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INTERACTION BETWEEN A VESICULAR ARBUSCULAR MYCORRHIZA AND A ROOT KNOT NEMATODE AND ITS EFFECT ON GROWTH AND CHEMICAL COMPOSITION OF TOMATO

by
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An important aspect of vesicular arbuscular (VA) mycorrhizal research, which is attracting attention, is the possible use of mycorrhizal fungi as biological control agents (Schenck, 1981; Dehne, 1982). Baltruschat *et al.* (1973) indicated that *Endogone mosseae* had an antagonistic effect on *Meloidogyne incognita* in tobacco. In a survey of soybeans in Florida, Schenck and Kinloch (1974) found that spore counts of endomycorrhizal fungi were consistently low when associated with high population densities of root knot nematodes. The sequence in which plants become colonised by mycorrhizal symbionts and infested by nematodes may affect the interaction between these organisms (Hussey and Roncadori, 1982). A study conducted earlier in this laboratory indicated that inoculation with VA mycorrhiza followed by root knot nematodes reduced galling on tomato (Bagyaraj *et al.*, 1979).

This paper reports further observations on the effects of successive application of a VA mycorrhizal fungus, *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. and Trappe and the root knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. on the development of each other and their effects on the growth and chemical composition of tomato plants.

Materials and Methods

The soil used in this experiment was phosphorus deficient (2.4 ppm extracted with NH_4F + HCl) lateritic soil of pH 4.8 and consisting of

22.8% clay, 4.4% silt and 72.8% sand. Four week old healthy tomato (*Lycopersicon esculentum* Mill. cv. Pusa Ruby) seedlings with no mycorrhizal colonization were transplanted, one seedling per pot, into 3 kg pots containing sterilised soil. Inocula as indicated below were then applied, each treatment being replicated four times:

Uninoculated (U),

Inoculated with mycorrhiza only (M),

Inoculated with mycorrhiza and 7 days later with nematode larvae (M + N),

Inoculated with a combination of mycorrhiza and nematode larvae (MN),

Inoculated with nematode larvae and 7 days later with mycorrhiza (N + M),

Inoculated with nematode larvae only (N).

The mycorrhizal fungus, *G. fasciculatum* used to inoculate tomato seedlings was cultured on sudan grass *Sorghum bicolor* (L.) Moench var. Sudanense. Pots were inoculated with mycorrhiza by adding 20 ml of the inoculum into holes (2 cm deep) around the roots of the seedlings. Inoculum consisted of chlamydospores (300 spores/20 ml), hyphae and infected roots.

Stock cultures of the nematode, *M. incognita*, were maintained on tomato. Nematode larvae hatching from hand picked egg masses were used for inoculation. Pots were inoculated with nematodes by pouring a 100 ml suspension of 300 larvae into holes (2 cm deep) around the roots of the seedlings.

The plants were grown for 90 days in a glasshouse with a temperature range of 25-30° C and the height of stem and length of roots were recorded. Dry weights of roots and shoots were determined. Percentage fungal infection of the roots was determined after clearing the roots and staining with trypan blue (Nicolson, 1960). Number of *G. fasciculatum* spores in soil were counted by the wet sieving and decantation technique (Gerdemann and Nicolson, 1963). Nematode larvae were also extracted by wet sieving and decantation from 25 ml soil samples and counted. The number of galls on the roots were counted.

The dry plant samples were ground and used for estimating various chemical constituents. The total phosphorus was estimated by vanadomolybdate phosphoric yellow method, total nitrogen by micro-kjeldahl method and potassium by flame photometry (Jackson, 1973). Zinc and manganese were estimated using an atomic absorption

spectrophotometer (Hitachi-model 508) following the procedure of Issac and Kerber (1971). Amino acids in plants from treatments U, M, N and M + N were determined with an automatic amino acid analyser (Hitachi-model KLA 3B) by the method outlined by Spackman *et al.* (1958), reducing and total sugars were estimated colorimetrically by the method described by Nelson (1944) and total phenols by the method of Bray and Thorpe (1954).

