Interaction of Population Levels of Fusarium oxysporum f. sp. vasinfectum and Meloidogyne incognita on Cotton

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Abstract: In autoclaved greenhouse soil without *Fusarium oxysporum* f. sp. *vasinfectum*, *Meloidogyne incognita* did not cause leaf or vascular discoloration of 59-day-old cotton plants. Plants had root galls with as few as 50 *Meloidogyne* larvae per plant. Root galling was directly proportional to the initial nematode population level. *Fusarium* wilt symptoms occurred without nematodes with 77,000 fungus propagules or more per gram of soil. As few as 50 *Meloidogyne* larvae accompanying 650 fungus propagules caused *Fusarium* wilt. With few exceptions, leaf symptoms appeared sooner as numbers of either or both organisms increased. In soils infested with both organisms, the extent of fungal invasion and colonization was well correlated with the extent of nematode galling and other indications of the *Fusarium* wilt syndrome. *Key Words:* nematode-fungus interaction, integrated pest management, host-plant resistance, *Gossypium hirsutum*, fungus and nematode populations.

Fusarium oxysporum f. sp. *vasinfectum* (Atk.) Snyd. and Hans. and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, have been associated with "frenching" or *Fusarium* wilt of cotton, *Gossypium hirsutum* L., since 1892 (1). Relations between these organisms are now well established (2, 4, 7, 8, 9). Much of the evidence for the role of root-knot nematodes in increased *Fusarium* wilt disease of cotton is based on suppression of the disease when soil is fumigated to reduce root-knot nematode populations (6, 7, 12, 13). Bowman and Bloom (3) used *M. incognita* to break the resistance of tomato to *F. oxysporum* f. sp. *lycopersici*. The root system of each plant was split. Wilt symptoms developed only on plants in soil infested with both organisms, even if fungus infested one half of the roots and nematode the other. Yang et al. (14) found *Fusarium* wilt of cotton to be more severe with high than with low populations of *M. incognita*. In their experiments, wilt occurred with *F. oxysporum* f. sp. *vasinfectum* alone at high spore numbers, but mechanical wounding increased the amount of disease. Perry (11) reported that root-knot larvae did not stimulate infection of cotton seedlings by the *Fusarium*. There were no more fungal hyphae near points of entry of larvae than elsewhere, and the fungus did not colonize mature gall tissue in preference to normal root tissue.

This study compares the influences on *Fusarium* wilt disease of cotton of different population levels of *F. oxysporum* f. sp. *vasinfectum* and *M. incognita*, separately and together. Plants exposed to the fungus, the nematode, and both are respectively referred to herein as Fo, Mi, and Fo-Mi plants.

MATERIALS AND METHODS

Hesperia sandy loam soil was autoclaved, allowed to cool, and infested with propagules of *F. oxysporum* f. sp. *vasinfectum* and larvae of *M. incognita*. Four dilutions of each organism, plus uninfested controls, were used in all 25 possible combinations. Spores from week-old colonies of *F. oxysporum* f. sp. *vasinfectum* were washed from the surface of potato-dextrose agar and counted with a counting chamber (hemocytometer). Dilutions were made with sterile distilled water, and final concentrations were determined with the counting chamber. Each dilution was sprayed into 54 kg of soil being tumbled in a modified cement mixer. Resulting fungal concentrations were about 647,000, 77,000, 5,100, 650 and 0 propagules/g of soil. Each treatment consisted of ten 10-cm-diam polystyrene pots each containing 486 cm^3 of the various soil-fungus concentrations. Three acid-delinted untreated 'Acala SJ-2' cotton seeds were planted 3 cm deep in each pot. This cultivar is highly susceptible to *F. oxysporum* f. sp. *vasinfectum* and *M. incognita*. In treatments that were to include nematodes, the soil was

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infested by pouring 25 ml of the appropriate dilution of *M. incognita* over the seeds before they were covered. The nematode population levels were 33,000, 3000, 360, 50, and 0 larvae/pot.

The 250 pots were placed in a randomized complete block design in a greenhouse maintained at about 17–19 C (night) and 23–25 C (day). The time required for the first appearance of chlorotic leaf symptoms of *Fusarium* wilt was recorded. Data recorded after 59 days included plant heights, root-knot galling, fresh-plant weight, and extent of colonization and vascular discoloration by *F. oxysporum* f. sp. *vasinfectum*. The height of each plant was measured from the ground to the growing tip. Each plant was severed at the ground, and the roots were carefully removed, evaluated for root-knot galling, and weighed. The shoots were weighed and the cut stems were evaluated for vascular discoloration on a scale of 0 (no discoloration) to 5 (completely discolored or dead). Root-knot galling of each plant was rated on a scale of 0 (no galls) to 4 (heavily galled). Stem portions of each living plant, taken at various distances from ground level, were plated on peptone-PCNB agar in petri dishes. *F. oxysporum* colonies isolated were tested for pathogenicity in the greenhouse. The plants were rated on the basis of the number of stem parts containing *F. oxysporum* f. sp. *vasinfectum:* 0 (none), 4 (all), 5 (plants dead).

