Influence of Vesicular-Arbuscular Mycorrhizae and Soil Phosphorus on Take-All Disease of Wheat

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ABSTRACT

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Wheat grown for 4 wk in P-deficient sandy soil (0.5 μg P/g soil) had significantly greater levels of root P as a result of either addition of 50 μg P/g soil as superphosphate or inoculation with the mycorrhizal fungus, *Glomus fasciculatus*. For plants grown in P-deficient soil, the formation of high levels (>70% root colonization) of vesicular-arbuscular mycorrhizae (VAM) increased root P content nearly to the level of P-amended plants, whereas 50 μg P/g soil severely inhibited VAM formation (<10%). Root exudation of amino acids and reducing sugars, which was lower in heavily mycorrhizal and P-treated plants than the untreated controls, was inversely correlated with root P content. When 4-wk-old plants of each P-VAM treatment combination were inoculated with *Gaeumannomyces graminis* var. *tritici* at two inoculum levels, P-deficient nonmycorrhizal plants developed more take-all at the high inoculum level (0.5% w/w, grams of oat kernel inoculum per gram of soil) than at the low inoculum level (0.1%). By comparison, P-treated plants developed fewer take-all symptoms, irrespective of inoculum density or presence of low levels of mycorrhizal infection. High levels of VAM infection in plants grown in P-deficient soil significantly decreased take-all at the low inoculum level only. The decrease in disease severity in P and VAM treatments was not a result of significant increases in root growth, but apparently was related to improved root P status and decreased root exudation prior to pathogen inoculation. The influence of soil phosphorus and mycorrhizae on take-all disease appears to be the same; that is, these factors increase the P status of the host, which leads to a decrease in net leakage of root exudates and thereby reduces pathogen activity.

In many cases, prior colonization of roots by vesicular-arbuscular mycorrhizae (VAM) increased host resistance to fungal pathogens (18). Chlamydospore formation by *Thielaviopsis basicola* was reduced 10-fold in mycorrhizal vs nonmycorrhizal roots of tobacco and extracts of mycorrhizal roots inhibited chlamydospore production in culture (1). The effect of VAM was attributed to a large increase in levels of free amino acids in roots, especially arginine, which was shown to affect chlamydospore production when added with nonmycorrhizal root extracts (2). When soil phosphorus was limiting, however, the increased tolerance of mycorrhizal citrus to *Phytophthora parasitica* root rot was an indirect effect due to VAM improvement of host P nutrition (5). Moderate levels of soil phosphorus, not inhibitory to mycorrhiza activity, equaled the effect of VAM in reducing disease severity.

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Clearly, the influence of VAM on fungal root pathogens may vary with the disease situation. Since mycorrhizae are normally responsible for an increase in uptake of phosphorus and other mineral nutrients, Gerdemann (8) suggested that studies on the influence of VAM on disease should distinguish whether the changes in host resistance are direct, or indirect as a result of improved host nutrition.

Take-all of wheat (*Triticum aestivum* L.), which is caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker, has long been recognized as a disease favored by inadequate plant nutrition (7), especially phosphorus deficiency (16,19,22). Several reports indicate that application of phosphorus reduces take-all disease in the field (16,19). Mycorrhizal inoculation of wheat, like many other economically important crops, stimulates both growth and yield of plants growing in field soil low in P (11). These observations suggest that VAM have the potential to suppress take-all in P-deficient soils.

The purpose of this study was to determine whether mycorrhizae play a role analogous to phosphorus in decreasing the severity of take-all of wheat and, if so, to identify how VAM and P influence disease development.

MATERIALS AND METHODS

Soil and soil treatments. Superstition sand soil (pH 8.2 in 1:2 0.01 M $CaCl_2$) containing 0.5 μg available P per gram of soil by Olsen analysis (4) was sterilized by autoclaving twice (121 C, 1.5 kg/cm^2) for 1 hr with a 24-hr interval between treatments. Phosphorus in the form of finely ground superphosphate ($Ca[H_2PO_4]_2 \cdot H_2O$) was incorporated into the soil at 0 and 50 μg P/g soil.

