Prevalence and Pathogenicity to Corn of Fusarium species from Corn Roots, Rhizosphere, Residues, and Soil

H. L. Warren and Thor Kommedahl

Scientific Journal Series Paper No. 8208, Minnesota Agricultural Experiment Station, St. Paul.

Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907; Department of Plant Pathology, University of Minnesota, St. Paul 55101, respectively.

Accepted for publication 11 April 1973.

ABSTRACT

Among species of *Fusarium* isolated from corn roots, plant residues, rhizosphere, and soil, in plots where corn had been grown annually for 10 years, *F. oxysporum*, *F. solani*, and *F. roseum* predominated, making up 62, 21, and 12%, respectively, of the colonies of *Fusarium* species isolated. Other species of *Fusarium* (*F. moniliforme*, *F. tricinctum*, and *F. episphaeria*) totaled 4% of the colonies of *Fusarium* isolated. In soil where fertilizers were applied and plant residues were removed, *F. roseum* was more numerous in the rhizosphere than in nearby soil, but where fertilizers were applied and plant residues were present, it was more numerous in nearby soil than in the rhizosphere. If roots were wounded, *F. oxysporum* in field soil significantly reduced dry weight of plants over nonwounded roots at 18, 24, and 29 C, but greater losses were sustained at the higher temperature. *F. roseum* reduced dry weight of plants at 18 and 24 C but not at 29 C. Both species, mixed in soil, reduced dry weight of plants at all three temperatures.

Phytopathology 63:1288-1290

Additional key words: ecology, temperature effect.

Fusarium oxysporum, *F. roseum*, and *F. solani* have been the predominant species of *Fusarium* associated with the roots of wheat in several regions (1, 4, 5) and *F. oxysporum* predominated in wounded roots of corn (3). This work was undertaken to determine the predominant species of *Fusarium* present on corn roots, in plant residues, in the rhizosphere, and in soil in which corn had been grown annually for 10 years; to determine the effects of fertilizers and plant residues upon these fungi; and to determine their pathogenicity at three different temperatures.

MATERIALS AND METHODS.—At Rosemount, Minnesota, corn (*Zea mays* L. 'Minhybrid 5302') was grown annually for a decade, during which time the corn crop residues (stubble and stalks) were either retained or removed, with and without application of fertilizer (5:20:20 of NPK at 243 kg/hectare). N was NH_4NO_3 equivalent, P was P_2O_5 equivalent, and K was K_2O equivalent. This arrangement gave four treatments: (i) no fertilizer nor residues; (ii) no fertilizer with residues; (iii) fertilizer but no residues; and (iv) fertilizer with residues.

To detect root surface flora, we put root samples (each 5.0 g) in water on a rotary shaker for 20 min to remove rhizosphere soil. Roots were surface-treated for 2 min in 70% ethanol, kept 2 min in 5% NaOCl, and placed on PCNB (pentachloronitrobenzene)-peptone agar adjusted to pH 5.2.

Five-gram soil samples obtained by combining three randomly selected samples (3- to 15-cm deep) per treatment were passed through a 2-mm sieve, then successively diluted in water to give a final dilution of 1:10,000. Final soil dilutions were mixed in a shaker for 30 sec; 1-ml aliquots were pipetted into petri dishes to which had been added 15 ml of cool PCNB agar.

Unless otherwise stated, plant residues were removed from the soil by means of a hand rake. To determine the quantity of plant residue that remained after this, we screened 2.8×10^4 cc of soil over a screen with meshes 2-cm wide, then dried the material so collected, at 110 C for 24 hr, and weighed it. As judged from the results of this, the raking removed only 68 to 78% of the residues present in the soil of plots where residues had ostensibly been removed. Thus, raking only removed the coarser residues of corn. On the other hand where residues were retained, less residue was present in soil where fertilizer was applied, possibly because the fertilizer promoted residue decomposition.

