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Interactions of arbuscular mycorrhizae, *Meloidogyne arenaria*, and phosphorus fertilization on peanut

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Abstract The individual and combined effects of two arbuscular mycorrhizal fungi (AMF), *Meloidogyne arenaria*, and phosphorus (P) fertilization, (0, 25, 75, and 125 μg/g soil) on peanut plant growth and pod yield were determined in greenhouse studies. Best growth and yield usually occurred at 75 or 125 μg P regardless of inoculation treatment. Peanut growth and yield were generally stimulated by AMF development, and growth alone was suppressed by *M. arenaria* at 0 and 25 μg P. In challenge inoculations, VAM increased peanut plant tolerance to the nematode and offset the growth reductions caused by *M. arenaria* at the two lower P levels. However, VAM and added P increased galling and *M. arenaria* egg production/g root, thereby increasing peanut susceptibility to nematode attack. *M. arenaria* had only a minimal effect on root colonization by AMF and sporulation by the fungi.

Key words *Arachis hypogaea* · Endomycorrhizae · Root-knot nematode

Introduction

Root systems of many crops commonly symbiotic with arbuscular mycorrhizal fungi (AMF) often are inhabited also by plant parasitic nematodes. The two organisms are obligate feeders and can affect plant health and each other's activities (Hussey and Roncadori 1982; Smith 1987, 1988). Consequently, AMF have the potential to serve both as biological fertilizers and biocontrol agents.

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Numerous coinoculation studies with AMF and plant parasitic nematodes have been conducted on a variety of crops (Hussey and Roncadori 1982; Smith 1987). Most investigations, however, involved species of *Meloidogyne* on crops such as cotton, onion, soybean, and tomato. The outcome of such challenge studies may vary depending upon the specific members involved in the tripartite relationship (Hussey and Roncadori 1982). Mycorrhizal plants are often more tolerant to nematode attack, showing less severe stunting than nonmycorrhizal plants, and in fewer cases, mycorrhizal plants exhibited altered susceptibility or resistance to nematode attack through suppressed galling or reproduction (Hussey and Roncadori 1982; Smith 1987, 1988). However, with hosts such as onion or tomato, AMF failed to change host tolerance or resistance to the root-knot nematode (MacGuidwin et al. 1985; Thomson-Cason et al. 1983). Rarely have AMF decreased plant tolerance and increased nematode susceptibility.

Since AMF improve plant P nutrition, the role of this nutrient has been investigated in recent interaction studies (Smith 1987, 1988). P fertilization generally improved plant tolerance to nematode attack, but also stimulated nematode reproduction. Furthermore, comparison of mycorrhizal and P-fertilized, nonmycorrhizal plants of similar size showed that the latter are more susceptible to nematode attack, indicating the likely involvement of factors other than P nutrition in the interaction (Smith 1987, 1988).

A preliminary study of the interactions of AMF, *Meloidogyne arenaria* (Neal) Chitwood, and P fertilization on peanut indicated that both tolerance and susceptibility were affected by these factors. The objectives of this study were to determine the individual and interacting effects of AMF, *M. arenaria*, and P fertilization on (1) peanut growth and yield, and (2) microorganism activities.

Materials and methods

Soil preparation

The experiment was conducted in a Marlboro loamy sand soil (Plinthic Paleudults series, 81% sand, 6% silt, 13% clay, and 1% organic matter) which had not been in agricultural production for at least the last 20 years. Extractable P determined by double-acid extraction and plasma emission spectroscopy was less than 5 μg/g soil. The soil was screened to remove large debris and mixed 1:1 (v:v) with washed builder's sand; the final texture was 90% sand, 3% silt, and 7% clay. During mixing, the pH was adjusted with horticultural grade lime and the soil amended to attain the following elemental concentrations (μg/g): A1 240, B 0.9, Ca 500, Fe 20, K 100, Mg 80, Mn 38, and Zn 9. $Ca(H_2PO_4)_2$. H_2O was incorporated into the mix at the following actual P levels (μg/g): $P_0 = 0$, $P_1 = 25$, $P_2 = 75$, and $P_3 = 125$. Pre- and postexperimental levels were: $P_0 = 4.5/6.5$, $P_1 = 19.2/14.9$, $P_2 = 58.4/46.4$, and $P_3 = 107.8/77.7$. Initial pH of the mixes was 6.4–6.6 and dropped to 6.2–6.3 after the study. The mixes were fumigated under a polyethylene cover with Dowfume MC-2, (Dow Chemical Co., Midland, Mich.) at the rate of 1 kg/500 kg for 48 h and vented for 2 weeks prior to use.

