Effect of Phosphorus Concentration and *Glomus intraradices* on Fusarium Crown and Root Rot of Tomatoes

Michel Caron, J. André Fortin, and Claude Richard

Research plant pathologist, Agriculture Canada Experimental Farm, P. O. Box 1070, L'Assomption, Québec J0K 1G0; professor and research scientist, Centre de Recherches en Biologie Forestière, Université Laval, Québec G1K 7P4; research plant pathologist, Agriculture Canada Research Station, 2560, boul. Hochelaga, Sainte-Foy, Québec G1V 2J3.

We thank M. Bernier-Cardou for assistance in statistical analyses and V. Furlan for helpful comments and suggestions in reviewing this manuscript. We also thank D. LeQuéré for technical assistance and M. Chartrand for typing the manuscript.

Contribution J1003 of the Saint-Jean Research Station and 283 of the Sainte-Foy Research Station.

Accepted for publication 4 February 1986.

ABSTRACT

Caron, M., Fortin, J. A., and Richard, C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. Phytopathology 76: 942-946.

The effect of colonization of tomato roots by *Glomus intraradices* on population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and on crown and root rot development in relation to increasing P concentrations in the substrate was investigated. An increase of available P in the substrate as well as in the P content of roots and leaves had no effect on the population of *F. o.* f. sp. *radicis-lycopersici* and root necrosis but resulted in a decrease of the root colonization by *G. intraradices*. Only the presence of *G. intraradices* resulted in a significant decrease in the population of *F. o.* f. sp. *radicis-lycopersici* and root necrosis at all P concentrations. At all P concentrations, the presence of *G. intraradices* had no significant effect on the mean dry mass of plants, whereas a significant decrease in plant dry mass was observed for plants inoculated with *F. o.* f. sp. *radicis-lycopersici*. It is concluded that the improved P nutrition from the endomycorrhizal fungus cannot explain the observed limitation in disease and pathogen development.

Additional key words: biological control, *Lycopersicon esculentum*, mycorrhizae.

RÉSUMÉ

Une étude a été faite sur l'influence de la colonisation des racines de tomate par le *Glomus intraradices* sur le *Fusarium oxysporum* f. sp. *radicis-lycopersici* et le développement de la pourriture du collet et des racines en relation avec une augmentation du niveau de P dans le substrat. La population fusarienne et la nécrose des racines n'ont pas été affectées par une augmentation du P disponible dans le substrat ainsi que du contenu en P des racines et du feuillage; cette augmentation de P a cependant entrainé une diminution de la colonisation racinaire par le *G. intraradices*. Seule la présence du *G. intraradices* a résulté en une diminution significative de la population fusarienne et de la nécrose des racines, quel que soit le niveau de P. Pour tous les niveaux de P, la présence du *G. intraradices* n'a pas eu d'effet significatif sur la masse sèche des plants en l'absence du *F. o.* f. sp. *radicis-lycopersici*, alors qu'une diminution de la masse sèche des plantes a été observée en présence du *F. o.* f. sp. *radicis-lycopersici*. La diminution du développement de la maladie et du champignon pathogène telle qu'observée, ne peut être expliquée par une amélioration de la nutrition en P de la plante associée à la colonization des racines par le champignon endomycorhizien.

Several reports have clearly established that vesicular-arbuscular mycorrhizal (VAM) fungi allow better absorption of nutrients by plants, and that phosphorus is generally found in higher concentrations in endomycorrhizal plants (1).

Interaction studies between VAM fungi and root fungal pathogens have shown that the effect of VAM fungi on pathogen and disease development could be related to enhanced P nutrition. Improved P nutrition of host plants was completely or partially responsible for an increase (8) or a decrease (7,10,12,14,18) in disease severity, whereas other reports have indicated no effect (2,6).

