Effect of Meloidogyne javanica on Rhizosphere Microflora and Fusarium Wilt of Tomato

G. B. Bergeson, S. D. Van Gundy, and I. J. Thomason

Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907; and Department of Nematology, University of California, Riverside 92502.

Journal Paper No. 3904, Purdue University.

Sincere appreciation is extended to D. C. Erwin and P. H. Tsao, Department of Plant Pathology, University of California, Riverside, for their help and advice, and to the University of California for financial support to the senior author during sabbatical leave.

Accepted for publication 20 March 1970.

ABSTRACT

Propagules of *Fusarium oxysporum* f. sp. *lycopersici* were more numerous in the rhizosphere of tomato infected with *Meloidogyne javanica* than in rhizospheres of noninfected plants. Combined infection by the fungus and nematode caused increased growth of the fungus in the roots but not in the foliage. Growth reduction due to fungus-nematode complex appeared to be independent of the amount or extent of mycelium growth in the stem. Numbers of actinomycetes were lower in the rhizosphere of galled roots. When actinomycetes increased after chitin soil amendments, there was a marked decrease in secondary invasion of root galls by other microorganisms. Phytopathology 60:1245-1249.

Effects of root exudate on the rhizosphere microflora are well known, but recent emphasis has been on their role in overcoming soil fungistasis. Schroth & Snyder (14) showed that germination of chlamydospores of *Fusarium solani* f. sp. *phaseoli* in soil occurred only within 1 mm of germinating bean seeds or growing root tips. They detected several sugars and amino acids in these exudates which caused a 20-40% germination of chlamydospores (0% for controls) when applied to the soil in a 1% concn. Even exudate from nonhost plants such as tomato, lettuce, and corn furnished sufficient nutrient to stimulate germination and increase the inoculum level in the soil (13). Schroth et al. (15) later identified 22 amino acids from bean seed and root exudate, and showed that the exudate from a single seed stimulated chlamydospore germination when added to the soil. Similarly, germination of microsclerotia of *Verticillium albo-atrum* are stimulated by the addition of glucose and sucrose to the soil according to Green & Papavizas (4).

Since root knot infection is known to increase concn of carbohydrates, amino acids, proteins, and lipids in roots (12), we sought initially to compare the effect of exudate from galled vs. nongalled roots on the germination of chlamydospores of *Fusarium oxysporum* f. sp. *lycopersici* Snyd. & Hans. While conducting preliminary experiments, quantitative changes in the population of rhizosphere actinomycetes were noted along with some peculiarities in the root knot-*Fusarium* complex. These were further investigated and reported herein.

MATERIALS AND METHODS.—*Fusarium-nematode interaction under optimum temp.*—River sand soil (steamed at 60 C for 30 min 3 months previously) was seeded with micro- and macroconidia of *F. oxysporum*. The inoculum was increased on V-8 juice agar from a single spore isolate previously checked for virulence on tomato, *Lycopersicon esculentum* Mill. 'Bonny Best'. This variety was used in all experiments. Spore suspensions were sprayed on soil being mixed in a cement mixer. The soil was then stored for at least 10 days to allow conidia to convert to chlamydospores (11). Just before use, the number of propagules/g of soil was determined by plating soil dilutions to a selective peptone-pentachloronitrobenzene (PCNB) agar according to the procedures of Nash & Snyder (11), but modified by increasing streptomycin to 1,000 ppm and adding neomycin to give 100 ppm (W. C. Snyder, *personal communication*). On the basis of these counts, the soil was mixed with noninfested soil to give propagules/g of soil of 9,500 and 19,000. Noninfested soil served as a control. Soil was placed in 7-quart plastic containers and transferred to constant-temp water tanks at 27 C (average min and max air temp were 20 and 31 C, respectively). Half of the containers were planted with washed tomato transplants which had been inoculated 2 weeks earlier while in a 3-4 petiole stage with 10,000 larvae of *Meloidogyne javanica* (Treub) Chitwood. Larvae were obtained by placing thoroughly washed, galled bean and tomato roots in tissue-paper-lined wire baskets nestled in glass funnels. These were placed in a mist chamber for 24 hr. Larvae emerging from egg masses on the roots were carried by water into the funnel stem and collected in a test tube. Noninfected plants of the same age were planted in the remaining containers. Infected plants were moderately galled (averaged 2.8 on a 0-4 scale) at time of transplanting. After 5 weeks, the containers were inverted and the plant removed with root ball intact. The root ball was squeezed to remove peripheral soil, and the plant was gently shaken 3 times. Soil remaining on the roots was considered to be rhizosphere soil and was removed by vigorous shaking. Actinomycetes were estimated from rhizosphere soil suspensions prepared by adding the equivalent of 1 g oven-dried soil to 100 ml sterile, distilled water, followed by 20 min mixing on a wrist action shaker. Further dilutions were made with sterile water, and 1 ml aliquots were mixed with 15 ml molten water agar (considered to be selective for actinomycetes) at 40-45 C in petri dishes (6). Another portion of the rhizosphere soil was air-dried, and the *Fusarium* propagules were determined on PCNB agar as previously described. Actinomycetes and *Fusarium* colonies were counted after 5 days at room