For vesicular-arbuscular mycorrhiza (VAM) inoculation, half of the soil at each P level was mixed with inoculum of *Glomus fasciculatus* (Thaxter) Gerd. and Trappe from a pot culture on *Citrus aurantium* L. at a rate of 20 g of soil inoculum per liter of soil. The inoculum consisted of soil that contained a mixture of chlamydospores (300 spores per gram of soil), hyphae, and infected roots. Soil at each P level, with and without inoculum, was potted in 50 65-cm^3 Leach tubes (Leach Cone-Trainer Nursery, Aurora, OR 97002). To establish the microflora associated with the pot culture inoculum in uninoculated treatments, a water extract of pot culture inoculum was prepared by leaching the inoculum (an amount equivalent to that received by mycorrhizal treatments) on a 38-μm sieve to exclude mycorrhizal propagules, and was added to untreated soil. Leech tubes were seeded with cultivar Fielder spring wheat and seedlings thinned to one per tube. Plants were grown for 4 wk in the glasshouse under a maximum light intensity of 1,000 μE/m^2/sec and 29/22 C day-night temperatures, and were watered daily with 14% Hoagland's solution minus P (10).

Influence of P and VAM on take-all. To investigate the influence of P and VAM on take-all disease of wheat, 40 plants from each P-VAM treatment combination were utilized. For inoculation with *G. graminis* var. *tritici*, oat-kernel inoculum of a single-ascospore isolate from diseased wheat plants in Washington state was prepared as described by Reis et al (16). The kernels were fragmented in a Waring Blendor and ground inoculum was mixed with soil of each P level (0 and 50 μg P/g soil) at rates of 0, 0.1, and 0.5% w/w (grams oat kernel inoculum per gram of soil). Mycorrhizal and nonmycorrhizal 4-wk-old wheat plants from each P treatment were transplanted into 500-cm^3 clay pots containing soil with or without pathogen inoculum at the same P level. Plants were grown for another 5 wk under the conditions described above.

VAM and disease assessment. For VAM assessment, entire root systems were cleared in 10% KOH and stained with trypan blue-lactophenol (13). The stained roots were randomly distributed under a grid of 1-mm^2 divisions and examined for the presence or absence of mycorrhizal arbuscules, vesicles, hyphae, and spores in 100 1-mm^2 sections of root tissue. Prior to inoculation with the pathogen, five 4-wk-old plants per treatment were evaluated. For 9-wk-old plants, dried roots from 10 plants were examined.

To quantify take-all disease, 10 9-wk-old plants per treatment were harvested and roots rated visually for the presence of take-all infection on a 0–4 scale in which 0 = no infected roots, 1 = a few small lesions on few roots, 2 = multiple small lesions on few roots, 3 = multiple small to large lesions on most roots, and 4 = multiple large lesions on all roots (20). Disease was also assessed by excising the visibly lesioned portions of the root system and determining the percentage dry weight of lesioned root tissue of the total root dry weight. No difference in root dry weight per unit root length of lesioned and healthy root tissue could be detected for the root systems that were sampled.

Influence of P and VAM on wheat root exudation. To identify factors affected by both P and VAM treatments that might influence subsequent take-all disease severity, 4-wk-old plants prior to pathogen inoculation were examined. Our recent studies on Sudangrass (*Sorghum vulgare* Pers.) indicated that either added soil phosphorus or mycorrhizal inoculation improved the P status of roots and as a result lowered rates of root exudation (9,15). For this reason, we analyzed P content of roots and root exudates of plants selected from each P-VAM treatment combination.

Phosphorus content of two replicate root tissue samples per treatment was determined by magnesium nitrate-nitric acid digestion and colorimetric assay by using the molybdenum blue method (4). Results were expressed relative to dry root weight.

For collection of root exudates, two replicate groups of five plants from each treatment were removed from Leach tubes and carefully washed free of soil. Each batch of plants was immediately placed in a beaker with the roots completely covered with aerated 0.5 mM $CaCl_2$ solution containing 0.05 g of rifampicin and 0.25 g of tetracycline per liter, and incubated for 2 hr. For Sudangrass roots, this antibiotic pretreatment reduced bacterial populations 100-fold compared to untreated roots during the subsequent period of exudate collection (9).

After antibiotic pretreatment, roots were rinsed in one volume of aerated 0.5 mM $CaCl_2$ solution for 5 min and allowed to stand in fresh aerated $CaCl_2$ solution for 22 hr under continuous low light at 23–24 C. The plants were removed from the beaker and dry weights of the root systems were determined. The exudate solution (200–250 ml) was immediately passed through a 0.45 μm filter to remove root debris and microorganisms. The filtered solution was stored at 5 C until it was roto-evaporated to a 20-ml volume then frozen until analyzed.

Concentrated exudate solution was tested for total amino acid and reducing sugar content by using the standard ninhydrin (21) and sulphonated α-napthol (6) procedures, respectively. These procedures were performed in duplicate for each replicate sample. The concentrations of amino acids and reducing sugars were expressed as microgram equivalents of leucine or milligram equivalents of glucose, respectively, per gram dry weight of root.

