Influence of *Meloidogyne incognita* on Fusarium Wilt of Tomato at or below the Minimum Temperature for Wilt Development[1]

J. J. Morrell and J. R. Bloom[2]

Abstract: 'Bonny Best' tomato plants were grown at 16, 21, or 24 C for 28 d in soil infested with either of two isolates of *Fusarium oxysporum* f. sp. *lycopersici* race 1 and *Meloidogyne incognita*. Significant levels of fusarium wilt occurred at all temperatures including 16 C, which has not been reported previously. One *Fusarium* isolate resulted in the highest levels of disease incidence at 21 and 24 C in the presence of root-knot nematodes, and at 24 C when the nematodes were not present. At 16 C there was no significant difference in the number of plants infected by the second *Fusarium* isolate alone or in combination with root knot nematodes, although the presence of nematodes resulted in a significant increase in the percentage of disease occurrence and vessel infection at 21 C. *Key words:* root-knot, *Fusarium oxysporum* f. sp. *lycopersici* race 1, *Lycopersicon esculentum*.

Clayton (1) reported the minimum temperature at which fusarium wilt developed in susceptible tomato 'Chalk's Early Jewel' and 'Mangus' was 21 C. Below this temperature, susceptible tomatoes remained free of the disease. Several workers have demonstrated that polygenic resistance to *Fusarium* can be broken in tomato in the presence of root-knot nematodes (3,5). Would it also be possible to induce fusarium wilt below 21 C in the presence of root-knot nematodes? If polygenic resistance to *Fusarium* can be broken by root-knot nematodes, can resistance induced by low temperatures also be broken? This study reports the results of experiments designed to determine if fusarium wilt will develop below 21°C in the presence of *Meloidogyne incognita* (Kofoid & White) Chitwood.

MATERIALS AND METHODS

Fusarium wilt susceptible 'Bonny Best' tomato seedlings were transplanted into sterilized (1% Na OCl) 946-ml plastic pots containing a mixture of steamed sand, soil, and peat (1:1:1). After transplanting, the pots (without drainage holes) were immersed in water in Cornell-type temperature tanks maintained at 16, 21, or 24 C. Soil temperatures, as measured at the center pot, ranged ± 1 C.

Four days after transplanting, the soil in each of the pots in each temperature treatment was infested with 10,000 *M. incognita* eggs suspended in 10 ml of sterile distilled water. The eggs were extracted from freshly harvested roots of tomato 'Rutgers' grown in infested soil as described by Hussey and Barker (4). Control pots received 10 ml of sterile distilled water.

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[1]Paper number 5918 in the Journal Series of the Pennsylvania Agricultural Experiment Station; and contribution number 1157, Department of Plant Pathology, Pennsylvania State University.

[2]Respectively, Graduate Research Assistant, Syracuse University, Syracuse, NY 13210; and Professor, Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802.

Application of *F. oxysporum* f. sp. *lycopersici* race 1 was delayed 2 wk from the time of nematode infestation to allow for nematode establishment (7). The experiment was conducted using two isolates of the fungus; one isolate was tested only at 16 and 21 C. Both isolates were obtained from lyophilized cultures maintained by the Fusarium Research Center at Pennsylvania State University. The isolates were grown for 8 d before inoculation. The first isolate was grown on potato-dextrose agar (PDA) plates and harvested by flooding the plates with sterile distilled water. The resulting suspension was then strained through cheesecloth to remove mycelial fragments. The second isolate was grown in potato-dextrose broth (PDB) shake cultures and strained to remove large mycelial fragments. Both suspensions were adjusted to a final concentration of 500,000 spores/ml using a hemacytometer, and 10 ml of this solution was delivered into holes in the soil surface of half the pots previously receiving the nematode inoculum and half of the control pots. Treatments consisted of control, nematode alone, *Fusarium* alone, and nematode plus *Fusarium*.

