Fusarium Wilt of Soybean in Delaware

N. P. FERRANT, Graduate Assistant, and R. B. CARROLL, Associate Professor of Plant Pathology, Department of Plant Science, University of Delaware, Newark 19711

ABSTRACT

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In 1976 and 1977, significant yield losses occurred in southern Delaware soybean fields. The major symptom was wilting of plants at different stages of development, and the pathogen was identified as *Fusarium oxysporum*. Isolations were made periodically from diseased plants and their rhizospheres collected from experimental plots established in a heavily infested field. *F. oxysporum* represented 84% of pathogens isolated from roots and stems. From soil, *Fusarium* species comprised 86%. *F. oxysporum* was isolated from the surface of 41% of the seed harvested and tested.

During the 1976 and 1977 growing seasons, diseased soybean plants were observed in 40 commercial fields in southern Delaware. Yield losses were often severe, as high as 35% in some cases, and the cause at that time was undetermined.

Wilt of soybean caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. has been investigated (1–3,5,13), and *F. oxysporum* has been studied as a primary pathogen and as part of a root rot complex (6). Although it can be isolated from diseased root and stem tissue, pathogenicity is not always demonstrated (9), and it may act as a secondary invader of host tissue (14). The purpose of our study was to investigate wilt of soybean in the field and to test the pathogenicity of the *F. oxysporum* isolates.

MATERIALS AND METHODS

Field survey, 1977. Late in the growing season, roots and basal stems of wilted soybean plants were collected from fields throughout southern Delaware. Samples were prepared for isolation by washing them in running tap water and surface-disinfesting 1-cm sections for 1 min in 70% ethanol and 1 min in an aqueous solution of 0.5% sodium hypochlorite before rinsing them in sterile distilled water. Sections were placed on potato-dextrose and lima bean agars and incubated at 25 C. Between July and September 51 plants were sampled.

Current address of the senior author: Asgrow Seed Co., Twin Falls, ID 83301.

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Field studies, 1978. Plots were established in a grower's field in Bridgeville, DE, where disease losses were serious the previous growing season. The experimental design was a randomized block with two treatments (fumigation and no fumigation) and four replications. Each plot was 6.1 × 12.2 m and contained 16 rows of soybeans spaced 0.8 m apart. Plots were separated by 6.1-m alleys. Soil fumigation was with 1,3 dichloropropene and chloropicrin (Telone C-17, Dow Chemical Co.). Plots were conventionally tilled and planted to Essex soybeans.

Biweekly from June to October, five plants were selected from each plot (a total of 40 plants), with emphasis on plants showing symptoms. Plant material below the third internode was brought to the laboratory where isolations from the roots and basal stems were conducted as described, except that sections were placed on each of four culture media: acidified potato-dextrose agar (9), Martin's peptone agar (10), pentachloronitrobenzene agar (10), and Martin's medium modified for *Pythium* (12). All plates were incubated at 25 C for 7 days.

Soil samples were collected from the rhizospheres of soybean plants that were removed for isolation by taking five 15.24 × 2.0 cm cores of soil adjacent to plant roots. These were combined to form one soil sample per replication. Soil was passed through a 2-mm screen, and oven dried for 24 hr at 40 C. Thirty grams of each dried sample was placed in 300 ml of 0.1% water agar, thoroughly shaken, and diluted to 1:1,000 and 1:10,000 with 0.1% water agar. One milliliter of each sample solution was pipetted into 9-cm diameter petri plates of the four culture media and replicated five times. Fungal isolates were identified after incubation at 25 C for 5 days.

From the final plant samples collected in October, seed was saved from each plot to determine the mycoflora present. The three groups assayed were seeds without any surface disinfestation, seeds that were surface disinfested, and seeds that were surface disinfested and then triturated in a Waring Blendor. Each sample consisted of five seeds per plate tested on each of the four culture media. This was replicated three times. Plates were incubated at 25 C and fungal isolates identified.

Disease survey, 1978. In September and October, samples of diseased soybeans and rhizosphere soil were collected from fields throughout the state, and isolations were made.

Pathogenicity. Twenty-two *F. oxysporum* isolates obtained from diseased soybeans were tested for pathogenicity on Essex soybeans. Inoculum was prepared by growing each isolate in 100 ml of Czapek-Dox broth for 6 days in shake culture at 25 C. Each isolate was then collected on No. 5 Whatman filter paper and rinsed with sterile distilled water. The inoculum was placed in a Waring Blendor with a small amount of sterile water and blended for 2 min at high speed. Sterile distilled water was then added to each inoculum suspension to give a final volume of 200 ml. Four replications of 50 ml each of inoculum were used. Four replications of 50 ml of sterile distilled water served as a control.