Although initial isolations were made on PCNB agar, colonies were subsequently transferred to homemade potato-dextrose agar (PDA) and incubated at about 24 C under fluorescent lamps for 16 hr/day before being identified.

Dry weights of roots and shoots were obtained by placing plant materials in paper bags and drying them for 48 hr in an oven at 70 C.

To test for pathogenicity, corn kernels were surface-treated in a 1% solution of NaOCl for 2 min, and sown in sand. When 7 days old, seedlings were dug from sand, washed, immersed in conidial suspensions (250,000 macroconidia/ml water) for 30 min, then transplanted to steamed or nonsteamed (field) soil. Roots were assumed to be injured by the digging and washing process. Seedlings were allowed to grow for 3 weeks in greenhouse rooms, each set at 18, 24, or 29 C.

Other methods in general were similar to those described previously (5).

RESULTS AND DISCUSSION.—*Fusarium spp. in roots, residues, rhizosphere, and soil.*—Table 1 shows that the prevalence of the three main *Fusarium* spp. was approximately equal on all four substrates. If prevalence on the four substrates is averaged, *F. oxysporum* (Schl.) Snyd. & Hans. made up 62%, *F. solani* (Mart.) Appel & Wr. 21%, and *F. roseum* (Lk.) Snyd. & Hans. 12% of the *Fusarium* species isolated during the 2-year period in plots with and without fertilizers or residues. The range in values for each percentage is relatively narrow and is shown to indicate that neither fertilizers nor residues appreciably influenced the prevalence of any of the three species during the 2 years of test. Similar reports were made for wheat in the same field (5) and for corn earlier (3).

Fusarium moniliforme (Sheld.) Snyd. & Hans., *F. tricinctum* (Cda.) Snyd. & Hans., and *F. episphaeria* (Tode) Snyd. & Hans. were usually isolated from residues or soil but never comprised more than 4% of the *Fusarium* species (Table 1).

From corn roots, *F. roseum* 'Graminearum' was the cultivar most frequently isolated; however, from corn residues, 'Avenaceum' was the most prevalent cultivar (23%, ranging from 20 to 29%) followed by Graminearum (18%, ranging from 13 to 20%), 'Culmorum' (9%, ranging from 5 to 15%), and 'Equiseti' (6%, ranging from 6 to 7%) - the ranges apply to plots with and without fertilizer or residues.

We isolated from residues in the fall and the following spring and found no appreciable differences in prevalence, indicated as follows: *F. oxysporum* 70 and 66%, *F. solani* 13 and 15%, and *F. roseum* 16 and 16%, for fall and spring isolations, respectively, for a 2-year period.

If retention of residues had increased the relative prevalence of pathogenic *Fusarium* species, one might expect that minimum tillage would have increased their inoculum potential, especially of *F. roseum* 'Graminearum'. This did not occur, which supports the thesis of Huber (2) that the growing crop exerts the main influence on soil microflora and that crop residues aid in persistence of conditions established during crop growth.

Rhizosphere ratios vs. treatments.—Because treatments had no appreciable effect on relative prevalence of *Fusarium* spp., detailed data were not shown in Table 1. However, some differences were apparent in rhizosphere ratios (R:S) that were related to treatment, especially for *F. roseum* (Table 2). Propagules of *F. roseum* were 4.5 times more numerous in the rhizosphere than in nearby soil, where fertilizer was applied and residues removed; whereas, propagules were only 0.3 times as numerous in the rhizosphere soil where no fertilizer was present and residues were retained. Removal of residues undoubtedly removed inoculum of *F. roseum* to shift the R:S to the rhizosphere. This change in R:S was not observed for the other two species, probably because they are not pathogens. *Fusarium roseum* probably invaded plant tissue when plants were alive, whereas the other species probably colonized residues after death of the plants. This is reflected also in Table 1 where *F. roseum* was more prevalent on residues than on roots, rhizosphere, or soil.

Pathogenicity of species at three temperatures.—Because *F. oxysporum* so consistently predominated among the *Fusarium* species in roots, residues, rhizospheres, and soil, this species was tested for pathogenicity alone and in combination with the two next-most-prevalent species and at three temperatures.