The plants were maintained at the stated temperatures for 28 d after *Fusarium* infestation. Stems of plants which succumbed before the harvest date were examined for the presence of vascular discoloration and cultured on PDA for the presence of *Fusarium*.

On the harvest date all plants were cut at the groundline. The stems were dipped in 70% ethanol and flamed to remove excess alcohol. Several sections were cut from the base of the stem and placed on PDA plates. Stem sections on PDA were observed for up to 21 d for the presence of *Fusarium*. A free-hand section was cut from each of the first three internodes to quantitatively determine the longitudinal extent of vascular discoloration. These sections were examined under a microscope and counts made of the total number of vascular bundles exhibiting discoloration. The fresh mounted tissue sections were incubated 24 h at room temperature (18 C) then stained in 1% cotton blue in lactophenol for 30 s, cleared in concentrated phenol, and the number of vascular bundles and infected vessels (those containing blue stained hyphae) counted.

After analysis of variance using an arcsin transformation, mean separation procedures were accomplished using Duncan's modified least significant difference test. Unless otherwise stated, all mean separations were done at k = 100 (9).

RESULTS AND DISCUSSION

Control tomato plants and plants grown in soil infested only with nematodes showed no symptoms of fusarium wilt, indicating the soil and the nematode inoculum were free of *F. oxysporum* f. sp. *lycopersici*. The roots of all plants grown in nematode infested soil were galled, although the degree of galling was not quantified.

In experiments using fungus isolate 1, the presence of root-knot nematodes resulted in a higher occurrence of fusarium wilt disease than with the fungus alone (Table 1). The increase of fusarium wilt of tomato when grown in the presence of root-knot nematodes was indicative of an interaction between the two organisms.

Table 1. The occurrence of *Fusarium oxysporum* f. s. *lycopersici* (isolate 1) and the percentage of vessel infection in tomato plants treated as indicated.*

	Treatment	% incidence†	Vascular bundles % infected/ plant
16 C	Control	0 D	0.0 E
	Meloidogyne incognita (Mi)	0 D	0.0 E
	Fusarium (F)	30 C	8.8 D
	F + Mi	55 B	30.0 B
21 C	Control	0 D	0.0 E
	Mi	0 D	0.0 E
	F	30 C	14.0 C
	F + Mi	90 A	71.5 A
24 C	Control	0 D	0.0 E
	Mi	0 D	0.0 E
	F	60 B	35.0 B
	F + Mi	90 A	75.0 A

*Based on results of plating stem sections on potato dextrose agar.

†Values followed by the same letter are not significantly different from one another according to Duncan's modified least significant difference test at k = 100. Values for 16 and 21 C, 20 replications each, represent means for experiments completed at two different dates. Values for 24 C are means of 10 replications.

The highest percentage of disease following infestation with the nematodes plus the fungus occurred at 21 and 24 C. When the fungus was used alone, the highest percentage of disease occurred at 24 C. When the percentage of infected vascular bundles per plant per treatment was compared, a similar relationship in disease occurrence was encountered, although the differences were much greater (Table 1).

The use of stem section culturing provides a means of assessing the occurrence of the fungus, but not disease severity. Conway and MacHardy (2) used a vessel counting technique to determine the extent of colonization by the fungus. The number of infected vascular bundles per stem was found to be related to the degree of colonization by the fungus, which could be used as a measure of severity of infection. If a plant with a higher percentage of infected vascular bundles could be considered to be more severely infected, then the presence of root-knot nematodes and the causal fungus resulted in a significant increase in the severity of fusarium wilt at 16, 21, and 24 C. Since the fungus infected 'Bonny Best' at 16 C, a fact not previously reported, it appears the isolates have a lower temperature tolerance than isolates used in previous temperature related studies.

The results obtained using *Fusarium* isolate 2 were less pronounced than those of the first isolate. The combination of root-knot nematodes with the fungus showed an increase in the occurrence of fusarium wilt at 21 C, but not at 16 C (Table 2). The reduced level of interaction between root knot nematodes and *Fusarium* at the lower temperature, expressed as lower percentages of disease occurrence suggests that the nematodes did not have the ability to predispose the tomatoes to isolate 2 of the fungus at 16 C (Figure 1).