Results

Mycorrhizal plants without nematodes produced significantly more shoot dry weight than the other treatments (Table I). Although root length was greater in mycorrhizal plants, root dry weights did not differ significantly. The mycorrhizal spore counts were not significantly different (Table I). The percentage mycorrhizal infection of root was highest in plants inoculated with mycorrhiza first and 7 days later with nematodes, followed by mycorrhiza only. Plants inoculated

Table I - *Growth characteristics and percentage mycorrhizal infection of tomato plants, and spore counts in soil as influenced by inoculation with mycorrhiza and root knot nematodes.*

Treatment	Root		Shoot		Mycorrhizal	
	Dry Wt (g)	Length (cm)	Dry Wt (g)	Height (cm)	Infection %	Spore count/ 25 ml soil
U	2.4	18.5	8.4	111.0	0	0
M	2.8	22.0	10.4	130.0	95	131
M+N	3.1	11.3	6.1	106.8	98	258
MN	1.8	15.0	5.5	125.0	78	177
N+M	2.4	14.0	5.7	95.0	84	207
N	3.0	14.5	5.4	86.5	0	0
L.S.D. at P = 0.05	NS	2.3	1.8	23.1	ND	NS

U = Uninoculated; M = Inoculated with mycorrhiza only immediately after transplanting; M+N = Inoculated with mycorrhiza immediately after transplanting and nematodes seven days later; MN = Inoculated with nematodes and mycorrhiza simultaneously immediately after transplanting; N+M = Inoculated with nematodes immediately after transplanting and mycorrhiza seven days later; N = Inoculated with nematodes only immediately after transplanting; (Also see text) ND = Not determined; NS = Not significant.

with a combination of mycorrhiza plus nematode larvae simultaneously had the least mycorrhizal infection of the root.

Plants inoculated with mycorrhiza first and 7 days later with nematode larvae had significantly lower number of larvae in the soil (Table II). Plants inoculated with nematodes only had the highest larval count. Although the differences in gall counts were not significant, plants inoculated with mycorrhiza first and later with nematodes had the least number of galls and their size was also small compared with the other inoculations with nematodes.

The amounts of nitrogen, phosphorus, potassium, zinc, manganese and calcium per plant are shown in Table III. There was a significant reduction in plant N content because of inoculation with *M. incognita*

Table II - *Nematode larvae in soil and root galls on tomato plants as influenced by inoculation with mycorrhiza and root knot nematodes.*

Treatment	Nematodes/ml soil	Galls/plant
U	0	0
M	0	0
M+N	11	263
MN	38	277
N+M	14	427
N	50	524
L.S.D. at P = 0.05	2.1	NS

Table III - *N, P, K, Zn, Mn and Ca contents of tomato plants as influenced by inoculation with mycorrhiza and root knot nematodes*

Treatment	N mg/plant	P mg/plant	K mg/plant	Zn µg/plant	Mn µg/plant	Ca µg/plant
U	159.9	40.0	2.3	82	58	31
M	173.0	56.5	3.4	92	56	64
M+N	136.7	46.1	1.7	68	53	16
MN	104.1	27.8	1.5	49	44	57
N+M	111.6	34.8	1.7	57	47	21
N	93.9	22.2	1.3	54	44	41
L.S.D. at P = 0.05	39.9	14.7	0.6	28	5	32

but not *G. fasciculatum* (Table III). Plants inoculated with mycorrhiza only had significantly more P and plants inoculated with nematodes only had significantly less P compared with the uninoculated plants. Plants with mycorrhiza only had significantly higher K than any of the other treatments. All treatments with nematodes had very low K while mycorrhiza caused an increase in the K content. The same was true with Ca; plants with mycorrhiza only had significantly more Ca than control plants. The highest Zn content was found in plants inoculated with mycorrhiza only. However mycorrhiza had no significant influence on the Mn content but plants with nematodes usually contained significantly less Mn.

A significant reduction in the total phenol content was associated with nematode inoculation (Table IV). Compared to uninoculated control plants, those inoculated with mycorrhiza or with mycorrhiza and nematodes simultaneously showed increases in the reducing sugar content. Lower amounts of reducing sugar were recorded from plants with nematodes only. Total sugars were highest in plants inoculated with mycorrhiza only and least in plants inoculated with nematodes first followed by mycorrhiza.

Plants inoculated with mycorrhiza contained larger amounts of amino acids specifically phenylalanine, isoleucine, threonine and serine than did uninoculated plants (Table V). Plants inoculated with mycorrhiza plus nematode larvae generally showed an increase in amino acids, especially glutamic and aspartic acids compared with

Table IV - *Phenol and sugar contents of tomato plants as influenced by inoculation with mycorrhiza and root knot nematodes.*

Treatment	Total phenols (µg/mg)	Total sugars (µg/mg)	Reducing sugars (µg/mg)
U	3.08	65.40	7.20
M	3.03	94.65	8.68
M+N	1.92	48.80	4.34
MN	1.12	73.20	10.89
N+M	1.78	28.35	4.98
N	1.84	47.50	4.68
L.S.D. at P = 00.5	0.47	4.62	1.02

