Influence of *Glomus fasciculatus* and Soil Phosphorus on Verticillium Wilt of Cotton

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ABSTRACT

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A sandy soil received superphosphate at 20 or 300 μg of P per gram of soil, and at each P regime soil was infested with *Verticillium dahliae* alone, *Glomus fasciculatus* alone, both fungi, or neither fungus. In plants fertilized with 20 μg of P per gram of soil, Verticillium wilt was more severe in cotton plants infected with the vesicular-arbuscular mycorrhizal fungus, *G. fasciculatus*, than in nonmycorrhizal plants. However, in plants fertilized with 300 μg of P per gram of soil, Verticillium wilt was equally severe in mycorrhizal and nonmycorrhizal plants. In plants fertilized with 20 μg of P per gram of soil, there were more propagules of *V. dahliae* in petioles of mycorrhizal plants than in petioles of nonmycorrhizal plants. In plants fertilized with 300 μg P per gram of soil, numbers of propagules of *V. dahliae* were not significantly different in mycorrhizal and nonmycorrhizal plants. Infection of cotton by *G. fasciculatus* was not affected by *V. dahliae* in plants fertilized with 20 μg of P per gram of soil, but infection was inhibited by P and further inhibited by *V. dahliae* in plants fertilized with 300 μg of P per gram of soil. Concentrations of P in leaves of mycorrhizal and nonmycorrhizal plants fertilized with 20 μg of P per gram of soil were similar, but concentrations of P were lower in plants infected with *V. dahliae* alone than in plants infected with both *V. dahliae* and *G. fasciculatus*.

Additional key words: endomycorrhizae, soil fungi.

Cotton (*Gossypium hirsutum* L.), like the majority of cultivated plants, is normally infected with vesicular-arbuscular (VA) mycorrhizal fungi. Moreover, mycorrhizal infections result in growth increases in cotton (10,13), especially in soil of low fertility where the symbiont increases efficiency of nutrient absorption by roots. VA mycorrhizae can explore greater amounts of soil and absorb more phosphorus and certain other minerals than nonmycorrhizal roots (7,15).

Several studies indicate that root infection by mycorrhizal fungi or the associated increase in mineral absorption can influence diseases caused by soilborne fungi. Mycorrhizal roots of a cultivar of soybean susceptible to *Phytophthora* were more susceptible than nonmycorrhizal roots to *P. megasperma* (14). In contrast, VA mycorrhizal fungi caused a decrease in production and germination of chlamydospores of *Thielaviopsis basicola* (1) and increased resistance of tobacco to disease caused by *T. basicola* (2). Nonmycorrhizal cotton roots were more severely damaged than mycorrhizal roots by *T. basicola* (16). In the only report on the influence of VA mycorrhizae on a vascular wilt, wilting of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* was reduced when plants were preinfected with the mycorrhizal fungus *Glomus mosseae* (5).

This study was initiated to determine the influence of VA mycorrhizae on Verticillium wilt of cotton caused by *Verticillium dahliae* Kleb. Because mycorrhizal infection increases phosphorus absorption by roots, two soil phosphorus levels were employed to study the influence of the mycorrhizal fungus on disease and the effects of phosphorus fertilization on Verticillium wilt and the *Verticillium*-mycorrhiza interaction.

MATERIALS AND METHODS

A sandy soil was autoclaved for two 1-hr periods with a 24-hr interval between autoclavings and amended with single superphosphate [$Ca(H_2PO_4)_2 \cdot H_2O$] at 20 or 300 μg of P per gram of soil. The soil contained 7 μg of P per gram of soil before the addition of phosphorus. Soil was infested with *Glomus fasciculatus* (Thaxter) Gerd. and Trappe, *V. dahliae* (defoliating, microsclerotial isolate V3H), *G. fasciculatus* plus *V. dahliae*, or neither fungus (Table 1). Inoculum of *G. fasciculatus* consisted of 10 g of soil, roots and spores (20–30 spores per gram) from a pot containing sudan grass (*Sorghum vulgare* Pers.) infected with *G. fasciculatus*. All soils not receiving *G. fasciculatus* received soil and roots of sudan grass grown free of mycorrhizal fungi. Microsclerotia of *V. dahliae* were mixed into the soil for a final concentration of 100 microsclerotia per gram of soil. In addition, part of the soil fertilized with 20 μg of P per gram of soil received 300 microsclerotia per gram of soil as inoculum of *V. dahliae*.