The objective of this work was to study the effect of *Glomus intraradices* Schenck & Smith at various colonization levels of tomato roots (*Lycopersicon esculentum* Mill.) on the development of tomato crown and root rot, and on the population of *Fusarium oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis & Shoemaker in relation with increasing P concentrations in the substrate.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Seeds of tomato (cultivar Vendor) were surface disinfested for 1.5 min in 3% sodium hypochlorite to eliminate surface contaminants, blotted dry on sterilized filter papers, and germinated for 5 days at 26 C on potato-dextrose agar (PDA) in petri dishes. Germinated seeds without contaminants were planted in pasteurized coarse sand in six different trays, each corresponding to one P concentration. The seedlings were grown in a greenhouse, watered as needed with demineralized distilled water, and fertilized with 300 ml of the appropriate P solution 2 wk after seeding.

Three-week-old seedlings were carefully removed from the trays and the root system gently washed free of sand with demineralized distilled water. The seedlings were transplanted to 18-cm-diameter pots containing 2.3 L of pasteurized, calcined montmorillonite clay (Turface, IMC Imcore, Mundelein, IL) (4); the initial available P content of the soil was 57 $\mu g \cdot g^{-1}$. The substrate in each pot was watered 1 hr before transplanting, with 500 ml of demineralized distilled water followed by 100 ml of the corresponding P solution.

Each seedling was inoculated with *G. intraradices* by placing 1 g of mycorrhizal leek roots (*Allium porrum* L. cultivar Alaska) 5 cm below the soil surface. To introduce into the nonmycorrhizal treatment the microflora usually associated with pot culture inoculum, 1 ml of spore-free washing, obtained by wet-sieving mycorrhizal roots through a 45-μm-mesh sieve, was added to the

control pots together with 1 g of autoclaved mycorrhizal leek roots. The seedlings were cultured in the greenhouse, watered as needed with demineralized distilled water, and fertilized with 100 ml·wk^{-1} of the corresponding P solution.

The six P concentrations used in this experiment were obtained by adding 41, 82, 164, 328, 656, or 1,312 μg·g^{-1} P as NaH_2PO_4 $2H_2O$ to Long Ashton solution as modified by Plenchette (17). Each pot received 100 ml·wk^{-1} of nutrient solution of its corresponding P concentration.

F. o. f. sp. *radicis-lycopersici* (MC-27) was grown on PDA. Conidia were harvested in sterilized distilled water from 26-day-old cultures incubated at 26 C under fluorescent lights (40 μE·m^{-2}·s^{-1}). The conidial suspension was sieved (45 μm). The inoculum concentration was 2×10^4 conidia per milliliter and consisted of microconidia and macroconidia in a 5:2 ratio.

Four weeks after inoculation with *G. intraradices*, the upper part of the root system of each plant was uncovered. Each plant was then inoculated with *F. o.* f. sp. *radicis-lycopersici* by spreading 1 ml of the conidial suspension on the bare roots, which were recovered immediately with the substrate. Uninoculated control plants received 1 ml of sterilized distilled water. The inoculation treatments were: *G. intraradices* only, *F. o.* f. sp. *radicis-lycopersici* only, *G. intraradices* and *F. o.* f. sp. *radicis-lycopersici*, and control. Each treatment was replicated six times for each P concentration. The plants were cultured in the greenhouse, watered and fertilized as previously described, and harvested 6 wk after inoculation with *F. o.* f. sp. *radicis-lycopersici*. For each plant, the percentage of root necrosis was estimated on the basis of the proportion of the root system with surface necrosis at harvest.

