Effects of Vesicular-Arbuscular Mycorrhizal Fungi on Infection of Tamarillo (*Cyphomandra betacea*) by *Meloidogyne incognita* in Fumigated Soil

KAREN M. COOPER, Division of Horticulture and Processing, and GORDON S. GRANDISON, Entomology Division, Department of Scientific and Industrial Research, Auckland, New Zealand

ABSTRACT

Cooper, K. M., and Grandison, G. S. 1987. Effects of vesicular-arbuscular mycorrhizal fungi on infection of tamarillo (*Cyphomandra betacea*) by *Meloidogyne incognita* in fumigated soil. Plant Disease 71:1101-1106.

The effects of vesicular-arbuscular mycorrhizal (VAM) fungi on infection of tamarillo (*Cyphomandra betacea*) plants by root-knot nematodes (*Meloidogyne incognita*) were studied in fumigated soils with and without supplementary phosphate fertilizer. Nematodes severely reduced plant growth. In dually-inoculated plants, mycorrhizal infection improved plant growth and suppressed nematode reproduction and development in roots. Nematode infection and development were less in plants preinfected with mycorrhizal fungi than in plants inoculated simultaneously with both organisms. The benefit achieved by mycorrhizal inoculation could not be duplicated by adding phosphate fertilizer and was not therefore due merely to improved phosphorus nutrition of the host.

Tamarillo or tree tomato (*Cyphomandra betacea* (Cav.) Sendtn.) is a native of Peru and Brazil. It is grown in many parts of the world but has so far achieved commercial significance only in New Zealand (2,9). The soils used for tamarillo production in New Zealand are generally naturally deficient in plant nutrients, and substantial growth increases can be achieved by inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi (4,5).

VAM fungi and soilborne pathogens commonly occur together in the roots or rhizosphere of the same plant. Interactions between VAM fungi and soilborne fungal and nematode pathogens are variable (8,17,22–25). However, recent experiments have shown that infection of plants of tomato, white clover, and lucerne by VAM fungi decreases the incidence of disease, limits nematode development and activity in plant roots, and minimizes growth suppression by root-knot nematodes (6,13).

Mycorrhizae-induced resistance to pathogens has been linked to better host nutrition and improved phosphorus uptake by VAM plants (17,31). A preliminary study on the effect of VAM fungi on the infection of tamarillo by root-knot nematodes (*Meloidogyne incognita* (Kofoid & White) Chitwood) in fumigated soils indicated a protective influence of VAM fungi on tamarillo roots that was independent of phosphate nutrition. This study was expanded and is reported here.

MATERIALS AND METHODS

Plant material. Plants were raised from seed in a soil-pumice sand potting mix (pH 6.0, 10 μg/g Olsen P in soil) that had been fumigated with methyl bromide at a concentration of 100 g/m^3 for 18 hr at atmospheric pressure and 20 C. Four weeks after sowing, seedlings were transplanted into 600-ml pots (one seedling per pot) containing a mix of fumigated Kumeu sandy clay loam and pumice sand (2:1). This soil had an analysis of pH 6.0, P 20, K 260, Mg 305 μg/g in soil. Soil in half of the pots was supplemented with finely ground superphosphate fertilizer (10% P) equivalent to 60 kg P/ha or 60 mg P/L of soil. The final analysis of this phosphorus supplemented soil was pH 6.2, P 38, K 300, and Mg 310 μg/g in soil. Nutrient solution containing all major and micronutrients, except phosphorus (15), was added weekly.

Inoculum preparation. A mycorrhizal inoculum of chopped roots (1 cm long) and soil sievings (53–710 μm fraction) (10) was prepared from onion and sorghum stock culture plants infected with *Gigaspora margarita* Becker & Hall, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *G. fasciculatum* (Thaxter sensu Gerd.) Gerdemann & Trappe, *G. tenue* (Greenall) Hall (14), or with a mixture of VAM fungi adapted to high phosphate levels and known to infect plant roots in soils fertilized with phosphate-based fertilizers (3). Stock culture plants were first checked for absence of pathogens and were used only where mycorrhizal root length was more than 70% and soil contained healthy VAM fungal spores. Because of the occurrence of suspected nonsporing fungi in the high-phosphorus fungal inoculum, no attempt was made to assess inoculum viability or to standardize inoculum potential of the individual VAM fungal species other than by quantity of well-infected root segments and soil sievings. Roots and sievings were prepared individually for each fungus, then combined and mixed well. At transplanting, each plant received 0.5 g fresh weight of chopped roots and 50-cm^3 soil sievings of the mixture of all mycorrhizal fungi. This inoculum was dispersed through the soil around and below the plant roots. All plants not inoculated with mycorrhizal fungi received 50-cm^3 soil sievings, which were passed through Whatman No. 542 filter paper to remove mycorrhizal fragments. This reintroduced some soil microflora to the fumigated soil. This is generally considered the most adequate form of control (18), although the problems associated with choosing a suitable control have been pointed out by Abbott and Robson (1).

