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Interaction between *Meloidogyne incognita* and *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici* on Tomato

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Abstract: *Agrobacterium tumefaciens* stimulated and *Fusarium oxysporum* f. sp. *lycopersici* inhibited development and reproduction of *Meloidogyne incognita* when applied to the opposite split root of tomato, *Lycopersicon esculentum* cv. Tropic, plants. The lowest rate of nematode reproduction occurred after 2,000 juveniles were applied and the fungus was present in the opposite split root. The effects of all three pathogens alone on the growth of roots and shoots of tomato plants were evident, but *M. incognita* had a greater effect alone than did either of the other pathogens. The length of split roots was reduced by the infection of *M. incognita* and *A. tumefaciens* or *F. oxysporum* f. sp. *lycopersici*. The number of galls induced by nematodes on roots was higher where the bacterium was applied and lower where the fungus was applied to the opposite split root.

Key words: *Agrobacterium tumefaciens, Fusarium oxysporum* f. sp. *lycopersici*, interaction, *Lycopersicon esculentum, Meloidogyne incognita*, split-root technique, tomato.

Interactions between phytopathogenic bacteria or fungi and species of *Meloidogyne* coinhabiting a particular host plant are not uncommon (2,3,6–10,12,13). *Pseudomonas solanacearum* in soil infested with *M. incognita* did not affect root galling of tomato, but it did cause a profound decrease in plant height and shoot and root fresh and dry weights (10). Similarly, a dramatic reduction in length and weight of roots and shoots occurred when sunflower plants were grown in soil infested with both *Agrobacterium tumefaciens* and *M. incognita* (2). When *Trichoderma* sp., *Fusarium* sp., and *Rhizoctonia solani* Kuhn and 6,000 *M. incognita* juveniles coinhabited roots of tomato plants, foliage and root weights were reduced 75 and 48%, respectively (7). Similarly, *M. incognita* and *Pythium aphanidermatum* on chrysanthemum plants reduced plant growth more than either pathogen alone (6).

In a recent survey of fields with stunted cotton plants infected with *M. incognita* in the Hula Valley, Israel, 60% of the plants had crown gall caused by *Agrobacterium radiobacter* var. *tumefaciens* (14).

Despite widespread occurrence of *Meloidogyne* spp., *Fusarium* spp., and *Agrobacterium* spp. in cultivated fields in the Middle East, little attention has been given to the potential for enhanced injury to tomato plants from interactions between these organisms. Therefore, this study was undertaken to determine 1) the population density changes of *Meloidogyne incognita* in infected tomato roots of one-half of a split-root system with *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici* in the opposite half of the system and 2) plant growth response of tomato to infection by the three organisms in the split-root arrangement under greenhouse conditions.

MATERIALS AND METHODS

Eighty-four black polythene bags, 15 cm d with four 1-cm-d pores in each side, were filled with steam-sterilized sandy clay loam soil (50% sand, 16% silt, 34% clay), 800 g per bag. Tomato, *Lycopersicon esculentum* Mill. cv. Tropic, seeds were germinated in vermiculite and transplanted when 2 weeks old. The root systems of the seedlings were split longitudinally and halves were planted in separate bags that had been stapled together.

Agrobacterium tumefaciens (Smith & Townsend) Conn. or *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen and *M. incognita* were added to the soil 1 week after tomato seedlings were transplanted. The *A. tumefaciens* inoculum was prepared from a sterilized suspension of a single colony where it was placed in a number of

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Erlenmyer flasks containing nutrient glycerol medium and was shaken at 50 rpm for 24 hours at 25 C. The contents of the flasks were centrifuged at 6,000 rpm. The supernatant was discarded and the pellets were suspended in sterilized water. A concentration of 1 × 10^6 CFU/g soil was prepared and used as inoculum.

Potato dextrose medium was prepared in Erlenmyer flasks, seeded with *Fusarium oxysporum* f. sp. *lycopersici*, and incubated at 25 C for 15 days. The fungal mat from each flask was collected and blended in 500 ml distilled water for 15 seconds to obtain a concentrated suspension which was used as inoculum.

