Localized Influence of *Meloidogyne incognita* on Fusarium Wilt Resistance of Flue-Cured Tobacco

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ABSTRACT

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Coker 298, a flue-cured tobacco cultivar resistant to Fusarium wilt and susceptible to *Meloidogyne incognita*, was grown in a split-root system and inoculated with both *Fusarium* and root-knot nematodes in various combinations to examine the influence of the nematode on wilt resistance. Only plants inoculated with both pathogens on the same half-root system developed severe wilt symptoms. Thus, *M. incognita* did not induce a systemic alteration of Coker 298 Fusarium wilt resistance.

Meloidogyne incognita (Kofoid and White) Chitwood is most devastating when interacting with other microorganisms (10). Plants normally resistant to certain fungi or bacteria become susceptible to these organisms when infected with root-knot nematodes (5). One of the first observed disease interactions and one known to occur on cotton, tomato, and other crops is that between *forma speciales* of *Fusarium oxysporum* and *Meloidogyne* spp. (1,7,9). The incidence and severity of Fusarium wilt are increased in nematode-infected plants compared to nematode-free plants.

The predisposition of tomato cultivars to Fusarium wilt by the root-knot nematode has been reported to be a systemic alteration which was not restricted to the galled roots (2,11). For a full understanding of the disease complex, it is essential to know if the tomato-*Meloidogyne* interaction is unique or if the nematode induces a systemic alteration of resistance in other plants as well. The present study was undertaken to determine whether the predisposition of a wilt-resistant cultivar of tobacco by *M. incognita* is systemic or restricted to the galled area of the roots.

MATERIALS AND METHODS

Coker 298 (Fusarium wilt-resistant; root-knot nematode-susceptible), a flue-cured tobacco cultivar, was transplanted into sand in 5-cm-diameter clay pots 3 wk after the seeds were sown. Three wk later, the roots were washed and divided to establish a split-root system in adjacent 1,000-ml plastic pots containing a sand-soil (1:1, v/v) mixture. Two glass tubes were positioned 6–8 cm deep in each pot to provide avenues for inoculation. Each pot was placed on an inverted saucer to prevent cross-pot contamination. Plants were watered daily. Every 2 wk 60 ml of liquid fertilizer was added to each pot (25 g KNO_3 + 45 g $MgSO_4 \cdot 7H_2O$ + 140 g VHPF, Miller Chemical & Fertilizer Corp., Hanover, PA 17331, suspended in 16 L of H_2O).

M. incognita was maintained on Homestead tomatoes. The washed, infected roots were soaked in 1.0% sodium hypochlorite for 3 min with continuous stirring (6). The suspension was poured into nested sieves and the eggs were collected on the bottom 26-μm sieve. The eggs to be applied to appropriate half-root systems were thoroughly washed with tap water, counted, and diluted to give inocula containing either 25,000, 50,000, or 100,000 eggs in 20 ml of tap water.

A stock culture of *Fusarium oxysporum* (Schlect) f. sp. *nicotianae* (Johns.) Snyd. & Hans. stored in sterile soil was used. The soil was plated on potato dextrose agar and the fungus was transferred to liquid Armstrong's *Fusarium* medium (12) as soon as mycelium appeared. Five days in shake culture (27 C) produced a heavy suspension of microconidia and hyphal fragments with few macroconidia or chlamydospores. These propagules were trapped and washed on filter paper, resuspended in tap water, and counted by using a hemacytometer.

The following inoculations were made by removing the glass tubes and pouring the inoculum into the holes in the potting medium. In experiment 1, 9-wk-old plants were inoculated with fungal propagules (100×10^6/20 ml H_2O) and/or nematode eggs (25,000, 50,000, or 100,000 eggs/20 ml H_2O). In experiment 2, certain of the 9-wk-old plants were inoculated with fungal propagules (30×10^6/20 ml H_2O) and/or nematode eggs as above. Some of the plants inoculated only with nematodes at that time were inoculated with *Fusarium* propagules (100×10^6/20 ml H_2O) 4 wk later. This 4-wk period allowed the nematodes to become well established in the roots before the fungus was added. A minimum of nine plants per treatment (Tables 1 and 2) were randomized on greenhouse benches and all plants were in a split-root system.