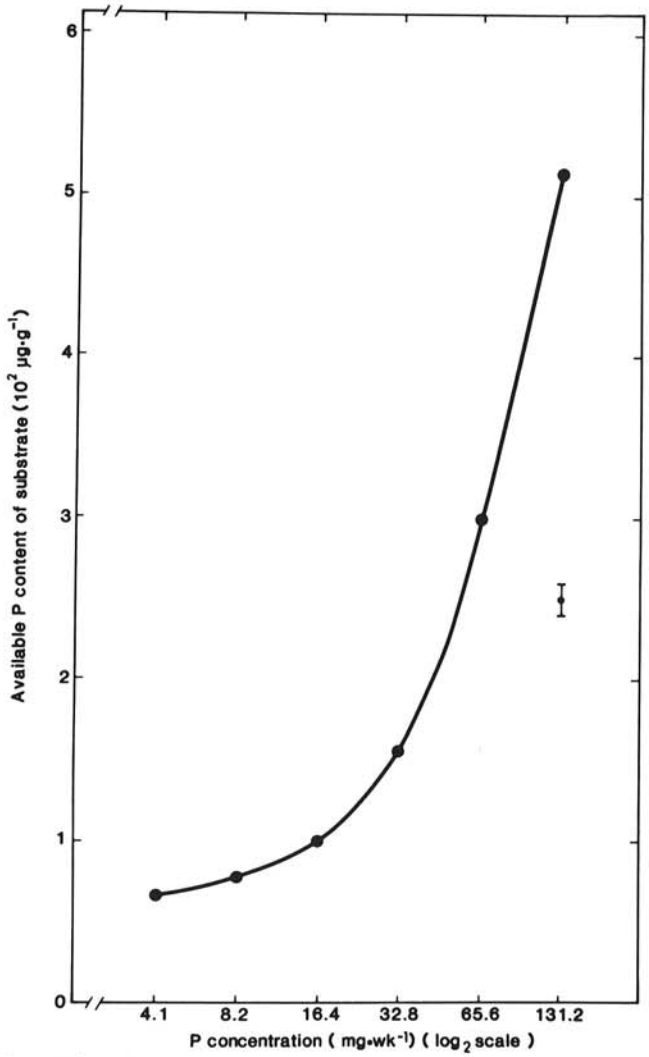


Fig. 1. Effect of applied P on the available P content of the substrate.

Root pieces of each plant, with and without necrosis, were plated on sucrose nutrient agar (15) to detect the presence of *F. o.* f. sp. *radicis-lycopersici* in the roots.

The population of *F. o.* f. sp. *radicis-lycopersici* was evaluated in each pot by shaking 2 g of substrate free of plant material in 100 ml of water agar (0.12%) in a 125-ml flask for 1 hr and leaving the substrate to settle for 10 min, after which 1 ml of the supernatant was diluted in 9 ml of water agar (0.12%). From each flask and each subsequent dilution, 1 ml of the suspension was spread on each of four petri dishes containing Komada's selective medium for *F. oxysporum* (13). The petri dishes were stored in the dark for 10 days at 24 ± 1 C, after which colonies of *F. o.* f. sp. *radicis-lycopersici* were counted and the number of propagules per gram of dry mass of substrate was determined.

Root samples from each plant were stained according to Phillips and Hayman (16) with acid fuchsin instead of cotton blue (3). The root endomycorrhizal colonization index (17) of each plant was established from three sites 3 mm apart on each of 40 1-cm-long root segments randomly selected.

Dry mass of roots and top of each plant were determined after drying at 65 C for 24 hr. Moisture content of the substrate from each pot also was obtained by drying a 10-g sample at 65 C for 24 hr. The substrate pH of each pot also was determined. The available P content of the substrate in each pot was extracted in 0.1 *N* HCl and 0.3 *N* NH_4F at the end of the experiment and determined using the vanadomolybdophosphoric yellow color method in nitric acid system (11). The P content of leaves and roots of a compound sample for each inoculation treatment-P concentration combination was extracted by wet-oxidation process (9) and determined by atomic absorption.