temp. Plants were processed as follows: petioles and leaves were cut from the stem, oven-dried, and weighed. Stems were detached from the roots and cut into 2-cm segments, dipped in 95% ethanol, flamed, and placed in their natural sequence on water agar plates. After 5-7 days at room temp, the density of the *Fusarium* mycelium on each segment was rated on a 0-4 scale. Fresh root segments were collected from each plant and homogenized in sufficient sterile water to make a $1:10^5$ dilution, and 1 ml aliquots were plated to PCNB agar. The remainder of the roots were oven-dried and weighed.

RESULTS.—Plant growth was substantially reduced by all treatments (Table 1). However, the interaction between *Fusarium* and root knot was additive rather than synergistic. Reduction and necrosis of roots were particularly striking, but this may have been aggravated by poor drainage in the containers. Mycelium density of stem sections was greater in the absence of root knot infection; whereas *Fusarium* propagules/g of root were greatly increased by root knot infection. *Fusarium* propagules in the rhizosphere soil of galled plants were consistently higher, but differences were not significant. There was a highly significant reduction of actinomycetes in rhizosphere soil of all treatments with root knot nematode.

Fusarium-nematode interaction under sub-optimum air temp.—Results of the preceding experiment made it desirable to restrict the *Fusarium* infection to the root system to determine if synergism is confined to this area. This was attempted by growing plants at a soil temp optimum for *Fusarium* wilt development (27 C), but at an air temp (16-20 C) suboptimum for foliage infection. Heating coils with thermostatic controls were placed in large, sand-filled metal containers (58 × 48 × 23 cm) located in a controlled climate chamber. Clay pots (15 cm diam) were filled with *Fusarium*-seeded soil (14,000 propagules/g) and an equal number with noninfested soil. Three-week-old tomato plants inoculated 1 week previously with 12,000 *M. javanica* larvae were transplanted to half the pots in each treatment. At transplanting they received an additional 4,500 larvae. Noninfected plants were transplanted to the remaining half of the pots. Treatments were as follows: (A) neither fungus nor nemas; (B) nemas alone; (C) fungus alone; and (D) fungus plus nemas. Nine pots from each treatment were buried in the sand-filled containers and covered with 1-2 inches of Perlite to minimize heat loss from the soil. A thermometer was placed 3 cm above the pot surface, and the air temp adjusted so that the ambient foliage temp would not exceed 20 C during daytime or 16 C at night. At foliage height, the illumination was 4,100 ft-c with a 16-hr photoperiod. A duplicate set of treatments was placed in the greenhouse for comparison. The average min and max greenhouse temp were 23 and 32 C, respectively. After 5 weeks, the plants and rhizosphere soil were processed as before, except that actinomycetes were not enumerated. For a better indicator of foliage infection, the mycelium density rating of stem sections was multiplied by the percentage of the stem invaded by mycelium. This is referred to as the foliage infection index.