The nematode inoculum of 12-hr-old juveniles of *M. incognita* was extracted under intermittent mist from infected roots of cucumber (*Cucumis sativus* L.) or tomato (*Lycopersicon esculentum* L.). Two thousand juveniles per plant were pipetted 1 cm deep in the soil over the plant roots.

Experimental design. There were six treatments: not inoculated with either mycorrhizal fungi or nematodes, inoculated with mycorrhizal fungi at transplanting, inoculated with nematodes at transplanting, inoculated with mycorrhizal fungi and nematodes at transplanting, inoculated with nematodes 4 wk after transplanting, and inoculated with mycorrhizal fungi at transplanting and with nematodes 4 wk later. Each treatment was replicated six times. Pots were maintained in randomized, replicated blocks in a greenhouse during summer months (November through January) (16-hr day, 15–25 C).

Harvesting of plants. Plants were harvested 12 wk after transplanting, which potentially allowed for the development of at least two life cycles of nematodes. Dry weights of shoots were recorded after oven-drying and fresh weights of roots after spin-drying to remove excess moisture. Root systems were cut into lengths of 1–2 cm, thoroughly mixed, and divided on a fresh weight basis for measurements of tissue

phosphorus, mycorrhizal root length, and nematode infection.

Estimation of mycorrhizal root length and nematode infection. To assess mycorrhizal infection, root systems were cleared in 10% KOH and stained in trypan blue in lactophenol (0.05%, w/v) (20). Mycorrhizal infection was assessed as a percentage of aggregate length of the entire root system by a modification of the line intercept method (11). Vertical and horizontal grid lines were scanned and mycorrhizal infection was scored on a scale of 0–5 at each point where roots intersected a line. Mycorrhizal infection was then expressed as a percentage of root length.

To assess nematode infection, roots were stained in acid fuchsin in lactophenol (30) and homogenized in water in a tissue blender to release nematodes from plant tissue. The nematodes (juveniles, third-stage juveniles, and adults) in an aliquot were counted.

Determination of plant phosphorus content. Individual shoots, or a weighed portion of each root, were dried, ground, and digested in nitric acid (21). The percent phosphorus content of the shoot or root dry matter was then determined colorimetrically (19).

RESULTS

Plant growth. Mycorrhizal inoculation markedly improved shoot and root growth of tamarillo plants in the unfertilized soil but less so in fertilized soil (Table 1, Figs. 1 and 2). Inoculation of nonmycorrhizal plants with nematodes at transplanting depressed root and shoot growth at both soil phosphate levels (Table 1, Fig. 1A,B). However, with delayed inoculation, nematodes significantly depressed shoot and root growth only in soil not receiving additional phosphate.

Plants inoculated with both mycorrhizal fungi and nematodes achieved root and shoot weights similar to those of nematode-free plants (Table 1, Figs. 1 and 2). This was not affected by the time of nematode inoculation. Root development was more extensive and decay and gall formation less when plants were infected with mycorrhizal fungi before inoculation with nematodes than when plants were inoculated with mycorrhizal fungi and nematodes simultaneously (Fig. 2A,B).

Mycorrhizal infection. Roots of all plants inoculated with mycorrhizal fungi were infected. Mycorrhizal root length varied from 26 to 32% at the time of delayed nematode inoculation (4 wk after transplanting) and from 67 to 79% at harvest. There were no significant differences between treatments, and mycorrhizal root length was unaffected by nematode inoculation.

Nematode infection. Total numbers of nematodes (juveniles, third-stage juveniles, and adults) in nematode-inoculated, nonmycorrhizal plants increased with additional phosphate and were greater in the delayed inoculation treatment (Fig. 3A). However, mycorrhizal inoculation suppressed nematode reproduction, and this suppression was greatest when nematode inoculation was delayed.

Nematode numbers per gram fresh weight of root were lower after the delayed inoculation time (possibly reflecting increased root masses between times of first and second inoculation) but were unaffected by added phosphate (Fig. 3B). Mycorrhizal inoculation suppressed nematode numbers after both inoculation times and in both fertilized and unfertilized soils.

The numbers of adult nematodes extracted from roots of mycorrhizal plants were substantially lower than in nonmycorrhizal plants (Fig. 4A,B). Indeed, there was no adult development in most mycorrhizal plants.

Plant phosphorus content. In soil to which no phosphate fertilizer had been added, mycorrhizal inoculation increased

and nematode inoculation decreased the phosphorus content (%P in dry matter) of shoots and roots (Table 2). Mycorrhizal inoculation of nematode-infected plants increased shoot and root phosphorus contents to levels similar to those in plants inoculated only with mycorrhizal fungi. In soil given additional phosphate, phosphorus content of shoots and roots was similar in all treatments and generally remained unaffected by nematode or mycorrhizal inoculation.

