thompson (16) reported abundant external mycelium and ectocarpic spores and sporocarps of *G. mosseae* on nutrient-film-cultured maize (*Zea mays*) roots. However, neither reported quantitative data on spore production.

Bagyaraj and Manjunath (2) demonstrated host plant effects on inoculum production in soil-based pot culture. From

TABLE 2. Total root length, root colonization, and spore production of industrial sweet potato and bahia grass inoculated with three *Glomus* spp.[a]

Treatment	Total root length (cm)[b]	% Root colonization[b]	Spores[b]	
			No./plant	No./cm of colonized root
G. deserticola + sweet potato	476	54	2,062	7.8
G. etunicatum + bahia grass	202Y	47X	767X	6.5X
G. etunicatum + sweet potato	753X	44X	1,218X	3.6Y
G. intraradices + bahia grass	242B	17A	630B	13.4B
G. intraradices + sweet potato	492A	21A	4,478A	50.7A

[a] The inoculated plants were grown in aeroponic culture for at least 12 weeks.

[b] Means for each fungal treatment within the same column not followed by the same letter were significantly different by Scheffe's test at $P \leq 0.05$.

TABLE 3. Mean percent root colonization of bahia grass inoculated with cold-stored aeroponically produced inocula

Inoculum			% Root colonization by using inoculum stored for following period (mo)[a]:			
Fungus	Host	Carrier	2	4	6	9
G. deserticola	Bahia grass	Water	37	28	43	24
G. deserticola	Sweet potato	Vermiculite	31	12	39	26
G. deserticola	Sweet potato	Water	29	12	0	0
G. etunicatum	Bahia grass	Water	43	35	53	46
G. etunicatum	Sweet potato	Vermiculite	48	31	50	41
G. etunicatum	Sweet potato	Water	48	27	54	50

[a] For *G. deserticola*, the *P* values for bahia grass versus sweet potato are 0.0027 (2 months), 0.0007 (4 months), 0.0001 (6 months), and 0.0007 (9 months); the *P* values for water versus vermiculite are 0.2212 (2 months), 0.0545 (4 months), 0.0001 (6 months), and 0.0001 (9 months). For *G. etunicatum*, the *P* values for bahia grass versus sweet potato are 0.0420 (2 months), 0.3672 (4 months), 0.8911 (6 months), and 0.8391 (9 months); the *P* values for water versus vermiculite are 0.2447 (2 months), 0.9734 (4 months), 0.3899 (6 months), and 0.0799 (9 months).

our study, within each fungal species industrial sweet potato produced significantly more roots and spores per plant than did bahia grass, although the percent root colonization was similar (Table 2). Thus, industrial sweet potato seems to be a preferred candidate for mass production of VAM inoculum in aeroponic culture.

Infectivity of root inocula. At harvest, noninoculated controls were free of mycorrhizae. There were no differences in root colonization due to source of inoculum or inoculation rate for each VAM fungus treatment. Colonization of the assay plant, bahia grass, averaged 38%, 45%, and 28% for *G. deserticola*, *G. etunicatum*, and *G. intraradices*, respectively.

Infectivity of *G. etunicatum* spores. After 2 weeks, the percent germination of *G. etunicatum* spores collected from soil was significantly higher than that of spores collected from aeroponic culture (64% and 46%, respectively).

Results of the infectivity bioassay indicated that within each harvest date there were no differences in root colonization due to inoculation rate. At the first harvest (8 weeks), bahia grass inoculated with spores from soil was better colonized than bahia grass inoculated with spores from aeroponic culture (15% and 11%, respectively). However, no differences between the inoculum sources were detected after 12 weeks, when the mean colonization was 26%.

Storage study. In general, cold storage of *G. etunicatum* root inoculum did not affect its infectivity for up to 9 months, regardless of inoculum carriers and host species (Table 3). Although a significant difference ($P = 0.0420$) was detected for host species when inocula stored for 2 months was used, this difference might not be biologically significant.

For *G. deserticola* inoculum, bahia grass was a superior host to industrial sweet potato, regardless of storage time. No differences were detected for the inoculum carriers until the inoculum had been stored for 6 months or more. Inocula produced from industrial sweet potato and stored in sterile water lost their infectivity after 6 months of cold storage, whereas others retained their viability (Table 3).

Owing to different environmental conditions during the various experiments, comparison of root colonization over time is not possible. These different environmental conditions might have resulted in different mean percent root colonization at different storage periods.

Inoculum carriers and storage temperature, humidity, and time are important factors affecting the infectivity of mycorrhizal inoculum after storage. In general, infectivity decreases as storage time and temperature increase (3, 7, 12). Ectomycorrhizal and plant-pathogenic fungi have been successfully preserved at low temperature in sterile water (8, 14). Recently, Mugnier and Mosse (12) indicated that spores of *G. mosseae* retained their viability after cold (4°C) storage in a moist atmosphere for 4 years. Moreover, studying factors affecting inoculum infectivity after storage, Daft et al. (3) reported that spore infectivity was higher when the spores had been stored under wet or moist conditions. Our results indicate that a cold, moist environment maintains inoculum infectivity, although the reason for the loss of infectivity of the *G. deserticola*-industrial sweet potato inoculum after cold storage for 6 months is unclear.

Vermiculite is a common inoculum carrier for vegetative ectomycorrhizal inocula (9). Our results indicate that moist vermiculite can also be used as an inoculum carrier for VAM inocula.

This study extends previous findings (15) which demonstrated that VAM fungi colonize roots and sporulate rapidly in aeroponic culture. In addition, we have demonstrated that both colonized roots and spores produced in aeroponic chambers can serve as infective VAM inocula. Moreover, aeroponically produced *G. deserticola* and *G. etunicatum* inocula can be cold stored in either sterile water or moist vermiculite for at least 4 and 9 months, respectively.

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