Interaction Between *Meloidogyne arenaria* and *Glomus fasciculatus* in Grape

R. A. Atilano, J. A. Menge, and S. D. Van Gundy[1]

Abstract: Root zones of grape (*Vitis vinifera* cv Thompson Seedless) cuttings were infested with chlamydospores of *Glomus fasciculatus* or eggs of *Meloidogyne arenaria* or both. Growth of grapevines was greatest in mycorrhizal (*G. fasciculatus*) plants. Mycorrhizal development and growth of mycorrhizal and nonmycorrhizal plants were reduced in the presence of *M. arenaria*. At low initial nematode inoculum (PI) levels (approx. 200 eggs/plant), the presence of mycorrhizae enhanced plant growth during 1 yr, but no significant benefit was achieved by mycorrhizae where PI was high (approx. 2,000 eggs/plant). Final nematode populations were highest in mycorrhizal plants. *Key words: Vitis vinifera,* vesicular-arbuscular mycorrhizae, endomycorrhizal fungus, root-knot nematode.

Research on the role of vesicular-arbuscular mycorrhizae (VAM) in the growth of plants has greatly increased in recent years and the subject has been extensively reviewed (8,9,17). It is generally recognized that VAM enhance nutrient uptake of plants and therefore improve plant growth. Contrasting types of interactions have been reported between phytopathogenic nematodes and VAM. Fox and Spasoff (6) observed that both *Endogone gigantea* and *Heterodera solanacearum* were inhibited when combined in tobacco. Hussey and Roncadori (12) reported that *Pratylenchus brachyurus* reproduction was suppressed in VAM (*Gigaspora margarita*) cotton roots, but that the nematodes did not affect VAM development. Baltruschat et al. (3) found that *Meloidogyne incognita* development was retarded in VAM (*E. mosseae*) tobacco plants. Schenck et al., (23) suggested that *E. macrocarpa* suppressed the population of *M. incognita* in the soybean cultivar, 'Pickett.' Kellam and Schenck (14) demonstrated a reduction in the number of galls produced by *M. incognita* in VAM (*Glomus macrocarpus*) soybeans, and growth of VAM + *M. incognita* plants was greater than

Received for publication 17 December 1979.

[1]Departments of Nematology and Plant Pathology, University of California, Riverside, CA 92521. Current address of senior author: University of Florida, Agricultural Research Center, 3205 S.W. 70th Ave., Ft. Lauderdale, FL 33314. We thank Drs. H. Ferris and J. R. Rich for assistance in initiating these studies.

growth of plants treated with *M. incognita* alone. They also noted that VAM development was not affected by the presence of the root-knot nematode. Roncadori and Hussey (21) found that the detrimental effects on growth of cotton caused by *M. incognita* were nullified by the presence of *G. margarita* even though the nematode population was unaffected. Other studies indicated that several nematode species may retard mycorrhizal development (4,6,20,22), or that the nematodes may benefit from the presence of mycorrhizae (1,23).

Root-knot nematodes are the most important nematode pests of grape in coarse-textured soil (19). Growth responses in grape plants due to VAM have been reported, but the sources of the mycorrhizal fungi and the fungal species involved were not identified (5,18). The only report of VAM interaction with nematodes on grape was by Deal et al. (5) who gave a descriptive account of the association.

The objectives of the present study were to 1) demonstrate a growth response of grape in the presence of a specific mycorrhizal fungus; 2) compare the growth response of mycorrhizal and nonmycorrhizal grapes to root-knot nematodes; 3) compare root-knot nematode reproduction on mycorrhizal and nonmycorrhizal plants; and 4) determine the extent of development of the mycorrhizal fungus in the presence and absence of root-knot nematodes. A portion of this work was reported previously (1).

METHODS AND MATERIALS

One-node grape (*Vitis vinifera* L. cv. 'Thompson Seedless') cuttings were placed in pasteurized sand and rooted under intermittent mist in a glasshouse. Three weeks later the rooted cuttings were transplanted into 7.5-cm lengths of 3.5-cm-d polyvinyl chloride tubes containing pasteurized sandy loam (75% sand, 24% silt, 1% clay) alone, or infested with *Glomus fasciculatus* (Thaxter) Gerd. & Trappe, and placed in a controlled environment chamber (12-h d, 28 C). The soil was infested by thoroughly mixing in a sufficient amount of finely chopped roots, obtained from pot cultures of *G. fasciculatus* on sudan grass (*Sorghum vulgare* L.), to give 1.3 (experiment I) or 5 (experiments II and III) chlamydospores/g soil.

