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Growth and Sporulation of Vesicular-Arbuscular Mycorrhizal Fungi in Aeroponic and Membrane Systems

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Abstract

The application of vesicular-arbuscular (VA) mycorrhizal fungi to agriculture has been limited by inadequate methods for inoculum production. The VA mycorrhizae are usually produced on roots of host plants in soil-based pot cultures; however, the quality and quantity of this inoculum can vary widely due to complex interactions among host plant, mycorrhizal fungus, and soil parameters. In this paper we report the culture of VA mycorrhizae in aeroponic and membrane systems. In the aeroponic system colonization and sporulation were superior to that reported for soil-based pot culture. Uniform colonization (>75%) of *Paspalum notatum* roots by *Glomus mosseae* and abundant sporulation (5 chlamydospores cm^{-1} colonized root) were obtained after 8 wk in aeroponic culture. Colonization of roots by *G. intraradices* reached 50% after 12 wk in aeroponic culture and a mean of 8 chlamydospores cm^{-1} of colonized root were observed. This method should allow more efficient and uniform culture of VA mycorrhizae. Root colonization and sporulation by *G. mosseae* in the membrane system were less than that observed in the aeroponic system.

Keywords: *Glomus mosseae*, *Glomus intraradices*, aeroponics, culture, inoculum, sporulation, vesicular-arbuscular mycorrhizae.

1. Introduction

Most economically important crop plants are colonized by a group of ubiquitous soil fungi which penetrate roots and form morphologically distinct structures called arbuscules and vesicles within the cortex (Harley and Smith, 1983). These symbiotic associations are termed vesicular-arbuscular

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(VA) mycorrhizae. The VA mycorrhizae can improve plant growth in low-fertility soils. This observation has stimulated interest in their possible use as "biological fertilizers". However, a major limitation to the utilization of VA mycorrhizae in crop production is the inability to produce sufficient amounts of fungal inoculum for field application. The VA mycorrhizal fungi have not been grown successfully in pure culture and are considered obligate symbionts (Hepper, 1984). Due to this limitation, these fungi are usually maintained and increased in pot cultures (Ferguson and Woodhead, 1982). Pot cultures are comprised of host plants, mycorrhizal fungi, and soil microflora and microfauna, and are influenced by the physical and chemical properties of the potting medium. It is not surprising that the quality and quantity of propagules produced for inoculum by this method vary widely due to the many interactions among these variables.

Several alternatives to the pot-culture system have been proposed. The VA mycorrhizae have been formed on root-organ cultures (Miller-Wideman and Watrud, 1984; Mosse and Hepper, 1975). However, colonization and sporulation are limited in these monoxenic systems. The VA mycorrhizae have also been established in solution culture (Crush and Hay, 1981: Elmes and Mosse, 1984; Howeler, et al., 1982; Mosse and Thompson, 1984). Elmes and Mosse (1984) reported approximately 50% colonization of *Zea mays* roots after 9 wk in nutrient flow culture.

In this paper we report two methods of producing VA mycorrhiza inoculum without a potting medium. One method used an aeroponic system described by Zobel et al. (1976). The system was adapted for mycorrhiza production by the utilization of seedlings with roots pre-colonized by a VA mycorrhizal fungus and the use of a modified Hoagland's nutrient solution (1950) with a very low phosphorus level. The second method utilized a semi-permeable membrane system to physically separate roots from soil (Brown and Anwar-Ul-Haq, 1984; Cappy and Brown, 1980).

2. Materials and Methods

Aeroponic System

To obtain colonized seedlings, chlamydospores of a VA mycorrhizal fungus, *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe or *G. intraradices* Schenck & Smith, were mixed with pasteurized (75°C for 3 hr) loamy sand (siliceous hyperthermic Grossarenic Paleudult). The infested soil was seeded with bahiagrass (*Paspalum notatum* Flugge) and placed in a non-shaded glasshouse (maximum photosynthetic photon flux density was 1200μmol $s^{-1}m^{-2}$ and the temperature range was 25–35°C). Seedlings were removed

from the soil after 3 wk and the roots were washed and trimmed to a length of 5 cm. Mycorrhizal colonization was confirmed by the autofluorescence method of Ames et al. (1982).

