Effect of Two Plant-Parasitic Nematodes on Fusarium Dry Root Rot of Beans

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ABSTRACT

A study was made of the role of the plant parasitic nematodes, *Pratylenchus penetrans* and a mixture of *Meloidogyne* spp. including *M. arenaria* and *M. javanica*, in the dry root rot of beans caused by *Fusarium solani* f. sp. *phaseoli*. When 'Red Kidney' bean plants were subjected to a low inoculum level of the fungus, more plants were affected by the dry root rot among plants infected by either nematode genus than among nematode-free plants. When subjected to a high inoculum level of the fungus, all plants, whether nematode-free or nematode-infected, became infected by the fungus. Nematode infection had no effect on the severity of the fungus disease at either level of fungus inoculum.

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Additional key words: nematode-fungus interactions, *Fusarium solani* f. sp. *phaseoli*, *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa, *Meloidogyne* spp., *Phaseolus vulgaris* L.

Some plant diseases have a complex etiology that involves nematodes which frequently act as initiators, cooperators, synergists, aggravators, or otherwise (13). Powell (10) thoroughly reviewed the involvement of plant-pathogenic nematodes in the etiology of plant diseases in which fungi had previously been recognized as the causal agents. He discussed some of the possible mechanisms of these fungus-nematode interrelationships, but admitted that the basis for many of them was still poorly understood.

It has been frequently reported that plants infected by a nematode prior to, or simultaneously with infection by a fungus, showed diagnostic symptoms of the fungus disease earlier than when only the fungus was involved (1, 4, 5, 8, 9, 11). Moreover, the incidence of fungus disease commonly was higher when the nematode was present.

The purpose of this study was to investigate whether infection of bean (*Phaseolus vulgaris* L. cv 'California Light Red Kidney') plants by the nematodes *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa and *Meloidogyne* spp., including *M. arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood, affects the development of dry root rot.

MATERIALS AND METHODS.—*Pratylenchus penetrans* was maintained and increased on alfalfa (*Medicago sativa* L.) callus on Krusberg's medium (7). Nematodes were extracted through a single milk filter by a piepan technique for 15-24 hr. The suspension of nematodes in water was used as inoculum at levels of either 1,000 or 10,000 nematodes per pot.

Meloidogyne spp. were maintained and increased on roots of tomato (*Lycopersicon esculentum* Mill. cv 'Rutgers') plants. Inoculum was prepared by washing heavily infected roots, 44- to 99-days old, cutting them into small pieces, covering them with water in a conical flask, and agitating the flask on a wrist-action shaker for 4 days. The suspension collected, containing both eggs and larvae of the nematode, was used as inoculum at levels of either 500 or 2,500 eggs and larvae per pot.

Fusarium solani f. sp. *phaseoli* was maintained on potato dextrose agar. Inoculum was prepared by washing test tube culture slants 7- to 45-days old with tap water and collecting the washings. The suspension collected, which contained only macroconidia, was used as inoculum at levels of either 50,000 or 500,000 macroconidia per pot.

The low and high levels of inoculum of the nematodes and fungus that were used had been determined by preliminary tests to produce light to medium and heavy infection, respectively.

Ten-cm clay pots were used in all experiments. These were completely filled with a greenhouse mix consisting of equal parts (v/v) of coarse sand, peat moss, and top soil. Each pot was fitted with a collar of polyethylene-lined paper adjusted to fit just inside the pot and to extend about 7.5 cm above the soil surface (14). The soil was wetted and allowed to drain for 1-2 hr. Then a narrow stake was used to punch 10 to 12 holes from the soil surface to the bottom of the pot. Three or four bean seeds (*Phaseolus vulgaris* L. cv 'California' Light Red Kidney') were placed on the soil surface, and the nematodes, when required, were added to about 40-50 ml of water and poured into the holes in the soil. The soil was then covered with 6 cm of 1:1 (v/v) mixture of greenhouse soil and vermiculite, which nearly filled the collar. Ten days were allowed for initial growth of the seedlings and then the fungus inoculum was added. The required number of fungus spores was pipetted into about 150 ml of tap water and this suspension poured onto the soil-vermiculite mix in the collar (14). In order to obtain uniform bean plants for the experiments, when the seedlings emerged, they were thinned to one per pot.

In an experiment with a particular nematode species, the seven treatments were: the fungus alone at two levels of inoculum; the four possible combinations of each of the two levels of fungus with each of the two levels of nematode inoculum; and uninoculated check plants. Root symptoms were observed on five or six plants from each treatment on

each of five dates at 4-day intervals beginning the fourth day after the *Fusarium* was added. The roots of each plant were washed and examined for severity (extent of lesion development) of the fungus disease and the number of infected plants was determined.