Four (experiments II and III) or seven (experiment I) weeks later, eggs of *Meloidogyne arenaria* (Neal) Chitwood were collected from tomato by a method used by McClure et al. (15), except that the sodium hypochlorite concentration was 0.005%. An aqueous egg suspension was injected into the root zones of half of the plants growing in *G. fasciculatus*-infested soil and in half of the plants growing in noninfested soil. Inoculum levels of 1,000, 200, and 2,000 eggs per plant were used in experiments I, II, and III, respectively. Five days after the addition of eggs, the plants were transplanted into 2-liter pots containing soil of the same type and placed on a glasshouse bench. Plants were fertilized with 250 ml of 1:3 (mineral solution:deionized water) Hoagland's solution at 2-wk intervals. Phosphorus was omitted from the solution during alternate applications. In experiments II and III the shoots were harvested by pruning the plants to the 4th node at 3, 4, 5, 8, and 12 months following infestation with *M. arenaria* eggs. Shoots were dried in a forced air oven at 65 C for 72 h before obtaining dry weights.

Mycorrhizal development was estimated by chlamydospore counts, since spore counts of *G. fasciculatus* have been found to be proportional to root colonization (2,16). Chlamydospores and nematodes were extracted from soil samples composed of two (2.5 cm-d × 15 cm) cores obtained from opposite sides of each plant approx 5 cm from the plant stem. In experiment I the number of chlamydospores obtained from soil samples by wet sieving (7,10) was determined. In experiments II and III the fresh weights of the roots were determined. The roots were then macerated for 1 min in 150 ml tap water with a Hamilton Beach® 14-speed blender set at high speed. The macerate was poured onto a 150-μm-pore sieve stacked on a 45-μm-pore sieve and flushed with water. The residue collected on the 150-μm sieve was blended for 30 s and sieved again. The residues collected on the 45-μm sieve were retained, diluted to a known volume and the chlamydospores counted. The root systems remaining in the pots were washed free of soil, blotted dry,

and weighed; nematodes were then extracted by misting over a 72-h period (24) and counted.

Experiment I was begun in July 1975 and harvested in January 1976. Experiments II and III were begun in May 1976 and terminated in May 1977. Six, seven, and ten replications were used for experiments I, II, and III, respectively. Plants were arranged in a randomized complete block design. A logarithmic transformation was made of all count data before analysis. Unless otherwise indicated, significance is based on $P \leq 0.05$.

RESULTS

Shoot growth at the termination of all experiments was always greatest ($P \leq 0.01$) in plants treated with *G. fasciculatus* alone. In experiment I, mean shoot dry weights were 5.52, 1.52, 0.74, and 0.96 g for plants treated with *G. fasciculatus*, *M. arenaria* + *G. fasciculatus*, *M. arenaria*, and untreated plants, respectively. At the first shoot harvest in experiments II and III (Fig. 1A and B), plants treated with *G. fasciculatus* or *M. arenaria* + *G. fasciculatus* were significantly larger than plants not treated with *G. fasciculatus*. Growth of plants inoculated with low PI (200 eggs/plant, Fig. 1A) was not significantly retarded, compared to control plants, until the last harvest. Similarly, there was no significant difference between plants treated with *M. arenaria* + *G. fasciculatus* and plants treated with only *G. fasciculatus* until after the fourth harvest, but the *M. arenaria* + *G. fasciculatus* plants grew significantly larger than plants treated with only *M. arenaria* throughout the harvest period except at the second harvest.

Shoot growth of plants inoculated only with the high PI (2000 eggs/plant, Fig. 1B) was significantly less than the growth of control plants at the fourth harvest and later. Growth was not reduced until the fifth harvest where plants were treated with both nematode and fungus. Shoot growth of plants treated with *M. arenaria* + *G. fasciculatus* was significantly less than that of plants with *G. fasciculatus* alone at the second harvest, and except for early in the experiment (first and third harvests), plants in the *M. arenaria* + *G. fasciculatus* treatment were not significantly different from plants with *M. arenaria* only.