Inoculum increase and inoculation

Spores of VAM fungi were produced on *Sorghum vulgare* (Stumpf) Haines 'Roxburgi' in greenhouse pot culture and were extracted using a modified centrifugation-flotation technique (Jenkins 1964). Approximately 400 spores of *Gigaspora margarita* Becker & Hall or *Glomus etunicatum* Becker & Gerd. were added to appropriate pots (4 l) which were half filled with mix. Microflora in all treatments were standardized by adding a 25-ml aliquot of a combined spore suspension filtrate collected from both endophytes after passage through Whatman no. 1 filter paper. Following addition of inoculum, the pots were filled with soil and five seeds of peanut (*Arachis hypogeae* L. 'Starr') were planted per pot. Seven days after planting, the seedlings were thinned to one per pot. Sixteen days after planting, 9 000 *M. arenaria* eggs were added to each appropriate pot by pouring a suspension into a shallow trench around the base of the plant and replacing the soil. *M. arenaria* eggs were produced in the greenhouse on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') and collected according to the method of Hussey and Barker (1973). Ten days after planting, a commercial preparation of *Bradyrhizobium japonicum* Kirch. (lot no. LX 305, The Nitragin Co., Milwaukee, Wis.) was added to the soil surface of each pot and watered thoroughly.

Experimental design and collection of data

Treatments in this factorialized experiment consisted of individual inoculation with each fungal endophyte, *M. arenaria*, and joint inoculations with each fungus and *M. arenaria* at each P level. Each treatment was replicated eight times and the test was arranged in a randomized complete block design in the greenhouse. As the study was repeated with similar results, data from only one test are reported. Plants were sprayed with insecticides on a regular basis throughout the study to control mites.

Plants were harvested 101 days after planting and root and shoot fresh weights were recorded. The number of galls on each root system was expressed according to the following scale: 0 = 0, 1 = < 25, 2 = 26–100, 3 = 101–300, and 4 = > 300. After root samples for mycorrhizal assay were excised, *M. arenaria* eggs were collected from the remaining roots according to the procedure of Hussey and Barker (1973). Samples for AMF assay were collected by cutting a transverse 5- to 10-mm-wide band across the root system at a point 5–7 cm below the root crown. The sample size was approximately 2–5% of the total root system weight. The roots were processed using a modified clearing and staining technique (Phillips and Hayman 1970), and AMF development as a percentage of root length was estimated using the stereoscopic microscope and expressed as 0 = 0%, 1 = < 10%, 2 = 11–40%, 3 = 41–70%, 4 = 71–90%, and 5 = > 95%. Spore populations in the soil were determined using the modified centrifugation-flotation technique (Jenkins 1964). Analysis of variance was used to analyze data and treatment means were separated according to Duncan's multiple range test.

Results

Plant responses

Peanut growth and yield were affected by AMF development, *M. arenaria*, and P fertilization. P fertilization increased growth and yield with the maximal effect at the highest P levels (Table 1). AMF development also increased shoot growth and pod weight but there was a significant interaction with P fertilization since beneficial effects occurred only at the 0 and 25 μg P levels. Root weight was not greatly affected by AMF. *M. arenaria* suppressed both root and shoot growth but mostly in the nonfertilized mix. Pod yield was not affected by nematode parasitism. Coinoculation with either endophyte and *M. arenaria* offset the root and shoot stunting caused by the nematode but had little influence on mycorrhizal growth stimulation or pod yield. A significant interaction also occurred between coinoculation and P fertilization with mycorrhizal-induced tolerance occurring only at 0 and 25 μg P. However, root weights of plants grown at 75 and 125 μg P were increased by *M. arenaria* parasitism in both mycorrhizal and nonmycorrhizal plants. Much of the increase appeared to be gall tissue rather than root tissue, thus it was of no functional value to the plant.

Both AMF development and P fertilization increased nematode galling (Table 1). Maximal galling occurred primarily at the highest P levels and was greatest when plants were mycorrhizal.

Effects on microorganism reproduction and AMF root colonization

Nematode reproduction was related to AMF development and increased P fertilization (Table 2). Both total egg production per root system and eggs/g root were stimulated by AMF at 0 and 25 μg/g P. The highest egg population densities occurred in mycorrhizal and nonmycorrhizal plants grown at the 75 and 125 μg P fertilization levels.

AMF root colonization and fungal sporulation were adversely affected by increased P fertilization but relatively unaffected by *M. arenaria* parasitism (Table 3). Root colonization was suppressed at the highest P level, whereas, sporulation began to decline with any addition of P fertilizer.