DISCUSSION

Inoculation of tamarillo plants by VAM fungi increased the host's resistance to *M. incognita*. When mycorrhizal fungi and root-knot nematodes were added simultaneously at transplanting, root and shoot growth of nonmycorrhizal seedlings were depressed by 79–91%, but the depression of growth of mycorrhizal seedlings was either not significant or was less than 20%. Preinfection with mycorrhizal fungi did not prevent this growth depression although damage to roots (decay and gall formation) was often considerably less. This ability of mycorrhizal plants to grow well despite infection by nematodes is generally considered to be the principal effect of VAM fungi on the interaction of host plants with parasitic nematodes (6,13,17).

Nematode reproduction was also suppressed by inoculation with mycorrhizal fungi. The plant's resistance to nematode development was increased further when plants were preinoculated with mycorrhizal fungi even though only 26 to 32% of the root length was mycorrhizal at the time of nematode penetration. Development and reproduction of nematodes is often inhibited in mycorrhizal plants (6,13,16,26–29), and preinfection of plants with VAM fungi may well afford the plant maximum protection from damage by root-knot nematodes.

The benefits of improved growth and lower nematode numbers afforded by inoculation with mycorrhizal fungi applied to both the high- and low-phosphate soils and to plants with adequate tissue phosphorus content. Indeed, the reduction in numbers of juveniles and adults was greater in the fertilized than in the unfertilized soil. Because mycorrhizal fungi adapted to infecting plants in soils fertilized with phosphate-based fertilizers (3) were included in the mycorrhizal inoculum to ensure adequate mycorrhizal infection in all mycorrhizal treatments, these benefits can be attributed directly to mycorrhizal infection and not just to improved phosphorus nutrition as has often been suggested (7,12,17,31).

Nevertheless, because VAM fungi differ in their ability to infect roots and survive in phosphate-fertilized soil (3), it is likely that the population of VAM fungi in the roots of plants in the

Table 1. Influence of vesicular-arbuscular mycorrhizal fungi and *Meloidogyne incognita* singly and in combination on the shoot dry weight and root fresh weight of tamarillo with and without added phosphate fertilizer

Treatment[a]		Added superphosphate (kg P ha^{-1})[b]			
		Shoot dry weight (g)		Root fresh weight (g)	
Myc.	Nem.	0	60	0	60
−	−	0.30 r	0.91 st	2.50 s	5.46 t
+	−	1.03 t	1.14 t	6.85 uv	7.46 v
Inoc. time a					
−	+	0.03 p	0.18 q	0.22 p	0.87 q
+	+	0.83 s	0.79 st	5.70 tu	5.28 t
Inoc. time b					
−	+	0.18 q	0.64 s	1.52 qr	3.86 st
+	+	0.75 s	0.91 st	4.96 t	5.54 tu