The roots of mycorrhizal seedlings placed in the aeroponic system were inserted, with an approximate spacing of 10 cm, through an aluminum foil-covered light screen. The aeroponic chamber contained 55 L of the following modified Hoagland's nutrient solution in mg L^{-1}, adjusted to pH 6.5 with 0.1 N H_2SO_4: $Ca(NO_3)_2$, 246; KNO_3, 152; $MgSO_4$, 72; FeEDTA, 11; NaCl, 3; H_3BO_3, 0.86; $MnCl_2 \cdot 4H_2O$, 0.54; KH_2PO_4, 0.40; $ZnSO_4 \cdot 7H_2O$, 0.07; $CuSO_4 \cdot 5H_2O$, 0.02 and $H_2MoO_4 \cdot H_2O$, 0.006. After 2, 4, 8 and 12 wk in the aeroponic chamber, 3 seedlings from each fungal treatment were harvested and roots were cleared in hot, 10% KOH for 15 min and stained with 0.05% trypan blue overnight. The roots were divided into three sections; the top 5 cm and the remainder of the root subdivided into two equal portions. The percentage of root length colonized by mycorrhizal fungi in each section was estimated by a gridline intersect method (Giovannetti and Mosse, 1980). After each harvest, the nutrient solution was changed and the roots of seedlings that remained in the aeroponic system were trimmed to a length of 5–10 cm to prevent the roots from growing into the nutrient solution.

Membrane system

Root pouches for the membrane system were made from Versapor 1200 acrylic-coated nylon membranes with a 1.2μm pore size (Gelman Instrument Co., Ann Arbor, MI). The pouches were made by folding the membranes lengthwise. The side and bottom of each pouch was sealed with contact cement (Ross Chemical Co., Detroit, MI). The final dimensions of the pouches were 9.0 × 4.5 cm or 20.0 × 6.5 cm for the first two or last harvest, respectively. The root system of a bahiagrass seedling colonized by *G. mosseae* (see above) was placed within each pouch. The pouch was then buried in pasteurized soil in a 15 cm-diameter pot. The top 1–2 cm of the pouch extended above the soil line. The pots were placed in the glasshouse and 3 seedlings were harvested after 2, 4, and 12 wk. The pouches were removed from the soil and the root systems were assessed for mycorrhizal colonization by the procedures described above.

3. Results and Discussion

Roots grown aeroponically were colonized rapidly by *G. mosseae* (Fig. 1A, Fig. 2A). Within 2 wk the lower portions of the roots were colonized at a low level and by 8 wk the root systems were uniformly colonized by this fungus. Chlamydospores of *G. mosseae* were observed after only 4 wk. By

Table 1. Chlamydospore formation by *Glomus mosseae* and *G. intraradices* on roots of *Paspalum notatum* in aeroponic culture after 8 and 12 wk, respectively.[a]

	Chlamydospores cm^{-1} colonized root	
Root position[b]	*G. mosseae*	*G. intraradices*
Top	4.2±1.4[c]	10.9±6.0
Middle	5.0±1.8	7.9±2.6
Bottom	6.5±2.7	4.5±2.7

[a] Seedlings were exposed to the VA mycorrhizal fungi for 3 wk before colonized seedlings were transferred to aeroponic culture.

[b] The roots were divided into three sections; the top (T) 5 cm and the remainder of the root subdivided into middle (M) and bottom (B) portions of equal length.

[c] Values represent the mean for 3 seedlings ± S.E.M.

8 wk chlamydospores were abundant, with a mean of 5 chlamydospores cm^{-1} colonized root (Table 1, Fig. 2B). This amount of sporulation was greater than that reported by Sylvia and Schenck (1983) for *G. mosseae* grown under similar conditions in soil-based pot culture after 18 wk. The high standard errors in Table 1 indicate that the chlamydospores were not uniformly distributed along the roots; rather they were aggregated. Spore distributions in soil-based pot cultures were also aggregated (St. John and Koske, 1985). Chlamydospores ranged in diameter from 60 to 125μm. Thin-walled extramatrical vesicles or immature spores, ranging in diameter from 30 to 50μm, were also observed (Fig. 2C).