RESULTS AND DISCUSSION

After 4 wk of growth, significant differences in the P content of wheat roots occurred as a result of either the addition of 50 μg P/g soil or inoculation with *G. fasciculatus* (Table 1). While soil P amendment greatly increased plant growth and root P content, VAM formation was severely inhibited compared to that of inoculated plants grown in P-deficient soil. The inhibition of VAM infection in P-sufficient plants is widely recognized (12,17). In P-deficient plants, mycorrhiza formation was dramatically higher and, as a result of infection, the P content of roots increased to a level more comparable with P-amended plants (Table 1).

Nine-week-old plants grown in untreated soil and inoculated at 4

TABLE 1. Vesicular-arbuscular mycorrhiza formation, root phosphorus content, and total amino acids and reducing sugars in root exudates of wheat uninoculated and inoculated with *Glomus fasciculatus* and grown for 4 wk at two soil phosphorus levels

Treatment	VAM[w] formation (%)	P in dry root (%)	Total dry wt (mg)	Root exudates: Amino acids (μg/g dry root wt)	Root exudates: Reducing sugars (mg/g dry root wt)
0 P[x] - NM[y]	0	0.123 a[z]	186 a[z]	746 a[z]	4655 a[z]
0 P - VAM[y]	71	0.161 b	166 a	443 b	2785 a
50 P - NM	0	0.187 c	411 b	245 b	2773 a
50 P - VAM	8	0.180 c	408 b	267 b	3183 a

[w] Percentage of root length with mycorrhizal structures present.
[x] Concentrations (0 and 50 μg P/g soil) of P added to soil as superphosphate.
[y] NM = nonmycorrhizal, VAM = inoculated with *G. fasciculatus*.
[z] Values are the means of two replications. Column means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

TABLE 2. Influence of soil phosphorus and vesicular-arbuscular mycorrhizae on the severity of take-all disease of wheat caused by *Gaemannomyces graminis* var. *tritici*

Treatment	VAM formation (%)[u]		Roots lesioned (%)[v]		Disease rating (0–4)[v]	
	NM[w]	VAM[w]	NM[w]	VAM[w]	NM[w]	VAM[w]
0 P[x] - 0[y]	···	92 a[z]	0 a[z]	0 a[z]	0 a[z]	0 a[z]
0 P - 0.1	···	94 a	46 b	33 b*	3.2 b	2.3 b*
0 P - 0.5	···	94 a	53 c	44 c	3.4 b	3.0 c
50 P - 0	···	14 b	0 a	0 a	0 a	0 a
50 P - 0.1	···	11 b	30 d	31 b	2.2 c	2.2 b
50 P - 0.5	···	12 b	33 d	30 b	2.4 c	2.3 b

[u] Percentage of root length with mycorrhizal structures present.
[v] For disease assessment, roots were rated visually on a 4–0 scale, and the percentage dry weight of lesioned root tissue of the total root dry weight determined.
[w] Means for VAM (mycorrhizal with *Glomus fasciculatus*) treatments followed by an asterisk (*) are significantly different from the respective NM (nonmycorrhizal) mean, $P = 0.05$.
[x] Concentrations (0 and 50 μg P/g soil) of P added to soil as superphosphate.
[y] Percentage inoculum level (grams of inoculum per gram of dry soil) of *G. graminis* var. *tritici*.
[z] Values are the mean of 10 replications. Column means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

wk with *G. graminis* var. *tritici* developed more take-all at the high inoculum level (0.5% w/w) than at the low level (0.1%) as determined by either visual or quantitative disease assessment (Table 2). For plants grown at 50 μg P/g soil, disease severity was uniformly low regardless of pathogen inoculum concentration or presence of VAM. The lack of a mycorrhizal response in P-amended soil was due to the continued P inhibition of infection first observed in 4-wk-old plants (Table 1). Mycorrhizal infection of less than 15% apparently had no significant effect on disease development compared to nonmycorrhizal treatments (Table 2). High levels of VAM (>90% root colonization) in plants grown in P-deficient soil were correlated with significant decreases in disease severity at the low inoculum level, but not at the higher level, although there were still marked differences in the percentage of roots lesioned.

There was a significant decrease in the dry weight of diseased plants grown with added P (Table 3). The reduction in growth reflected a decrease in both root and shoot dry weight and occurred whether or not the plants were mycorrhizal. In soil without added P, there was also a substantial reduction in root dry weight of both mycorrhizal and nonmycorrhizal plants with take-all although the differences were not significant. Decreases in shoot growth of these plants were less apparent and were small for mycorrhizal treatments. For this reason, the reduction in total dry weight of mycorrhizal plants by take-all was minimal.