RESULTS AND DISCUSSION

Soil without the fungus: No Mi plants had chlorotic leaves or vascular discoloration regardless of nematode levels (Table 1). In the 59-day experimental period, Mi plant heights were not reduced significantly. Root galling was most severe at the highest nematode level, although some occurred at the lowest level (Table 2). None of the stem parts contained the fungus (Table 1).

Soil without the nematode: At the highest level, Fo plants developed chlorotic leaf symptoms in an average of 44 days after planting (Table 1). At lower levels symptom appearance took longer. At the lowest levels, Fo plants had no symptoms at harvest, 59 days after planting (Table 2). The *Fusarium* was detected in the stem only at the two highest propagule levels. Some vascular discoloration occurred at the three highest levels (Table 1). Thus, *F. oxysporum* f. sp. *vasinfectum* was present in the stems of plants at the 5,100-propagule concentration, even though no fungus was isolated after the Fo plants were harvested. It seems possible that if only a few spores and hyphal strands were present, they may have died in the plant or did not grow onto the isolation medium. Similarly, plants infected with *Verticillium dahliae* Kleb. commonly have vascular discoloration but no leaf symptoms, and the fungus is often undetectable on agar medium later in the season (5). In our tests, the *Fusarium* at high levels was able to penetrate, colonize, and produce disease symptoms without the aid of nematodes.

Effects of combined inocula: With both inocula at the lowest level, some Fo-Mi plants developed symptoms of *Fusarium* wilt. Leaf symptoms appeared earlier as the level of either organism increased (Table 1). These results support the conclusion of numerous workers that *F. oxysporum* f. sp. *vasinfectum* and *M. incognita* have an interacting influence on *Fusarium* wilt (4, 6, 7, 10, 11). We suggest that a low population of either organism in combination with a high population of the other could result in marked adverse plant reactions. These results also suggest that populations of both organisms are high where cotton disease is severe.

Even at the lowest levels of *Fusarium* the height and fresh weight of Fo-Mi plants were reduced if the nematode levels were high (Table 2). At the three highest nematode levels, plant height decreased with increasing fungus levels. The shortest plants were those exposed to the two highest levels of combined organisms (Table 2). The number of stem parts containing *Fusarium* increased with either nematode or fungus inoculum level (Table 1). Even if the nematode level was high, not many stem parts contained the fungus if the soil level of the fungus was low. At each *Fusarium* level the vascular discoloration generally increased with nematode level (Table 1). The effect was most pronounced at the three highest *Fusarium* levels. In general, the data for vascular discoloration relate well to the data for infection and leaf symptoms. The fungus was most prevalent in plants that had leaf symptoms in the

TABLE 1. Effects of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* population level on the expression of disease symptoms of *Fusarium* wilt of cotton plants.[a]

Fusarium propagules/g of soil	Nematodes per plant					
	33,000	3,000	360	50	0	r[e]
	Time (days) required for chlorotic leaf symptoms					
647,000	21	28	35	37	44	−0.98*
77,000	24	24	28	43	47	−0.93*
5,100	28	34	44	53	57	−0.97*
650	52	52	58	56	59[b]	−0.85
0	59[b]	59[b]	59[b]	59[b]	59[b]	—
LSD 0.05 = 8.0						
r[f]	−0.93*	−0.92*	−0.86	−0.90*	−0.84	
	Vascular discoloration rating[c]					
647,000	5.0	4.8	4.2	3.0	2.7	0.98*
77,000	5.0	5.0	4.4	3.0	2.1	0.96*
5,100	4.7	3.7	3.3	1.1	0.3	0.97*
650	2.4	2.7	0.4	0.7	0.0	0.86
0	0.0	0.0	0.0	0.0	0.0	—
LSD 0.05 = 1.1						
r[f]	0.95*	0.98*	0.90*	0.92*	0.82	
	Rating of plants containing *F. oxysporum* f.sp. *vasinfectum*[d]					
647,000	5.0	4.7	4.2	2.6	1.9	0.96*
77,000	5.0	5.0	4.3	3.8	1.9	0.96*
5,100	4.8	3.4	2.8	0.9	0.0	0.98*
650	1.7	2.3	0.5	0.5	0.0	0.84
0	0.0	0.0	0.0	0.0	0.0	—
LSD 0.05 = 1.2						
r[f]	0.93*	0.98*	0.92*	0.82	0.78	

[a]Each value is the mean for 10 plants.
[b]No symptoms from planting to harvest (59 days).
[c]Scale: 0 = no discoloration; 5 = completely discolored or plants dead.
[d]Scale: 0 = none; 4 = all; 5 = plants dead.
[e]Correlation coefficient between log (X + 1) of number of nematodes per plant and disease symptoms.
[f]Correlation coefficient between log (X + 1) of number of *Fusarium* propagules per gram of soil and disease symptoms data.
*Significantly (5% level) different from zero.

fewest days and in plants with the most vascular discoloration. Perry recovered *F. oxysporum* from all plants that had leaf symptoms and vascular discoloration (11). It is likely that his inoculum levels were much higher than some of the levels used in our experiment.