Treatments were 1) no organisms (C) in either half-root system; 2) *M. incognita* (N) juveniles (J2) on one half-root system and no organisms in the other half-root system; 3) the fungus (F) in one half-root system and no organism in the other half-root system; 4) the bacterium (B) in one half-root system and no organism in the other half-root system; 5) *M. incognita* in one half-root system and the fungus in the other half-root system; and 6) the nematode in one half-root system and the bacterium in the other half-root system.

Nematodes were applied at two levels, 1,000 (N1) and 2,000 (N2) newly hatched J2/half-root system. *Agrobacterium tumefaciens* was applied at the rate of 10^6 colony forming unit/g soil, and 10 ml *F. oxysporum* f. sp. *lycopersici* suspension was added per half-root system. All treatments were replicated four times, except the fungus treatments which were replicated three times. Juveniles of *M. incognita* were extracted from galled roots with a water mist system. The organisms collected during the first 24 hours were discarded. Juveniles collected in the next 24 hours were used as inoculum.

Bags containing the various treatments were arranged in a randomized complete block design in a greenhouse maintained at 25 ± 2 C. Plants were watered daily, and Hoagland's solution was applied biweekly. After 45 days, plant height and weight and root length and weight were recorded. Each half-root system was stained in 0.01% acid fuchsin in lactophenol (4), and nematode stage and egg-mass and gall numbers were recorded. Soil nematode population densities in each bag were determined with an Oostenbrink apparatus (5).

Data were analyzed by analysis of variance (11), and differences between means were determined using least significant difference (LSD).

TABLE 1. Means of root weight and length and shoot weight and height of Tropic tomato plants with split-root systems inoculated with *Meloidogyne incognita* and *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici*.

Treatment†	Root weight (g)	Root length (cm)	Shoot weight (g)	Shoot height (cm)
C1	1.2	13.8	2.3	15.8
C2	1.2	13.8		
C	0.6	12.0	1.8	11.0
N1	1.1	7.0		
C	0.4	9.0	1.5	9.7
N2	0.7	6.0		
C	0.6	11.2	2.2	12.0
B	1.0	11.0		
N1	0.9	6.0	1.4	9.0
B	0.9	10.9		
N2	0.5	5.0	1.1	7.2
B	0.5	9.0		
LSD (0.05)	0.48	3.50	0.78	2.46
C1	2.4	18.6	3.3	18.0
C2	2.7	18.3		
C	0.9	11.6	3.2	13.3
N1	2.1	10.3		
C	0.9	10.0	1.8	12.3
N2	1.4	8.0		
C	2.6	11.7	3.2	12.3
F	2.2	11.3		
N1	1.6	11.6	2.9	13.8
F	0.7	13.3		
N2	0.8	7.3	1.2	10.2
LSD (0.05)	1.03	3.08	NS	2.52

Numbers are means of four replications in the test with the bacterium three replications in the test with the fungus.

† C = control (no organisms); N1 = 1,000 *M. incognita* J2/half-root system; N2 = 2,000 J2; B = 10^6 colony forming units of *A. tumefaciens*/g soil; F = 10 ml *F. oxysporum* f. sp. *lycopersici* suspension.

RESULTS

The effects of bacterium or fungus and nematodes on the growth of roots and shoots of tomato plants were obvious. Root weights were lower ($P = 0.05$) when plants were inoculated with 2,000 J2 than with

TABLE 2. *Meloidogyne incognita* population density increases on split-root systems of Tropic tomato plants with *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici* on the opposite half-root system.

		M. incognita (N)			
Treatment†	Galls (N)	In root	In soil	Total (Pf)	Rate of increase (Pf/Pi)
N1 and C	195	2,077	210	2,287	2.29
N1 and B	390	2,456	400	2,856	2.86
N2 and C	275	3,845	760	4,605	3.30
N2 and B	404	4,155	850	5,005	2.50
LSD (0.05)	19.9	14.6	177.1	11.2	
N1 and C	190	2,066	240	2,306	2.31
N1 and F	63	1,850	207	2,057	2.06
N2 and C	273	3,840	770	4,610	2.31
N2 and F	100	1,846	850	2,655	1.31
LSD (0.05)	6.6	19.5	12.8	26.4	

Numbers are means of four replications in the test with the bacterium, three replications in the test with the fungus.
† C = control (no organisms); N1 = 1,000 *M. incognita* J2/half-root system; N2 = 2,000 J2; B = 10^6 bacterial colony forming units of *A. tumefaciens*/g soil; F = 10 ml *F. oxysporum* f. sp. *lycopersici* suspension.