The fungus alone caused a significant reduction (Table 2) of shoot growth under greenhouse conditions, but not in the controlled climate chamber. Nematodes alone did not significantly affect shoot growth in either location. The fungus-nematode combination caused a striking synergistic reduction of 61 and 55% in the greenhouse and controlled climate chamber, respectively. Root wt was significantly reduced by treatments B, C, and D in both locations. *Fusarium* propagules in rhizosphere soil were increased by root knot infection in both locations, but the increase was significant in only one. There were no statistical differences between the foliage infection indices of fungus alone vs. the nematode-fungus treatment in either location.

Rhizosphere studies in root observation boxes.—Young tomato plants with or without a 2-week-old root knot infection were transplanted to root observation boxes (25 × 18 × 29 cm) filled with either *Fusarium*-seeded soil (18,000 propagules/g of soil) or noninfested soil. The two lateral walls were removable, and the ends were slotted to hold a piece of glass between the soil and wall. Eight boxes, two compartments and two plants/compartment, were placed in

TABLE 1. Effect of *Meloidogyne javanica* infection on number of *Fusarium oxysporum* f. sp. *lycopersici* propagules in root, stem, and rhizosphere; on actinomycetes in rhizosphere; and on wilt severity in the host[a]

Treatment	Dry wt, g		*Fusarium*		Rhizosphere population/g of soil (× 10^3)	
	Shoot	Root	Propagules/g of root (× 10^3)	Mycelium density index in stem[b]	*Fusarium*	Actinomycete
Control	8.6	3.5	0	0.0	0.0	23.0
Nematode[c] alone	2.8	1.8	0	0.0	0.0	2.0**
Fungus[d] alone	3.3	0.8	6	3.1	0.4	19.0
Fungus[e] alone	2.8	0.7	40	3.2	0.6	26.4
Nematode[c] + fungus[d]	1.7	1.4	1,000**[f]	2.5	16.0	7.0**
Nematode[c] + fungus[e]	2.6	1.2	3,000**	2.1*	2.5	9.6**

[a] Average of four replications (colony counts based on four plates/replication).
[b] Index based on a visual 0-4 rating of mycelium density of plated stem sections.
[c] Inoculum level = 10,000 larvae/pot.
[d] Inoculum level = 9,500 propagules/g of soil.
[e] Inoculum level = 19,000 propagules/g of soil.
[f] * = Significant at 5% level. ** = Significant at 1% level.

TABLE 2. Effect of *Meloidogyne javanica* on *Fusarium oxysporum* f. sp. *lycopersici* in foliage and rhizosphere of host and on wilt development under two temp regimes[a]

Treatment	Dry wt, g		*Fusarium* propagules/g of rhizosphere soil ($\times 10^3$)	Foliage infection index[b]
	Shoot	Root		
Greenhouse[c]				
Control	9.2	2.00	0	0
Nematode[d]	7.6	1.28*	0	0
Fungus[e]	7.0*[g]	1.35*	6.2	240
Nematode + fungus	3.4**	0.81*	15.5	229
Controlled climate[f]				
Control	6.4	1.85	0	0
Nematode[d]	5.9	1.55*	0	0
Fungus[e]	7.4	1.43**	5.3	129
Nematode + fungus	2.4**	0.85**	17.3*	141

[a] Average of nine replications (colony counts based on four plates/replication).
[b] Index based on mycelium density of plated stem section × per cent of stem infected.
[c] Average daily temp range = 23-32 C.
[d] Nematode inoculum = 16,500 larvae/pot.
[e] Fusarium inoculum = 14,000 propagules/g of soil.
[f] Air temp day 20, night 16 C, soil temp 27 C.
[g] * = Significant at 5% level. ** = Significant at 1% level.

the greenhouse with average min and max temp of 23 and 32 C, respectively. Each treatment had eight replications. After 3 weeks the boxes were tilted over at a 45 degree angle, and the wood and glass sides removed to expose the soil profile. Roots on or near the surface were carefully lifted with dissecting needles, and a paper towel was inserted between the root and the soil surface. Soil clinging to the roots was brushed off onto the paper with a small brush. Two exploratory and one final sample were taken at weekly intervals. Soil was processed for actinomycetes and *Fusarium* counts as previously described.