High populations of *Fusarium* did not inhibit galling on Fo-Mi plants (Table 2). There was some variation at the two lowest fungus and nematode levels.

Population levels of *M. incognita* and *Fusarium* were strongly associated with the criteria used to evaluate *Fusarium* wilt of cotton. At each *Fusarium* population level a correlation coefficient was calculated between the log (X + 1) of the numbers of nematode per plant and the plant measurements reported in Tables 1 and 2. Similarly at each nematode population level a correlation coefficient was calculated between log (X + 1) of the number of *Fusarium* propagules per gram of soil and the plant measurements. The correlation coefficients are reported in Tables 1 and 2.

At the three highest *Fusarium* population levels all plant measurements except root weight had relatively good correlations with nematode population levels. The r in these cases was generally greater than 0.900 or less than −0.900. At *Fusarium* levels of 650 and 0, correlations did not generally differ significantly from zero. At the 77,000, 5,100, 650, and 0 levels of *Fusarium* the 360 and 50 levels of nematode appear to have had a stimulative effect on root weights

TABLE 2. Effects of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* population level on root-knot galling and growth of cotton plants.[a]

Fusarium propagules/g of soil	Nematodes per plant					
	33,000	3,000	360	50	0	r[c]
	Plant height (cm)					
647,000	16.5	18.8	24.8	30.4	33.8	−0.98*
77,000	17.9	14.4	26.2	31.5	35.1	−0.91*
5,100	21.1	21.9	29.7	36.0	37.7	−0.94*
650	30.2	27.5	34.3	32.9	38.5	−0.86
0	31.6	31.7	34.0	33.3	36.2	−0.93*
LSD 0.05 = 5.2						
r[d]	−0.92*	−0.90*	−0.88*	−0.45	−0.49	
	Top fresh weight (g)					
670,000	0.0	0.5	2.4	5.6	9.4	−0.98*
77,000	0.0	0.0	2.1	6.1	7.4	−0.95*
5,100	1.0	3.8	5.2	9.1	11.2	−0.98*
650	6.6	6.3	9.3	9.0	11.6	−0.94*
0	8.3	9.1	9.9	9.6	10.7	−0.95*
LSD 0.05 = 2.7						
r[d]	−0.92	−0.96*	−0.91*	−0.85	−0.55	
	Root fresh weight (g)					
647,000	0.4	0.7	2.4	2.9	4.1	−0.98*
77,000	0.5	0.5	1.8	3.7	2.5	−0.76
5,100	1.0	2.5	2.5	2.9	2.2	−0.52
600	2.5	2.2	3.9	3.9	2.5	−0.21
0	3.8	4.5	4.8	5.5	3.9	−0.14
LSD 0.05 = 1.4						
r[d]	−0.97*	−0.96*	−0.91*	−0.88*	−0.14	
	Root-knot galling rating[b]					
647,000	3.6	3.8	4.0	2.5	0.0	0.87
77,000	4.0	4.0	4.0	2.9	0.0	0.89*
5,100	4.0	3.7	3.5	1.6	0.0	0.96*
650	3.7	3.7	2.9	2.2	0.0	0.96*
0	4.0	3.3	2.8	2.2	0.0	0.98*
LSD 0.05 = 0.5						
r[d]	−0.43	0.90	0.91*	0.37	—	

[a]Each value is the mean for 10 plants.
[b]Scale 0 = no gall to 4 = heavily galled.
[c]Correlation coefficient between log (X + 1) of number of nematodes per plant and disease symptoms.
[d]Correlation coefficient between log (X + 1) of number of *Fusarium* propagules per gram of soil and plant growth or root-knot galling.
*Significantly (5% level) different from zero.

(generally greater than with 0 nematodes). That doubtless contributed to the nonsignificant correlations between root weight and nematode population levels of *Fusarium* population levels below 647,000.

For plant measurements and *Fusarium* population levels there were generally good correlations at nematode population levels 33,000, 3,000, and 360 for all plant measurements including root weight.

Disease was not severe unless both organisms were present. High levels of resistance to *F. oxysporum* are not available in cotton cultivars, although excellent resistance to *M. incognita* is available. Soil fumigation for control of *F. oxysporum* f. sp. *vasinfectum* is too costly. In our experiment, increasing the nematode population drastically increased the probability of *F. oxysporum* colonizing and producing disease symptoms on young cotton plants. Therefore, a part of our approach to the control of *Fusarium* wilt should be reducing the activities of *M. incognita* through soil

fumigation, use of nematode-resistant cultivars, or a combination of the two.

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