TABLE 1. Fusarium wilt rating of Coker 298 tobacco plants in a split-root system 6 wk after simultaneous inoculation with *Meloidogyne incognita* eggs and 100×10^6 *Fusarium oxysporum* f. sp. *nicotianae* propagules

Treatment (half-root/half-root)	Plants rated (no.)	Mean wilt rating[a]
None/None	9	0.0
Fungus/None	18	0.1 ± 0.3
Fungus/Fungus	10	0.0
25,000 eggs/Fungus	10	0.1 ± 0.3
50,000 eggs/Fungus	10	0.0
100,000 eggs/Fungus	10	0.0
25,000 eggs + Fungus/None	10	2.2 ± 1.0[b]
50,000 eggs + Fungus/None	10	2.4 ± 1.5[b]
100,000 eggs + Fungus/None	10	2.3 ± 1.0[b]

[a] Wilt rating: 0 = no apparent disease; 1 = very slight vascular discoloration but no other symptoms; 2 = moderate to extensive vascular discoloration with slight leaf yellowing, wilting, and distortion; 3 = extensive vascular discoloration with wilting and pronounced leaf distortion; 4 = plant dead or permanently wilted. Mean wilt rating = (sum of individual ratings)/(number of plants rated).

[b] Significantly different from 0 rating at $P = 0.01$.

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Greenhouse temperatures ranged from 25 to 30 C during these experiments.

Six weeks after the first inoculations were made, all plants were rated for Fusarium wilt severity according to the following scale: 0 = no apparent disease; 1 = very slight vascular discoloration, but no other symptoms; 2 = moderate to extensive vascular discoloration with slight leaf yellowing, wilting, and distortion; 3 = extensive vascular discoloration with wilting and pronounced leaf distortion; 4 = plant dead or permanently wilted (4). The mean wilt rating is the sum of ratings of individual plants per total number of plants rated.

RESULTS AND DISCUSSION

The incubation period for plants inoculated simultaneously with both pathogens on the same half-root system was 3 wk. In experiment 2, plants inoculated with *Fusarium* on the half-root system which had been inoculated with nematodes 4 wk previously, developed wilt symptoms in 9 days. Only one of 88 plants inoculated with the fungal and nematode pathogens on opposite root halves developed Fusarium wilt symptoms, while two of 48 plants inoculated with *Fusarium* alone developed symptoms. The results of experiment 1 are summarized in Table 1 and of experiment 2 in Table 2.

M. incognita increased the susceptibility of Coker 298 tobacco to Fusarium wilt in these experiments, but this influence was confined to the sections of the root systems infected with the nematode. The high numbers of nematodes added produced heavy galling and should have induced a systemic alteration of resistance if such an alteration indeed occurs in tobacco. Bowman and Bloom (2) inoculated tomato plants with 9,000 *M. incognita* eggs (as described by Walker [14]) in a split-root system. Similarly, Sidhu and Webster (11) added 12 *M. incognita* egg masses to the primary root system of tomato plants which were air layered to establish four additional root systems along their stems. In both cases, Fusarium wilt symptoms developed regardless of the site of fungal inoculation. The entire plant was predisposed to disease incited by the wilt organism. Estores and Chen (3), also using tomato, found a systemic influence of root-knot nematodes on *Pratylenchus penetrans*. The conflict between those reports and the present study may reflect a basic difference in tomato and tobacco physiology or in the overall reaction of each to infection by root-knot nematodes. This difference should be noted since most comprehensive examinations of the physiology of galls induced by root-knot nematodes and the gall's influence on resistance have employed tomato host plants. Before generalizations can be made, subsequent interaction studies must employ several cultivars of more than one host genus.

Further studies on disease interactions may provide a better understanding of resistance, susceptibility, and host specificity since the root-knot nematode dramatically alters these characteristics.

