

Interaction of Fusarium Wilt and Nematodes in Cobb Soybean

DONALD R. SUMNER, Professor of Plant Pathology, and NORMAN A. MINTON, Research Nematologist, Department of Plant Pathology, ARS-USDA, Coastal Plain Experiment Station, Tifton, GA 31793-0748

ABSTRACT

Sumner, D. R., and Minton, N. A. 1987. Interaction of Fusarium wilt and nematodes in Cobb soybean. *Plant Disease* 71:20-23.

A host differential greenhouse study showed that Fusarium wilt of Cobb soybean was caused by *Fusarium oxysporum* f. sp. *tracheiphilum* race 1. In experiments with three field soils, internal gray to black stem discoloration in Cobb was most severe in soils infested with *F. o. f. sp. tracheiphilum* race 1, *Belonolaimus longicaudatus*, and *Pratylenchus brachyurus*. Wounding roots with a knife 18-20 days after planting did not increase wilt or internal stem discoloration.

Fusarium wilt of soybean has been observed in many states (1,3,5,15) and

Accepted for publication 10 September 1986
(submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1987.

sometimes can cause substantial reductions in yield (7). Fusarium wilt in soybean (*Glycine max* L.) cultivar Cobb was observed at the Coastal Plain Station in 1982 and 1983 in an experiment with double-cropped corn/soybean. Interveinal chlorosis and necrosis and internal stem discoloration developed during September and October each year and was more severe in plots not treated with nematicides (14). Nematodes are known to increase severity of Fusarium wilt in soybean (15) and cowpea (*Vigna unguiculata* (L.) Walp) (3).

This research was initiated to identify the formae speciales of *Fusarium oxysporum* causing wilt in Cobb soybean in the field and to determine if nematodes were related to the incidence and severity of wilt.

MATERIALS AND METHODS

Cobb soybean plants showing symptoms of interveinal chlorosis and necrosis were collected in 1982 and 1983. Stems from plants with gray to black vascular discoloration were cut into 1-cm sections from 0 to 25 cm above the ground. Tissues were surface-disinfested 30 sec in 0.5% NaOCl, blotted on sterile filter paper, and incubated in petri dishes of water agar. Hyphal tips were transferred to potato-dextrose agar (PDA). Cultures of *Fusarium* spp. were then transferred to carnation-leaf agar and identified (8).

Experiment 1. In the first greenhouse experiment, Tifton loamy sand (fine, loamy, siliceous, thermic Plinthic Paleudults) was collected 15 cm deep in

January 1984 from each of three adjacent fields at the Coastal Plain Experiment Station. The last crops grown in these fields in 1983 were as follows: field A, tobacco (*Nicotiana tabacum* L.); field B, corn (*Zea mays* L.); field C, corn and soybean. The major nematodes affecting these crops were *Meloidogyne incognita* Kofoed & White (root-knot nematode) in all fields and *Belonaimus longicaudatus* Rau (sting nematode) in fields B and C. Other nematodes present in all fields were *Paratrichodorus minor* (Colbran) Siddiqi, *Pratylenchus* spp., and *Cricone-mella* spp. *Helicotylenchus* spp. also were present in field B. Soybean growing in field C in 1982 and 1983 was infected with *F. oxysporum*.

Soils from the different fields were used as whole plots (three replicates) in a split-plot experiment with a randomized complete block design. Subplots were four soil treatments: natural soil, natural soil infested with *F. oxysporum* isolated from Cobb soybean, heat-treated soil (30 min at 65–68 C in wooden trays in an oven), and heat-treated soil infested with *F. oxysporum*. Sub-subplots were cultivars: Yelredo, Yelnando, Cobb, and D78-4668 soybeans and Brown Sugar Crowder cowpea. Yelredo is susceptible to *F. oxysporum* (*F. o.* f. sp. *glycines*) and to race 1 but not race 2 of *F. o.* f. sp. *tracheiphilum*. Brown Sugar Crowder is susceptible to both races of *F. o.* f. sp. *tracheiphilum* but resistant to *F. o.* f. sp. *glycines*. Yelnando is resistant to both races of *F. o.* f. sp. *tracheiphilum*, but its susceptibility to *F. o.* f. sp. *glycines* is not known. The susceptibility of Cobb and D78-4668 to formae speciales of *F. oxysporum* was not known. Cobb is considered resistant to *M. incognita* (13) but sustains a low level of galling and reproduction.