[a] Mycorrhizal fungi added at transplanting. Inoculation time a = nematodes added at transplanting. Inoculation time b = nematodes added 4 wk after transplanting. Values are means of six replicates.
[b] Within shoot or root weights, values sharing the same letter do not differ significantly ($P \leqslant 0.05$) (analysis of variance, square-root transformation).
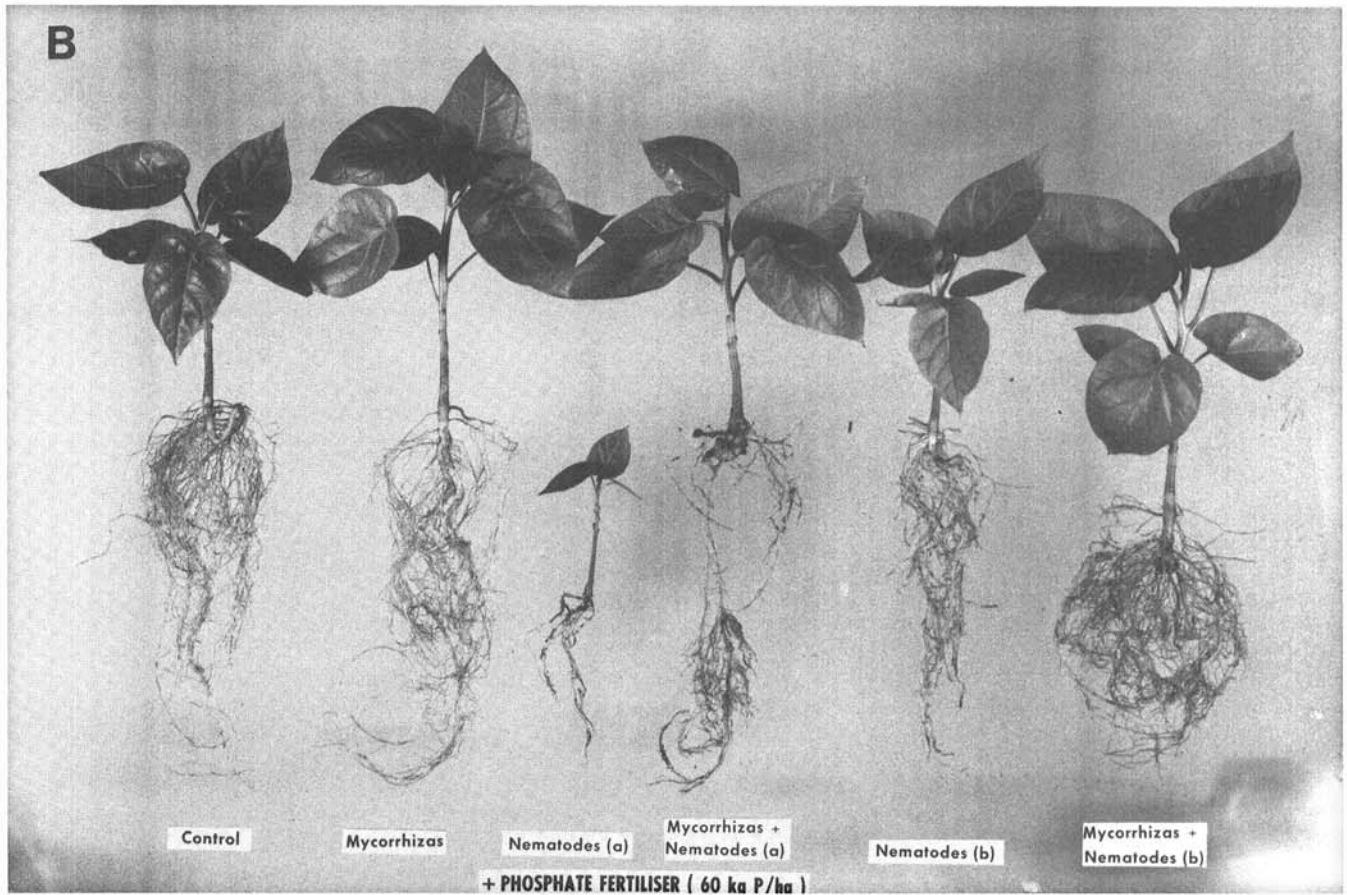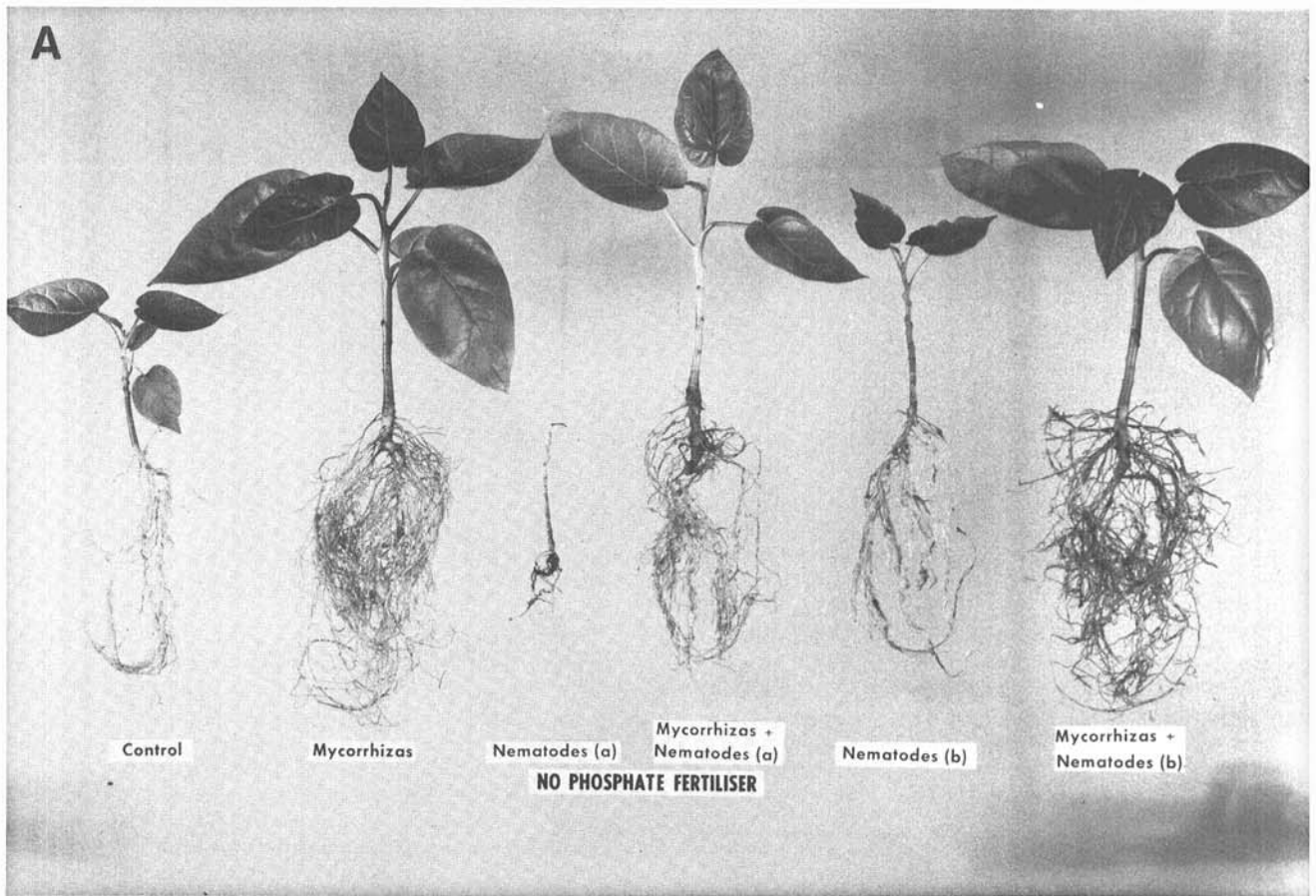
Fig. 1. Growth of tamarillo as influenced by vesicular-arbuscular mycorrhizal fungi, root-knot nematode (*Meloidogyne incognita*), or phosphate fertilizer (mycorrhizal fungi added at transplanting). **(A)** Unfertilized soil; **(B)** soil fertilized with 60 kg P⁻¹. a = Nematodes added at transplanting; b = nematodes added 4 wk after transplanting.