To produce microsclerotia, 1 ml of a conidial suspension of *V. dahliae* was pipetted onto sterilized cellophane disks that covered the surface of potato-dextrose agar in 90-mm diameter petri dishes. The cultures were incubated at 23 C. After 3 wk, microsclerotia were scraped off the cellophane and blended at full speed for 20 sec in 100 ml of sterile distilled water in a Sorvall Omni-Mixer cup. The suspension was passed through sieves and only microsclerotia between 100 and 200 μg in diameter were used.

Three or four cotton seeds (cultivar SJ-2) were sown in 8-cm diameter clay pots containing soil from the various treatments (Table 1). Seedlings were thinned to one plant per pot and kept in a glasshouse at 22–23 C. All plants were watered when necessary with 14% Hoagland's solution minus phosphorus (8). Treatments were replicated 10 times and the experiment was repeated once.

Thirteen weeks after the seedlings germinated, numbers of propagules of *V. dahliae* in infected plant tissue were estimated by a modification of Buchenauer and Erwin's method (3). Petioles collected from the third and fourth uppermost leaves from each plant were surface disinfected in 1.0% sodium hypochlorite for 3 min. Each group of petioles was cut into smaller sections and blended in 200 ml of sterile distilled water in a Sorvall Omni-Mixer at full speed for three 20-sec periods. The suspension was diluted 1:6 with water, and 1 ml was spread on each of six petri dishes of sodium polypectate agar (9). Plates were incubated at 23 C for 6 days. Colonies of *V. dahliae* produced microsclerotia and were easily recognized.

At the end of the experiment, the percentage of roots infected with *G. fasciculatus* was estimated. Randomly selected root samples from each pot were stained with 0.05% trypan blue in

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lactophenol (12), placed on a grid of 1-mm^2 divisions, and examined with a dissecting scope for the presence of arbuscles, vesicles, spores, and hyphae of *G. fasciculatus* in 100 or more 1-mm^2 sections of root tissue.

Dry weights and mineral content of cotton leaves were determined as described by Labanauskas et al (11).

RESULTS

In soil fertilized with 20 μg of P per gram of soil, heights and top and root weights of cotton plants infected with *G. fasciculatus* alone were significantly greater ($P = 0.05$) than those of nonmycorrhizal plants (Table 1). However, this growth response due to the mycorrhizal association was not evident with 300 μg of P per gram of soil, because the greater amount of soil phosphorus caused greater heights, top weights, and root weights whether or not *G. fasciculatus* was present. Despite the greater growth of mycorrhizal plants fertilized with 20 μg of P per gram of soil, *G. fasciculatus* did not significantly improve growth in plants infected with *V. dahliae* at either soil phosphorus concentration (Table 1).

Verticillium wilt of plants grown in soil amended with 20 μg of P and 100 microsclerotia of *V. dahliae* per gram of soil was significantly ($P = 0.05$) more severe in plants infected with *G. fasciculatus* (Table 2). The degree of vascular discoloration was three times greater in mycorrhizal plants than in nonmycorrhizal plants, and 14 times more propagules of *V. dahliae* were recovered from petioles of mycorrhizal plants than from nonmycorrhizal plants. In soil amended with 20 μg of P and 300 microsclerotia per gram of soil, vascular discoloration in plants was uniformly severe whether or not the plants were mycorrhizal, but greater numbers of propagules of *V. dahliae* were recovered from petioles of mycorrhizal plants than from petioles of nonmycorrhizal plants. In plants fertilized with 300 μg of P per gram of soil, vascular discoloration and numbers of propagules of *V. dahliae* recovered from petioles of mycorrhizal and nonmycorrhizal plants did not differ significantly. Symptoms of diseased plants correlated well with vascular discoloration and propagule counts. Mycorrhizal plants fertilized with 20 μg of P per gram of soil and both mycorrhizal and nonmycorrhizal plants fertilized with 300 μg of P per gram of soil had the earliest and most severe wilt symptoms.

Root infection by *G. fasciculatus* in cotton fertilized with 20 μg of P per gram of soil was not reduced by *V. dahliae* (Table 3). However, the amount of infection was reduced in plants fertilized with 300 μg of P per gram of soil and was further reduced in plants fertilized with 300 μg of P per gram of soil and infected with *V. dahliae*.

