

Relationship between hyphal and arbuscular colonization and sporulation in a mycorrhiza of *Paspalum notatum* Flugge

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SUMMARY

Experiments were conducted to determine correlation between sporulation by an arbuscular mycorrhizal (AM) fungus to proliferation of arbuscules or intercellular hyphae. *Paspalum notatum* Flugge seedlings were inoculated with the AM fungus *Gigaspora margarita* Becker & Hall and treated with either tap water, nutrient solution without P, or nutrient solution with P to manipulate colonization levels. Plants receiving the nutrient solution without P tended to have greater colonization than plants receiving water only (e.g. 62 vs 54%, respectively at week 7) but lesser percentage root length with arbuscules (e.g. 27 vs 38%, respectively at week 7). Mycorrhizas receiving the nutrient solution without P sporulated sooner (week 7) than the water only treatment and had larger spore populations (e.g. 20.9 vs 14.2 spores cm⁻³ at week 13). Nutrient solution with P did not completely inhibit colonization, and though these plants had total colonized root lengths similar to those of the water treatment, spore populations were much less (2.9 cm⁻³ at week 13). Spore populations correlated equally well with total root length as with root length colonized and root length with arbuscules in the water and nutrient solution without P treatments. Populations were not correlated to these measures for the nutrient solution with P, indicating that the host may have limited the ability of a unit length of colonization to produce spores in this treatment.

Key words: Vesicular-arbuscular mycorrhiza, *Gigaspora margarita*, sporulation, arbuscular colonization.

INTRODUCTION

Production of soil-borne spores and colonization of roots by arbuscular mycorrhizal (AM) fungi is influenced by the nutrition of the host plant. The detrimental effect of P addition upon mycorrhizal colonization has long been known (Mosse, 1973). Addition of nutrient solutions without P have increased both colonization and sporulation in a wide variety of AM fungi (Verkade & Hamilton, 1983; Thompson, 1987; Douds & Schenck, 1990).

The previous work (Douds & Schenck, 1990) measured only percentage root length colonized after 10–12 wk of nutrient addition and could only conclude that sporulation was proportional to colonization level. The experiments reported here were conducted to characterize more closely the effect of plant nutrition upon AM fungus colonization. Sporulation and colonization were manipulated through the addition of nutrient solutions to glasshouse pot cultures. Overall colonization, arbuscular colonization, intensity of colonization, and spore populations were measured four times over a 3 month period. Responses of these parameters were compared and correlated to gain an understanding of the regulation of sporulation of AM fungi.

MATERIALS AND METHODS

Experimental material

Plants were grown in 165 cm³ conical plastic containers ('Super-cell C-10', Stuewe & Sons, Corvallis, OR 97333, USA*) in a 0.5:1:1:0.75 [v/v] mixture of field soil [Comly silt loam (fine-loamy, mixed, mesic Typic Fragiudalf)], sand, vermiculite, and calcined clay ('Turface', Applied Industrial Materials Corp., Deerfield, IL 60015, USA). Sixty cm³ of pot culture inoculum, containing approximately 330 spores of *Gigaspora margarita* Becker & Hall (INVAM 185) and colonized root pieces, was added as a band 7.5 cm above the bottom of the cone. One seedling of *Paspalum notatum* Flugge was transplanted into each cone.

Plants were grown in a glasshouse under natural photoperiods from 30 August to 3 December, 1991. A minimum temperature of 15 °C was maintained with heating and daily maximum temperatures ranged up to 32 °C. Three nutrient treatments were applied (20 ml 3 × wk⁻¹): (1) tap water only, (2)

* Mention of brand or firm name does not constitute an endorsement by the US Department of Agriculture over others not mentioned.

single strength Hoagland's solution without P [H-P], and (3) single strength Hoagland's solution with P [H+P] (Hoagland & Arnon, 1938).

Collection of data

Five plants from each of the nutrient treatments were harvested 4, 7, 10, and 13 wk after planting. Spores of *G. margarita* were isolated from a 43 cm³ section within the inoculated zone of the middle of the cone and quantified (Douds & Schenck, 1990). Colonization of roots in the section sampled for spores was quantified. All roots in that section were first assayed for total colonization (hyphae and arbuscules) via the gridline intersect method (Newman, 1966) following clearing and staining (Phillips & Hayman, 1970). Subsamples of at least 25% of each of these root samples were mounted in glycerol on microscope slides. Two slides were prepared for each plant. This subsample was used to measure arbuscular colonization (McGonigle *et al.*, 1990). Intensity of the AM fungus colonization also was measured on this sample to indicate development of intercellular hyphae. An integral value from 0 to 5, proportional to the cortical area stained for AM fungi (1 = 1–20%, 2 = 21–40%, 3 = 41–60%, etc.), was recorded for 100–200 intersects of the ocular cross hairs.

