Influence of Vesicular Arbuscular Mycorrhizae on the Growth of Apple and Corn in Low-Phosphorous Soil

R. P. Covey, B. L. Koch, and H. J. Larsen

Associate plant pathologist, associate horticulturist/associate soil scientist, and assistant plant pathologist, Washington State University, Tree Fruit Research Center, Wenatchee 98801.

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ABSTRACT

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Growth responses of apple and corn plants to inoculation with *Glomus mosseae* were observed in a fumigated low phosphorous soil. In contrast, roots of these crops did not become infected after inoculation with *G. microcarpus* and no growth responses were observed. Corn was apparently protected from arsenic toxicity by vesicular arbuscular (VA) mycorrhizae. Apple seedlings were more VA mycorrhiza-dependent than were corn plants in this study.

Additional key words: Malus domestica, Zea mays.

Plants with vesicular arbuscular (VA) endomycorrhizae frequently manifest an increased growth response compared with similar plants without VA mycorrhizae. Improved growth is generally attributed to more efficient uptake of various elements in the soil, especially phosphorous (P) (4–6,18). Enhancement of apple tree growth by VA mycorrhizae, as well as changes in chemical composition of tree tissues, were reported by Mosse (16,17). In fumigated soil, VA mycorrhizae increase growth and zinc uptake of apple seedlings (2).

Methyl bromide (MB) fumigation is currently recommended to alleviate an apple replant problem in Washington (1). In some cases similar treatments in citrus and peach orchards resulted in poor growth, apparently due to the lack of VA mycorrhizae (10,12,14,22). This condition was not observed on apple in Washington because most of the apple trees are apparently infected with mycorrhizal fungi while in the nurseries (*unpublished*). In discussing the matter with various nurserymen, we found that fumigation with general biocides (eg, MB) is not widespread and trees apparently become mycorrhizal in the nursery beds. Some nurserymen, however, are becoming interested in applying MB. This study was undertaken to determine whether MB fumigation of soil would cause a problem in growing apple trees.

Because the problems on citrus and peach apparently relate to modifications in P uptake, the effect of VA mycorrhiza on the growth of apple in a soil supplemented with various concentrations of P was studied. Corn was included in the study since information is available on the effects of P (25) and the formation of VA mycorrhizae (4,16) on this crop. Arsenic was included in the treatment because many of the soils in older orchards are contaminated with spray residues of this element (1) and it is chemically closely related to P. Also, Trappe et al (23) found that As tended to suppress formation of VA mycorrhizae on apple. A preliminary report of this research has been published (13).

MATERIALS AND METHODS

Test soil. The possible response of plants to VA mycorrhiza was increased by use of a soil of volcanic origin that is low in P content. The soil was obtained from forested land at the edge of an orchard near White Salmon, WA, and represented a composite profile obtained between depths of 15 and 76 cm. Soil test results from the Washington State University Soil Testing Laboratory indicated pH 6.7, in 0.014% calcium chloride, and 2.3% organic matter. With bicarbonate extraction (19) P content was 8.7 mg/kg and K was 200 mg/kg. Acetate extraction (20) indicated P at 0.6 mg/kg and K at 192 mg/kg. Calcium (Ca) and magnesium (Mg) contents were 5.88 and 0.53 meq., respectively.

Soil fumigation and nutrient amendments. The soil was fumigated with 454 g of MB in bulk containers (122 × 122 × 92 cm) sealed with 4-mil polyethylene plastic. After 2 wk the plastic was opened at the top, and the soil was vented for 4 wk and thoroughly mixed before further treatment.

All soil amendments were made by spraying preweighed lots of soil (926 kg) during agitation in a cement mixer. All lots were amended with sufficient K_2SO_4 and $Mg(C_2H_3O_2)_2 \cdot 4H_2O$ to increase the soil concentration of K and Mg by 200 mg/kg. Individual lots were amended with $NaH_2PO_4 \cdot H_2O$ or As_2O_5 or both to obtain the desired increase in concentration of P and As, respectively. In experiments involving inoculation with mycorrhizal fungi, levels of 0, 50, 100, and 200 mg/kg of As were used. Soil P was increased by 0, 50, 100, 200 mg/kg when corn, *Zea mays* L., was the test plant and by 0, 100, 200, and 400 mg/kg when apple, (*Malus domestica* Borkh.) seedlings were used. Phosphorous and As concentrations were tested singly and in all combinations.