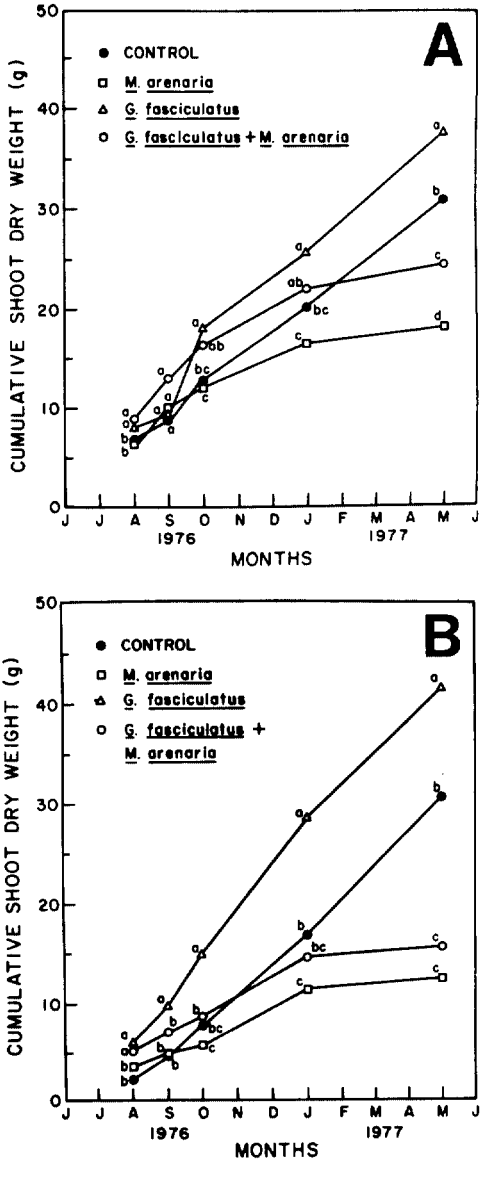

Fig. 1. Cumulative shoot dry weights of mycorrhizal and nonmycorrhizal grapevines infected and noninfected with *Meloidogyne arenaria*. Shoot weights on a given date marked with the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test. (A) Experiment II, initial nematode inoculum (PI) = 200 eggs/plant. (B) Experiment III, PI = 2,000 eggs/plant.

At the termination of experiments II and III, the galled roots of plants infected by *M. arenaria* were greatly decomposed and had very few feeder roots. Root weights of plants treated with *G. fasciculatus* tended to be greater than those of control plants except in the presence of *M. arenaria* (Fig.

2A). Roots of plants treated with *M. arenaria* only were approximately the same weight as roots in the *M. arenaria* + *G. fasciculatus* treatment.