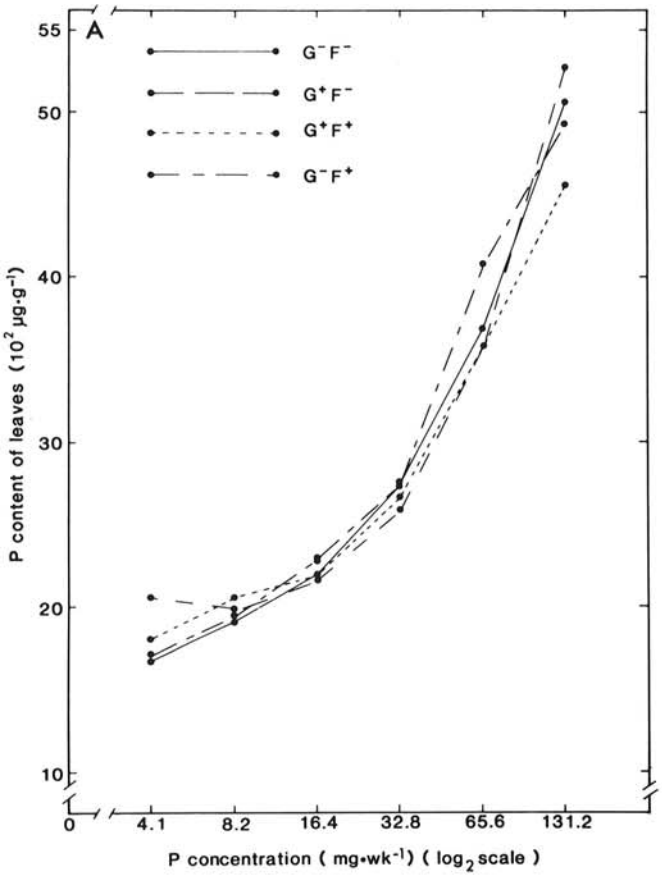
The experimental design was a split plot in six blocks with the four inoculation treatments randomized among the main plot and the six P concentrations randomized among the subplots. The variances of the percentage of root necrosis, the logarithm of the root endomycorrhizal colonization index, the square root of the number of propagules, the available P content of the pasteurized, calcined montmorillonite clay, and the dry masses of plants were analyzed. Logarithm and square root transformations were used to reduce the effect of data variability. The interaction between P concentrations and treatments for the P content of calcined montmorillonite clay, the root endomycorrhizal colonization index, the percentage of root necrosis, and the number of propagules of *F. o.* f. sp. *radicis-lycopersici* were investigated by the method of orthogonal polynomials. Treatment means for dry masses were compared using orthogonal comparisons.

RESULTS

Control plants for treatments without inoculation with *G. intraradices* and treatments without inoculation with *F. o.* f. sp. *radicis-lycopersici* were free of mycorrhizae, and of propagules of *F. o.* f. sp. *radicis-lycopersici* and root necrosis, respectively; thus, data from treatments for controls for *G. intraradices* (*F. o.* f. sp. *radicis-lycopersici* inoculated alone and absence of both microorganisms) were not included in the root endomycorrhizal colonization index analysis of variance. Likewise, data from treatments for controls for *F. o.* f. sp. *radicis-lycopersici* (*G. intraradices* inoculated alone and absence of both microorganisms) were not included in the number of propagules of *F. o.* f. sp. *radicis-lycopersici* and percentage of root necrosis analysis of variance.

As there were no significant differences ($P > 0.05$) among the four inoculation treatments and no significant interactions ($P > 0.05$) between any inoculation treatment and any concentration of applied P, the effect of the increasing concentration of applied P on the available P content of the substrate was represented by a single curve (Fig. 1). The available P content of the substrate varied from 65 μg·g^{-1} at 4.1 mg of P per week up to more than 500 μg·g^{-1} at 131.2 mg of P per week (Fig. 1).

Similarly, the P content of leaves increased for all four inoculation treatments with increasing concentration of applied P (Fig. 2A). The P content of roots also increased (Fig. 2B), but roots