VA mycorrhizae. *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *G. microcarpus* Tul. and Tul. were maintained on *Coleus* sp. grown in the greenhouse. The *G. mosseae* culture was obtained from the late S. Graham, Washington State University, and the *G. microcarpus* was isolated from soil under an apple tree growing at the research center in Wenatchee. Inoculum consisted of 28 g of a mixture of ground roots and sterile sand (1:1, v/v). The control received 100% autoclaved sand in place of the inoculum. The inoculum was separated from the fruit or roots by placing a layer of soil about 1 cm below either the corn or the root of the apple seedling immediately before planting. Mycorrhizal treatments consisted of soil infested with each of the mycorrhizal fungi alone, soil infested with both species, and uninfested soil. All mycorrhizal treatments were tested against all soil amendment treatments.

Growing conditions and chemical assay. Corn (cv. Sugar King) and apple (cv. Northwest common) seedlings were grown in 15 ×19-cm pots. Corn was planted about 1 cm deep and the soil surface was kept damp until germination. Four corn kernels were planted per pot, and the stand was thinned to the best single plant per pot after the plants were established. Apple seedlings, about 5 wk old, were transplanted into the test soil. These seedlings were

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produced from aseptically germinated seed and had been grown in autoclaved vermiculite. All experimental plants were grown in a greenhouse and the individual pots were watered as needed. Nitrogen was added every 2 wk at the rate of 50 ml of an NH_4NO_3 solution of 2.65 g/L per pot.

Corn was harvested after 55 days and apples after 142 days. Unless otherwise stated, data on plant growth was based on oven dry weight of the shoot at the end of the experiment. Mycorrhizal infection was confirmed by microscopic examination of harvested roots (21).

Arsenic and phosphorous concentrations of the shoots were determined on individual plants for corn and on bulked samples for apples because the apple sample was small. A modified Gutzeit method using silver diethyldithiocarbamate (24) was employed for As analysis. Phosphorous was determined colorimetrically after conversion to molybdivanadophosphoric acid (9).

A randomized block design with five replications was used in these experiments. Data was analyzed by the General Linear Models procedure designed by Statistical Analysis Systems (8). All differences reported for these experiments were statistically significant, $P = 0.01$.

RESULTS

The mean height of the uninoculated corn growing in soil without P supplement was 47.4 cm and that of corn inoculated with *G. mosseae* and grown in soil supplemented with 200 mg P per kilogram was 172.9 cm. Dry weight of corn was significantly increased by inoculation with *G. mosseae* and by increased P (Table 1). Inoculation with *G. microcarpus* had no effect on dry weight of corn, but the mixed inoculation of the two VA mycorrhizal fungi increased dry weight over *G. mosseae* alone. If the various mycorrhiza treatments and As concentrations are plotted against the slope of the dry weight vs P curve and the data are analyzed by using simultaneous interval estimation, it becomes apparent that As has less effect on mycorrhizal corn than on corn without mycorrhizae. This is illustrated in Fig. 1, which shows the effect of increasing As concentration and mycorrhizal treatments on the slope of the P vs dry weight of corn curve. Increasing As from 0 to 200 mg/kg of soil resulted in only slight decreases in the slope of the P vs dry weight when the corn had been inoculated with *G. mosseae* or *G. mosseae* plus *G. microcarpus*. The slope of this curve was significantly reduced with increasing As concentration from 50 to 100 mg/kg of soil, compared with either control plants or those inoculated with *G. microcarpus* alone.