TABLE 2. Fusarium wilt rating of Coker 298 tobacco plants in a split-root system 6 wk after simultaneous inoculation with *Meloidogyne incognita* eggs and 30×10^6 *Fusarium oxysporum* f. sp. *nicotianae* propagules. Certain plants not inculated at that time or which were inoculated with nematode eggs only, received 100×10^6 *F. oxysporum* f. sp. *nicotianae* propagules 4 wk later. All plants were rated at the same time

Treatments (half-root/half-root)	Plants rated (no.)	Mean wilt rating[a]
None/None	15	0.0
Fungus/None[b]	10	0.0
25,000 eggs/None[b]	15	0.0
50,000 eggs/None[b]	15	0.0
100,000 eggs/None[b]	15	0.0
25,000 eggs/Fungus[b]	9	0.0
50,000 eggs/Fungus[b]	10	0.0
100,000 eggs/Fungus[b]	10	0.0
25,000 eggs + Fungus/None[b]	9	1.1 ± 1.3
50,000 eggs + Fungus/None[b]	10	2.7 ± 1.0[d]
100,000 eggs + Fungus/None[b]	10	2.1 ± 1.0[d]
Fungus/None[c]	10	0.0
25,000 eggs/Fungus[c]	10	0.0
50,000 eggs/Fungus[c]	9	0.0
100,000 eggs/Fungus[c]	10	0.0
25,000 eggs + Fungus/None[c]	9	2.3 ± 1.0[d]
50,000 eggs + Fungus/None[c]	10	1.5 ± 1.0[d]
100,000 eggs + Fungus/None[c]	10	1.6 ± 1.4[d]

[a] Wilt rating: 0 = no apparent disease; 1 = very slight vascular discoloration but no other symptoms; 2 = moderate to extensive vascular discoloration with slight leaf yellowing, wilting, and distortion; 3 = extensive vascular discoloration with wilting and pronounced leaf distortion; 4 = plant dead or permanently wilted. Mean wilt rating = (sum of individual ratings)/(number of plants rated).

[b] Simultaneous inoculation of plants.

[c] Fungus inoculations made 4 wk after plants had been inoculated with nematodes.

[d] Significantly different from 0 ratings at $P = 0.01$.

LITERATURE CITED

1. ATKINSON, G. F. 1892. Some diseases of cotton. Pages 61–65 in: Alabama Agric. Stn. Bull. 41.
2. BOWMAN, P., and J. R. BLOOM. 1966. Breaking the resistance of tomato varieties to Fusarium wilt by *Meloidogyne incognita*. (Abstr.) Phytopathology 56:871.
3. ESTORES, R. A., and T. A. CHEN. 1972. Interactions of *Pratylenchus penetrans* and *Meloidogyne incognita* as coinhabitants of tomato. J. Nematol. 4:170-174.
4. GRITTON, E. T., G. L. JONES, N. T. POWELL, and D. F. MATZINGER. 1965. Inheritance of resistance to Fusarium wilt in flue-cured tobacco. Crop Sci. 5:547-550.
5. HIRANO, K. 1975. Interrelationships between plant parasitic nematodes and other plant parasitic organisms. Rev. Plant Prot. Res. 8:55-68.
6. HUSSEY, R. S., and K. R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
7. MARTIN, W. J., L. D. NEWSOM, and J. E. JONES. 1956. Relationships of nematodes to the development of Fusarium wilt in cotton. Phytopathology 46:285-289.
8. PORTER, D. M., and N. T. POWELL. 1967. Influence of certain *Meloidogyne* species on Fusarium wilt development in flue-cured tobacco. Phytopathology 57:282-285.
9. POWELL, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Ann. Rev. Phytopath. 9:253-274.
10. SIDHU, G., and J. M. WEBSTER. 1977. Predisposition of tomato to the wilt fungus (*Fusarium oxysporum lycopersici*) by the root-knot nematode (*Meloidogyne incognita*). Nematologica 23:436-442.
11. TUITE, J. 1969. Plant Pathological Methods. Burgess Publishing Co., Minneapolis, MN. 239 pp.
12. VAN GUNDY, S. D., J. D. KIRKPATRICK, and J. GOLDEN. 1977. The nature and role of metabolic leakage from root-knot nematode galls and infection by *Rhizoctonia solani*. J. Nematol. 9:113-121.
13. WALKER, J. C. 1971. Fusarium wilt of tomato. Phytopathol. Monogr. 6. The American Phytopathological Society, St. Paul, MN. 56 pp.