TABLE 1. Relative prevalence of *Fusarium* spp. isolated from roots, residues, rhizosphere, and soil in a field cropped annually to corn for a decade with or without fertilizer or residues

Corn substrate for *Fusarium* spp.	Relative prevalence of *Fusarium* spp.[a]		
	F. oxysporum	*F. solani*	*F. roseum*
Roots[b]	65 (53-70)	22 (17-30)	10 (9-11)
Residues[c]	68 (60-74)	14 (12-16)	17 (12-21)
Rhizosphere[d]	60 (58-63)	21 (18-26)	10 (7-12)
Soil[e]	55 (53-58)	25 (21-28)	13 (10-15)

[a] Avg and range (in parentheses) in field plots with and without residues and with and without fertilizer (NPK, 5:20:20, at 243 kg/hectare).

[b] Based on 180 plants (30 roots, three times/year, 2 years).

[c] Based on 400 corn residue sections (200 in fall and 200 in spring). *F. moniliforme* plus *F. tricinctum* = 2% of total *Fusarium* spp.

[d] Avg of 36 petri plates for each of 2 years of sampling.

[e] Avg of three samples each time and sampled four times/year in each of 2 years (total 24 soil samples). *F. tricinctum* = 4% and *F. episphaeria* = 2% of total *Fusarium* spp.

Previous work with *F. oxysporum* on corn had indicated this species to be a wound parasite, especially at higher temperatures (3). Consequently, corn roots were wounded and compared with nonwounded roots prior to inoculation with *F. oxysporum* and the effect of inoculation was measured as dry weights of roots and shoots of 1-month-old plants.

When corn roots were wounded, there was a significant (5% level) reduction in dry weight of plants at 18, 24, and 29 C, whether soil (steamed or not) was inoculated with *F. oxysporum* or not. However, the reduction in dry weights of roots or shoots was greater where inoculum was present. Instead of giving the data on dry weights per treatment, the information is shown as percentages of

TABLE 2. The rhizosphere to soil ratio (R:S) of corn in field plots in which fertilizer was either applied or withheld, and in which corn crop residues were either removed or retained during the 2-year period

Treatment		R:S of *Fusarium* spp.		
Fertilizer[a]	Residue[b]	*F. oxysporum*	*F. roseum*	*F. solani*
None	Removed	1.2	1.8	1.2
None	Retained	1.2	0.3	1.5
Applied	Removed	0.8	4.5	1.2
Applied	Retained	1.9	1.1	1.9

[a] NPK (5:20:20) at 243 kg/hectare.

[b] Although residue was removed by raking, organic matter was not removed completely and was found to be 4.9%, 6.4%, 5.3%, and 5.8%, respectively, for the four treatments, based on sampling at soil depths from 5-35 cm (12 samples/treatment).