The final and most extensive sampling of rhizosphere soil (Table 3) showed that root knot infection caused a highly significant reduction of actinomycetes and a significant increase of *Fusarium* propagules. Two earlier samplings showed a similar pattern, but differences were not significant due to large variation and fewer replications.

Influence of chitin amendments.—Two soil types, a silty loam and a river sand soil, were amended with crude chitin at 0 and 1 g/kg of soil and with steer manure at 0, 5, 25, and 125 g/kg of soil to stimulate actinomycete reproduction. Amendments were mixed with the soil for 15 min in a cement mixer, then stored in 5-gal crocks in the greenhouse for 3 weeks to allow microflora to stabilize. Soil was sampled prior to treatment to determine initial numbers of actinomycetes. After storage the soil was transferred to 15-cm plastic pots. Half the pots in each treatment were planted with 4-week-old okra plants (*Hibiscus esculentus* L. 'Clemson Spinless') inoculated 2 weeks previously with 11,000 *M. javanica* larvae. Noninfected okra was transplanted to the other half of the pots. After growing 6 weeks in the greenhouse, plants were removed, rhizosphere soil was collected, and actinomycetes were enumerated. The roots were washed and examined for galling and necrosis.

Pretreatment estimation of actinomycetes in the silt-loam and sandy soil was made at dilutions of 10^4, 10^5, and 10^6. The silty-loam soil had a count of 460,000/g of soil, whereas the number in sandy soil was below the detection level at the 10^4 dilution. The results (Table 4) show that chitin has a greater influence than steer manure on the stimulation of actinomycetes in sandy soil. Numbers of actinomycetes were suppressed

TABLE 3. Comparison between numbers of actinomycetes and *Fusarium oxysporum* f. sp. *lycopersici* propagules in rhizosphere of *Meloidogyne javanica* infected vs. noninfected tomatoes grown in root observation boxes

Treatment	Actinomycetes/g of soil ($\times 10^3$) Samplings			*Fusarium* propagules/g of soil ($\times 10^3$) Samplings		
	1[a]	2[b]	3[c]	1[a]	2[b]	3[c]
Nematode[d] + fungus[e]	60	43	67**[f]	5.2		60*
Fungus[e] alone	89	146	275	0.4		14

[a] Average of two replications.
[b] Average of four replications.
[c] Average of eight replications.
[d] Nematode inoculum = 17,000 larvae/plant.
[e] Fungus inoculum = 18,000 propagules/g of soil.
[f] * = Significant at 5% level. ** = Significant at 1% level.

TABLE 4. Effect of chitin and steer manure on actinomycete population in a blow sand soil

Treatment	Application rate, g/kg of soil	Actinomycetes/g of soil ($\times 10^3$)
Chitin	1	330**[a]
Steer manure	5	69
Steer manure	25	214**
Steer manure	125	13*
Control (root knot-infected)	0	10*
Control (noninfected)	0	81

[a] * = Significant at 5% level. ** = Significant at 1% level.

at the highest level of steer manure, but were present in significantly greater numbers at the medium level. Response of actinomycetes to steer manure amendments in the silt-loam soil was erratic and inconclusive; however, a twofold increase occurred in the presence of chitin. Many of the okra plants, both infected and noninfected, died when transplanted, presumably from transplant shock, although the highest rate of steer manure was obviously toxic. Of the surviving root knot-infected plants, those growing in chitin amended soil were larger and their root systems were much less necrotic (Fig. 1) than those grown in nonamended soils, even though the amount of galling was about the same. Because of the uneven number of plants surviving in different treatments, no attempt was made to statistically analyze growth difference.