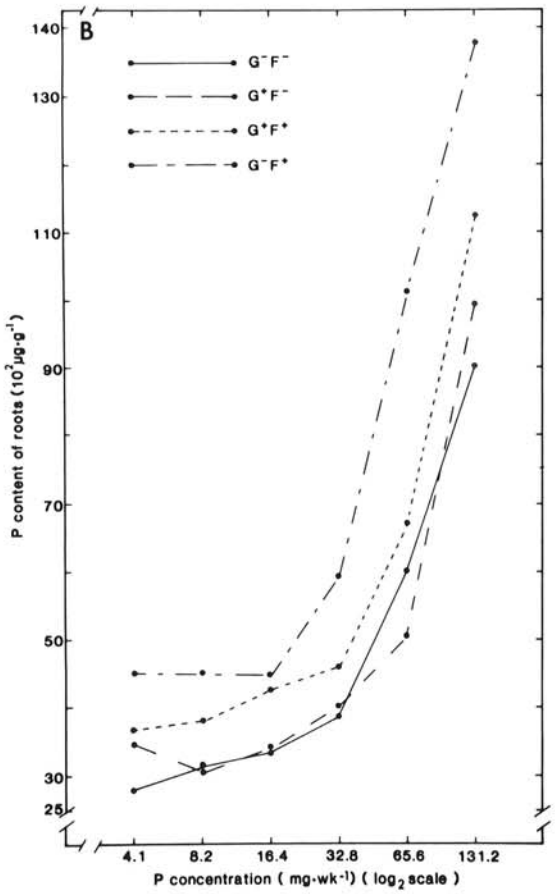
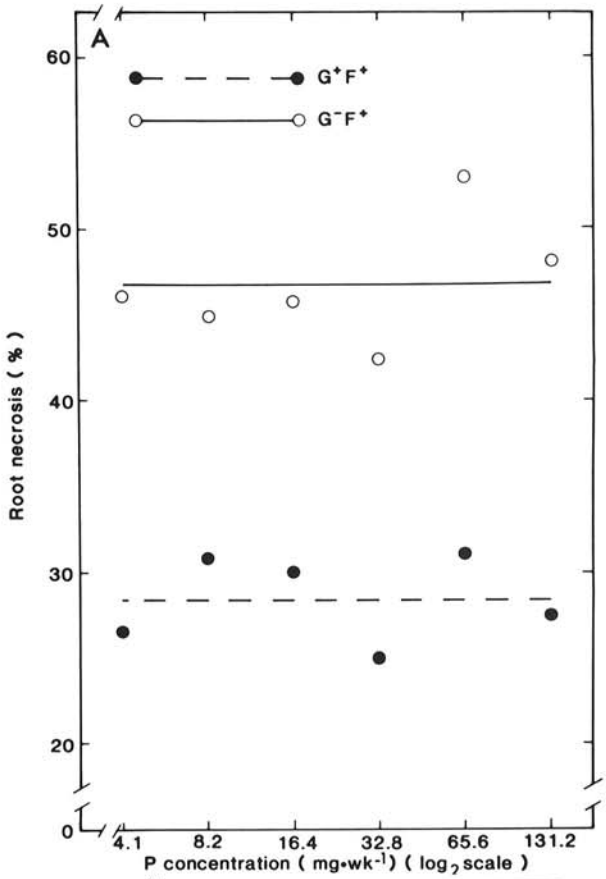


Fig. 2. Effect of applied P on the P content of: **A,** leaves, **B,** roots of tomato plants inoculated (F^+) or uninoculated (F^-) with *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the presence (G^+) or absence (G^-) of *Glomus intraradices*.