Soybean seeds were germinated in flats of soil that had been fumigated with sodium *N*-methyldithiocarbamate (Vapam) to decrease natural fungal populations. Plants at the first trifoliolate stage were removed from the flats, rinsed in running tap water, immersed in groups of five roots in the vials of inoculum for 5 min, and transplanted in 12.7-cm diameter pots containing fumigated soil. The inoculum was also poured around the base of the plants, and the pots were randomized on the greenhouse bench.

Disease ratings were made 7 wk after inoculation. Stem length measurements and fresh root weights were also recorded.

Reisolation of the inoculated organism was attempted on acidified potato-dextrose agar.

RESULTS

Symptomatology. Symptoms in the field consisted of randomly scattered round to elongate patches of yellow to brown plants with interspersed healthy green plants. Soybeans were killed at various stages of development. Individual plants showed stunting, chlorosis, sudden wilting, epinasty, and extensive defoliation. Pinkish spore masses were present on the surface of basal stems of some

older plants. Roots showed brown to black cortical decay, with vascular discoloration extending into the stem. In some plants, reddening of the pith was followed by tissue disintegration.

Field survey, 1977. *Fusarium* spp. were the predominant root-infecting fungi isolated from 51 diseased soybean plants collected in 1977. *Fusarium* sp. was isolated from plants collected on all dates and was present in 200 (18.9%) of 1,058 root sections.

The 200 *Fusarium* isolates obtained from the field survey were grouped into 12 categories according to macroscopic culture characteristics. One single spore isolate from each category was sent to the Fusarium Research Center at Pennsylvania State University for identification. Of the 12, eight were identified as *F. oxysporum*, two as *F. solani*, and two as *F. roseum*. These isolates were then used as standards for identifying other *Fusarium* isolates from diseased soybean plants. In all cases, the fungus was transferred to homemade potato-dextrose agar, grown at room temperature (20–24 C) for 5 days under Heidt 40-W fluorescent lights (12 hr) 30.5 cm from the surface of the petri plates, and compared macroscopically and microscopically with the identified *Fusarium* isolates.

Field studies, 1978. *Fusarium* spp. were isolated from 3,837 (53%) of 7,200 root sections, and 84% of these isolates were *F. oxysporum*. Of 2,880 dilution plates (1:1,000 and 1:10,000) prepared from rhizosphere soil, 86% of the possible soilborne pathogens isolated were *Fusarium* sp.

Of 480 seeds harvested from the experimental plots and tested, 195 (41%) carried *F. oxysporum* on the seed coat. *F. oxysporum* could not be isolated from seed that was first surface-disinfested and then plated or from seed that was surface-disinfested, ground, and then plated.

The seasonal fluctuation in the number of *Fusarium* sp. isolated from rhizosphere soil and the number of *F. oxysporum* isolates obtained from soybean roots are presented in Figs. 1 and 2, respectively. A significantly larger number of *Fusarium* sp. was isolated from soil collected during August than from soil collected during June, July, September, or October. *F. oxysporum* was isolated from a significantly greater number of roots and basal stem sections of soybeans collected during late September and early October.

The number of times *Fusarium* sp. or *F. oxysporum* was isolated from soybean roots and basal stems did not differ significantly ($P = 0.05$) between fumigated plots and plots that were not fumigated (Table 1). However, a significantly greater number of *Fusarium* sp. was obtained from soil in the nonfumigated than in fumigated plots ($P = 0.01$).

Disease survey, 1978. All plants had symptoms of *F. oxysporum* infection—wilting, epinasty, vascular discoloration, and cortical decay of the root.

Isolations from root and basal stem sections of diseased plants showed that 70.2% contained *F. oxysporum*. Of all potential pathogenic fungi isolated from the rhizospore soil of diseased plants, 49.3% were *Fusarium* spp.

Pathogenicity. Nine of the 22 *F. oxysporum* isolates caused significant ($P = 0.05$) reduction in stem length and fresh root weight, compared with the controls (Table 2). The disease ratings for these isolates were significantly higher for inoculated plants, as would be expected. Symptom expression was not as severe as that seen in the field, but symptoms were similar and included chlorosis, stunting, epinasty, wilting, vascular discoloration in the stem, and root decay. Healthy plants were commonly found among