Table 1 Influence of arbuscular mycorrhizal fungi, *Meloidogyne arenaria* (*MA*), and phosphorus fertilization (*P*) on peanut growth, yield and gall development. Row means followed by different letters are significantly different according to Duncan's multiple range test ($P=0.05$). The unamended soil contained 5 μg P/g

Parameter measured	Added P (μg/g soil)	No mycorrhizal fungus		*Gigaspora margarita*		*Glomus etunicatum*	
		No MA	MA	No MA	MA	No MA	MA
Shoot weight (g)	0	62 d	39 e	246 c	227 c	296 a	269 b
	25	169 c	139 c	296 b	322 a b	356 a	337 a
	75	353 a	274 b	346 a	348 a	375 a	366 a
	125	409 a b	353 b c	380 a b c	394 a b c	412 a	349 c
Root weight (g)	0	116 a b	84 c	115 a b	106 b c	104 b c	138 a
	25	180 a	164 a b	112 b	175 a	121 b	219 a
	75	142 c	169 b c	130 c	210 a b	171 b c	252 a
	125	169 b	247 a	134 b	246 a	161 b	206 a b
Pod weight/plant (g)	0	4.8 c	2.6 c	18.4 a b	16.1 b	19.9 a	19.3 a
	25	12.3 b	9.9 b	19.9 a	20.6 a	20.6 a	21.4 a
	75	21.8 a	19.5 a	24.1 a	19.5 a	24.5 a	20.6 a
	125	27.2 a	24.1 a	24.6 a	22.9 a	22.6 a	23.3 a
Gall index[a]	0	—	1.00 b	—	1.63 a	—	1.50 a
	25	—	1.13 b	—	1.87 a	—	2.13 a
	75	—	1.75 a	—	1.75 a	—	2.38 a
	125	—	1.50 b	—	3.13 a	—	2.50 a

[a] Gall index: 0 = 0, 1 = 1–25, 2 = 26–100, 3 = 101–300, 4 = > 300 galls

Table 2 Effects of arbuscular mycorrhizal fungi and phosphorus fertilization on *Meloidogyne arenaria* reproduction on peanut. Column means followed by different letters are significantly different according to Duncan's multiple range test ($P=0.05$). The unamended soil contained 5 μg P/g

Fungus	Phosporus fertilization level (μg/g soil)			
	0	25	75	125
Eggs per root system ($\times 10^3$)				
None	33.6 b	69.7 b	251.1 a	387.0 a
Gigaspora margarita	130.2 a	298.6 a	362.5 a	241.3 b
Glomus etunicatum	128.5 a	222.0 a	396.5 a	379.0 a b
Eggs per g root ($\times 10^3$)				
None	0.43 b	0.44 b	1.65 a	1.57 a
Gigaspora margarita	1.33 a	1.93 a	1.67 a	1.06 b
Glomus etunicatum	1.00 a	1.12 a	1.58 a	1.97 a

Table 3 Effects of *Meloidogyne arenaria* and phosphorus fertilization on root colonization and sporulation by arbuscular mycorrhizal fungi on peanut. The unamended soil contained 5 μg P/g

Fungus	*M. arenaria*	Phosphorus fertilization level (μg/g soil)			
		0[a]	25	75	125
Root colonization index[a]					
Gigaspora margarita	0	5.0	5.0	5.0	1.9
	+	5.0	5.0	4.6	2.4
Glomus etunicatum	0	4.1*	4.0	4.0*	1.6
	+	4.8	4.3	2.8	1.6
Spores per 110 cm^3 soil					
Gigaspora margarita	0	1812*	828	660	96
	+	920	686	332	124
Glomus etunicatum	0	4255*	1944	1440	732
	+	6220	1930	732	120

[*] Significant difference between means for the two fungi in treatments with (+) or without (0) *M. arenaria* ($P=0.05$)
[a] Percentage of root length colonized: 0 = 0, 1 = ≤ 10, 2 = 11–40, 3 = 41–70, 4 = 71–95, 5 = > 95

Discussion

Although maximal growth and yield of peanut occurred at P levels of 75–125 μg/g soil, the greatest response to P was evident below 75 μg/g. Similar effects of P fertilization on peanut growth and peg formation were reported by Bell et al. (1989). In contrast to our study, where AMF stimulation of plant growth and yield was noted up to 25 μg/g P, Bell et al. (1989) observed an AMF response in peanut at P levels as high as 60 μg/g soil.