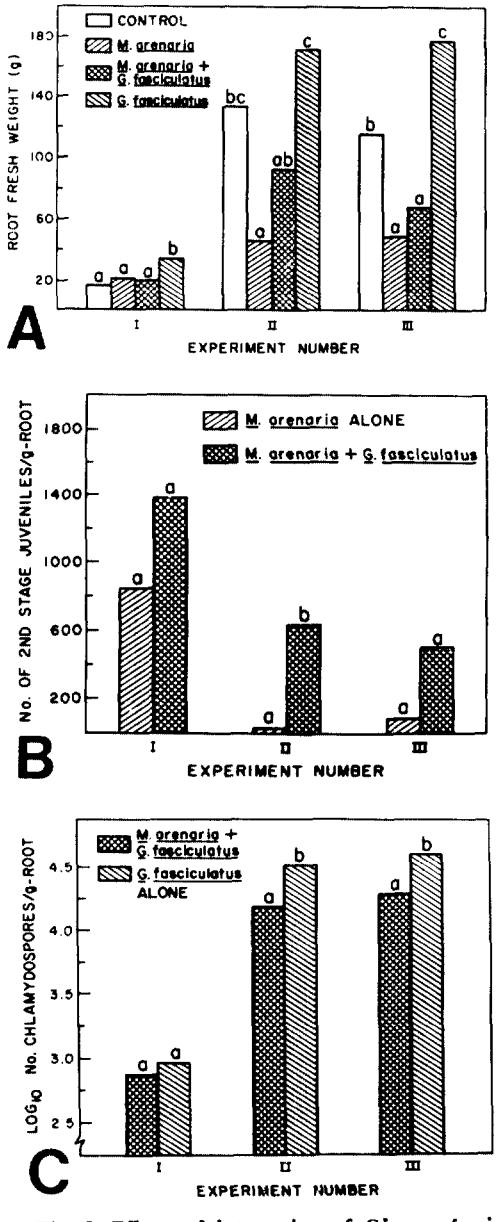

Fig. 2. Effects of interaction of *Glomus fasciculatus* and *Meloidogyne arenaria* in grapevines at termination of experiments I, II, III. Initial nematode inoculum was 1,000, 200, and 2,000 eggs per plant in experiments I, II, and III, respectively. Bars marked with the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test. (A) Root fresh weights. (B) Number of *M. arenaria* second stage juveniles (J2). (C) Number of *Glomus fasciculatus* chlamydospores observed.

The nematode populations were generally low at the end of experiments II and III, probably due to the root decomposition which had occurred in nematode-infected root systems. The number of second stage juvenile (J2) root-knot nematodes collected was consistently higher in the presence of *G. fasciculatus* (Fig. 2B), but significantly higher only where PI was relatively low (experiment II). The number of *G. fasciculatus* chlamydospores present at the end of each experiment was consistently lower in the presence of *M. arenaria* (Fig. 2C), and the reduction in the number of spores was significant in experiments II and III. At the end of experiment I a slightly positive linear relationship ($r = 0.6$) occurred between number of spores and the number of J2 present, but no significant correlation occurred between these two parameters at the end of experiments II and III.

Root systems of all plants not treated with *G. fasciculatus* in experiment I contained *G. fasciculatus* chlamydospores at the end of the experiment. However, the highest counts of the contaminant spores were only one-third the number from plants treated with *G. fasciculatus*. Three replicates in experiment II and five replicates in experiment III showed similar contamination when these experiments were terminated.

DISCUSSION

Root-knot nematodes seem to be the nematodes most apt to affect VAM greatly. The ability of *M. incognita* to suppress mycorrhizal development has been observed by several investigators (4,20,22,23), and the present studies demonstrate a similar effect induced by *M. arenaria*. Schenck and Kinloch (22) encountered several nematode genera in soybean fields, but only *Meloidogyne* spp. appeared to be associated with the inhibition of mycorrhizal fungi.

The degree to which plant parasitic nematodes, especially *Meloidogyne* spp., affect mycorrhizae may depend on the effects that the nematodes have on the plants, rather than effects exerted directly on the fungi. Of four parasitic nematode genera, each represented by one species, studied by Van Gundy and Kirkpatrick (25), *M. javanica* was found to cause the most severe

growth inhibition of tomato plants. After *Meloidogyne* spp. invade the vascular cylinder, the root tissues around developing females usually enlarge to form "knots" or "galls." The transport of water and metabolites through the altered roots is disrupted (13), and this may interfere with the movement of metabolites required by mycorrhizal fungi. The disease syndrome initiated by root-knot nematodes very often includes the invasion of affected root tissue by secondary pathogens which cause decay of root tissues (11), including the cortical tissue colonized by VAM fungi.

Mycorrhizae may condition a variety of factors, as yet unclear, which influence nematode activity in roots. Several investigators (3,6,12,14) have indicated an inhibition of nematode activity in mycorrhizal plants, others (21) have demonstrated no difference between mycorrhizal and nonmycorrhizal plants with respect to nematode reproduction. Results presented here show another type of relationship. The number of *M. arenaria* J2 produced on mycorrhizal plants in three experiments was consistently greater than the number produced on plants treated with only *M. arenaria*. This third relationship may indicate that mycorrhizal plants provide nutrients that are of a greater quality or quantity than those provided by nonmycorrhizal plants. However, this explanation should be accepted with caution until further studies confirm or unify current inconsistencies in the relationship between nematode numbers and mycorrhizal plants.

The nematode inoculum used in these experiments was favored by the restricted nature of the root system in pot culture which greatly favors a continuous high rate of nematode infection of roots and perhaps a higher level of plant growth suppression than in a field situation. In spite of the bias toward nematode infection, the presence of mycorrhizae enhanced growth of grapevines infected with nematodes, at least at relatively low PI levels. A justified speculation is that mycorrhizae probably aid grapes and other plants to offset some of the deleterious effects of plant parasitic nematodes, but long-term experiments are necessary to fully assess the nematode mycorrhizal relationship in field situations.