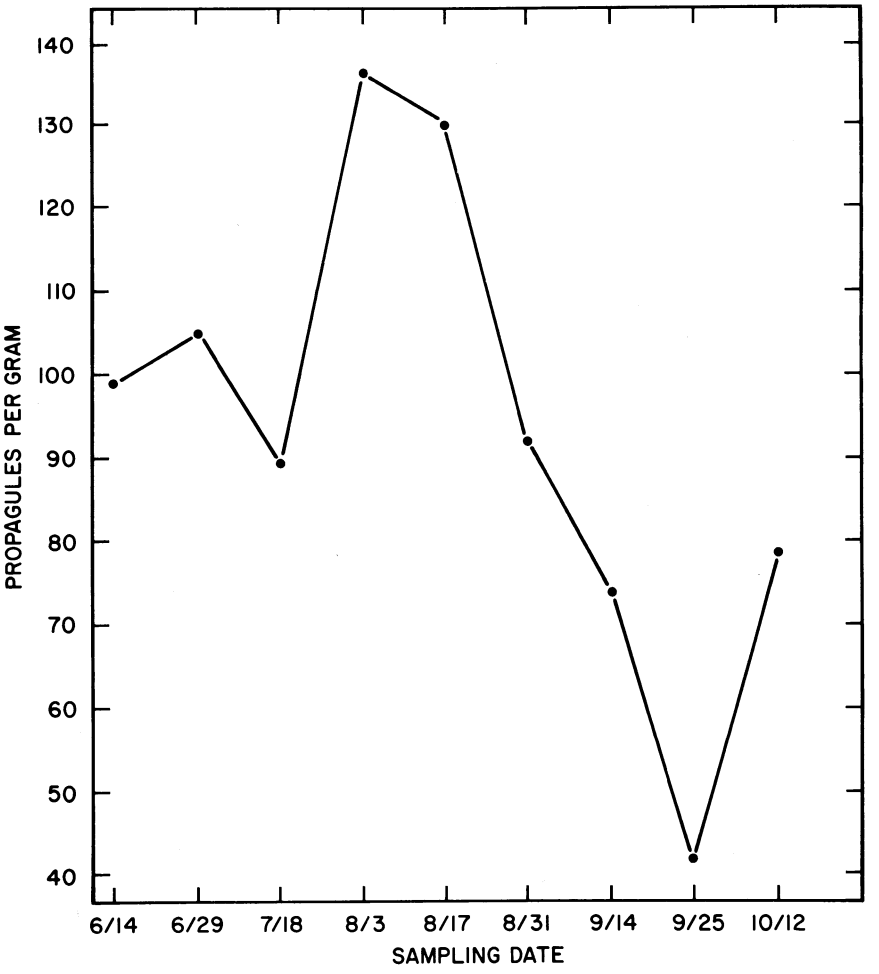

Fig. 1. Seasonal fluctuation in numbers of *Fusarium* spp. isolated from soybean rhizosphere. Mean number of propagules per gram at 1:1,000 dilution averaged over eight replications.

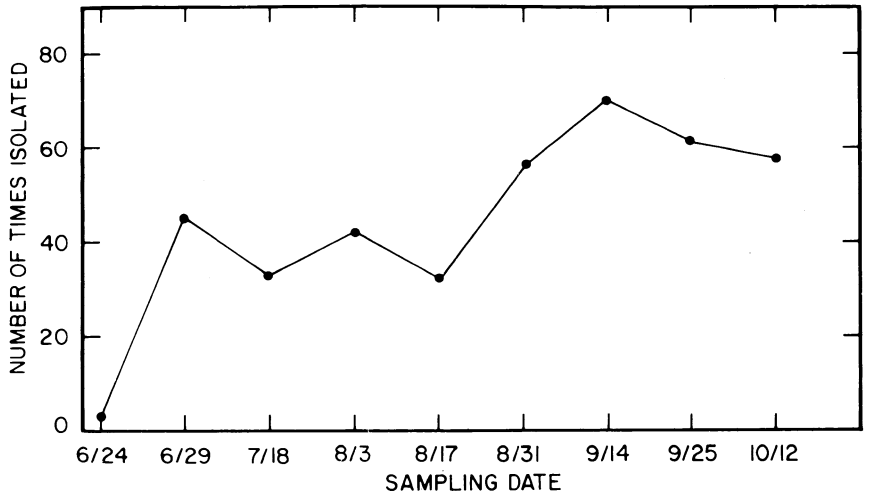

Fig. 2. Seasonal fluctuation in frequency of *Fusarium oxysporum* isolated from soybean roots. Mean number per replication of 100 root sections averaged over eight replications.

Table 1. Effect of fumigation on isolation of *Fusarium* from soybean roots and rhizosphere soil

Soil treatment	Roots[a]		Rhizosphere soil[b]
	Fusarium spp.	*F. oxysporum*	*Fusarium* spp.
Not fumigated	48.5	46.8	103.2**[c]
Fumigated	45.3	43.0	79.3

[a] Mean number of times isolated from 100 root sections averaged over four replications and nine sampling dates.
[b] Mean number of propagules (1:1,000 dilution) averaged over four replications and nine sampling dates.
[c] **Indicates significant difference ($P = 0.01$).

