Failure of Root-knot Nematode to Affect Fusarium Wilt Resistance of Tomato

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ABSTRACT

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Manapal (resistant to race 1 and susceptible to race 2 of *Fusarium oxysporum* f. sp. *lycopersici*) and Florida MH-1 (resistant to both races) tomato seedlings were either (i) root-dip-inoculated with either race 1 or 2 and transplanted into soil infested with root-knot nematode larvae (*Meloidogyne incognita*), or (ii) inoculated first with root-knot nematodes and 2 weeks later with either race 1 or 2. All nematode-inoculated plants were moderately galled. Manapal seedlings inoculated with race 1 and nematodes did not succumb to Fusarium wilt, but developed severe wilt when inoculated with race 2 with or without nematodes. Florida MH-1 seedlings did not develop Fusarium wilt whether inoculated with race 1 or 2 either with or without nematodes. Therefore, it was concluded that root-knot nematodes, whether applied simultaneously with the Fusarium inoculum or 2 weeks prior to the Fusarium inoculum, did not reduce the resistance of Manapal to race 1, nor the resistance of Florida MH-1 to race 1 or 2.

Additional key words: Fusarium oxysporum f. sp. *lycopersici, Lycopersicon esculentum, Meloidogyne incognita.*

Jenkins and Coursen (10) reported that monogenic resistance (*I* gene) of tomato (*Lycopersicon esculentum* Mill.) to Fusarium wilt, incited by *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyd. & Hans., was rendered ineffective by inoculation with root-knot nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood and *M. hapla* Chitwood] (4). Several researchers (3, 5, 14) reported similar results with other *I* gene cultivars and Goode and McGuire (8) with an I_2 gene breeding line which was resistant to races 1 and 2.

In contrast, other reports (2, 9, 12) indicate that monogenic resistance to Fusarium wilt was not affected by the root-knot nematode, and McClellan and Christie (13) reported that the susceptibility of Marglobe (a polygenic tolerant cultivar) was not increased by root-knot nematodes. Furthermore, there are no reports of wilt of *I* or I_2 gene cultivars grown in fields infested with races of the Fusarium wilt pathogen and root-knot nematodes.

Because of these inconsistencies in the literature and the continuing effectiveness of monogenic resistant cultivars for field control of Fusarium wilt in the presence of high populations of root-knot nematodes, an experiment was carried out in temperature- and light-controlled growth rooms to determine if root-knot nematode infection would affect the resistance to Fusarium wilt in cultivars containing the *I* or I_2 gene for Fusarium wilt resistance.

MATERIALS AND METHODS

A steam-pasteurized mix of peat, virgin Myakka fine sand, and vermiculite (2:1:1, v/v) amended with 6-6-6 fertilizer (82% ammonia-nitrogen), superphosphate, fritted micronutrients (FTE 503), and calcitic limestone (enough to obtain a pH of 5.5) was used. Each experimental unit was a 22.2 × 16.8 × 5.7-cm plastic tray which contained 12 plants. Each treatment was comprised of four replicates which were maintained at a constant 28 C and a 12-hour daily photoperiod (10,760 lux) provided by cool-white fluorescent tubes.

Two standard cultivars were used: Manapal with the *I* gene and resistance to *F. oxysporum lycopersici* race 1, but susceptibility to race 2, and Florida MH-1 with the I_2 gene and resistance to both races. Half of the 3-week-old seedlings of each cultivar were inoculated simultaneously with root-knot nematodes (46 or 23 larvae per plant) and race 1 or 2 of *Fusarium* (via root-dip inoculation) and half were inoculated first with nematodes, then 2 weeks later with *Fusarium*. The treatments for each cultivar were: (i) nematodes (46/plant) + race 1 (simultaneously or 2 weeks after inoculation with nematodes); (ii) nematodes (23/plant) + race 1 (simultaneously or 2 weeks later); (iii) nematodes (46/plant) + race 2 (simultaneously or 2 weeks later); (iv) nematodes (23/plant) + race 2 (simultaneously or 2 weeks later); (v) race 1 alone, no nematodes (one set was root dip-inoculated at the time. that plants of treatments i through iv were simultaneously inoculated with *F. oxysporum* f. sp. *lycopersici* and nematodes and a second set was inoculated 2 weeks later by pouring the *Fusarium* inoculum onto cut roots); (vi) race 2 alone, no nematodes (inoculation procedure same as treatment v); (vii) nematodes (46/plant) alone; (viii) nematodes (23/plant) alone; (ix) supernatant from centrifuged nematode suspension; and (x) noninoculated.

The fungus inoculum was produced on PDA plates maintained at 28 C and 1507 lux continuous illumination for 2 weeks. The spores and mycelium then were

suspended in deionized water and roots of the seedlings were inoculated by dipping them into the inoculum. The inoculum concentrations for race 1 and race 2 were 14.3×10^6 spores/ml and 15.7×10^6 spores/ml, respectively, plus noncounted mycelial fragments. The *Fusarium* inoculum used to inoculate seedlings 2 weeks after inoculation with nematodes was prepared similarly, but contained 27.6×10^6 spores of race 1 or 46.3×10^6 spores of race 2.