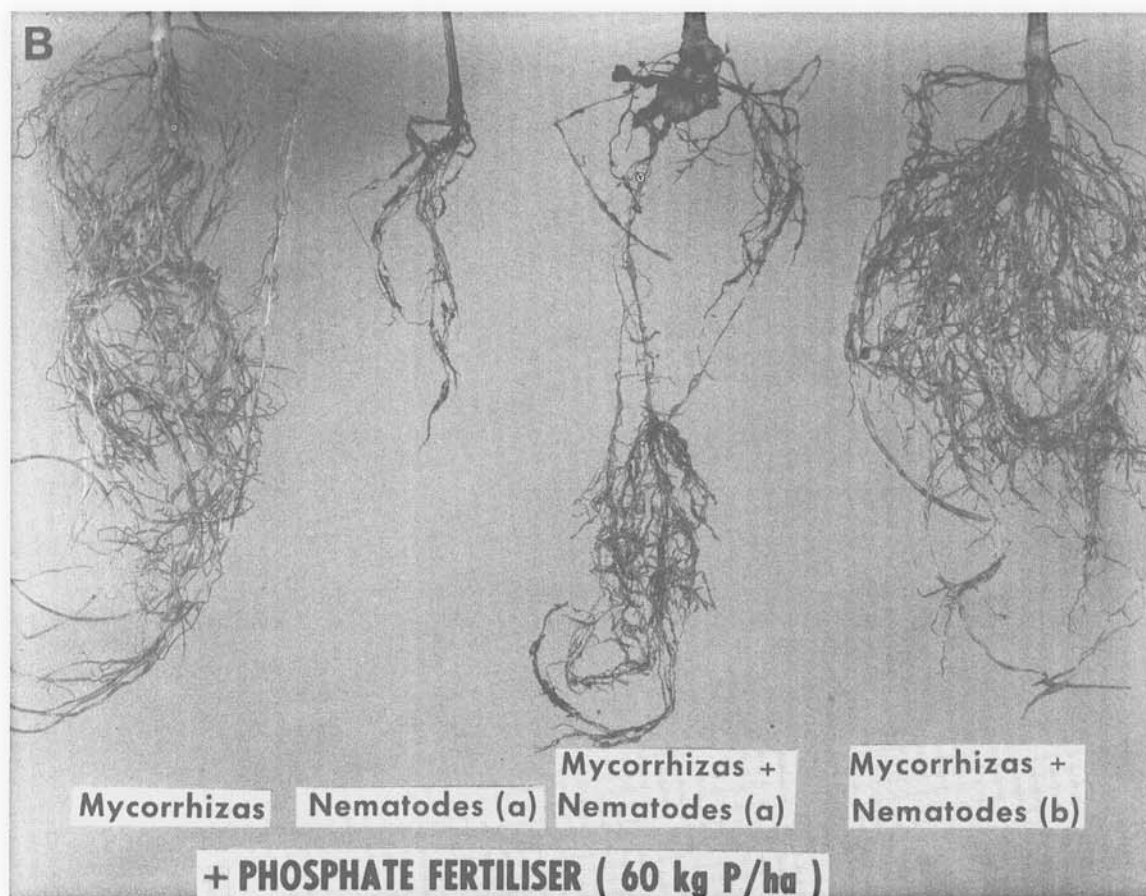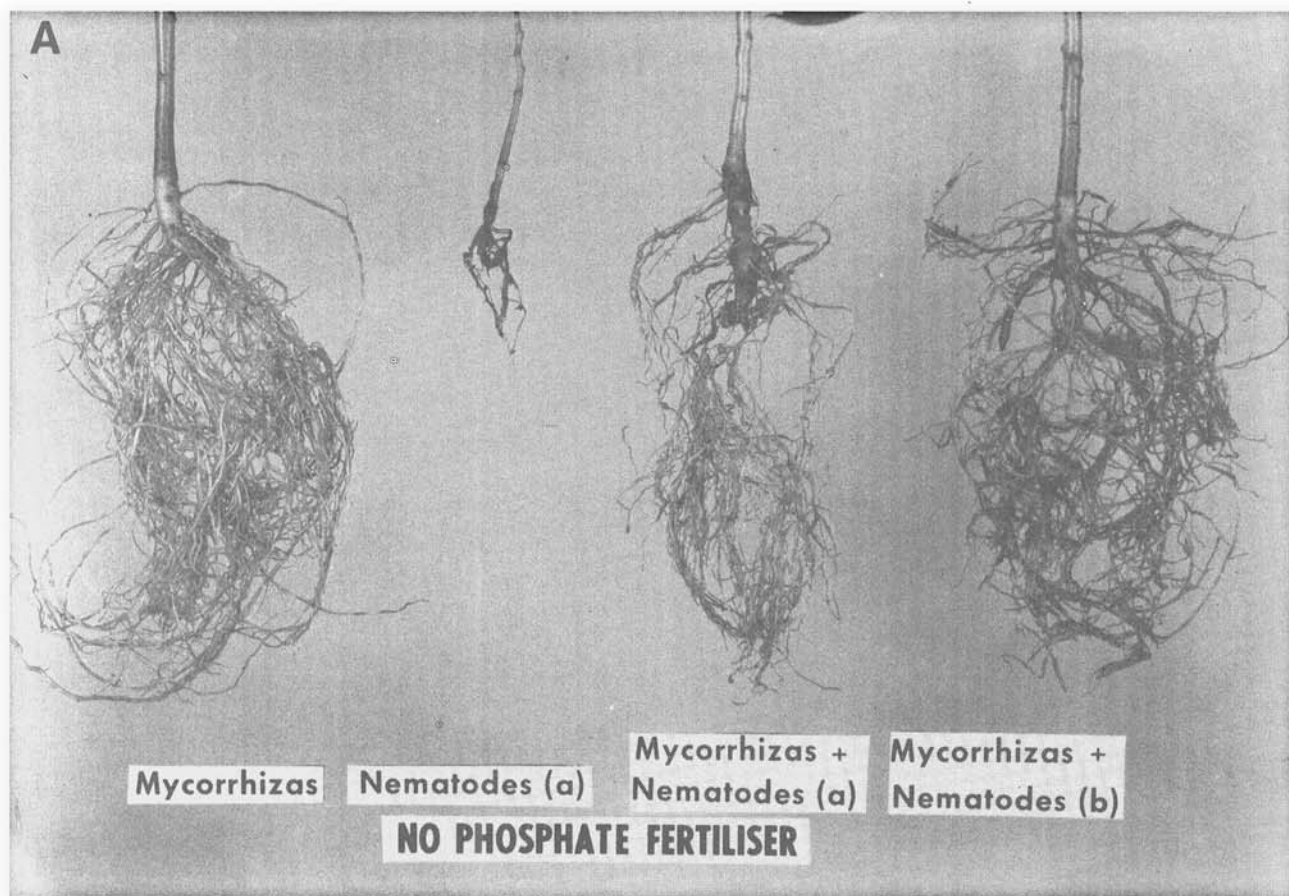
Fig. 2. Root development of tamarillo as influenced by vesicular-arbuscular mycorrhizal fungi, root-knot nematode (*Meloidogyne incognita*), or phosphate fertilizer (mycorrhizal fungi added at transplanting). **(A)** Unfertilized soil; **(B)** soil fertilized with 60 kg P^{-1}. a = Nematodes added at transplanting; b = nematodes added 4 wk after transplanting.
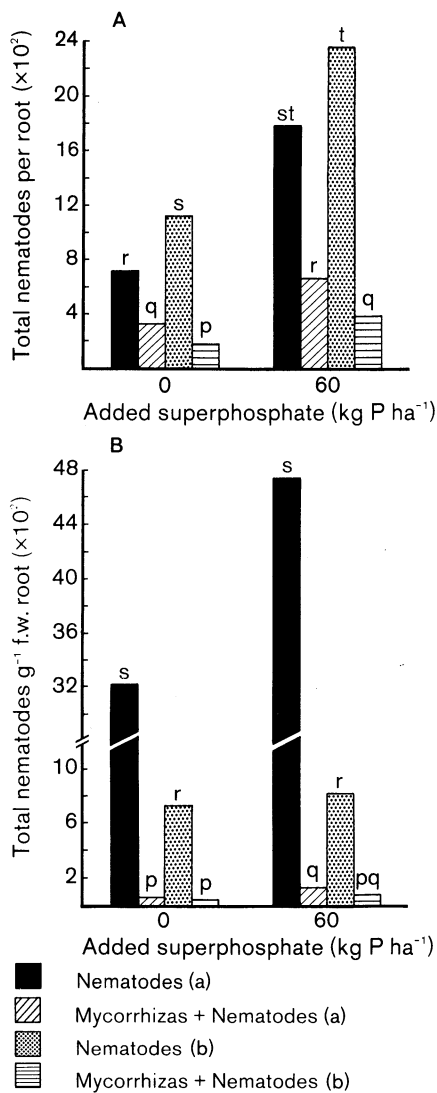
Fig. 3. Nematode infection in roots of tamarillo 12 wk after inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi or with both VAM fungi and root-knot fungi (mycorrhizal fungi added at transplanting). **(A)** Total nematodes (juveniles and adults) per root system; **(B)** total nematodes (juveniles and adults) per