The percent P in the corn shoots was influenced by both mycorrhizal and P treatments (Table 2). Addition of 200 mg of P per kilogram of soil increased the P content of corn seedlings, but seedlings grown in either 50 or 100 mg of P per kilogram of soil did

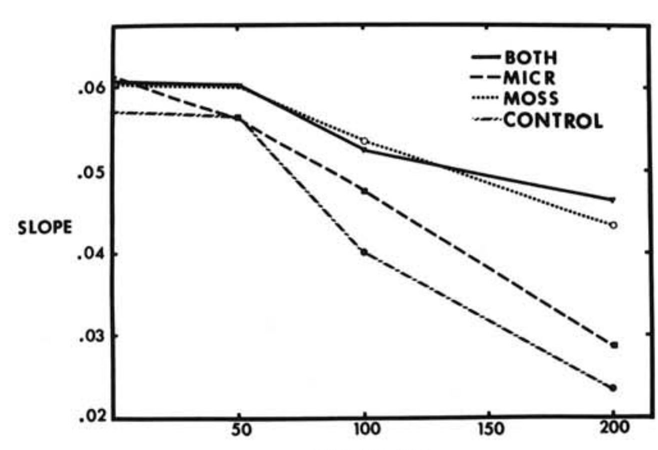

Fig. 1. Effect of inoculation with *Glomus microcarpus* and *G. mosseae* (Moss), alone and combined, and arsenic concentration on the slope of the curve representing growth of corn vs phosphorous concentration.

TABLE 1. Effect of vesicular-arbuscular mycorrhizal fungi inoculation and phosphorous and arsenic concentrations in the soil on dry weight of corn shoots

Amendment and concentration[a]		Mycorrhizae and dry weight[b] (g) of the shoot				
Phosphorous	Arsenic	Control	*Glomus microcarpus*	*Glomus mosseae*	Both	Mean of phosphorous treatment
0	0	2.44	2.15	4.23	4.14	
0	50	2.12	2.43	5.24	4.59	
0	100	2.36	2.47	4.68	6.00	
0	200	1.90	1.90	6.00	6.97	
	Mean	2.21	2.24	5.04	5.42	3.73
50	0	13.55	13.78	15.62	17.75	
50	50	9.47	9.76	13.16	15.15	
50	100	6.37	6.29	12.22	12.30	
50	200	3.98	3.43	13.65	16.46	
	Mean	8.34	8.32	13.66	15.42	11.43
100	0	17.08	19.33	20.46	19.17	
100	50	12.42	20.40	21.06	21.93	
100	100	14.84	15.75	20.30	21.22	
100	200	8.63	8.49	17.10	19.51	
	Mean	15.49	15.99	19.73	20.46	17.92
200	0	28.23	28.87	27.43	27.57	
200	50	26.41	26.65	27.34	26.69	
200	100	21.65	24.90	24.37	23.53	
200	200	16.85	19.65	20.56	20.32	
	Mean	23.28	25.02	24.92	24.53	24.44
Mean	Mean	12.33	12.89	15.84	16.46	

[a]Concentration of soil amendment in milligrams per kilogram.
[b]LSD at $P = 0.001$; mycorrhiza, phosphorous, and arsenic = 0.66; second order interactions = 1.32.

TABLE 2. Effect of mycorrhizal inoculation and phosphorous and arsenic amendments on the percent of concentration of corn shoots

Amendment and concentration[a]		Mycorrhiza and percent phosphorous[b] in shoot				
Phosphorous	Arsenic	Control	*Glomus microcarpus*	*Glomus mosseae*	Both	Mean of phosphorous treatment
0	0	0.08	0.07	0.13	0.14	
0	50	0.06	0.12	0.14	0.13	
0	100	0.06	0.06	0.15	0.16	
0	200	0.05	0.06	0.19	0.17	
	Mean	0.06	0.08	0.15	0.15	0.11
50	0	0.10	0.10	0.12	0.11	
50	50	0.09	0.10	0.15	0.13	
50	100	0.07	0.07	0.17	0.18	
50	200	0.06	0.06	0.21	0.17	
	Mean	0.08	0.08	0.16	0.14	0.12
100	0	0.10	0.09	0.10	0.10	
100	50	0.09	0.09	0.10	0.10	
100	100	0.10	0.09	0.12	0.11	
100	200	0.10	0.09	0.17	0.16	
	Mean	0.10	0.09	0.12	0.12	0.11
200	0	0.14	0.15	0.17	0.19	
200	50	0.13	0.13	0.13	0.13	
200	100	0.11	0.13	0.14	0.15	
200	200	0.11	0.11	0.19	0.21	
	Mean	0.12	0.13	0.16	0.17	0.14
Mean of mycorrhiza	Mean	0.09	0.10	0.15	0.14	

