Effect of Root-Knot Nematodes on Fusarium Wilt of Watermelon

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ABSTRACT

Eight cultivars of watermelon with known reactions to Fusarium wilt were grown in soils collected from 21 fields with a history of watermelon production in eight growing areas of South Georgia. More plants wilted in soils when *Meloidogyne incognita* was present. Wilt symptoms were increased more in resistant than in susceptible cultivars in soils naturally infested with root-knot nematodes. Wilt severity was significantly correlated with initial populations of root-knot larvae, inoculum density of all *Fusarium oxysporum* in the soil, and wilt severity in the previous crop of watermelons, but not with soil pH or the number of years since the previous crop of watermelons was grown. In another study, Dothan loamy sand was artificially infested with 3% cornmeal-sand cultures of *F. oxysporum* f. sp. *niveum* 1:400 or 1:16,000 (v/v) or noninfested. Each treatment was then infested with *M. arenaria, M. javanica, M. hapla*, or noninfested. Root-knot nematodes did not significantly increase wilt at 17, 584, or 218 propagules of total *F. oxysporum* per gram of air-dry soil. However, at 650 propagules per gram of total *F. oxysporum, M. arenaria* reduced foliage weights 11-13% in susceptible 'Jubilee', moderately resistant 'Charleston Gray', and resistant 'Crimson Sweet', and significantly increased wilting and root necrosis in Charleston Gray. Total plant weight of Charleston Gray was significantly less than Crimson Sweet with *M. hapla*, but not with other root-knot nematodes or in the controls at the high level of *F. oxysporum*. Significantly more root galls were caused by *M. arenaria* and *M. javanica* than by *M. hapla*.

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Fusarium wilt of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] caused by *Fusarium oxysporum* Schl., emend Snyd. & Hans. f. sp. *niveum* (E. F. Sm.) Snyd. & Hans., is an endemic problem in South Georgia. More wilt symptoms and death of plants are noted in susceptible cultivars, but occasionally severe wilt is observed in resistant cultivars. The fungus survived in soil in the midwestern USA for 16 years (7). Several authors also have reported that more than one race of the pathogen exists (1, 2). Severity of wilt symptoms varies among cultivars according to inoculum density of the fungus in soil (12), but inoculum density alone does not explain the variation in wilt observed in many fields. Soil and air temperature, soil moisture, light, and wind velocity are other factors reported to influence development of wilt symptoms (6).

Root-knot nematodes (*Meloidogyne* spp.) are known to cause severe galling in watermelons (9, 10, 13, 14, 15, 17). Estimated losses in watermelon due to nematodes alone in the USA are 5% (11). Many soils in Georgia are heavily infested with one or more species of root-knot nematodes. This study was undertaken to determine whether the Fusarium wilt-root-knot complex (8) exists in watermelon.

MATERIALS AND METHODS.–Soil was collected randomly from each of 21 fields with a history of watermelon production in eight growing areas of South Georgia in November and December 1971. Some fields were planted to watermelons in 1971, and others had not been cropped to watermelons for 2 to 10 years. One or two liters of soil were taken from the top 20 cm at 15 to 20 different sites at each location. Each soil was thoroughly mixed with N, K_2O, and P_2O_5 at 24, 47, and 71 mg/liter, respectively, and placed in three 51- × 35- × 9-cm trays within 3 days after collection. Controls were natural Dothan loamy sand (DLS) free of root-knot nematodes and the wilt pathogen, or artificially infested with 1:400 or 1:4,000 (v/v) *F. oxysporum* f. sp. *niveum* grown on 3% cornmeal-sand (v/v). A pathogenic monosporidial isolate of the fungus, recovered from a wilted watermelon plant in 1971, was used. Random samples of soil were collected from each tray and used for pH, nematode, and total *F. oxysporum* assays. Soil was mixed 1:1 (v/v) with water for pH determinations. Initial populations of root-knot larvae and other stylet-bearing nematodes were determined by the centrifuge-sugar-flotation method (3). In addition, 15-cm clay pots were filled with soil from each location and planted to tomato, *Lycopersicon esculentum* Mill. 'Rutgers', seedlings. Fifty-four days later, tomato roots were carefully freed from soil, washed, and rated for nematode damage according to a standard root-gall index. A scale of 1 to 5 was used with 1 = no galls, 2 = 1 to 25, 3 = 25 to 50, 4 = 50 to 75, and 5 = 75 to 100% of all roots galled. Inoculum density (ID) in propagules/g (PG) of air-dry soil of *F. oxysporum* in the soil was estimated, with soil dilutions on peptone-pentachloronitrobenzene agar as modified by Papavizas (5).