gram fresh weight of root. a = Nematodes added at transplanting; b = nematodes added 4 wk after transplanting. Bars showing the same letter do not differ significantly ($P \leqslant 0.05$) by analysis of variance, square-root transformation.
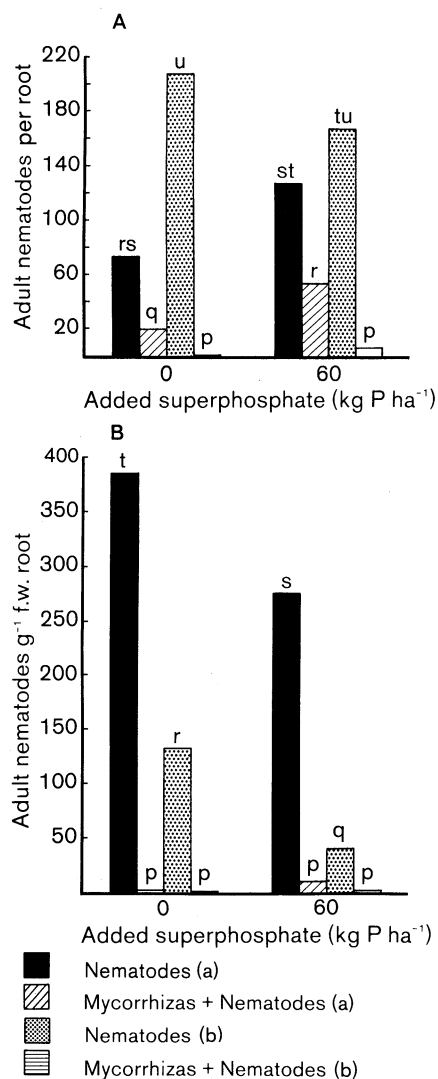
Fig. 4. Adult nematodes in roots of tamarillo 12 wk after inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi or with VAM fungi and root-knot nematode (mycorrhizal fungi added at transplanting). **(A)** Adult nematodes per root system; **(B)** adult nematodes per gram fresh weight of root. a = Nematodes added at transplanting; b = nematodes added 4 wk after transplanting. Bars showing the same letter do not differ significantly ($P \leqslant 0.05$) by analysis of variance, square-root transformation.