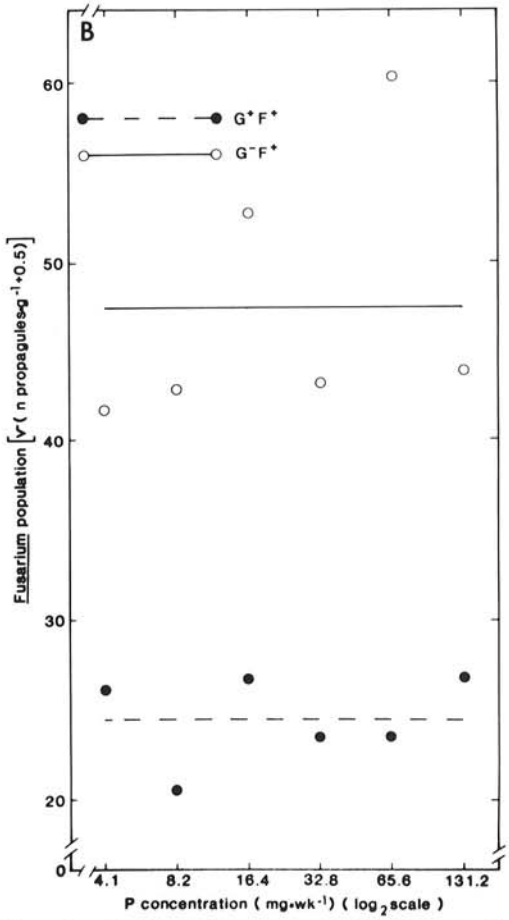


Fig. 3. Effect of applied P on: **A,** root necrosis (%) of tomato plants, **B,** the population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the substrate after inoculation of tomato plants with *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the presence (G^+F^+) or the absence (G^-F^+) of *Glomus intraradices*.

of plants inoculated with *F. o.* f. sp. *radicis-lycopersici* had a greater P content with or without *G. intraradices* (Fig. 2B).

The presence of *G. intraradices* resulted in a significant decrease ($P<0.01$) in the percentage of root necrosis and in the number of propagules of *F. o.* f. sp. *radicis-lycopersici* at all concentrations of applied P (Fig. 3). There were no significant differences ($P>0.05$) in the percentage of root necrosis and in the number of propagules of *F. o.* f. sp. *radicis-lycopersici* among concentrations of applied P and no significant interactions ($P>0.05$) between any inoculation treatment and any concentration of applied P, and none of the correlation coefficient was significant (*t* test, $P>0.05$).

There was a significant decrease ($P<0.01$) in the root endomycorrhizal colonization indices with increasing concentrations of applied P (Fig. 4). The root endomycorrhizal colonization indices, obtained in the presence of *F. o.* f. sp. *radicis-lycopersici*, were significantly higher ($P<0.01$) at all P concentrations. There were no significant interactions ($P>0.05$) between the root endomycorrhizal colonization indices and the increasing concentrations of applied P.

Orthogonal comparisons of plant dry mass means from inoculated treatments showed that, at all P concentrations, inoculation with *G. intraradices* had no significant effect ($P>0.05$) on plant dry mass, whereas the presence of *F. o.* f. sp. *radicis-lycopersici* resulted in a significant decrease ($P<0.05$) in plant dry mass (Table 1).