Although the presence of the nematodes did not significantly increase the disease-producing ability of isolate 2 at 16 C, the level necessary to be of significance was correspondingly higher than would be necessary if the incidence of fusarium wilt was

Table 2. The occurrence of *Fusarium oxysporum* f. s. *lycopersici* (isolate 2) and the percentage of vessel infection in tomato plants treated as indicated.*

	Treatment	% occurrence†	% vascular bundles infected
16 C	Control	0% C	0.00% C
	Meloidogyne incognita (Mi)	0 C	0.00 C
	Fusarium (F)	56 B	23.44 B
	F + Mi	67 B	31.48 B
21 C	Control	0 C	0.00 C
	Mi	0 C	0.00 C
	F	59 B	25.93 B
	F + Mi	79 A	55.74 A

*Values followed by the same letter are not significantly different from one another according to Duncan's modified least significant difference test at k= 100. Analysis of 27 replications.

†Based on results of plating stem sections on potato dextrose agar.

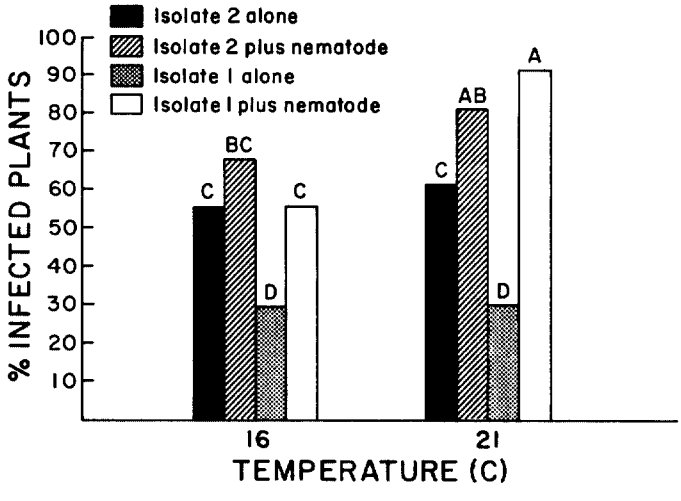

Fig. 1. Effect of root knot nematode (*Meloidogyne incognita*) and *Fusarium oxysporum* f. sp. *lycopersici*, isolates 1 and 2 on the occurrence of fusarium wilt in tomato plants. Bars subtending the same letter are not significantly different according to Duncan's modified least significant difference test using k = 100. Values obtained for the isolate 1 reresent the means of 20 observations, while those for the isolate 2 represent means of 27 observations.

lower for the fungus treatment alone. Thus, isolate 2 was probably more virulent than isolate 1, and its effect upon the host was only slightly enhanced by the presence of the root-knot nematode. This would be in agreement with the suggestion by Kapplemen (6) that nematodes act to render weakly virulent isolates more virulent, but have little effect on highly virulent isolates. This does not explain the corresponding increase in the interaction at 21 C. A possible explanation for this effect may be that the nematodes existed at a low level of metabolic activity at 16 C and fewer nematodes penetrated the roots (8). Although the level of activity was sufficient to increase the effect of a weakly virulent isolate, it was not sufficient to increase the effect of a highly virulent isolate.

It is evident from the results of both experiments that the fungal isolates infected tomato 'Bonny Best' at a temperature five degrees lower than previously reported for tomatoes (1). The reason for this deviation from previous reports could be that the isolate of *Fusarium* used by Clayton was less active at temperatures below 21 C than the isolates used in these experiments. In addition, it is likely that 'Bonny Best,' although susceptible to fusarium wilt, differed physiologically from the cultivars used by Clayton. Thus, its response at lower temperatures may have varied widely from the responses of the cultivars used by Clayton.

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