Colonization of roots by *G. intraradices* in the aeroponic system proceeded at a slower rate than that observed for *G. mosseae*, but by 12 wk a mean of 50% of the root length was colonized (Fig. 1B, Fig. 3A). Chlamydospores were first observed after 8 wk and by 12 wk there was a mean of 8 chlamydospores cm^{-1} colonized root length (Table 1). Sporulation occurred primarily within the root and spore diameters ranged from 55 to 110μm (Fig. 3B).

The membrane system allowed for the complete recovery of intact, clean roots from the soil. However, root colonization and sporulation by *G. mosseae* were less than that observed in the aeroponic system. The mean percentage of root length colonized by *G. mosseae* was 39, 40, and 56% after 2, 4, and 12 wk in culture, respectively. After 12 wk there were 3 ± 1 chlamydospores 10 cm^{-1} of colonized root length.

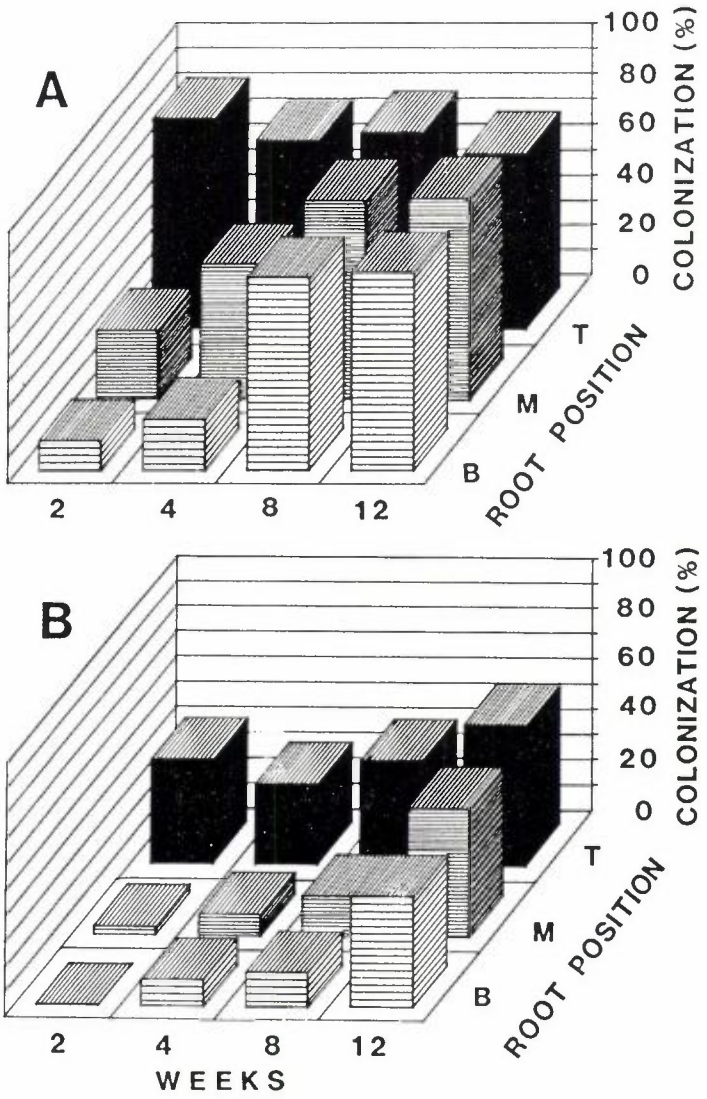
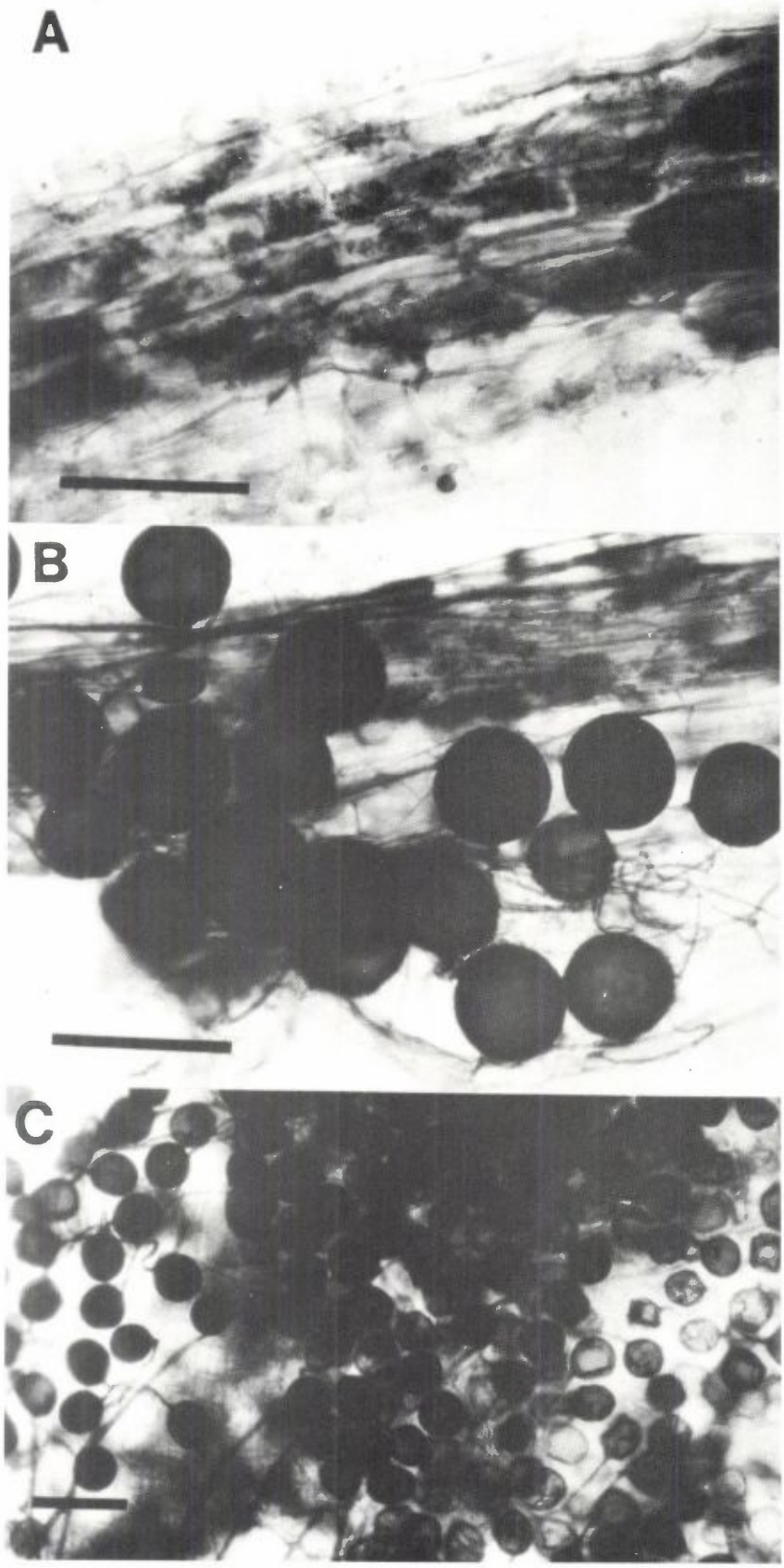


Figure 1. Colonization of *Paspalum notatum* roots by (A) *Glomus mosseae* and (B) *G. intraradices* in aeroponic culture. Seedlings were exposed to the VA mycorrhizal fungi for 3 wk in soil before transfer to aeroponic culture. At each harvest, roots were divided into three sections; the top (T) 5 cm and the remainder of the root subdivided into middle (M) and bottom (B) portions of equal length. Bars represent the mean colonization of 3 seedlings.