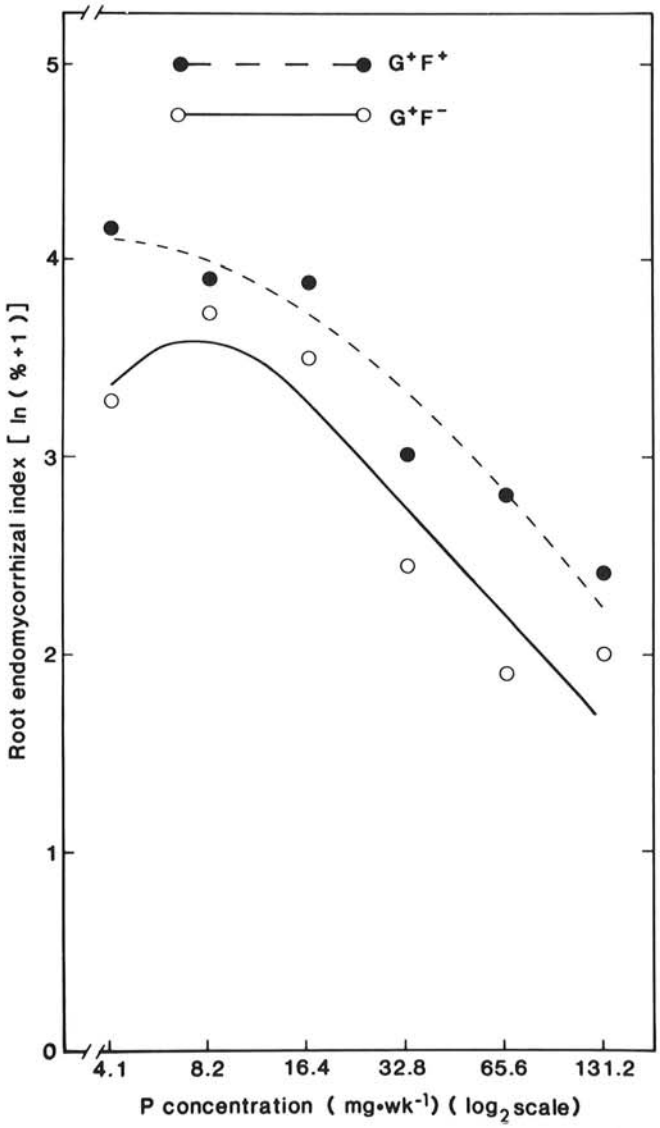


Fig. 4. Effect of applied P on the root endomycorrhizal colonization index of tomato plants inoculated with *Glomus intraradices* with (G^+F^+) and without inoculation of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (G^+F^-).

DISCUSSION

An increase of the phosphorus nutrition of the plants, as indicated by higher P content of the roots and the leaves following an increase in the available P in the substrate, did not alter the pathogen population nor the development of the disease as suggested by others (7,10,12,14,18), but it did result in a decrease in root colonization by *G. intraradices*.

The presence of *G. intraradices* in the substrate as indicated by tomato root colonization, even in the presence of high levels of P, resulted in the development of less root necrosis and it limited the population of *F. o.* f. sp. *radicis-lycopersici*. Earlier reports that plant tolerance or reduced pathogen activities could not be related solely to a better P nutrition of the plants were based on a single P concentration (12,14,18) or on an early, and single, addition of different P concentrations (7,10). This study clearly demonstrates that a higher concentration of P in the plant is not responsible for lower disease development and lower population of *F. o.* f. sp. *radicis-lycopersici*. It confirms that root necrosis and population of *F. o.* f. sp. *radicis-lycopersici* could be reduced even at low levels of endomycorrhizal colonization (4).

The increase in root colonization by *G. intraradices* in the presence of *F. o.* f. sp. *radicis-lycopersici* was not observed in our previous studies (4,5). Nevertheless, such an increase was also reported by Kaye et al (12) in poinsettia inoculated with *Glomus fasciculatum* (Thaxt. sensu Gerd.) Gerd. & Trappe and *Pythium ultimum* Trow. This phenomenon cannot be explained at present.

The presence of *G. intraradices* had no effect on the plant dry masses, as expected (4,5), whereas the presence of *F. o.* f. sp. *radicis-lycopersici* resulted in decreases of the plant dry masses at all P concentrations.

Graham and Menge (10) reported a decrease in the take-all disease of wheat with a decrease in root exudation due to an increase in the P status of the host in the presence of *G. fasciculatum*. However, the increased P status of the tomato plants obtained in the absence of *G. intraradices* by increasing the concentration of applied P, strongly suggests that a decrease in root exudation due to an increase in the P status of the host is not associated with the disease suppression incited by *G. intraradices*. The possibility remains that competition takes place between the two organisms for nutrients contained in the root exudates, although it is very likely that competition alone would not explain the decreases in root necrosis and in the population of *F. o.* f. sp. *radicis-lycopersici* where low root endomycorrhizal colonization indices were observed.

We have suggested (4) that interaction between the endomycorrhizal fungus and the root pathogen might occur in the remote extramatrical phase of the VAM fungus. This work emphasizes the necessity of further investigations on the possible production of antibiotics or other inhibitory compounds produced by VAM fungi or their hosts and on the ecological conditions that could enhance such advantageous effects.