TABLE 1. Effect of *Glomus fasciculatus*, *Verticillium dahliae*, and two levels of soil phosphorus on growth of cotton[a]

	Phosphorus per gram of soil[c]					
	20 μg			300 μg		
Treatment[b]	Height (cm)	Dry top weight (g)	Dry root weight (g)	Height (cm)	Dry top weight (g)	Dry root weight (g)
Uninfected	44.7 x	12.4 x	3.2 yz	53.9 w	21.9 v	7.3 w
V. dahliae (100 MS/g of soil)	35.6 yz	9.0 xy	2.4 z	35.1 yz	4.0 z	4.7 xy
G. fasciculatus	50.0 w	17.5 w	5.4 x	55.3 w	23.3 v	9.5 v
V. dahliae (100 MS/g of soil) + *G. fasciculatus*	33.3 z	5.7 yz	2.9 z	40.6 xy	5.1 z	5.6 x
V. dahliae (300 MS/g of soil)	30.4 z	4.3 z	1.8 z	...	...	...
V. dahliae (300 MS/g of soil) + *G. fasciculatus*	31.0 z	5.9 yz	3.3 yz	...	...	...

[a] Values represent the mean of 10 replicates. Means in each column at both phosphorus levels followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] MS = microsclerotia of *V. dahliae* added to soil.
[c] Phosphorus was added to the soil as $Ca(H_2PO_4)_2 \cdot H_2O$.

TABLE 2. Effect of *Glomus fasciculatus* and two levels of soil phosphorus on the severity of Verticillium wilt of cotton[a]

	Phosphorus per gram of soil[c]			
	20 μg		300 μg	
Treatment[b]	Vascular discoloration index[d]	Propagules per gram of petiole tissue[e]	Vascular discoloration index	Propagules per gram of petiole tissue
Uninfected	0 z	0 z	0 z	0 z
V. dahliae (100 MS/g of soil)	18.5 y	791 z	79.3 vw	23,575 wx
G. fasciculatus	0 z	0 z	0 z	0 z
V. dahliae (100 MS/g of soil) + *G. fasciculatus*	53.6 wx	11,010 y	86.6 v	21,444 wx
V. dahliae (300 MS/g of soil)	46.4 x	17,037 xy	...	...
V. dahliae (300 MS/g of soil) + *G. fasciculatus*	69.8 vwx	26,731 w	...	...

[a] Values represent the mean of 10 replicates. Means in each column at both phosphorus levels followed by the same letter are not significantly different ($P = 0.05$) acording to Duncan's multiple range test.
[b] MS = microsclerotia of *V. dahliae* added to soil.
[c] Phosphorus was added to the soil as $Ca(H_2PO_4)_2 \cdot H_2O$.
[d] Vascular discoloration index $= \frac{\text{Sum of vascular discoloration rating (0–3)}}{\text{Number of nodes on each plant} \times 3} \times 100$,
based on a vascular discoloration scale of 0 = no discoloration to 3 = 100% discoloration of a cross section of the xylem tissues at each node.
[e] Petioles were blended, diluted, and plated on sodium polypectate agar.

Although concentrations of phosphorus in leaves of nonmycorrhizal and mycorrhizal plants were similar, concentrations of phosphorus were significantly greater in leaves of plants infected with both *G. fasciculatus* and *V. dahliae* than in plants infected with either fungus alone (Table 4). In plants fertilized with 20 μg of P per gram of soil and not infected with *V. dahliae*, zinc concentrations were higher in mycorrhizal plants than in nonmycorrhizal plants, but potassium concentrations were less in mycorrhizal plants than in nonmycorrhizal plants.

DISCUSSION

Adequate phosphorus nutrition of cotton, whether due to phosphorus fertilizer in the soil or to increased phosphorus uptake by mycorrhizal fungi, resulted in more severe wilt of plants than did inadequate phosphorus nutrition. In plants fertilized with a low level of soil P, 20 μg of P per gram of soil, Verticillium wilt was more severe in mycorrhizal than in nonmycorrhizal cotton plants; this is apparent from measurements of vascular discoloration and propagule counts and from the reduction of growth in plants infected with *V. dahliae*. For example, the average total weight of plants infected with *V. dahliae* alone was 73% of the weight of uninfected plants, but the average weight of plants infected with both *V. dahliae* and *G. fasciculatus* was only 39% of the weight of plants infected with *G. fasciculatus* alone.