DISCUSSION.—The number of *F. oxysporum* propagules was greater around galled roots than nongalled roots in three separate experiments. This increase may be accounted for by assuming that (i) the fungus multiplies more rapidly than normal in galled roots (Table 1) and as shown by Melendez & Powell (9), and releases a larger number of propagules when the roots decompose which is also accelerated by root knot infection; and/or (ii) exudates from galled roots provide a greater stimulus for germination of chlamydospores. According to Schroth & Hendricks (13), germinated chlamydospores are able to use exudate as a substrate for further growth and reproduction. The latter possibility may be a heretofore unrecognized factor contributing to the commonly observed synergism between these two pathogens. Subsequent to the initiation of our studies, Beute & Lockwood (1) reported that synergism between *F. solani* and bean yellow mosaic virus was due to greater amino acid content in the root exudate of virus-infected plants which stimulated growth of *Fusarium* in the rhizosphere.

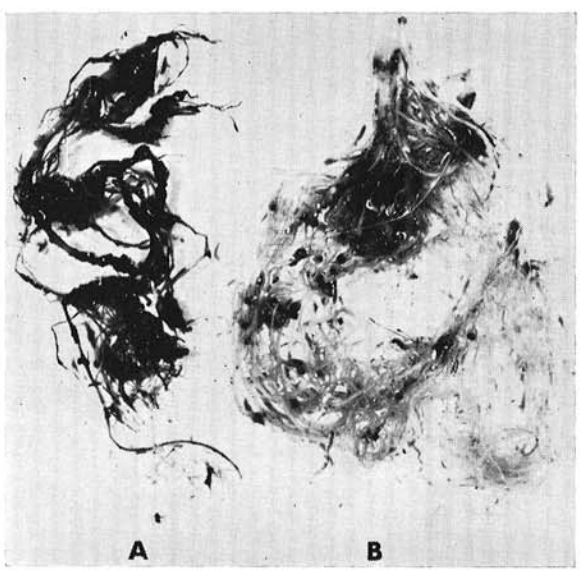

Fig. 1. Comparison of root necrosis between *Meloidogyne javanica*-infected okra planted **A)** in nonamended soil; **B)** chitin-amended soil.

Root knot infection did not increase the amount or extent of mycelium growth in the stems. It appears that synergistic action is confined to the root system, and that growth reduction caused by the pathogen complex is independent of foliage infection. Our attempt to test this idea by restricting the *Fusarium* infection to the root system by lowering the air temp as reported by Clayton (2) was only partially successful, since the foliage infection index was reduced only about 50%. In spite of this reduction, the nematode-fungus combination caused as much growth reduction as under conditions optimum for development of foliage infection. Either the nematode-fungus interaction causes sufficient root injury to account for reduced foliage growth, or a toxin of root origin is translocated to the foliage.

Depression of actinomycete numbers in the rhizosphere of galled roots was a consistent phenomenon in our experiments. A possible explanation is that exudates from galled roots favor competitors or antagonists of actinomycetes. A direct depressing effect by the exudate per se is a less likely reason. It is probable that many microorganisms that are thus favored become secondary invaders of galled roots (8). When chitin was added, conditions for actinomycetes growth became favorable and the effect on actinomycetes by galled root exudate was nullified. In our experiments, root knot-infected okra transplanted into chitin-amended soil showed much less secondary invasion of the root system as compared with those grown in nonamended soil. This response was limited to a soil with a low initial actinomycetes population. Mankau & Das (7) reported reduced root knot damage in chitin-amended soils, but they attributed this to an effect on the larvae per se. Since the larvae in our experiments were already within the roots, and development of infection and the nematodes was normal, a direct effect of chitin on the nematode would not explain our results. A somewhat similar condition exists in PCNB-induced disease accentuation as reported by Farley & Lockwood (3). They suggest that "PCNB decreases competition for nutrients in soil by suppressing PCNB-sensitive actinomycetes and fungi. The reduced competition allows populations of root-infecting fungi insensitive to PCNB to increase selectivity". Tobacco varieties resistant to fusarium wilt have a higher population of antagonistic actinomycetes in the rhizosphere than do susceptible varieties, and this effect is attributed to differences in root exudate (16). Horst & Herr (5) reported that the numbers of actinomycetes in the rhizosphere of corn seedlings that were antagonistic to *Fusarium roseum* f. sp. *cerealis* could be changed by foliar application of urea. Mitchell & Alexander (10) reported that significant control of *F. solani* was obtained by adding chitin (500 lb./acre) to the soil. They concluded that chitin preferentially encourages organisms such as actinomycetes that produce antibiotics or show mycolytic activities against the *Fusarium*.

