Interaction of Fusarium spp. and Certain Plant Parasitic Nematodes on Maize

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Paper No. 8110 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul. Department of Plant Pathology, University of Minnesota, St. Paul 55101. A portion of the senior author's Ph.D. thesis.

Accepted for publication 8 June 1973.

ABSTRACT

The maize root rot complex caused by *Fusarium* spp. was studied in the presence of various plant parasitic nematode species including *Meloidogyne incognita, Pratylenchus penetrans, P. hexincisus, P. scribneri, Paratylenchus nanus* and *Tylenchorhynchus martini*. Average dry root and shoot weight of maize seedlings inoculated with both *M. incognita* and *F. moniliforme* were less than those of seedlings inoculated with either organism alone. *Fusarium moniliforme* alone decreased root and shoot weights of maize seedlings more than when the fungus was combined with either *Pratylenchus scribneri* or *P. penetrans*. Saprozoic nematodes may play a role in decreasing populations of *F. moniliforme*.

Phytopathology 64:14-17

Additional key words: inoculum, nematode-fungus relationship.

Taylor & Schleder (14) reported that *Helicotylenchus* spp. *Pratylenchus* spp., *Tylenchorhynchus* spp., and *Xiphinema americanum* were present in more than 60% of the soil samples collected from fields of maize (*Zea mays* L.) throughout Minnesota, but that *Meloidogyne* spp. were encountered less frequently. Large populations of *Pratylenchus hexincisus* Taylor & Jenkins and *Helicotylenchus pseudorobustus* were present in fields cropped continuously to maize in Minnesota (D. H. MacDonald *unpublished*). *Helicotylenchus* and *Pratylenchus* species have been reported to cause yield losses in maize in various states (2, 8, 9, 13).

The interaction of nematodes and fungi on a variety of host plants has been reviewed by Powell (12, 13). It is apparent that the interaction of nematodes and fungi on maize has received little attention. Kisiel et al. (6) concluded that *Tylenchorhynchus claytoni* and *Tylenchus agricola* may not necessarily increase the invasive potential or root rot severity caused by *Fusarium roseum* or *Pythium ultimum*. This study was to determine the interaction of some species of nematodes on the incidence and severity of root rot caused by *Fusarium moniliforme* Sheldon.

MATERIALS AND METHODS.—Difco potato-dextrose agar and acidified potato-dextrose agar were used to isolate and culture fungi from roots of maize. Peptone-pentachloronitrobenzene (PCNB) agar, a selective medium was used for the isolation of *Fusarium* spp. from soil.

Nematodes were extracted from soil by Seinhorst elutriation (using 200- and 270-mesh sieves) and from debris by the Cornell piepan technique (5). Migratory endoparasitic nematodes emerged from the maize roots that were incubated for 48 hr in an antibiotic solution contained in deep (20-mm) petri dishes and placed on a reciprocating shaker.

Fungal inoculum was added to the soil 1 week prior to planting maize and the addition of nematodes. *Fusarium moniliforme* isolates were from maize roots and reinoculated to maize to establish pathogenicity (10).

'Minhybrid 417', a Fusarium root rot-susceptible maize hybrid (10) was used in these experiments.

A *Meloidogyne incognita* (Kofoid & White) Chitwood isolate known to parasitize maize was used as the nematode inoculum in the first experiment. One hundred cc of *F. moniliforme* inoculum or steamed sand and/or 10 g of chopped nematode-infected maize roots (3,500 juveniles) were mixed with 400 cc of steamed soil. The experiment consisted of 10 replicates of the following treatments: fungus alone, nematode alone, fungus plus nematode and control. Plants were grown for 3 wks at 27 C. A 0-5 scale in which 0 = no galls, 1 = 1-19 galls, 2 = 20-29 galls, 3 = 30=39 galls, 4 = 40-49 galls, and 5 = more than 50 galls per plant was used to rate maize roots infested with *M. incognita*.

Pratylenchus scribneri Steiner inoculum consisted of 200 nematodes extracted from maize roots and placed at the time of planting beneath a maize kernel in a 10-cm diam pot containing 400 cc of steamed soil. This experiment consisted of 10 replicates of the following treatments: fungus alone (50 cc of sand-cornmeal-*F. moniliforme*), nematode alone, fungus plus nematode, and control. The plants were grown for 3 wks in one experiment and 4 wks in another at 25 C. A 1-4 root rot index was used where 1 = no rot, 2 = slight rot (25%), 3 = moderate rot (26-50%), and 4 = more than half of the roots rotten or a dead plant.