Possible reasons for the increase in disease severity in mycorrhizal plants include: (i) an increase in the number of avenues for penetration by *V. dahliae*, since *G. fasciculatus* may produce chlamydospores in cortical tissue in such abundance that the cortex ruptures; (ii) dilution of the concentration of potassium in mycorrhizal plants; (iii) a larger population of *V. dahliae* in mycorrhizal plants due to their improved nutrient status; and (iv) more movement of microconidia in the mycorrhizal plants where the greater amount of tissue resulted in greater transpiration. These experiments support the latter three hypotheses since the severity of disease increased as the phosphorus nutrition of the plants improved. Both mycorrhiza and heavy phosphorus fertilization improved vigor and growth of cotton and at the same time caused an increase in the severity of Verticillium wilt. Adequate amounts of all nutrients except phosphorus were supplied to all plants, but growth was impaired in nonmycorrhizal plants fertilized with 20 μg of P per gram of soil, and poor plant growth resulted in less severe Verticillium wilt. The high concentration of potassium in nonmycorrhizal plants was perhaps due to luxury consumption, since phosphorus was the limiting factor for growth. The concentration of potassium was generally reduced in mycorrhizal plants. This could be a factor in the increased severity of disease in mycorrhizal plants, since cotton plants with high levels of potassium are more resistant to *Verticillium* (6).

V. dahliae did not affect mycorrhizal development in soil with 20 μg of P per gram of soil, since infection by *G. fasciculatus* was not reduced at either low or high inoculum densities of *V. dahliae*. Furthermore, concentrations of phosphorus and zinc were not reduced in mycorrhizal plants infected with *V. dahliae*. With 300 μg of P per gram of soil, infection by *G. fasciculatus* was reduced in plants infected with *V. dahliae* because very high levels of phosphorus accumulated in disease tissue. It is well known that high phosphorus levels inhibit mycorrhizae formation (4).

TABLE 3. Effect of *Verticillium dahliae* and soil phosphorus on mycorrhizal infection of cotton[a]

Treatment[b]	Percent of root tissue infected with *Glomus fasciculatus*[c]	
	20 μg of phosphorus per gram of soil[d]	300 μg of phosphorus per gram of soil
Uninfected	0 z	0 z
V. dahliae (100 MS/g of soil)	0 z	0 z
G. fasciculatus	72.2 w	39.5 x
V. dahliae (100 MS/g of soil) + *G. fasciculatus*	71.8 w	20.0 y
V. dahliae (300 MS/g of soil)	0 z	...
V. dahliae (300 MS/g of soil) + *G. fasciculatus*	74.0 w	...

[a] Values represent the mean of 10 replicates. Means in each column at both phosphorus levels followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] MS = microsclerotia of *V. dahliae* added to soil.
[c] Percent infection was determined by the presence of arbuscles, vesicles, spores, and hyphae of *G. fasciculatus* in 100 or more 1-mm^2 sections of stained root tissue.
[d] Phosphorus was added to the soil as $Ca(H_2PO_4)_2 \cdot H_2O$.

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TABLE 4. Effect of *Glomus fasciculatus* and *Verticillium dahliae* on concentrations of phosphorus, potassium, and zinc in leaves of cotton plants fertilized with two levels of phosphorus[a]

Treatment[b]	Phosphorus per gram of soil[c]					
	20 μg			300 μg		
	P (%)	K (%)	Zn (ppm)	P (%)	K (%)	Zn (ppm)
Uninfected	0.12 yz	1.08 x	10.4 z	0.41 v	0.90 xyz	15.6 x
V. dahliae (100 MS/g of soil)	0.09 z	0.90 xyz	9.2 z	1.05 u	1.52 w	20.0 u
G. fasciculatus	0.11 z	0.87 yz	13.2 y	0.42 v	0.77 z	16.8 wx
V. dahliae (100 MS/g of soil) + *G. fasciculatus*	0.16 x	0.87 yz	18.0 vw	1.12 t	2.23 v	19.6 uv
V. dahliae (300 MS/g of soil)	0.15 xy	1.06 xy	10.2 z	...	...	...
V. dahliae (300 MS/g of soil) + *G. fasciculatus*	0.21 w	1.00 xy	13.0 y	...	...	...

[a] Values represent the mean of 10 replicates. Means in each column at both phosphorus levels followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[b] MS = microsclerotia of *V. dahliae* added to soil.
[c] Phosphorus was added to the soil as $Ca(H_2PO_4)_2 \cdot H_2O$.

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