Five to 39 days after the soils were collected, five seeds of each of eight cultivars of watermelon with a known reaction to wilt and muskmelon (*Cucumis melo* L. 'Hales Best Jumbo') and summer squash

(*Cucurbita pepo* L. 'Early Summer Yellow Crookneck') were planted in each tray. Watermelon cultivars 'Smoky Lee' and 'Crimson Sweet' are very resistant and resistant, respectively, to Fusarium wilt; Charleston Gray, moderately resistant; 'Blackstone' and 'Congo', slightly resistant; and 'Jubilee', 'Garrisonian', and 'New Hampshire Midget' are susceptible. Muskmelon and squash are resistant to the watermelon wilt pathogen. A split-plot experiment, in a randomized complete block design, with three replications, was used. Soils were whole plots, and cultivars were subplots. Plants were grown in a greenhouse at soil temperatures of 9 to 37 C and were fertilized as needed to promote vigorous growth. The number of wilted plants was recorded every 7 to 10 days. Plants were considered "wilted" when they were flaccid, gray-green, and showing severe necrosis on lower leaves. Vines were 75- to 125-cm long and most plants were flowering when the experiment was terminated after 51 to 54 days. Roots of plants were carefully freed from soil, washed, and indexed for root galls. Soil in each tray was re-assayed for root-knot larvae and other stylet-bearing nematodes.

In a second experiment, noninfested DLS was artificially infested with *F. oxysporum* f. sp. *niveum* and root-knot nematodes. Cornmeal-sand (CMS) inoculum of the fungus was mixed with DLS and fertilizer 1:400 or 1:16,000 (v/v). Sterile CMS was added to noninfested control soil 1:400 (v/v). Soil dilutions indicated that the inoculum densities of total *F. oxysporum* in the soil were ca. 17,580, 650, and 216 PG, respectively, 4 days after soils were amended. Fungal populations were comparable to those found in soil collected immediately under a wilted susceptible plant, in soil 3 to 10 years after the last watermelon crop, and in soil not previously planted to a host that would support *F. oxysporum*, respectively.

Groups of forty-eight, 15-cm clay pots were filled with ca. 1,500 ml of each amended soil in each pot, and a 2- to 3-cm deep circular depression 4- to 5-cm diam was made in the surface of each. Five seeds of Jubilee, Charleston Gray, or Crimson Sweet were placed at the bottom of each depression. A tap water suspension of 10 egg masses each of *Meloidogyne arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, *M. hapla* Chitwood, or no nematodes was poured over the seeds and immediately covered with soil. Well-developed egg masses, hand-picked from 50-day-old populations on heavily galled Rutgers tomato roots, were used. The mean number of eggs per egg mass (384, 391, and 387, respectively, for *M. arenaria, M. javanica*, and *M. hapla*) was estimated by the sodium hypochlorite method (4).

A split-split plot experiment was used in a randomized complete block design, with four replications of one pot per treatment. Levels of *F. oxysporum* were whole plots, nematode species were subplots, and cultivars were sub-subplots. Plants were greenhouse-grown for 55 days at soil temperatures of 18 to 41 C and evaluated every 7 to 10 days for wilt. Then the fresh weight of the foliage in each pot was recorded. Roots were carefully freed from soil, washed, blotted dry, and weighed. Invasion and reproduction of nematodes were determined by the percentage of the root system galled, and egg-mass production. The percentage of root necrosis was also noted, as an additional indication of *F. oxysporum* virulence.

All data were statistically analyzed by one or more of the following computer programs: least-squares analysis of variance, simple correlation, or multiple linear regression. The words "significant" and "highly significant" are used to indicate differences at 0.05 and 0.01 levels of probability, respectively.