Soil analyses were conducted at the beginning and end of the experiment. Soil from sections of the cone above and below the midsection sampled for spores in the last harvest was pooled. Soil pH (water), available P (Bray I), NO₃⁻, and NH₄⁺ were quantified using methods outlined in Black *et al.* (1965).

Data were analyzed using analysis of variance and linear regression. Percentage colonization data were first transformed (arcsin). Parameters for which significant treatment effects were found were further characterized using Tukey's method of multiple comparisons.

RESULTS

Soil analyses revealed an increase of one pH unit over the course of the experiment (Table 1). Available N and P decreased in the water only treatment, while NO₃⁻-N increased in both nutrient treatments because NO₃⁻ is the form of N in Hoagland's solution. Phosphorus accumulated in the soil mix receiving H+P.

Paspalum notatum plants showed a marked response to nutrient addition. Addition of H-P significantly increased growth over that of water only, and the addition of H+P caused a further increase in growth of roots and shoots (data not shown).

Addition of H+P significantly inhibited colonization of *P. notatum* by *G. margarita* (Fig. 1a). Plants receiving H-P were more colonized than

plants receiving water only at 7 wk. A reversal occurred with arbuscular colonization (Fig. 1b). Plants receiving water had arbuscules in significantly greater proportion of their root length than did plants receiving H-P, even though the latter had equal or greater total percentage root length colon-

Table 1. Results of soil analyses

Soil sample	pH*	Nutrient concentration (µg g ⁻¹)		
		P†	NO ₃ -N‡	NH ₄ -N§
Initial	5.8	40	9	10.0
After 13 wk				
Tap water	6.8	29	4	0.5
Hoagland's -P	6.7	33	24	1.6
Hoagland's +P	6.8	115	27	0.4

* In water.

† Bray I (NH₄F+HCl) extraction.

‡ Water + CaSO₄ extraction.

§ 1 M KCl extraction.

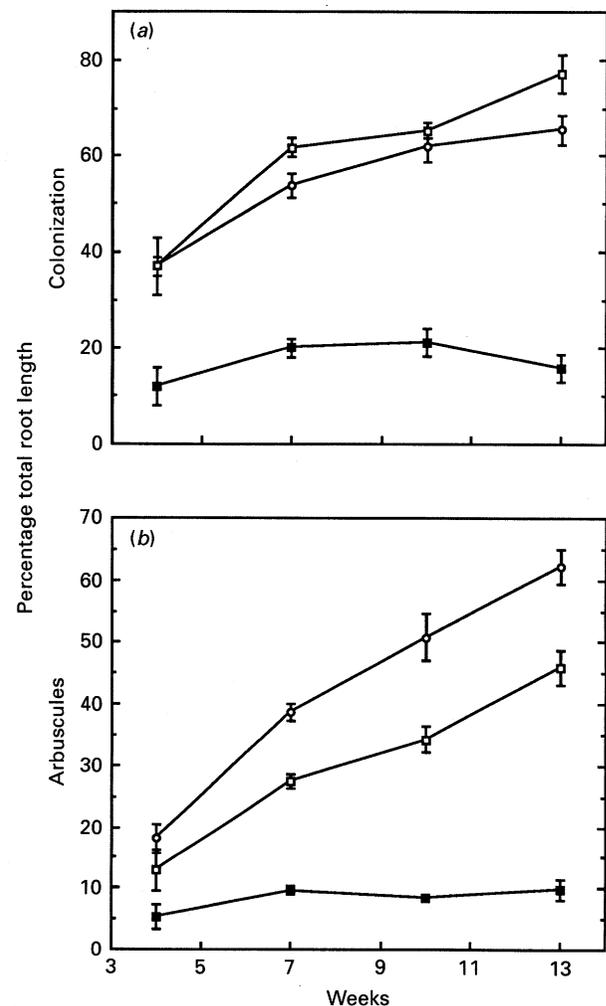


Figure 1. Percentage root length of *Paspalum notatum* receiving one of three nutrient solutions colonized by *Gigaspora margarita*: (a) all fungal structures and (b) arbuscules only. Each point is the mean of five observations \pm SEM. \circ — \circ , Water; \square — \square , Hoagland's -P; \blacksquare — \blacksquare , Hoagland's +P.