TABLE 1. Orthogonal comparisons for mean dry masses of tomato plants inoculated (F^+) or not (F^-) with *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the presence (G^+) or absence (G^-) of *Glomus intraradices*

P concentration (mg added per week)	Comparison for *Glomus*		Comparison for *Fusarium*	
	G^-F^- & G^-F^+ Mean	G^+F^- & G^+F^+ Mean	G^-F^- & G^+F^- Mean	G^-F^+ & G^+F^+ Mean
4.1	12.5	12.4	13.7	11.1
8.2	13.2	13.2	14.3	12.1
16.4	13.5	13.1	14.2	12.4
32.8	13.4	13.7	14.7	12.4
65.6	12.9	13.6	14.8	11.7
131.2	11.9	13.3	13.7	11.6
Mean	12.9	13.2	14.2	11.9
F	n.s.[a]		**[b]	
Standard error = 1.307				

[a] Not significant.
[b] Indicates statistical significance, $P = 0.01$.

The reduction in root necrosis and the population of *F. o.* f. sp. *radicis-lycopersici* is attributed to the presence of *G. intraradices*. The effect of this VAM fungus on the disease and the pathogen development is more important than the effect of an increase in P nutrition. The observed reduction of the disease and the pathogen development cannot be explained by the improved P nutrition attributed to the presence of the endomycorrhizal fungus.

LITERATURE CITED

1. Abbott, L. K., and Robson, A. D. 1984. The effect of VA mycorrhizae on plant growth. Pages 113-130 in: VA Mycorrhiza. C. L. Dowell and D. J. Bagyaraj, eds. CRC Press, Cleveland, OH.
2. Bååth, E., and Hayman, D. S. 1983. Plant growth responses to vesicular-arbuscular mycorrhiza. XIV-Interactions with *Verticillium* wilt on tomato plants. New Phytol. 95:419-426.
3. Berch, S. M., and Kendrick, B. 1982. Vesicular-arbuscular mycorrhizae of Southern Ontario ferns and fern-allies. Mycologia 74:769-776.
4. Caron, M., Fortin, J. A., and Richard, C. 1985. Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomatoes. Plant Soil 87:233-239.
5. Caron, M., Fortin, J. A., and Richard, C. 1986. Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes over a 12-week period. Can. J. Bot. 64:552-556.
6. Davis, R. M. 1980. Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* root rot of citrus. Plant Dis. 64:839-840.
7. Davis, R. M., and Menge, J. A. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on Phytophthora root rot of citrus. Phytopathology 70:447-452.
8. Davis, R. M., Menge, J. A., and Erwin, D. C. 1979. Influence of *Glomus fasciculatus* and soil phosphorus on Verticillium wilt of cotton. Phytopathology 69:453-456.
9. Gorsueh, T. T. 1959. Radiochemical investigations on the recovery for analysis of trace elements in organic and biological materials. Analyst 84:136-173.
10. Graham, J. H., and Menge, J. A. 1982. Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat. Phytopathology 72:95-98.
11. Jackson, M. L. 1958. Phosphorus determinations for soils. Pages 134-182 in: Soil Chemical Analysis. Prentice-Hall, Inc
12. Kaye, J. W., Pfleger, F. L., and Steward, E. L. 1984. Interaction of *Glomus fasciculatum* and *Pythium ultimum* on greenhouse-grown poinsettia. Can. J. Bot. 62:1575-1579.
13. Komada, H. 1975. Development of a selective medium for quantitative isolatïon of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.
14. Krishna, K. R., and Bagyaraj, D. J. 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Can. J. Bot. 61:2349-2351.
15. Nirenberg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Can. J. Bot. 59:1599-1609.
16. Phillips, J. M., and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.
17. Plenchette, C., Furlan, V., and Fortin, J. A. 1982. Effect of different endomycorrhizal fungi in five host plants grown on calcined montmorillonite clay. J. Am. Soc. Hortic. Sci. 107:535-538.
18. Zambolim, L., and Schenck, N. C. 1983. Reduction of the effects of pathogenic root-infecting fungi on soybean by the mycorrhizal fungus, *Glomus mosseae*. Phytopathology 73:1402-1405.