1,000 J2. At either level alone, *M. incognita* affected root weight more than did the bacterium or the fungus. The combination of nematodes and either bacterium or fungus had the largest reduction in root weights (Table 1).

Root length also was reduced ($P = 0.05$) by inoculation with either level of *M. incognita* and *A. tumefaciens* or *F. oxysporum* f. sp. *lycopersici* (Table 1). The nematodes appeared to inhibit the growth of split-root systems. Plants with *A. tumefaciens* or *F. oxysporum* f. sp. *lycopersici* in one half-root system and *M. incognita* in the opposite half-root system had the shortest root systems (Table 1).

TABLE 3. Development and reproduction of three stages of *Meloidogyne incognita* on split-root systems of Tropic tomato plants with *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici* on the opposite half-root system.

Treatment†	Young stages (N)	Females (N)	Egg masses (N)	Rate of maturity‡	Rate of reproduction§
N1 and C	1,998	79	33	0.09	0.59
N1 and B	2,380	76	42	0.07	0.61
N2 and C	3,760	85	52	0.06	0.62
N2 and B	4,100	55	36	0.03	0.62
LSD (0.05)	16.8	9.0	7.6		
N1 and C	2,011	55	25	0.06	0.60
N1 and F	1,740	110	27	0.13	0.50
N2 and C	3,780	60	30	0.04	0.60
N2 and F	1,716	130	26	0.15	0.54
LSD (0.05)	47.5	11.5	5.6		

Numbers are means of four replications in the test with the bacterium, three replications in the test with the fungus.
† C = control (no organisms); N1 = 1,000 *M. incognita* J2/half-root system; N2 = 2,000 J2; B = 10^6 colony forming units of *A. tumefaciens*/g soil; F = 10 ml of *F. oxysporum* f. sp. *lycopersici* suspension/half-root system.
‡ Rate of maturity = (females + [egg masses + females]/total [A + B + C + D + females + egg masses]) in soil and root.
§ Rate of reproduction = (females + egg masses)/(females + [females + egg masses]).

Both halves of the control root system were similar in weight and length. Moreover, the weight of the control root system was less than the weight of its opposite half-root system inoculated with 1,000 or 2,000 J2 of *M. incognita*. These results may be attributed to the nematode galls (Table 2).

The soil infested with the bacterium stimulated the formation of root galls on the opposite half-root system where nematodes had been added (Table 2), whereas the opposite occurred with the fungus. Similar results were obtained for length of root system (Table 1). *Meloidogyne incognita* affected root length more than *A. tumefaciens* or *F. oxysporum* f. sp. *lycopersici*.

The population level and rate of buildup of *M. incognita* on one half-root system was highest ($P = 0.05$) when *A. tumefaciens* was present on the other half-root system (Table 2). This resulted in a slight increase in the rate of *M. incognita* reproduction in the presence of the bacterium (Table 3), but differences were not significant.

On the other hand, the fungus on half

of the root system resulted in the lowest population density and rates of increase of *M. incognita* on the opposite half-root system. The lowest rate of nematode population increase was obtained at the 2,000 J2 inoculum level, a rate of 1.31 compared with 2.30 in the absence of fungus (Table 3). The rate of maturity of *M. incognita* was changed only slightly by the presence of either bacterium or fungus (Table 3).

Discussion

Apparently the effect of soil-borne plant pathogen infection is not always confined to the locality of infection but may be systemically translocated to other parts of the host. In this study, tissues remote from the site of nematode infection were rendered more susceptible to the fungal or bacterial infection. The same may be true for other pathogens tested, because the presence of *A. tumefaciens* appeared to enhance the rate of *M. incognita* population increase, its final population level, and the number of nematode galls, whereas these same characteristics were inhibited by the presence of the fungus.

The present findings also support previous work (1) in that when one part of the root system of a wilt-resistant variety of tomato is exposed to *F. oxysporum* f. sp. *lycopersici* and the other part to *M. incognita,* the plant becomes diseased.

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