Four hundred cc of soil infested with an average of 320 nematodes of *Pratylenchus penetrans* (Cobb) Filipjev & Stekhoven which had been maintained on vetch (*Vicia villosa* Roth), was mixed with 100 cc of steamed sand or 100 cc of the sand-cornmeal-*F. moniliforme* inoculum and placed in 10-cm pots. One maize kernel was planted per pot. Each of the following treatments were replicated three times: nematode alone, fungus alone, nematode plus fungus, and control. The plants were grown for 3 wks in greenhouse units maintained at 22, 25, and 27 C with 16 hr of supplemental light per day.

Field soil which had been cropped to maize and which contained populations of *Tylenchorhynchus martini* Fielding (375/250 cc soil sample), *Pratylenchus hexincisus* Taylor & Jenkins (100/250 cc soil sample), and *Paratylenchus nanus* Cobb (10/250 cc soil sample) was used as the nematode inoculum. Other species of plant parasitic nematodes were present in trace amounts. One hundred cc of fungus inoculum of *F. moniliforme* or *F. oxysporum* Schlecht. or 50 cc of each was added to 400 cc of either nematode-infested field soil or steamed field soil. The treatments were replicated three times and the experiment repeated by replanting maize in the same soil 4 wks after the first set of seeds were planted. After a total of 8 wks, including 6 wks during which maize seedlings were present, the soil and roots were analyzed for nematodes.

RESULTS.—*Meloidogyne incognita*.—The roots of the plants grown in nematode-infested or nematode plus fungus-infested soil were lightly galled (average root ratings of 2.9 and 1.5, respectively). The dry wts of the plants in the nematode and nematode plus fungus treatments were significantly lower ($P = 0.01$) than the plants grown in the control and/or fungus infested soil (Fig. 1).

Pratylenchus scribneri.—With the exception of one comparison, there were no differences in growth between plants inoculated with only the fungus and those inoculated with both the nematodes and the fungus. Furthermore, the average shoot weight and stalk diameter of the plants grown in the fungus plus nematode-infested soil tended to be greater than those of plants grown in the fungus-infested soil (Table 1). In a similar study in which the nematode inoculum consisted of 400 *P. scribneri* per plant, the dry wt of maize roots was reduced by 12% by *F. moniliforme*, 39% by *P. scribneri* and 39% by the combination of both pathogens compared to the control (11). In both experiments, recovery of nematodes from the soil and roots was very low (0-5% of the initial population).

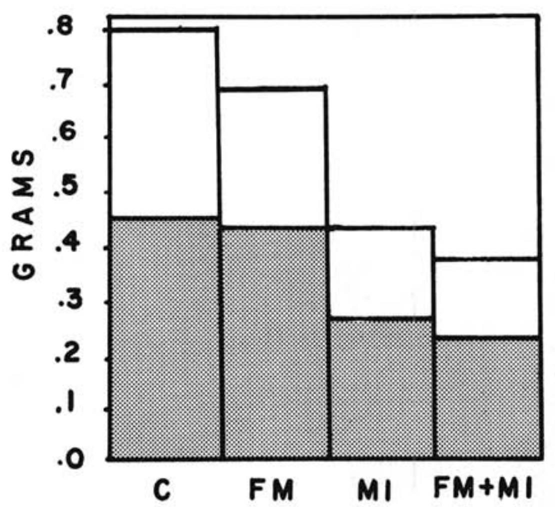

Fig. 1. The dry root (dark bar) and shoot (light bar) weights in grams of 3-week-old maize seedlings grown in steamed soil infested with *Fusarium moniliforme* (FM) or *Meloidogyne incognita* (MI) or both. Control is C. $P = 0.01$ for nematode and nematode plus fungus compared to control.

TABLE 1. Effect of *Fusarium moniliforme* and *Pratylenchus scribneri* on maize seedlings grown for 3 and 4 weeks in a greenhouse with an average temperature of 25 C

Treatment[a]	Fresh wt (g)	Root dry wt (g)	Shoot dry wt (g)	Stalk diam (mm)	Root rot index[b]
3 weeks					
Control	16.02 c[c]	0.463 c	2.52 c	5.8	1.0
Fungus	8.10 d	0.357 c	0.19 d	4.4	2.2
Nematode	19.10 c	0.460 c	2.74 c	6.4	1.0
Fungus plus nematode	10.02 d	0.295 c	1.24 d	5.1	2.4
4 weeks					
Control	33.92 c	1.752 c	2.82 c	8.8	1.0
Fungus	14.92 d	0.626 d	0.88 d	5.2	2.6
Nematode	35.84 c	1.444 c	3.00 c	7.8	1.0
Fungus plus nematode	22.30 d	0.790 c	1.62 d	7.2	2.8

[a] Each treatment is the average of 10 replicates, one plant per replicate.

[b] Root rot index, 1-4 scale.