unfertilized soil did differ from those in fertilized soil. In work subsequent to that reported here, individual fungi were found to vary in their ability to compensate for plant growth depressions caused by root-knot nematode and to suppress nematode development in roots, and these responses differed between low-phosphate and phosphorus-supplemented soils (K. M. Cooper, *unpublished*). Of seven fungal inocula tested, three (*G. fasciculatum*, *G. mosseae*, and *G. macrocarpum* Tul. & Tul.) exerted the same type of protective influence on tamarillo roots as a mixed fungal inoculum in a low-phosphate soil, whereas only one VAM fungal population, which adapted to phosphorus-fertilized soils, was comparable in phosphorus-supplemented soil. An inoculum containing several VAM fungi appears to afford tamarillo plants maximum protection against root-knot nematodes.

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. Powell and D. J. Bagyaraj, eds. CRC Press, Boca Raton, FL. 234 pp.
2. Bollard, E. G. 1981. Prospects for horticulture: A research viewpoint. N.Z. Dep. Sci. Ind. Res., Discuss. Pap. 6. 212 pp.
3. Cooper, K. M. 1978. Adaptation of mycorrhizal fungi to phosphate fertilizers. Page 107 in: Plant Nutrition 1978. A. R. Ferguson, R. L. Bieleski, and I. B. Ferguson, eds. N.Z. Dep. Sci. Ind. Res. Inf. Ser. 134.
4. Cooper, K. M. 1983. VA mycorrhizal fungi can improve the growth of horticultural crops. Orchardist N.Z. 56:410-415.
5. Cooper, K. M. 1983. Mycorrhizal fungi—their role in plant establishment. Proc. Int. Plant Propag. Soc. 32:396-404.
6. Cooper, K. M., and Grandison, G. S. 1986. Interaction of vesicular-arbuscular mycorrhizal fungi with root-knot nematode on cultivars of tomato and white clover susceptible to *Meloidogyne hapla*. Ann. Appl. Biol. 108:1-11.
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. Dehne, H. W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115-1119.
9. Fletcher, W. A. 1979. Growing Tamarillos. N.Z. Minist. Agric. Fish. Bull. 307. 27 pp.
10. Gerdemann, J. W., and Nicolson, T. H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46:235-244.
11. Giovanetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.
12. 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.
13. Grandison, G. S., and Cooper, K. M. 1986. Interaction of vesicular-arbuscular mycorrhizae and cultivars of alfalfa susceptible and resistant to *Meloidogyne hapla*. J. Nematol. 18:141-149.
14. Hall, I. R. 1984. Taxonomy of VA mycorrhizal fungi. Pages 58-94 in: VA Mycorrhiza. C. L. Powell and D. J. Bagyaraj, eds. CRC Press, Boca Raton, FL. 234 pp.