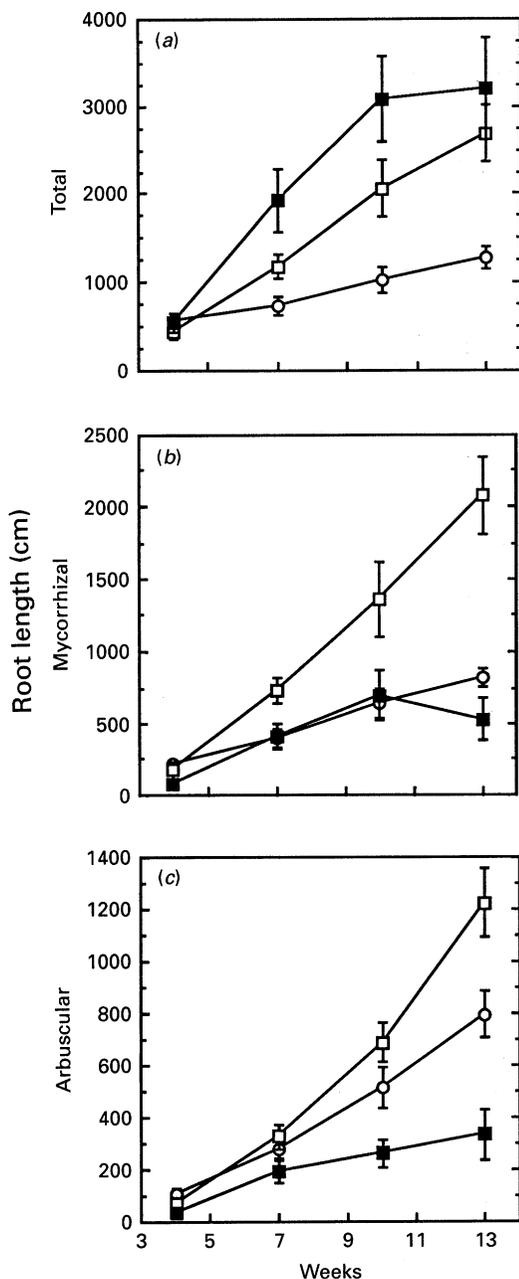


Figure 2. (a) Total root length, (b) mycorrhizal root length and (c) root length with arbuscules of *Paspalum notatum* receiving one of three nutrient solutions colonized by *Gigaspora margarita*. Each point is the mean of five observations \pm SEM. Symbols same as in Fig. 1.

ized. Plants in the H+P treatment produced the greatest total root length and plants in the H-P treatment had the greatest colonized root length and length of root with arbuscules (Fig. 2). Plants from the water and H+P treatments had equal total root lengths colonized throughout the first 10 wk (Fig. 2c).

Plants in the water and H-P treatments had significantly greater intensity of AM colonization than plants in the H+P treatment throughout the experiment (Table 2). Only at week 13, however, did roots from the H-P treatment exhibit significantly greater intensity of colonization, both overall and

Table 2. Intensity of AM fungal colonization. Points along the root system were scored from zero to five, proportional to the area of the cortex appearing to be occupied by AM fungal hyphae stained with trypan blue*

Treatment	Intensity of colonization	
	All points	Colonized points only
Water	2.4 b	3.3 b
Hoagland's -P	2.9 a	3.7 a
Hoagland's +P	0.4 c	2.7 c

* Data represent the means of 100–200 points on five root systems. Plants were grown for 13 wk under one of three nutrient regimes. Numbers in a column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's method of multiple comparisons).

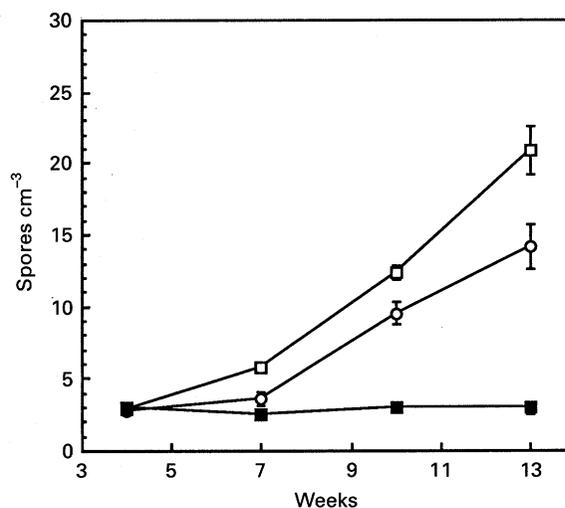


Figure 3. Populations of spores of *Gigaspora margarita* in symbiosis with *Paspalum notatum* receiving one of three nutrient solutions. Each point is the mean of five observations \pm SEM. Symbols same as in Fig. 1.

considering only the colonized segments, than roots from the water only treatment.