[c] Numbers followed by the same letter are not statistically different from each other according to Duncan's multiple range test ($P = 0.05$). Differences in stalk diameter and root rot index were nonsignificant.

At the time of planting, the fungal population varied from 23,000 to 135,000 propagules/g of soil. After 4 wks, the population was in the range of 4-50,000 propagules/g of soil in the fungus-infested soil and 2-9,000/g in the nematode plus fungus-infested soil. Saprozoic nematodes (presumably the descendants of nematodes added as contaminants along with the plant parasitic forms) were abundant (950/250 cc of soil) in the soil infested with nematodes plus fungus, but were absent in the soil to which only the fungus had been added. In the nematode-alone treatment, the saprozoic nematodes numbered 105/250 cc soil sample.

Pratylenchus penetrans.—With the exception of the nematode plus fungus treatment at 27 C, all of the other inoculated plants had lower shoot and root weights than plants in their respective controls (Fig. 2). The differences were significant ($P = 0.05$) for the fungus alone, but not for the nematode alone or for the combination of both except at 22 C, when compared to the controls at each temperature.

In the nematode-alone treatment an average of 60 nematodes of *P. penetrans* (18% of the original inoculum) were recovered from the roots of maize plants grown at 27 C, 70 (21%) from plants grown at 25 C, and only 10 (3%) from roots of plants grown at 22 C. Nematodes were not recovered from the roots of any of the plants grown in the nematode plus fungus-infested soil or from the control.

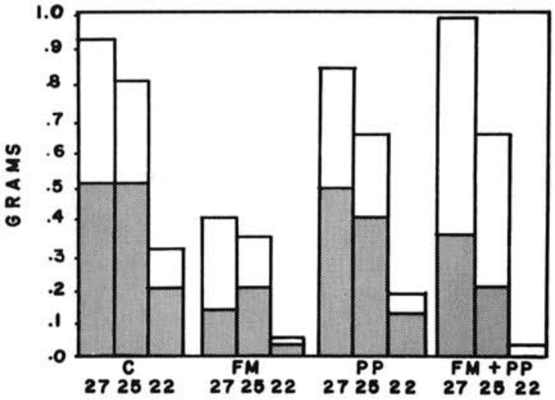

Fig. 2. The dry root (dark bar) and shoot (light bar) weights in grams of 3-week-old maize seedlings grown in steamed soil at three temperatures (22, 25, 27 C). Soil infested with *Fusarium moniliforme* (FM) and/or *Pratylenchus penetrans* (PP). C is control (steamed soil only). Average of three replicates. Differences significant ($P = 0.05$) for fungus alone at all temperatures and for fungus plus nematode only at 22 C compared to controls.

Tylenchorhynchus martini, Paratylenchus nanus, and Pratylenchus hexincisus.—The addition of both *F. moniliforme* and *F. oxysporum* or either fungus alone to previously steamed field soil was associated with a reduction in the dry weights of roots and shoots of maize plants grown at 27 or 25 C, but not at 22 C. The addition of the fungus inoculum to field soil was associated, for the most part, with slight decreases in root weight and measurable increases in shoot weight of plants grown at 27 or 25 C, but not at 22 C (Table 2). Of the three species of plant parasitic nematodes present initially in the field soil to which the fungus inoculum was added, only the population of *Tylenchorhynchus martini* increased (from 375 to 455/250 cc of soil) during the course of this experiment. The *Paratylenchus nanus* population remained constant at 10/250 cc while the 100 *Pratylenchus hexincisus* originally present per 250 cc of soil disappeared.

DISCUSSION.—Fungus hyphae have been shown to readily penetrate egg masses, but initial experiments in this study with *M. incognita* were unsuccessful when the nematode inoculum consisted of one, three, or five egg masses placed beneath the maize kernel. Therefore, this nematode was added to the soil in chopped galled roots which may have been infected with *Fusarium* spp. as well as other soil-inhabiting saprophytes and plant pathogens. Because of Mayol & Bergeson's work (7), it seems reasonable to speculate that the reduction in dry weights of roots and shoots of maize seedlings attributed to the nematode alone might have been less or perhaps nonexistent if members of the soil microflora had been excluded. Only *M. incognita* appeared to have the potential for significantly increasing the severity of root rot due to *F. moniliforme*. Because of the variability of *M. incognita* isolates in parasitizing maize (1), the interaction of this nematode and *F. moniliforme* on this crop will probably be shown to be important only in limited areas.

Although *P. scribneri* did [in one study (11)], significantly reduce the growth of maize seedlings, the activities of this nematode apparently do not increase the severity of root rots caused by *F. moniliforme* at populations of 200 nematodes/plant. The low number of *P. penetrans* recovered from the roots of maize plants that had been grown in soil to which *F. moniliforme* had not been added may reflect the inability of many individuals in the population to change hosts (from vetch to maize). Although *T. martini* and possibly *P. nanus* fed and reproduced on the maize roots, our studies, like most of those of Kisiel et al. (6), did not detect any increase in root rot due to these migratory ectoparasitic nematodes.