RESULTS.—*P. penetrans*.—All plants inoculated with *Fusarium* at a high inoculum level, showed symptoms of dry root rot. No influence of nematodes could be detected on development of the rot, which was equally severe in nematode-infected and nematode-free plants.

Of the plants inoculated with *Fusarium* at the low inoculum level, fungus lesions were observed on 11% of those inoculated with the fungus alone, 32% of those also inoculated with *P. penetrans* at low inoculum level, and 61% of those also inoculated with *P. penetrans* at high inoculum level (Table 1). Although there was a higher number of *Fusarium*-diseased plants among the nematode-infected plants than among nematode-free plants, the severity of the dry root rot did not appear to be influenced by *P. penetrans* infection. Two more identical experiments produced similar results.

Meloidogyne spp.—All plants inoculated with *Fusarium* at the high inoculum level, showed symptoms of dry root rot whether the plants were nematode-infected or nematode-free. No influence of nematodes could be detected on development of the fungus rot, which was severe in all cases.

Of the plants inoculated with *Fusarium* at the low inoculum level, fungus lesions were observed on 14% of those inoculated with the fungus alone, 40% of those also inoculated with *Meloidogyne* spp. at low inoculum level, and 50% of those also inoculated with *Meloidogyne* spp. at high inoculum level (Table 2). Although there was a higher number of *Fusarium*-diseased plants among the nematode-infected plants than among nematode-free plants, the severity of the dry root rot did not appear to be influenced by nematode infection. Two more identical experiments produced similar results.

TABLE 1. Incidence of dry root rot symptoms when 10-day-old healthy or *Pratylenchus penetrans*-infected 'Red Kidney' bean seedlings were inoculated with macrospores of *Fusarium solani* f. sp. *phaseoli*

Treatment[a]	Plant with root rot lesions after 20 days/ No. plants inoculated	Average fungus disease rating per infected plant[b]	Infected plants (%)
Control	0/20	0.0	0.0
Low fungus	3/28	1.0	11.0
Low fungus + Low *P. penetrans*	9/28	1.1	32.0
Low fungus + high *P. penetrans*	17/28	1.1	61.0
High fungus	28/28	4.6	100.0
High fungus + low *P. penetrans*	27/27	4.4	100.0
High fungus + high *P. penetrans*	28/28	4.5	100.0

[a]Low fungus = 50,000 spores; high fungus = 500,000 spores; low *P. penetrans* = 1,000 nematodes; high *P. penetrans* = 10,000 nematodes.

[b]Fungus disease index: 0 = no disease; 1 = 1-5 lesions; 2 = 6-10 lesions; 3 = 11-15 lesions; 4 = 16-20 lesions; 5 = more than 20 lesions, tending to coalesce.

TABLE 2. Incidence of dry root rot symptoms when 10-day-old healthy or *Meloidogyne* spp.-infected 'Red Kidney' bean seedlings were inoculated with macrospores of *Fusarium solani* f. sp. *phaseoli*

Treatment[a]	Plants with root rot lesions after 20 days) No. plants inoculated	Average fungus disease rating per infected plant[b]	Infected plants (%)
Control	0/20	0.0	0.0
Low fungus	4/28	1.0	14.0
Low fungus + low *Meloidogyne* spp.	11/28	1.3	40.0
Low fungus + high *Meloidogyne* spp.	14/28	1.6	50.0
High fungus	28/28	5.0	100.0
High fungus + low *Meloidogyne* spp.	28/28	5.0	100.0
High fungus + high *Meloidogyne* spp.	28/28	5.0	100.0

[a]Low fungus = 50,000 spores; high fungus = 500,000 spores; low *Meloidogyne* = 500 eggs and larvae; high *Meloidogyne* = 2,500 eggs and larvae.

[b]Fungus disease index: 0 = no disease; 1 = 1-5 lesions; 2 = 6-10 lesions; 3 = 11-15 lesions; 4 = 16-20 lesions; 5 = more than 20 lesions, tending to coalesce.