[a]Concentration of soil amendments in milligrams per kilogram.
[b]LSD at $P = 0.01$; mycorrhizal and phosphorous treatments = 0.1; second stage interactions = 0.02.

not contain more P than the plants without added P. Inoculation with *G. mosseae* alone or combined with *G. microcarpus* resulted in high concentrations of P in the corn seedlings at all levels of additional P except 100 mg/kg of soil. Inoculation with *G. microcarpus* alone did not increase levels of P in the corn seedlings.

The mean heights of apple seedlings as a result of various treatments ranged from 2.3 to 58.0 cm. As with corn, significant increases were observed in dry weights of apple seedlings inoculated with *G. mosseae* alone or combined with *G. microcarpus* (Table 3). Even at the higher P concentrations, uninoculated plants or those inoculated with *G. microcarpus* grew only slightly. The dry weight of the seedlings at the start of the experiment was about 0.20 g. Thus, the largest control plants were only about three times their initial weight although the largest plants inoculated with *G. mosseae* increased by nearly 50 times.

Increased P concentration in the soil also significantly increased apple dry weight except that the differences between 100 and 200 mg of P per kilogram were not significant. The pattern for plants inoculated with *G. mosseae* was similar. When plants were inoculated with both species of VA mycorrhizal fungi, increasing P concentration again increased dry weight at 100 and 200 mg of P per kilogram but not at 400 mg of P per kilogram. In apple plants inoculated with *G. mosseae* singly or in combination, the P concentration was twice that in the uninoculated plants or those inoculated with *G. microcarpus* alone (Table 4). At each level of P, plants inoculated with both VA mycorrhizal fungi had slightly higher concentrations of P than plants inoculated with *G. mosseae* alone. Over the range of 0 to 200 mg/kg, increasing P content in the soil increased the P concentration in the plants. Plants grown in 400 mg of P per kilogram of soil, however, had slightly lower concentrations of P than those grown in 200 mg/kg. None of the treatments resulted in more than a trace (<0.1 mg/kg) of As in the plant tissue.

Examination of apple seedling roots inoculated with *G. microcarpus* showed no mycorrhizal structures, and infection on corn ranged from 0 to a trace with no apparent relation to chemical supplement. Almost all of the corn and all except one of the apple roots inoculated with *G. mosseae* were infected.

TABLE 3. Effect of vesicular-arbriscular mycorrhizal fungi inoculation and phosphorous and arsenic concentration in the soil on dry weight of apple shoots

Amendment and concentration[a]		Mycorrhiza and dry weight[b] (g) of the shoot				
Phosphorous	Arsenic	Control	*Glomus microcarpus*	*Glomus mosseae*	Both	Mean of phosphorous treatment
0	0	0.19	0.25	1.73	1.77	
0	50	0.22	0.26	2.17	3.22	
0	100	0.30	0.24	2.75	2.60	
0	200	0.29	0.24	3.29	4.20	
	Mean	0.25	0.25	2.49	2.95	1.48
100	0	0.24	0.22	6.05	5.35	
100	50	0.30	0.23	5.03	5.60	
100	100	0.33	0.27	4.82	5.35	
100	200	0.36	0.24	5.92	5.12	
	Mean	0.31	0.24	5.45	5.35	2.84
200	0	0.37	0.23	4.66	5.83	
200	50	0.29	0.25	5.24	9.14	
200	100	0.30	0.26	5.42	4.41	
200	200	0.37	0.28	5.25	8.20	
	Mean	0.33	0.26	5.14	6.89	3.16
400	0	0.59	0.52	8.84	7.88	
400	50	0.54	0.40	8.21	11.55	
400	100	0.46	0.64	7.49	7.03	
400	200	0.44	0.51	9.72	3.90	
	Mean	0.51	0.52	8.57	7.59	4.29
Mean of mycorriza	Mean	0.35	0.32	5.41	5.70	

[a]Concentration of soil amendment in milligrams per kilogram.
[b]LSD at $P=0.01$; mycorrhizal and phosphorous treatments = 0.70; second order interactions = 1.41.