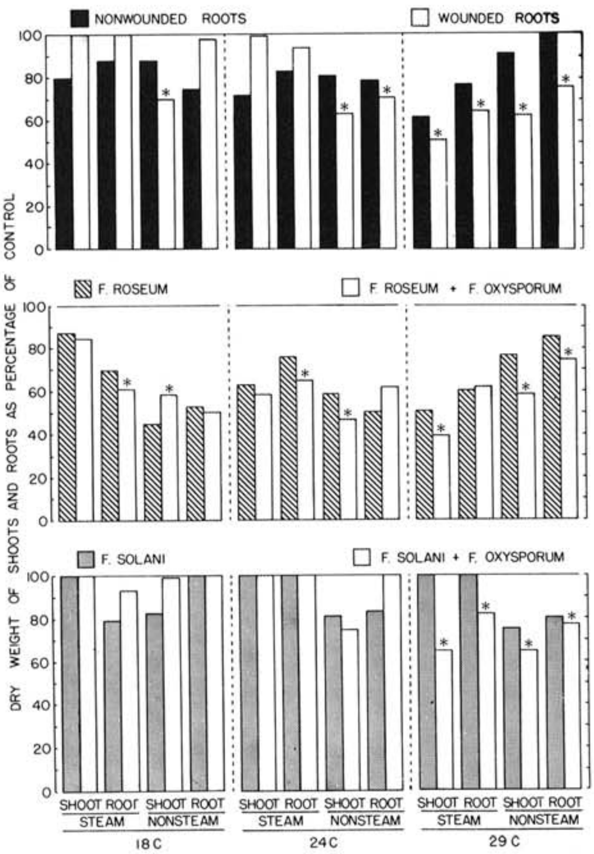

Fig. 1. The effect of *Fusarium oxysporum* alone and combined with *F. roseum* or *F. solani* on shoot and root dry weights for 1-month-old plants at three temperatures in either steamed or nonsteamed soil in the greenhouse. Values given are percentages of control which consisted of wounded but noninoculated roots, except in the top graph where wounded and nonwounded roots were compared. Asterisks over bars indicate significance at the 5% level (Duncan's multiple range test) between bars in each pair of bars. 'Graminearum' was the cultivar of *F. roseum* tested.

control plants from the effects of wounding, temperature, and fungus combination (Fig. 1).

One might conclude from Fig. 1, that wounding of roots at 18 or 24 C enhanced root growth. This is not so. The values given are based on percentages of the control plants; the growth of plants in the control when roots were wounded varied from 60 to 87% of that from plants where roots were not wounded. Moreover, with the change in temperature from 18 to 29 C, the root weights changed from 60 to 100 mg/plant and the shoot weights from 61 to 270 mg, in noninoculated, steamed soil, and nonwounded roots.

Fusarium oxysporum significantly reduced dry weights of shoots and roots at 29 C, when roots were wounded, but the reduction was greater in nonsteamed than in steamed soil. Nonwounded roots had lower dry weights in steamed than in nonsteamed soil. Thus, *F. oxysporum* seemed to cause greater loss in growth of plants at 29 C than at lower temperatures, especially when roots were wounded.

All roots were wounded in tests with *F. roseum* and *F. solani*, alone or in combination with *F. oxysporum*. *Fusarium roseum* caused more damage than *F. oxysporum* to roots and shoots at all three temperatures, but the effect was greater at 18 and 24 C, especially in nonsteamed soil (Fig. 1). These two species in nonsteamed soil reduced dry weights significantly at 29 C, but not at lower temperatures. *Fusarium solani* alone or combined with *F. oxysporum* did not reduce dry weights significantly at 18 or 24 C; however, they reduced dry weights significantly in both steamed and nonsteamed soils at 29 C (Fig. 1).

We conclude that *F. oxysporum*, and possibly *F. solani*, may function as root rot fungi of corn when one or more of the following conditions prevail: (i) roots are wounded, either mechanically or by insects; (ii) other *Fusarium* spp., or even other fungi, are part of the complex; and (iii) temperatures are relatively high. Other factors, not studied, may also be involved.

LITERATURE CITED

1. GORDON, W. L. 1954. The occurrence of Fusarium species in Canada IV. Taxonomy and prevalence of Fusarium species in the soil of cereal plots. Can. J. Bot. 32:622-629.
2. HUBER, D. M. 1963. Investigations on root rot of beans caused by Fusarium solani f. phaseoli. Diss. Abstr. 25:17.
3. PALMER, L. T., & T. KOMMEDAHL. 1969. Root-infecting Fusarium species in relation to rootworm infestations in corn. Phytopathology 59:1613-1617.
4. SNYDER, W. C., & T. A. TOUSSOUN. 1965. Current status of taxonomy in Fusarium species and their perfect stages. Phytopathology 55:833-837.
5. WARREN, H. L., & T. KOMMEDAHL. 1973. Fertilization and wheat refuse effects on Fusarium species associated with wheat roots in Minnesota. Phytopathology 63:103-108.