The root-knot nematode larvae used for inoculation were separated from galled tomato roots with a Baermann funnel (1).

All plants were examined for wilt symptoms daily. When the experiment was terminated all surviving plants were examined externally and internally for wilt symptoms. Plants then were cut at the soil line and the fresh weights determined, and roots were washed and examined for root-knot galling. No attempt was made to determine infection by culturing from inoculated plants because it is well known that parasitic and saprophytic *Fusarium* often colonize tissues of resistant plants but do not necessarily incite disease (6, 7, 11).

Twenty-four seedlings of Bonny Best were root dip-inoculated with each race 1 inoculum to evaluate the virulence of the cultures. All developed wilt symptoms and more than 98% died.

RESULTS AND DISCUSSION

Inoculation of Manapal seedlings simultaneously with root-knot nematodes and race 1 did not affect the resistance conferred by the *I* gene (Table 1). No plants exhibited internal or external symptoms 5 weeks after inoculation with race 1 + nematodes or with race 1 alone. In contrast, every seedling inoculated with race 2 developed symptoms and up to 98% died (Table 1). Root-knot nematodes did not affect disease development incited by race 2.

Simultaneous inoculation of Florida MH-1 seedlings with race 1 or 2 and root-knot nematodes did not affect the resistance of this cultivar. All plants remained disease-free up to 5 weeks after inoculation.

Inoculation of Manapal and Florida MH-1 seedlings with root-knot nematodes 2 weeks prior to inoculation with race 1 or 2 should have permitted the nematodes to invade the roots and alter the disease reaction of the seedlings. However, prior inoculation with nematodes did not affect the resistance of Manapal to race 1 or Florida MH-1 to race 1 or 2.

Only 2 and 0%, respectively, of the low nematode (23/plant) + race 1 and high nematode (46/plant) + race 1-inoculated Manapal seedlings developed wilt symptoms (Table 1). In contrast, 100% of the race 2-inoculated Manapal seedlings developed wilt and 77% died (Table 1). Nematode inoculation did not increase the occurrence or severity of race 2-incited wilt.

Inoculation of Florida MH-1 seedlings, first with root-knot nematodes then 2 weeks later with race 1 or 2, did not decrease the resistance of the cultivar. Only 2% of the plants inoculated with race 1 developed symptoms, and none of those inoculated with both nematodes and race 1 did so. Furthermore, none of the race 2-inoculated seedlings, whether inoculated with nematodes or not, developed symptoms.

No wilt symptoms developed on any root-knot nematode inoculated, supernatant-treated, or noninoculated Manapal or Florida MH-1 plants throughout the experiment.

Galls developed on all plants inoculated with root-knot nematode larvae, regardless of *Fusarium* inoculation. These larvae developed into adults which laid eggs that hatched into second generation larvae and caused secondary galling. Roots of all noninoculated plants remained free of galling.

The race 1 and 2 isolates used in the experiment were virulent, the inoculum levels were high, the root-knot nematodes resulted in primary and secondary galling, and the plants were incubated under environmental conditions highly favorable for wilt development, but no disease developed on resistant cultivars.

Our results show clearly that the root-knot nematode does not reduce the resistance to Fusarium wilt conferred by the *I* or I_2 gene in tomato. Thus, it is our contention that utilization of monogenic resistance for control of Fusarium wilt in tomato has been and will continue to be successful even in areas such as Florida where root-knot nematode field populations often are sufficient to cause severe root galling.

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TABLE 1. Stability of resistance of Manapal tomato to *Fusarium oxysporum lycopersici* race 1 in the presence of *Meloidogyne incognita* as indicated by percentage diseased and dead plants and their fresh weights

Fusarium race[c]	Nematode population[d]	Simultaneous inoculation[a]			Split inoculation[b]		
		Diseased plants (%)	Dead plants (%)	Fresh weight (g)	Diseased plants (%)	Dead plants (%)	Fresh weight (g)
1	High	0	0	94	0	0	89
1	Low	0	0	82	2	0	100
1	Nil	0	0	87	0	0	87
2	High	100	98	9	100	67	22
2	Low	100	90	9	98	54	26
2	Nil	100	94	11	100	77	27

[a]Plants inoculated simultaneously with *Fusarium* and nematodes; data gathered 5 weeks later.
[b]Plants inoculated first with nematodes, 2 weeks later with *Fusarium;* data gathered 6 weeks after nematode inoculation.
[c]Root dip-inoculated with 14.3×10^6 and 15.7×10^6 spores/ml of race 1 or 2.
[d]High and low root-knot nematode populations = 46 or 23 larvae per plant.

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