Because the nonmycorrhizal apples failed to grow even when the soil was supplemented with 400 mg of additional P per kilogram, another study was made to determine if still more P (0, 200, 400, 600, 800, 1,200, 1,600 mg/kg of soil) would stimulate apple growth. Symptoms resembling zinc (Zn) deficiency had also been noted in some uninoculated seedlings (not necessarily related to P treatments); therefore, zinc sulfate at 5 mg of Zn per kilogram of soil was added with 0, 200, 400, 600, and 800 mg of P per kilogram of soil. The 1,200 and 1,600 mg P rates were omitted from the Zn-supplemented treatments because adequate soil was not available. No mycorrhizal treatments were included in this experiment. Dry weight of the plants was taken after they had grown on the greenhouse bench for 130 days.

Seedlings growing in soil supplemented with 400 mg of P per kilogram of soil were not significantly larger than those growing in the nonamended soil (Fig. 2). The plants growing in 600 and 800 mg of P per kilogram of soil were significantly larger than those at 400 mg of P per kilogram of soil, and plants supplemented at 800 mg of P per kilogram of soil were significantly larger than at any other concentration. P concentrations of 1,200 mg/kg of soil and more were apparently phytotoxic, as they reduced growth. The Zn supplements had no significant effect on growth except at 800 mg of P per kilogram of soil.

DISCUSSION

Our results indicate that VA mycorrhizae may be essential to the healthy growth of apple in some soils. Corn plants appear to be less dependent on mycorrhizae than are apple seedlings. In this study nonmycorrhizal corn had growth increases as P supplement was increased from 0 to 200 mg/kg of soil, but nonmycorrhizal apple had no growth increase over the P range of 0–400 mg/kg of soil.

The soil used in this study was selected for its low P content. Based on the ratio of bicarbonate to acetate-soluble P, this soil had a high P-fixing capacity. Even though the soil was supplemented up to 200 or 400 mg of P per kilogram of soil, the saturation point was not reached in either of the VA mycorrhiza experiments. This was especially evident in the apple experiment in which no growth was noted on the nonmycorrhizal plants and further exemplified by the later experiment in which maximum response was obtained by addition of 800 mg of P per kilogram of soil.

Menge et al (15) compared the growth of mycorrhizal and nonmycorrhizal citrus over a range of P concentrations. They found that mycorrhizal fungal infection was equivalent to the addition of 56 and 278 mg of P per kilogram of soil for citrange and sour orange, respectively. Applying similar reasoning to the current study, *G. mosseae* infection of apple is equivalent to between 400 and 800 mg of P fertilizer per kilogram of this particular soil. This also may explain the lack of response of apple to P fertilization

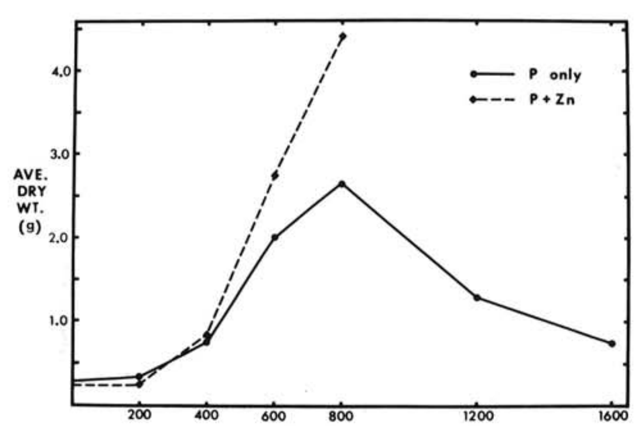

Fig. 2. Effect of phosphorous concentrations on the growth of nonmycorrhizal apple seedlings in a high phosphate-fixing soil with and without a zinc supplement. Differences as a result of zinc supplement are significant ($P=0.01$) only at 800 mg of P per kilogram of soil.

in Washington (3). Whether this high fertilizer equivalent is common for mycorrhizal apples in soil with lower P-fixing capacity will require further study.