Nematode disease ratings were made 30 days after planting in a series of nine similar experiments for both nematodes in all fungus-nematode combinations, as well as for nematode-alone controls. Counts of *P. penetrans* also were made in five of these experiments. At the high and low nematode concentrations in the nematode-alone controls *P. penetrans* produced lesions on 30 and 75% of the roots, respectively; *Meloidogyne* spp. produced galls on approximately 15 and 70% of the roots. At the end of the experiment, results of nematode counts showed that in roots of plants receiving low and high levels of *P. penetrans* inoculum there were 380 adn 2,850 eggs and larvae, respectively, per plant. Final populations of *P. penetrans* were lower in the plants inoculated with this nematode plus the high level of fungus inoculum than in those inoculated with this nematode alone. This reduction may have been due in part to the smaller root systems in plants inoculated with both organisms.

DISCUSSION.—*Fusarium solani* f. sp. *phaseoli* alone is a virulent pathogen (2, 3). In preliminary experiments, *P. penetrans* and *Meloidogyne* spp. were independently pathogenic to beans. When bean plants were subjected to low levels of fungus inoculum plus either *P. penetrans* or *Meloidogyne* spp., a

consistently higher number of infected plants showed Fusarium dry root rot symptoms than did nematode-free plants. Severity of the dry root rot, however, did not appear to be affected by the presence of nematodes. Thus nematode infection apparently influences the initiation of fungus infection but not the susceptibility of tissue to fungus development after the fungus is established.

Macroconidia of *F. solani* f. sp. *phaseoli* require nutrients, albeit in small amounts, for germination (6). Schroth et al. (12) demonstrated that a number of amino acids and sugars exuded from bean roots stimulate chlamydospores of this fungus to germinate, and that concentrations of these compounds in the exudate are sufficient to account for at least some of the germination observed in the vicinity of a suscept. It is possible that infection of the hypocotyls and roots of bean plants by *P. penetrans* and *Meloidogyne* spp. may effect an increase in the release of substances that stimulate germination of the fungus propagules in the vicinity of the plant roots and thus increase chances of fungus infection. Another possibility is that injury to roots effected by penetration of either nematode altered the physiology of root and hypocotyl cells and thus increased the possibility of fungus infection.

When high levels of fungus inoculum were used, all plants were infected by the fungus to the same degree. Consequently, the influence of the nematodes, whatever it may be, is obscured by the mass effect of the fungus inoculum and it was not possible to detect differences in the incidence or severity of fungus disease between nematode-infected and nematode-free plants. The virulence of the fungus and the levels of inoculum are considered to be critical to the results reported here.

LITERATURE CITED

1. BERGESON, G. B. 1963. Influence of Pratylenchus penetrans alone and in combination with Verticillium albo-atrum on growth of peppermint. Phytopathology 53:1164-1166.
2. CHATTERJEE, P. 1958. The bean root rot complex in Idaho. Phytopathology 48:197-200.
3. CHRISTOU, T., & W. C. SNYDER. 1962. Penetration and host-parasite relationships of Fusarium solani f. phaseoli in the bean plant. Phytopathology 52:219-226.
4. FAULKNER, L. R., W. J. BOLANDER, & C. B. SKOTLAND. 1970. Interaction of Verticillium dahliae and Pratylenchus minyus in Verticillium wilt of peppermint: influence of the nematode as determined by a double root technique. Phytopathology 60:100-103.
5. FAULKNER, L. R., & C. B. SKOTLAND. 1965. Interactions of Verticillium dahliae and Pratylenchus minyus in Verticillium wilt of peppermint. Phytopathology 55:583-586.
6. KO, W., & J. L. LOCKWOOD. 1967. Soil fungistasis: relation to fungal spore nutrition. Phytopathology 57:894-901.
7. KRUSBERG, L. R. 1961. Studies on the culturing and parasitism of plant parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissues. Nematologica 6:181-200.
8. MCCLELLAN, W. D., & J. R. CHRISTIE. 1949. Incidence of Fusarium infection as affected by root-knot nematodes. Phytopathology 39:568-571.
9. MCKEEN, C. D., & W. B. MOUNTAIN. 1960. Synergism between Pratylenchus penetrans (Cobb) and Verticillium albo-atrum R. and B. in eggplant wilt. Can. J. Bot. 38:789-794.
10. POWELL, N. T. 1963. The role of plant-parasitic nematodes in fungus diseases. Phytopathology 53:28-35.
11. SASSER, J. N., G. B. LUCAS, & H. R. POWERS, JR. 1955. The relationship of root-knot nematodes to black shank resistance in tobacco. Phytopathology 45:459-461.
12. 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.
13. STEINER, G. 1953. Changes in basic concepts in plant nematology. Plant Dis. Reptr. 37:203-205.
14. WALLACE, D. H., & R. E. WILKINSON. 1965. Breeding for Fusarium root rot resistance in beans. Phytopathology 55:1227-1231.