All treatments had approximately 2.9 spores cm^{-3} within the section sampled at 4 wk (Fig. 3), representing those spores in the original inoculum which still appeared healthy. Sporulation began before week 7 in plants receiving H-P. Mycorrhizas in the H+P treatment produced new spores only at a rate which replaced the aged, brown spores which were not counted.

Sporulation may be examined as a function of colonized root length (Giovanetti *et al.*, 1988) or root length with arbuscules (Table 3). Although colonization levels at week 7 for water and H-P plants differed by approximately 10%, sporulation per both measures of colonization was several fold greater for H-P mycorrhizas than those receiving water only. By the end of the experiment however, these measures of sporulation were not significantly different for the two treatments. Sporulation as a

Table 3. Sporulation as a function of total and arbuscular colonization*

Treatment	Spores cm ⁻¹ colonized root			Spores cm ⁻¹ arbuscular colonized root		
	7 wk	10 wk	13 wk	7 wk	10 wk	13 wk
Water	0.26 b	1.64 a	0.90 a	0.36 b	1.97 a	0.94 ab
Hoagland's -P	0.64 a	0.89 b	0.82 ab	1.47 a	1.67 a	1.34 a
Hoagland's +P	0.06 b	0.24 b	0.11 b	0.12 b	0.47 b	0.20 b

* Data represent the means of five observations. Numbers in the same column with the same letter are not significantly different ($\alpha = 0.05$, Tukey's method of multiple comparisons). Values were calculated by determining the net amount of spores produced within the sample zone since the previous sample, divided by total root length colonized or total root length with arbuscules within the sample zone.

function of total, mycorrhizal, or arbuscular root lengths was nearly linear for the water ($r^2 = 0.978$, 0.950 and 0.968, respectively) and H-P treatments ($r^2 = 0.947$, 0.977 and 0.995, respectively). Regressions for the H+P treatment concluded the slope was zero for length terms in all equations (spores = slope (length term) + intercept; $P > T = 0.300$, 0.343 and 0.387 for total, mycorrhizal and arbuscular root length, respectively), reflecting the nearly constant population of spores (Fig. 3).

DISCUSSION

Nutrient solution without P enhanced colonization and sporulation in the *P. notatum*-*G. margarita* symbiosis as reported earlier (Douds & Schenck, 1990). The nutrient poor situation in the water only treatment caused a response that has not been seen. Relative to the H-P treatment, percentage root length colonized decreased and percentage root length with arbuscules increased. This relation between total and arbuscular colonization has not been reported previously. Morandi, Bailey & Gianinazzi-Pearson (1984) noted that the percentage of cortical cells with arbuscules varied in a manner parallel to change in overall colonization of *Glycine max* by two *Glomus* species. These measures responded similarly to fertilizer addition in an experiment with *Phleum pratense* planted in dilutions of field soil inoculum (Clapperton & Reid, 1992). Total and arbuscular colonization both declined with decreasing light (Pearson, Smith & Smith 1991). The level of arbuscular colonization in the water treatment could be due to greater longevity or production relative to the H-P treatment. If a plant which is nutrient stressed, but not to the point where colonization is inhibited (Bethlenfalvay *et al.*, 1982), is less able to degenerate/digest arbuscules, they may remain functional longer, yielding the results seen here for the water only treatment. Further, Smith & Gianinazzi-Pearson (1990) speculated that arbuscules may be longer lived when the host is stressed by low light.

Spore populations were equally correlated to total root length as to total or arbuscular colonized root lengths in the water and H-P treatments, though the distribution of the intraradical fungus between arbuscules and hyphae in the two treatments was very different. The correlation of these factors to spore populations in the H+P treatment was much lower, due to the stable population of spores in that treatment.

Evidence of regulation of sporulation comes from examining the spore production per measures of colonization data (Table 3) Roots in the H+P treatment had mycorrhizal root lengths and intensities of colonized areas similar to those of the water treatment (Fig. 2b, Table 2), yet produced fewer spores overall and fewer on a per colonized root length basis. Evidently less carbon was made available to the fungus, per colonized root length, for spore production in the H+P treatment than in the other treatments, even though this treatment had the greatest shoot growth.

Previous work on the effect of high nutrient levels upon AM fungi has focused upon how P stimulates host mechanisms which inhibit colonization through alteration of membrane permeability (Graham, Leonard & Menge, 1981) or carbohydrate levels (Same, Abbott & Robson, 1983). The current work suggests there is another level of control, namely limitation by the host of the efficacy of intraradical structures, from the fungus' perspective, once established.

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