Table 2. Results of pathogenicity tests with *Fusarium oxysporum* isolates obtained from diseased soybeans

Isolate no.	Stem length[a] (cm)	Fresh root weight[b] (g)	Disease rating[a,c]
1	13.6*[d]	1.2	3.9
2	16.4	1.2	4.5*
3	13.9*	0.9*	4.1*
4	15.5	0.8*	3.8*
5	14.7*	1.0*	3.0
6	14.2*	0.9*	4.4*
7	16.2	1.0*	3.7*
8	9.7*	0.8*	5.7*
9	13.9*	1.5	4.4*
10	14.7*	1.1*	5.4*
11	15.5	1.2	4.6*
12	14.2*	0.8*	4.3*
13	13.7*	0.9*	5.5*
14	15.3*	1.0*	5.2*
15	12.3*	1.4	4.2*
16	13.7*	1.2	4.4*
17	14.8*	1.4	2.9
18	15.5	1.1*	3.9*
19	17.5	0.8*	3.2*
20	16.6	1.4	4.6*
21	12.1*	1.2	5.0*
22	15.3*	1.0*	6.0*
Control	18.4	1.6	2.0

[a] Mean of four replications of five plants each.
[b] Mean of four replications of three plants each.
[c] On a 1–10 scale where: 1 = healthy plant, 10 = dead plant.
[d] *Indicates significant difference ($P = 0.05$).

dead plants in the same pot. Defoliation did not occur, and spore masses were not produced.

All of the remaining isolates produced some symptoms in inoculated plants although the data collected were not significantly different from the controls in all categories. The pathogen was readily reisolated from inoculated plants.

DISCUSSION

Results of isolations from diseased soybean plants and of pathogenicity tests indicate that *F. oxysporum* is the pathogen that caused the severe disease in fields in southern Delaware. This corresponds with observations made previously (1–3,5,6) and supports Jester's hypothesis (8) that *F. oxysporum* is a primary pathogen of soybeans. Although others have proposed that *F. oxysporum* is a secondary invader following other weak pathogens (4,14), no other pathogen was consistently isolated from diseased plants in our study. *Fusarium* spp. other than *F. oxysporum* comprised only 4% of the total *Fusarium* isolates.

Nematodes have been associated with *Fusarium* wilt of soybean (11), but monthly soil sampling for nematodes revealed no correlation between nematode population and disease incidence in our study.

Symptom expression in vascular wilt diseases is greatly influenced by environment, and environmental factors could explain the variability in field observations (3,5,6,8). Although *F. oxysporum* was isolated as readily from diseased plants collected in 1978 as in 1977, losses to the disease were substantially greater in 1977. Since drought occurred in 1977, lack of moisture was a factor in disease development.

Dunleavy (5) reported that heavy rains and cool temperatures after planting increased seedling infection and that moisture stress thereafter aggravated the condition. Cromwell (3) only noted Fusarium blight in mature plants after drought.

Average temperature and rainfall in June at the Bridgeville test site did not differ in 1977 and 1978. However, only 12.01 cm of rain was recorded during July and August 1977, compared with 32.36 cm during that time in 1978. Disease losses were severe in July and August of 1977 but not in 1978. The average air temperature was also higher during July and August of 1977 than in 1978. During September and October of 1978, diseased soybeans were observed when a survey was made of fields in southern Delaware. Only 3.64 cm of rainfall was recorded during this period. Therefore, soil moisture level was an important factor in symptom expression.

Although the significance of the fluctuation of *Fusarium* population in the rhizosphere is difficult to assess from 1 yr of data, some interesting observations can be made. From 18 July to 17 August 1978, the population of *Fusarium* sp. in the soil was at its maximum, after which it steadily declined; 24.23 cm of rain fell in that month. Only 1.63 cm of rain was recorded during the 6 wk when the population declined. The frequency of isolation increased again during October. Perhaps senescence of plant material and an increase in saprobic strains of the fungus could account for this later rise in population.

Isolation of *F. oxysporum* from roots was at a maximum during the later part of the season when soil populations were declining. Increased water in the soil may have favored growth and infection by the pathogen, and the subsequent dry period then created a suitable environment for pathogen development and disease progression in the plant.

The less dramatic symptom expression in the greenhouse than in the field could be attributed to the artificial conditions of greenhouse testing. Temperature and humidity may not have been conducive to disease expression. Other researchers (3,9) have experienced difficulty in reproducing symptoms of *Fusarium* infection in plants grown in the greenhouse and have attributed it to a lack of controlled environmental conditions, varietal resistance, or nonpathogenic strains within a species of the pathogen (1).

Results of our fumigation test do not agree with Gray's findings (7) of various isolates of *Fusarium* sp. from roots of soybean plants in control plots but not in fumigated plots. We found no significant difference in the number of times *Fusarium* spp. or *F. oxysporum* were isolated from roots of plants in fumigated versus nonfumigated plots. The difference in results could be due to difference in efficacy of fumigants.

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