A
B
C

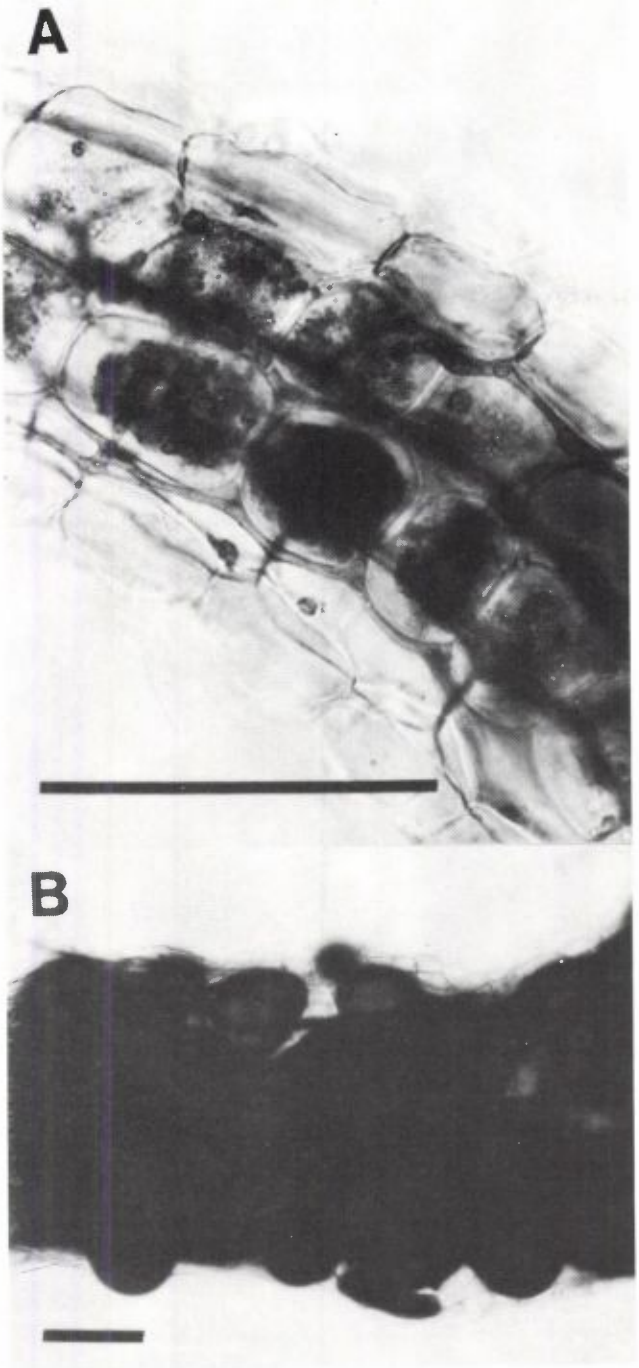


Figure 2. *Glomus mosseae* colonized *Paspalum notatum* roots after 8 wk in aeroponic culture. (A) Arbuscular development within cortical cells, (B) chlamydospores and external mycelia attached to the root, (C) extramatrical vesicles or immature chlamydospores on root surface. Bars = 100μm.

Figure 3. *Glomus intraradices* colonized *Paspalum notatum* roots after 12 wk in aeroponic culture. (A) Arbuscular development within cortical cells, (B) chlamydospore formation within the root. Bars = 100μm.

The rapid rates of colonization and sporulation achieved in the aeroponic system are likely attributable to high aeration and low-level phosphorus supply provided to the roots. The VA mycorrhizae produced aeroponically have several potential benefits when compared to pot culture-produced material. Intact root systems can be observed with minimal disruption, and soil-free material can be harvested readily for physiological or microscopic studies. Most of the spores remain attached to the roots, presumably because the system is not physically disturbed at harvest, as it is when sieving soil. Furthermore, the nutrient content of the solution can be adjusted readily to meet the needs of different plant and fungal species.

In this research our primary objective was to evaluate the aeroponic system for inoculum production. Pre-colonization of seedlings in soil was used to insure rapid mycorrhizal development. However, this procedure may introduce root pathogens into the culture. In the future we hope to eliminate this pre-colonization step from the process and inoculate plants directly in the aeroponic system.

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