RESULTS.–In the first experiment, root galls were observed on tomato or watermelon in 13 field soils. No root-knot larvae were recovered from seven field soils and the control DLS, nor were any galls observed on tomatoes or watermelons grown in those soils. Random samples of root-knot nematodes from 11 soils were identified as *M. incognita*. Many more plants showed symptoms of wilt in soils where root-knot nematodes were present. Wilt symptoms were increased more by root-knot nematodes in resistant, than in susceptible, cultivars (Table 1). The total number of wilted plants in all cultivars was significantly correlated with the root-gall index on tomatoes 8 to 13, and 24 to 28 days after planting, and the correlation was highly significant from 30 to 35, through 54, days. The initial population of root-knot larvae was highly correlated with the population recovered at the end of the experiment ($r = 0.86$) and with the root-gall index on tomatoes ($r = 0.58$) and cucurbits ($r = 0.79$). There was a significant correlation between initial populations of root-knot larvae and number of wilted plants at 24 to 28 days, and final populations of root-knot larvae and number of wilted plants, 30 to 35 days after planting. The correlation of both initial and final nematode populations with number of wilted plants was highly significant at 29 to 53 days. The final total population of stylet-bearing nematodes was significantly correlated with number of wilted plants.

Populations of *Criconemoides ornatus* Raski and initial total stylet-bearing nematodes were not correlated with wilt. *Belonolaimus longicaudatus* Rau, *Pratylenchus zeae* Graham, *Trichodorus christiei* Allen, and *Helicotylenchus* sp. Steiner were infrequently found in low numbers and were not included in the correlation analysis.

The pH of the soil solution ranged from 5.6 to 6.4 in the 21 soils and was not correlated with number of wilted plants. Also, no correlation was detected between the number of years since the previous crop of watermelons was grown and the number of wilted plants.

The correlation between wilt severity in the previous crop of watermelons and the total number of wilted plants in the eight watermelon cultivars was highly significant ($r = 0.61$). Inoculum density of *F. oxysporum* and wilt severity in the previous crop were significantly correlated ($r = 0.24$). Multiple linear regression showed that 37% of the variation in total wilted plants in the eight cultivars was related to

TABLE 1. The effect of *Meloidogyne incognita* on severity of wilt (caused by *Fusarium oxysporum* f. sp. *niveum*) in 21 soils with a history of watermelon production

Years out of watermelons	Number of fields	*Fusarium oxysporum*, PG[a]	Root-knot larvae/150 cc of soil		Root gall index 51-54 days[b]		Wilted watermelon plants, 49-53 days (%)[c]			
			Initial	49-53 days	Tomatoes	Watermelons	CG	CS	SL	Average of eight cultivars[d]
5-10										
Non-root-knot infested	3	1,005	0	20	1.0	1.2	4	7	0	5
Root-knot infested	3	1,953	0	6	3.3	2.0	35	41	28	36
3-4										
Non-root-knot infested	2	509	0	0	1.0	1.0	14	6	0	8
Root-knot infested	2	921	165	983	3.5	3.2	18	29	32	28
0-1										
Non-root-knot infested	5	1,297	0	4	1.0	1.0	19	22	15	29
Root-knot infested	6	1,534	23	2	3.3	1.1	13	20	16	19

[a] Propagules/g of air-dry soil.
[b] 1.0 = no galls, 5.0 = severely galled.
[c] CG = Charleston Gray, CS = Crimson Sweet, and SL = Smoky Lee; moderately resistant, resistant, and very resistant to wilt, respectively.
[d] Susceptible Jubilee, Garrisonian, and New Hampshire Midget; slightly resistant Blackstone and Congo; and CG, CS, and SL.

the wilt severity in the previous crop of watermelons (R^2 = 0.37), but that a highly significant additional 18% of the variation could be attributed to root-knot nematodes. In the wilt-resistant Crimson Sweet, more of the variation in wilt was explained by root-knot severity on tomatoes (R^2 = 0.077) than by wilt severity in the previous crop of watermelons (R^2 = 0.073). However, R^2 ranged from 0.1 to 0.5 for individual cultivars, indicating that factors other than those measured greatly influenced the variation in wilt. The increase in R^2 attributable to root-knot nematodes was greatest in Hales Best Jumbo (R^2 = 0.13) and Charleston Gray (R^2 = 0.10) and least in Smoky Lee (R^2 = 0.01). No other factor, except root-knot nematodes, significantly influenced severity of wilt in the muskmelon cultivar. Only one squash plant wilted or was damaged by root rot in any of the soils, indicating that the wilting and dying of the watermelon cultivars was primarily caused by *F. oxysporum* f. sp. *niveum*.