Table 2. Influence of vesicular-arbuscular mycorrhizal fungi, *Meloidogyne incognita*, or added phosphate fertilizer on shoot and root phosphorus content (%P in dry matter) of tamarillo

Treatment[a]		Added superphosphate (kg P ha^{-1})[b]			
		% P in root dry matter		% P in shoot dry matter	
Myc.	Nem.	0	60	0	60
−	−	0.26 q	0.50 st	0.19 q	0.24 qr
+	−	0.40 s	0.53 t	0.28 r	0.20 qr
Inoc. time a					
−	+	0.12 p	0.37 ps	0.06 p	0.27 r
+	+	0.31 qr	0.52 t	0.20 qr	0.21 qr
Inoc. time b					
−	+	0.16 p	0.49 st	0.08 p	0.26 r
+	+	0.36 rs	0.55 t	0.24 qr	0.19 qr

[a] Mycorrhizal fungi added at transplanting. Inoculation time a = nematodes added at transplanting. Inoculation time b = nematodes added 4 wk after transplanting. Values are means of six replicates.

[b] Within phosphorus contents of root and shoot, values sharing the same letter do not differ significantly ($P \leqslant 0.05$) (analysis of variance, square-root transformation).

15. Hewitt, E. J. 1952. Sand and water culture methods used in the study of plant nutrition. Page 189 in: Commonw. Bur. Hortic. Plantation Crops Tech. Commun. 22. Commonw. Agric. Bur. 239 pp.

16. Hussey, R. S., and Roncadori, R. W. 1978. Interaction of *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton. J. Nematol. 10:16-20.

17. Hussey, R. S., and Roncadori, R. W. 1982. Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth. Plant Dis. 66:9-14.

18. Menge, J. A., and Timmer, L. W. 1982. Procedures for inoculation of plants with vesicular-arbuscular mycorrhizae in the laboratory, greenhouse and field. Pages 59-68 in: Methods and Principles of Mycorrhizal Research. N. C. Schenck, ed. American Phytopathological Society, St. Paul, MN. 244 pp.

19. Murphy, J., and Riley, J. P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-36.

20. 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.

21. Reay, P. F., and Waugh, C. 1981. Mineral-element composition of *Lupinus albus* and *Lupinus augustifolia* in relation to manganese accumulation. Plant Soil 60:435-444.

22. Roncadori, R. W., and Hussey, R. S. 1977. Interaction of the endomycorrhizal fungus *Gigaspora margarita* and root-knot nematode on cotton. Phytopathology 67:1507-1511.

23. Schenck, N. C. 1981. Can mycorrhizae control root disease? Plant Dis. 65:230-234.

24. Schenck, N. C., and Kellam, M. K. 1978. The influence of vesicular-arbuscular mycorrhizae on disease development. Fla. Agric. Exp. Stn. Tech. Bull. 798. 16 pp.

25. Schönbeck, F. 1979. Endomycorrhiza in relation to plant diseases. Pages 271-280 in: Soil-borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, London. 686 pp.

26. Sikora, R. A. 1978. Einfluss der endotrophen Mykorrhiza (*Glomus mosseae*) auf das Wirt-Parasit-Verhältnis von *Meloidogyne incognita* in Tomaten. Z. Pflanzenkrankh. Pflanzenschutz 85:197-202.

27. Sikora, R. A. 1979. Predisposition to *Meloidogyne* infection by the endotrophic mycorrhizal fungus *Glomus mosseae*. Pages 399-404 in: Root-knot Nematodes (*Meloidogyne* species). Systematics, Biology and Control. F. Lamberti and C. E. Taylor, eds. Academic Press, London. 477 pp.

28. Sikora, R. A., and Schönbeck, F. 1975. Effect of vesicular-arbuscular mycorrhiza (*Endogone mosseae*) on the population dynamics of root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne hapla*). VIII Int. Cong. Plant Prot. Moscow 5:158-166.

29. Sitaramaiah, K., and Sikora, R. A. 1982. Effect of the mycorrhizal fungus *Glomus fasciculatus* on the host-parasite relationship of *Rotylenchulus reniformis* in tomato. Nematologica 28:412-419.

30. Southey, J. F. 1970. Laboratory Methods for Work with Plant and Soil Nematodes. 5th ed. Minist. Agric. Fish. Food Tech. Bull. 2. London. 148 pp.

31. Strobel, N. E., Hussey, R. S., and Roncadori, R. W. 1982. Interactions of vesicular-arbuscular mycorrhizal fungi, *Meloidogyne incognita* and soil fertility on peach. Phytopathology 72:690-694.