LITERATURE CITED

1. Atilano, R. A., J. R. Rich, H. Ferris, and J. A. Menge. Effects of Meloidogyne arenaria on endomycorrhizal grape (Vitis vinifera) rootings, J. Nematol. 8:278-279 (Abstr.).

2. Bagyaraj, D. J., and J. A. Menge. 1978. Interaction between a V. A. mycorrhiza and azotobacter and their effects on rhizosphere microflora and plant growth. New Phytol. 80:567-574.

3. Baltruschat, H., R. A. Sikora, and F. Schonbeck. 1973. Effect of VA-mycorrhiza (Endogone mosseae) on the establishment of Thielaviopsis basicola and Meloidogyne incognita in tobacco. 2nd Intern. Congr. Plant Pathol. Abstract No. 0661.

4. Bird, G. W., J. R. Rich, and S. U. Glover. 1974. Increased endomycorrhizae of cotton roots in soil treated with nematicides. Phytopathology 64: 48-51.

5. Deal, D. R., C. W. Boothroyd, and W. F. Mai. 1972. Replanting of vineyards and its relationship to vesicular-arbuscular mycorrhiza. Phytopathology 62: 172-175.

6. Fox, J. A., and L. Spasoff. 1972. Interaction of Heterodera solanacearum and Endogone gigantea on tobacco. J. Nematol. 4:224-225.

7. Gerdemann, J. W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47:619-632.

8. Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-418.

9. Gerdemann, J. W. 1975. Vesicular-arbuscular mycorrhizae. Pp. 575-591 *in* J. G. Torrey and D. T. Clarkson, eds. The organization and structure of roots. Academic Press, New York.

10. Gerdemann, J. W., and T. H. Nicholson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46:235-244.

11. Golden, J. K., and S. D. Van Gundy. 1975. A disease complex of okra and tomato involving the nematode, Meloidogyne incognita, and the soil inhabiting fungus, Rhizoctonia solani. Phytopathology 65:265-273.

12. Hussey, R. S., and R. W. Roncadori. 1977. Interaction of Pratylenchus brachyurus and Gigaspora margarita endomycorrhizal fungus on cotton. J. Nematol. 10:16-20.

13. Jenkins, W. R., and D. P. Taylor. 1967. Plant nematology. Reinhold Publishing Corp., New York.

14. Kellam, M. K., and N. C. Schenck. 1977. The effect of a vesicular-arbuscular mycorrhizal fungus, Glomus macrocarpus, on the amount of galling produced by Meloidogyne incognita on soybean. Proc. Amer. Phytopathol. Soc. 4:124 (Abstr.).

15. McClure, M.A., T. H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean Meloidogyne eggs. J. Nematol. 5:230.

16. Menge, J. A., D. I. Stierle, D. J. Bagyaraj, E. L. V. Johnson, R. Leonard, and M. Ratnayake. 1979. Phosphorus concentrations in plants are responsible for inhibition of mycorrhizal infection. New Phytol. 80:575-578.

17. Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopathol. 11:171-196.

18. Possingham, J. V., and J. G. Obbink. 1971. Endotrophic mycorrhiza and the nutrition of grape vines. Vitis 10:120-130.

19. Raski, D. J., W. H. Hart, and A. N. Kasimatis. 1973. Nematodes and their control in vineyards. Calif. Agric. Exp. Sta. Cir. 533.

20. Rich, J. R., and G. W. Bird. 1974. Association of early-season vesicular-arbuscular mycorrhizae with increased growth and development of cotton. Phytopathology 64:1421-1425.

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

22. Schenck, N. C., and R. A. Kinloch. 1974. Pathogenic fungi, parasitic nematodes, and endomycorrhizal fungi associated with soybean roots in Florida. Plant Dis. Reptr. 58:169-173.

23. Schenck, N. C., R. A. Kinloch, and D. W. Dickson. 1975. Interaction of endomycorrhizal fungi and root-knot nematode on soybean. Pp. 607-617 *in* F. E. Sanders, B. Mosse, and P. B. Tinker, eds. Endomycorrhizas. Academic Press, New York.

24. Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. Minis. Agric. Fish. Food Tech. Bull. No. 2. Her Majesty's Stationary Office, London.

25. Van Gundy, S. D., and J. D. Kirkpatrick. 1975. Nematode-nematode interactions on tomato. J. Nematol. 7:330-331 (Abstr.).