In summary, it appears that (i) in the rhizosphere soil of galled roots, actinomycete numbers are depressed and *Fusarium* is favored. Previous work (5, 10, 16) indicates that the two effects are correlated; (ii)

synergistic growth reduction by the interaction of the nematode and fungus occurs independent of the intensity of foliage infection; and (iii) chitin amendment to the soil reduces secondary invasion of galled roots, and is associated with an increase in actinomycetes.

LITERATURE CITED

1. BEUTE, M. K., & J. L. LOCKWOOD. 1968. Mechanism of increased root rot in virus-infected peas. Phytopathology 58:1643-1651.
2. CLAYTON, E. E. 1923. The relation of temperature to the fusarium wilt of the tomato. Amer. J. Bot. 10: 71-88.
3. FARLEY, J. D., & J. L. LOCKWOOD. 1969. Reduced nutrient competition by soil microorganisms as a possible mechanism for pentachloronitrobenzene-induced disease accentuation. Phytopathology 59:718-724.
4. GREEN, R. J., JR., & G. C. PAPAVIZAS. 1968. The effect of carbon source, carbon to nitrogen ratios, and organic amendments on survival of propagules of *Verticillium albo-atrum* in soil. Phytopathology 58: 567-570.
5. HORST, R. K., & L. J. HERR. 1962. Effects of foliar urea treatment on numbers of actinomycetes antagonistic to *Fusarium roseum* f. *cerealis* in the rhizosphere of corn seedlings. Phytopathology 52: 423-427.
6. LINGAPPA, Y., & J. L. LOCKWOOD. 1962. Chitin media for selective isolation and culture of actinomycetes. Phytopathology 52:317-323.
7. MANKAU, R., & S. DAS. 1969. The influence of chitin amendments on *Meloidogyne incognita*. J. Nematology 1:15 (Abstr.).
8. MAYOL, P. S., & G. B. BERGESON. 1970. The role of secondary invaders in *Meloidogyne incognita* infection. J. Nematology 2:80-83.
9. MELENDEZ, P. L., & N. T. POWELL. 1967. Histological aspects of the fusarium wilt-root knot complex in flue-cured tobacco. Phytopathology 57:286-292.
10. MITCHELL, R., & M. ALEXANDER. 1962. Microbiological processes associated with the use of chitin for biological control. Soil Sci. Soc. Amer. Proc. 26:556-558.
11. NASH, S. M., & W. C. SNYDER. 1962. Quantitative estimations by plate counts of propagules of the bean root rot fusarium in field soil. Phytopathology 52:567-572.
12. OWENS, R. G., & H. N. SPECHT. 1966. Biochemical alterations induced in host tissues by root knot nematodes. Contrib. Boyce Thompson Inst. 23:181-198.
13. SCHROTH, M. N., & F. F. HENDRIX, JR. 1962. Influence of nonsusceptible plants on the survival of *Fusarium solani* f. *phaseoli* in soil. Phytopathology 52:906-909.
14. SCHROTH, M. N., & W. C. SNYDER. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus, *Fusarium solani* f. *phaseoli*. Phytopathology 51:389-393.
15. SCHROTH, M. N., T. A. TOUSSOUN, & W. C. SNYDER. 1963. Effect of certain constituents of bean exudate on germination of chlamydospores of *Fusarium solani* f. *phaseoli* in soil. Phytopathology 53:809-812.
16. WALKER, J. C. 1965. Host resistance as it relates to root pathogens and soil microorganisms, p. 314-320. *In* K. F. Baker & W. C. Snyder [ed.] Ecology of soil-borne plant pathogens. Univ. Calif. Press, Berkeley, Los Angeles.