Levels of P in the leaves of apple seedlings (3) and corn (25) in excess of 0.1 and 0.25%, respectively, indicate that this element does not limit growth. Realizing that extrapolation from leaf levels to whole plant levels of P is of only limited value, it still can be pointed out that none of the nonmycorrhizal plants of either species reached a desirable level of P. P was probably the limiting factor in the later experiment in which growth increases were noted in apple seedlings when the P supplements were increased from 400 to 800 mg/kg. It is not unexpected that growth responses to P are seldom noted in Washington orchards (3), since most of the trees are probably mycorrhizal.

Arsenic at the higher concentrations used in this study should have depresssed the growth of apple but did not (1), probably because of the high fixing capacity of the soil.

Whether the apparent protection of corn plants from As toxicity by VA mycorrhizae (Fig. 1) is a reality or an artifact is difficult to ascertain. The VA mycorrhizal corn may just be more vigorous due to improved mineral nutrition and thus less affected by the toxicant. Alternately, the VA mycorrhizal root may be more selective in the elements absorbed than the uninfected root.

Both *G. mosseae* and *G. fasciculatus* (Thaxter sensu Gerd.) Gerd. & Trappe form VA mycorrhizae in pot culture on apple (2,7) and corn (7). Gerdemann and Trappe (7) reported infection of corn by *G. microcarpus*, but no such report of *G. microcarpus* on apple was found. Our initial intent was to compare *G. mosseae* and *G. fasciculatus* in this soil, and for the latter species, a recently obtained orchard isolate was used. The lack of growth response for either corn or apple inoculated with this isolate necessitated submission of a voucher specimen to J. M. Trappe for determination. Trappe identified this orchard isolate as the morphologically similar *G. microcarpus*, the spore size range of which overlaps that of *G. fasciculatus*. *G. microcarpus* was thus inadvertently used throughout these studies. Based on the growth data and root observations at the end of the experiment, *G. microcarpus* may be unable to infect apple and has only minimal ability to infect corn.

The results of our study show that a potential nutrition problem exists if the soil we studied were to be fumigated with a general biocide before being used to grow apple seedlings. The level of fumigant recommended for the apple replant problem in Washington (11) and those most likely to be used by nurserymen are toxic to VA mycorrhizal fungi (16). Tests are currently under way to determine the extent of the potential problem in soils from other fruit growing areas of the state.

TABLE 4. Effect of mycorrhiza inoculation and phosphorous and arsenic amendments on the percent phosphorous concentration in apple shoots

Amendment and concentration[a]		Mycorrhiza and percent phosphorous in shoot				
Phosphorous	Arsenic	Control	*Glomus microcarpus*	*Glomus mosseae*	Both	Mean of phosphorous treatment
0	0	0.04	0.04	0.08	0.08	
0	50	0.05	0.04	0.09	0.09	
0	100	0.04	0.04	0.10	0.09	
0	200	0.03	0.04	0.10	0.10	
	Mean	0.04	0.04	0.09	0.09	0.07
100	0	0.04	0.04	0.11	0.10	
100	50	0.04	0.05	0.10	0.10	
100	100	0.04	0.04	0.11	0.10	
100	200	0.04	0.04	0.11	0.10	
	Mean	0.04	0.04	0.11	0.10	0.07
200	0	0.04	0.04	0.12	0.12	
200	50	0.04	0.04	0.12	0.10	
200	100	0.04	0.04	0.14	0.12	
200	200	0.04	0.04	0.11	0.10	
	Mean	0.04	0.04	0.12	0.11	0.08
400	0	0.06	0.06	0.13	0.12	
400	50	0.06	0.06	0.14	0.13	
400	100	0.06	0.05	0.12	0.13	
400	200	0.06	0.06	0.13	0.13	
	Mean	0.06	0.06	0.13	0.13	0.10

[a] Concentration of soil amendments in milligrams per kilogram.

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