In the second experiment with controlled populations of *F. oxysporum* f. sp. *niveum*, root-knot nematodes increased severity of wilt and decreased foliage weights only at 650 PG of *F. oxysporum*. All cultivars were severely damaged with 17,584 PG of *F. oxysporum*, even when root-knot nematodes were absent, and no damage by root-knot nematodes was evident with 216 PG of total *F. oxysporum* in soil noninfested with *F. oxysporum* f. sp. *niveum* (Table 2).

Emergence was not significantly influenced by *F. oxysporum* populations. In contrast, emergence of Jubilee, but not of other cultivars, was significantly reduced by *M. arenaria* but not by the other nematodes.

The only significant increase in wilt and root necrosis was caused by *M. arenaria* 51 to 54 days after planting in Charleston Gray at 650 PG of *F. oxysporum*. With *M. arenaria*, 28% of the plants were wilted or had 50% root necrosis, as compared to no wilting or root necrosis without *M. arenaria*. Severity of wilt in Jubilee was also increased from 40 to 71% by *M. arenaria* at 650 PG of *F. oxysporum*, but no increase in wilt was noted in Crimson Sweet. Foliage weight was decreased 11 to 13% in all cultivars by *M. arenaria* at 650 PG of the fungus.

The increase in wilt caused by *M. arenaria* was not apparent 18 days after planting, when only 15% of the plants were wilting at 17,584 PG of the fungus, but from 25 through 54 days, differences were obvious at 650 PG (Fig. 1). Total plant weight of Charleston Gray was significantly less than Crimson Sweet with *M. hapla*, but not with other nematodes or in the control at 17,584 PG of *F. oxysporum*. There were no significant differences among any of the nematode-cultivar interactions at 216 PG of *F. oxysporum*.

Significantly more root galls were caused by *M. arenaria* and *M. javanica* than by *M. hapla* (Table 2). *Meloidogyne hapla* caused only slight galling on Charleston Gray and Crimson Sweet, and no galls were observed on Jubilee. No root galls were seen on control plants. So many plants were dead or dying at 17,584 PG of *F. oxysporum* when the experiment was terminated, that the root-gall indices were not as high as in the other treatments. With 216 PG of *F.*

TABLE 2. The influence of three root-knot (*Meloidogyne* spp.) nematodes on severity of watermelon wilt in three cultivars of watermelon at three levels of inoculum density of *Fusarium oxysporum* in Dothan loamy sand. Mean of four replications

Treatment means	Wilted plants (%) days 25	Wilted plants (%) days 51-54	Total fresh weight of plants, g	Root-gall index[z]
Fusarium oxysporum, PG[w]				
216	1 a	3 a	80 a	1.6 a
650	6 a	21 b	45 b	1.8 a
17,584	49 b	70 c	35 b	1.2 b
Nematode				
M. arenaria	23	33	52	2.2 a
M. javanica	17	29	54	1.9 a
M. hapla	15	28	52	1.0 b
None	17	32	54	1.0 b
	NS[y]	NS	NS	
Cultivar[x]				
Jub.	26 a	50 a	45 a	1.5 ab
CG	18 ab	29 b	54 b	1.4 b
CS	12 b	16 c	61 c	1.6 a

[w] PG = propagules of total *F. oxysporum* per gram of air-dry soil.

[x] Jub. = Jubilee, CG = Charleston Gray, and CS = Crimson Sweet; susceptible, moderately resistant, and resistant to Fusarium wilt, respectively.

[y] Numbers followed by the same letter are not significantly different ($P = .05$), NS = nonsignificant.

[z] 1.0 = no galls, 5.0 = severely galled.

oxysporum, *M. arenaria* caused significantly more galling than *M. javanica* and *M. hapla*, but there was no significant difference between the latter two nematode species. At 650 PG of *F. oxysporum*, galling caused by *M. arenaria* was not significantly different from that caused by *M. javanica*, but both nematode species caused significantly more galling than *M. hapla*. The average root-gall indices for *M. arenaria*, *M. javanica*, and *M. hapla* were 2.7, 2.3, and 1.0, respectively.