M. arenaria suppressed growth in the unamended soil but had no effect on yield. Tolerance to *M. arenaria* parasitism was increased by P fertilization or coinoculation with an AMF. Greater tolerance in mycorrhizal compared to nonmycorrhizal plants has been frequently reported in a number of different crop species and has been often linked to improved host P nutrition (Smith 1987, 1988).

Both AMF development and P fertilization influenced peanut susceptibility to nematode attack. Increased root galling and nematode reproduction/g root were evident with AMF symbiosis or higher P levels implicating P as a significant factor in predisposition of peanut to *M. arenaria*. Root gall formation alone is not an accurate measure of susceptibility as defined by Cook and Evans (1974) since it does not measure nematode reproduction, but it is an indication of an effect on the disease cycle.

Predisposition to penetration by root-knot nematodes associated with mycorrhizal development or P fertilization has been observed in clover (Cooper and Grandison 1986), cotton (Smith et al. 1986a,b), tamarillo (Cooper and Grandison 1987), and tomato (Thomson-Cason et al. 1983), although the effect on incidence of gall production was not determined in any of these studies. Gall numbers were highest on mycorrhizal peanut root systems in our study, but whether the increase was due solely to higher root P concentration in mycorrhizal than in nonmycorrhizal roots or to other changes is not certain, since tissue concentrations of P and other elements were not determined. Smith (1987, 1988) has discussed changes in nutrition (other than those due to improved P status) of mycorrhizal plants which were associated with decreases in susceptibility of host plants to nematode attack. However, except for P, changes in nutritional status associated with AMF that increase susceptibility of plants to nematodes are not well understood.

AMF development in peanut has been associated with increases in copper, iron, manganese, and zinc concentrations (Krishna and Bagaraj 1983b) as well as elevations in free amino nitrogen and protein fractions (Krishna and Bagaraj 1983a). Higher levels of phenols, which frequently have been implicated in nematode resistance, also have been reported (Krishna and Bagaraj 1984). However, effects of mycorrhizal development on incidence of galling vary with different endophyte-host-nematode combinations as indicated in reports of suppression of gall formation in soybean (Kellam and Schenck 1980) and tomato (Sikora 1978).

M. arenaria reproduction on peanut was also increased by AMF development and P fertilization. P fertilization stimulated reproduction of root-knot nematodes on bean (Zambolin and Oliveira 1986), cotton (Smith et al. 1986a,b), and tomato (Thomson-Cason et al. 1983). Increased nematode reproduction in mycorrhizal peanut parallels the response to P fertilization, suggesting that this symbiotic benefit may also increase susceptibility. However, most investigators reported decreased root-knot susceptibility or no effect due to AMF development on a variety of other crops (Carling et al. 1989; Smith 1987, 1988). The simultaneous increased tolerance and susceptibility based on nematode reproduction in a tripartite system reported here is uncommon, although Atilano et al. (1981) observed increased tolerance and susceptibility in mycorrhizal grape, the latter determined by density of second stage juveniles extracted from the root system.

Any measure of nematode activities short of determining reproduction may not be an accurate assessment of resistance as defined by Cook and Evans (1974). Use of such criteria as frequency of penetration or rate of juvenile development can be misleading, since similar numbers of root-knot nematode juveniles have been found in resistant and susceptible soybean cultivars; however, some of the juveniles may egress from roots of the resistant cultivar significantly lowering population levels within the roots (Herman et al. 1991). Furthermore, mature females may vary in numbers of eggs produced (Smith et al. 1986b) and, therefore, the possibility of egress of juveniles from roots of susceptible and resistant cultivars or varieties should be investigated with the specific crop species used to test AMF interactions with nematodes. The final measure of alteration of susceptibility or resistance should be based on nematode reproduction.

Root colonization and sporulation of both endophytes were consistently affected by P fertilization but less consistently by *M. arenaria*. Suppression of root colonization and spore production by AMF due to increased P fertilization is well known on a variety of crops (Hussey and Roncadori 1982; Smith 1987). However, AMF responses to nematode parasitism are unpredictable and often vary with each tripartite relationship (Hussey and Roncadori 1982).

Although AMF development has potential as a biological control for plant-parasitic nematodes, certain combinations of AMF and plant hosts may not be suitable candidates for this approach. For example, our study with peanut indicates that some tolerance to nematodes occurs in symbiotic plants but that this tolerance is accompanied by increased susceptibility and a subsequent buildup in the nematode population density. However, this particular tripartite system is unusual since improved P nutrition associated with mycorrhizal development increased susceptibility to the root-knot

nematode. This interaction may be useful as a model system to better understand the mechanisms of susceptibility and resistance in AMF-plant-parasitic nematode interactions.

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