Table V - *Amino acids in tomato plants (μg/mg samples) as influenced by inoculation with mycorrhiza and root knot nematodes.*

Treatment / Amino acids	U	M	Difference over U %	N	Difference over U %	M+N	Difference over U %
Alanine	2.29	3.73	63	3.52	54	5.25	129
Arginine	2.26	3.56	39	1.57	39	1.75	−32
Aspartic acid	6.18	9.95	61	6.03	−3	8.56	39
Cystic acid	Trace	Trace		Trace		Trace	
Cystine	Trace	Trace		Trace		Trace	
Glutamic acid	6.30	8.07	28	5.78	−8	12.08	92
Glycine	2.06	3.73	82	3.59	71	5.25	156
Histidine	0.78	1.45	85	0.66	−16	0.76	−4
Isoleucine	1.16	2.38	106	2.32	100	3.07	164
Leucine	1.16	Trace	−100	4.23	49	5.27	85
Lysine	3.26	4.76	46	3.41	5	3.66	12
Phenylalanine	0.50	1.47	197	2.00	304	2.62	428
Proline	3.97	6.41	62	3.07	−23	3.76	−5
Serine	2.49	4.33	74	3.46	39	4.33	74
Threonine	1.37	2.82	105	2.85	109	3.36	145
Tyrosine	Trace	Trace		1.33	133	1.85	185
Valine	2.38	3.51	48	4.91	106	4.35	83

plants inoculated with nematodes only. Plants with nematodes only had more tyrosine and phenylalanine and less proline, aspartic acid and histidine than did uninoculated control plants.

Discussion

Tomato plants inoculated with nematodes only were stunted in growth, with low shoot dry weight, while plants inoculated with mycorrhiza only showed the opposite trend. Though not statistically significant, addition of mycorrhiza prior to nematode inoculation increased shoot dry weight by 13% compared with plants treated with nematodes only. The mycorrhizal spore production in the presence of

nematodes was very high. Enhanced production of mycorrhizal spores in presence of nematodes has also been observed earlier by Roncadori and Hussey (1977). Schenck *et al.* (1975) using different levels of nematode population found that at lower levels *M. incognita* stimulated spore production by the mycorrhizal fungus, *Endogone herogama* in soybean. At very high nematode levels the spore production decreased. The present study indicates that nematodes are checked if mycorrhiza are present before they are able to infect the plants. This supports a similar observation made by Hussey and Roncadori (1982) in peaches.

Reduction in the severity of plant disease in mycorrhizal plants may be due to improved plant nutrition, especially phosphorus (Hussey and Roncadori, 1982) or altered biochemical constituents in the host plant (Sikora and Schonbeck, 1975). In the present study significantly more P, K and Ca were recorded in plants inoculated with mycorrhiza only. This accords with the findings of earlier workers who attributed plant improvement to an increased area of surface absorption in mycorrhizal roots (Hayman, 1980). It has been found that increased phosphorus made available to nematode infested plants either through supplemental P or VA mycorrhiza offset the nematode symptoms (Hussey and Roncadori, 1982).

No significant difference between the total phenol content of non-mycorrhizal and mycorrhizal plants was noted. Horsfall and Diamond (1957) reported that disease incidence is greater whenever the level of sugars in host plants is low which agrees with our findings since largest quantity was recorded in mycorrhizal plants. It was also observed by Batemann and Miller (1966) that resistant plants had higher sugar content. It therefore appears that high sugar content in mycorrhizal plants somehow affects the resistance of plants to nematodes. Mycorrhizal plants also had higher amino acid content compared to control confirming the observations of Nemec and Meredith (1981). Further, the mycorrhizal plants had higher concentrations of phenylalanine and serine which are known to reduce the growth and reproduction of the root knot nematodes (Krishnaprasad, 1971; Parvatha Reddy, 1974). Thus the presence of increased quantities of sugars, amino acids like phenylalanine and serine, and phosphorus may each or collectively play a role in suppressing the development of *M. incognita* in mycorrhizal tomato plants.

SUMMARY

Interaction between the vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum* and the root knot nematode *Meloidogyne incognita* was studied in tomato. Mycorrhizal inoculation was found to reduce the root knot infestation. Application of mycorrhiza first followed by nematodes reduced nematode infestation better than the simultaneous application of mycorrhiza plus nematodes or nematodes first followed by mycorrhiza. Mycorrhizal plants had increased quantities of phosphorus, potassium, calcium, total and reducing sugars, and amino acids phenylalanine and serine. The possible role of some of these chemical constituents in suppressing the development of root knot nematode is discussed.

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