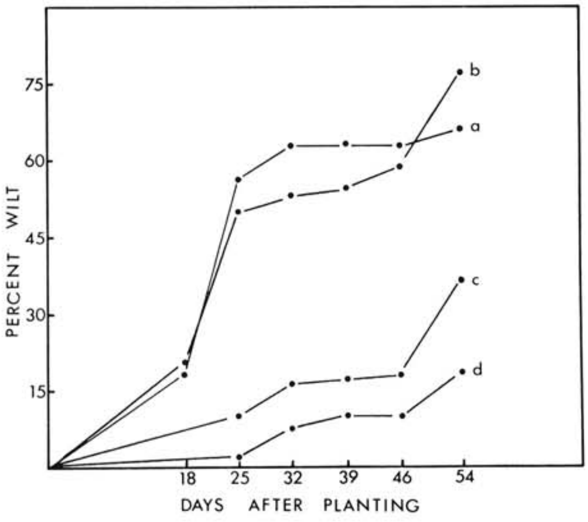

Fig. 1. The influence of *Meloidogyne arenaria* on Fusarium wilt in watermelon. Mean of three cultivars at two populations of *Fusarium oxysporum* f. sp. *niveum*. a = 17,584 propagules/g (PG) of *F. oxysporum* with *M. arenaria*; b = 17,584 PG of *F. oxysporum* with no root-knot nematodes; c = 650 PG of *F. oxysporum* with *M. arenaria*; and d = 650 PG of *F. oxysporum* and no root-knot nematodes.

There were significantly more root galls caused by *M. javanica* on Crimson Sweet than on Charleston Gray at both 216 and 650 PG of *F. oxysporum*, averaging 2.4 and 2.1, respectively. The number of root galls caused by *M. arenaria* was not significantly different among cultivars. Nevertheless, *M. arenaria* caused significantly more galling on Charleston Gray than *M. javanica* at 216 and 650 PG of *F. oxysporum*, but *M. javanica* caused significantly more galling than *M. hapla* only on Charleston Gray at 650 PG of *F. oxysporum*.

DISCUSSION.—Root-knot nematodes increased the susceptibility of resistant watermelon cultivars to Fusarium wilt. Similar Fusarium wilt-root-knot interactions were reported in tomato, cotton, and cowpea (8). However, root-knot nematodes increased wilt in watermelon only at intermediate populations of the fungus. Evidently an ID greater than 216 PG is necessary for nematodes to enhance wilt, whereas an ID above 1,000 to 2,000 PG is sufficient to cause severe damage in resistant cultivars even without the presence of root-knot nematodes (12). Nevertheless, in soils naturally infested with both root-knot nematodes and the wilt pathogen, interactions might occur at other fungus-nematode ratios in some highly resistant cultivars.

We found an ID of *F. oxysporum* of 1,900 and 3,400 PG in two soils immediately after a crop of cotton, but an average of only 12 and 8% wilt, respectively, in eight cultivars planted in those soils. Other crops susceptible to *F. oxysporum*, such as sweet potato, cowpea, bean, okra, and tomato, may also build up populations of the fungus that are not pathogenic on watermelon. In addition, saprophytic isolates of *F. oxysporum* are ubiquitous in soil, indicating the limitation of using ID to predict wilt severity.

All cultivars used in this study were susceptible to *M. incognita, M. arenaria*, and *M. javanica* (13). Winstead & Riggs (17) also reported that Charleston Gray, Blackstone, Congo, and New Hampshire Midget were susceptible to the same three nematode species but were resistant to *M. hapla*. Sasser (9, 10) reported that 'Dixie Queen' was resistant to *M. hapla* but susceptible to *M. arenaria, M. javanica*, and *M. incognita*. In our study, *M. hapla* did not reproduce on watermelons in artificially infested soil, but the nematode did reduce plant growth. The high daytime soil temperature maxima of 35 to 45 C during several weeks of the second experiment may have prevented reproduction of *M. hapla*. Walker (16) reported that *M. javanica* and *M. arenaria* were more resistant to high temperatures than *M. hapla*.

The most common root-knot nematode found in soils in the Southern Coastal Plain is *M. incognita*. Nevertheless, other species of root-knot nematodes occur throughout the watermelon-growing areas in southern Georgia and attack many agricultural crops grown in rotation with watermelon. *Meloidogyne arenaria* infects many plants. It is common in the peanut-growing area of South Georgia, and it is also a destructive parasite of tobacco. *Meloidogyne javanica* infects many of our agricultural plants, including peanuts, tobacco, soybean, corn, squash, cucumber, and tomato. *Meloidogyne hapla* has a limited distribution in Georgia but a wide host range.

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