Reis et al (16) proposed that certain macronutrients and micronutrients control take-all disease severity through either an increase in resistance of the host tissues to the pathogen or a greater plant tolerance of the pathogen as a result of more root formation. In our study, phosphorus increased root growth more than four-fold compared to plants grown without added P (Table 3). However, for diseased plants the increase in total dry weight of P-amended plants was primarily due to increased shoot growth. Likewise, VAM appeared to counteract the effect of take-all on plants grown in P-deficient soil; shoot growth was maintained at the level of uninfected plants (Table 3). Furthermore, at the time of host plant inoculation, the size of nonmycorrhizal and mycorrhizal plant root systems grown without added P was equal to and smaller than those of P-amended plants, but the P content of untreated mycorrhizal plants was closer to that of P-treated plants (Table 1). Significant reductions in take-all by VAM or P were correlated with higher root P content in these plants prior to pathogen inoculation (Tables 1 and 2). Thus, in this case, the decrease in disease severity associated with phosphorus or mycorrhizae appears to be due to a resistance mechanism related to P content of host roots rather than root growth before and during infection by the pathogen.

In 4-wk-old plants, the increases in P content of roots as a result of P treatment or VAM were correlated with significant decreases in amino acid exudation compared to untreated plants (Table 1). Exudation of reducing sugars followed the same trend, but differences were smaller and not significant. The relationship between phosphorus deficiency and increased root exudation has been observed for a wide variety of plants including *Pinus radiata* (3), Sudangrass, and citrus (15). Ratnayake et al (15) showed that changes in root exudation were correlated with phosphorus-induced decreases or increases in phospholipid content of cells and associated changes in root membrane permeability. In roots with low P status they observed a decrease in phospholipids with a corresponding increase root membrane permeability and root exudation. In roots with high P status, the opposite condition existed: higher phospholipid levels, a decrease in root membrane permeability, and less root exudation. Further studies on Sudangrass (9) indicated that membrane-mediated root exudation was responsible for the observed effect of phosphorus on VAM formation (12,17). In P-deficient Sudangrass, increased levels of exudation sustained high levels of infection which resulted in improved P nutrition and eventually a decrease in membrane permeability and root exudation compared to nonmycorrhizal plants. In the present experiment on wheat, the relationship between VAM improvement of P and lower rates of root exudation was confirmed (Table 1).

The importance of root exudates in the take-all infection process was pointed out by Pope and Jackson (14). They found that hyphae emerging from root or culture inocula had a positive growth response to wheat roots or their exudates. This response decreased as the distance between roots and inoculum increased. They concluded that changes in the quality or quantity of exudates reaching inocula of *Gaeumannomyces* might alter hyphal response

TABLE 3. Influence of soil phosphorus and vesicular-arbuscular mycorrhizae on growth of wheat infected with *Gaeumannomyces graminis* var. *tritici*

Treatment	Dry weight (g)[w]					
	Root		Shoot		Total	
	NM[x]	VAM[x]	NM[x]	VAM[x]	NM[x]	VAM[x]
0 P[y] - 0[z]	0.084 a	0.084 a	0.337 a	0.555 a	0.422 a	0.639 a
0 P - 0.1	0.020 a	0.025 a	0.333 a	0.531 a	0.378 a	0.606 a
0 P - 0.5	0.013 a	0.031 a	0.207 a	0.532 a	0.231 a	0.602 a
50 P - 0	0.349 b	0.435 b*	2.966 d	2.508 d*	3.315 d	2.903 c
50 P - 0.1	0.047 a	0.061 a	1.922 c	1.781 bc	2.084 c	1.992 b
50 P - 0.5	0.049 a	0.055 a	1.368 b	1.686 b	1.534 b	1.864 b

[w] Values are the mean of 10 replications. Column means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.
[x] Means for VAM (mycorrhizal with *Glomus fasciculatus*) treatments followed by an asterisk (*) are significantly different from the respective NM (nonmycorrhizal) mean, $P = 0.05$.
[y] Concentrations (0 and 50 μg P/g soil) of P added to soil as superphosphate.
[z] Percentage inoculum level (grams of inoculum per gram of dry soil) of *G. graminis* var. *tritici*.

and therefore the infection process.

Our observations also suggest that root exudation influences pathogen activity since phosphorus-induced decreases in root exudation were correlated with a subsequent decrease in disease severity. The influence of soil P and mycorrhizae on take-all appears to be the same; that is, these factors both increase the P status of the host, which ultimately lowers the quantities of root exudation (9,15). Thus, the reduction of take-all disease by VAM is indirect and results from improved phosphorus nutrition.

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