TABLE 2. Root and shoot dry weights of 3-week-old maize seedlings infected with *Fusarium moniliforme* and/or *F. oxysporum* grown at three temperatures in steamed or field soil[a]

	Root dry weight (g)					
	27 C		25 C		22 C	
	Steamed	Field	Steamed	Field	Steamed	Field
Control[b]	1.077	0.700	0.811	0.524	0.406	0.535
F. moniliforme	0.652	0.700	0.353	0.632	0.328	0.388
F. oxysporum	0.572	0.549	0.477	0.771	0.458	0.637
F. m. plus *F. o.*	0.465	0.656	0.648	0.728	0.381	0.377
	Shoot dry weight (g)					
Control[b]	1.614	0.716	1.028	0.503	0.327	0.277
F. moniliforme	1.344	1.074	0.643	1.109	0.223	0.141
F. oxysporum	1.007	1.049	0.782	1.086	0.358	0.128
F. m. plus *F. o.*	0.993	1.148	0.792	0.968	0.271	0.295

[a] Figures represent an average of two experiments and three replications per experiment. Field soil contained *Tylenchorhynchus martini, Pratylenchus hexincisus*, and *Paratylenchus nanus*.

[b] Field soil control: natural soil infested with plant parasitic nematodes and other soil microorganisms. Weights are significant ($P = 0.10$) for shoots and N.S. for roots.

The exact role that the saprozoic nematodes had in reducing the number of *M. incognita, P. penetrans,* and *P. hexincisus* is unknown but it is likely that the large number of these organisms in the fungus-amended soil did adversely affect the plant parasitic nematodes. These saprozoic nematodes, in addition to protecting ingested spores from various chemicals (4), also appear to play a significant role in reducing the number of fungus propagules per unit of soil (3). On the other hand, the complete absence of these organisms from the nonamended soil was not expected.

LITERATURE CITED

1. BALDWIN, J. G., & K. R. BARKER. 1970. Host suitability of selected hybrids, varieties and inbreds of corn to populations of Meloidogyne spp. J. Nematol. 2:345-350.
2. GRAHAM, T. W., & Q. L. HOLDEMAN. 1951. Nematode injury to tobacco, cotton and corn in relation to populations of root-knot and meadow nematodes. Phytopathology 41:14 (Abstr.).
3. JENSEN, H. J. 1967. Do saprozoic nematodes have a significant role in epidemiology of plant diseases? Plant Dis. Reptr. 51:98-102.
4. JENSEN, H. J., & S. R. SIEMER. 1969. Protection of fungus spores from certain biocides following ingestion by saprozoic nematodes. J. Nematol. 1:12-13 (Abstr.).
5. KABLE, P. F., & W. F. MAI. 1968. Influence of soil moisture on Pratylenchus penetrans. Nematologica 14:101-122.
6. KISIEL, M., K. DEUBERT, & B. M. ZUCKERMAN. 1969. The effect of Tylenchus agricola and Tylenchorhynchus claytoni on root rot of corn caused by Fusarium roseum and Pythium ultimum. Phytopathology 59:1387-1390.
7. MAYOL, P. S., & G. B. BERGESON. 1970. The role of secondary invaders in Meloidogyne incognita infection. J. Nematol. 2:80-83.
8. MILLER, R. E., C. W. BOOTHROYD, & W. F. MAI. 1963. Relationship of Pratylenchus penetrans to roots of corn in New York. Phytopathology 53:313-315.
9. NELSON, R. R. 1955. Nematode parasites of corn in the coastal plain of North Carolina. Plant Dis. Reptr. 39:818-819.
10. PALMER, L. T., & T. KOMMEDAHL. 1969. Root-infecting Fusarium species in relation to rootworm infestations in corn. Phytopathology 59:1613-1617.
11. PALMER, L. T., D. MAC DONALD, and T. KOMMEDAHL. 1967. The ecological relationship of Fusarium moniliforme to Pratylenchus scribneri in seedling blight of corn. Phytopathology 57:825 (Abstr.).
12. POWELL, N. T. 1963. The role of plant-parasitic nematodes in fungus diseases. Phytopathology 53:28-35.
13. POWELL, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annu. Rev. Phytopathol. 9:253-274.
14. TAYLOR, D. P., & E. G. SCHLEDER. 1959. Nematodes associated with Minnesota crops. II. Nematodes associated with corn, barley, oats, rye and wheat. Plant Dis. Reptr. 43:329-333.