Each soil treatment was mixed with fertilizer (37, 54, and 80 mg/kg NPK, the equivalent of 1, 120 kg of 5-10-15/ha) in a concrete mixer. Cultures of *F. oxysporum* grown on cornmeal-sand (3 g of cornmeal, 100 g of washed sand, 15 ml of deionized water) in flasks for 19–20 days were mixed with soil (1:433, v/v). Each soil treatment was placed in 15-cm-diameter black plastic containers, 800 ml per container, and five seeds of each cultivar were planted 2–3 cm deep in one pot of each replicate of each soil mixture.

Plants were grown in a greenhouse at night/day air and soil temperature ranges of 10–41 and 9–38 C, respectively. Soil was watered by hand as needed. Wilting plants were removed when the leaves were flaccid and did not regain turgor after watering. Stem tissues (1 cm) were surface-disinfested 30 sec in 0.5% NaOCl and incubated on water agar. Hyphal tips were transferred to PDA and then to carnation-leaf agar and identified. Remaining plants were removed 9 wk (replicates 1 and 2) and 12 wk (replicate 3) after planting. The plants were divided

into four categories: green, yellow, mild leaf scorch, and severe leaf scorch. Roots were then removed from the pots, washed, and rated for discoloration and decay on a scale of 1–5 (1 = none, 2 = <10%, 3 = 11–50%, 4 = >50%, and 5 = dead plant) and root galling on a scale of 1–5 (1 = no galls, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% of roots galled). The soil from each pot was mixed, and nematodes were extracted from 150 cm³ of soil by centrifugal-sugar flotation (10).

Experiment 2. Heat-treated Dothan loamy sand was used in a split-split-plot experiment with a randomized complete block design. Whole plots (three replicates) were root wounding vs. not wounding, subplots were isolates of fungi, and sub-subplots were cultivars of soybean and cowpea. Five isolates of *F. oxysporum* and one isolate each of *Rhizoctonia solani* Kühn AG-4, the *Rhizoctonia*-like binucleate fungus CAG-5, and *Macrophomina phaseolina* (Tassi) Goid. were used. The isolate of *R. solani* was from soil from field C. All other fungi were isolated in October 1984 from Cobb soybean plants with interveinal chlorosis and necrosis. Cultivars used in subplots infected with *F. oxysporum* isolates and the control were Yelredo and Yelnando soybean and Brown Sugar Crowder, California Blackeye No. 5, and Groit cowpea. Groit is susceptible to *F. o.* f. sp. *tracheiphilum* race 1 but resistant to race 2, whereas California Blackeye No. 5 is resistant to race 1 and susceptible to race 2. Both cultivars are resistant to *F. o.* f. sp. *glycines*. Cultivars Cobb, Yelredo, Hutton, and Bragg soybean and Groit cowpea were planted in subplots infested with other fungi. Cornmeal-sand inoculum was mixed 1:250 (v/v) with soil in each subplot. Other methods were the same as those used in experiment 1.

Roots were wounded when plants were in the three- to four-leaf stage, 18–20 days after planting. A sterile knife was inserted 10 cm deep into the soil parallel to the stem in two places on opposite sides of each plant. The knife was rinsed in 0.5% NaOCl and flamed in ethanol after all replications of each subplot were completed. Plants in nonwounded whole plots were not disturbed. All plots were watered immediately after wounding. The same parameters were measured as in the first experiment.

Data were analyzed with least-squares analysis of variance and stepwise multiple regression statistical procedures.

RESULTS

Fungi isolated from field-grown soybean plants. *F. oxysporum* was isolated from stems of Cobb soybean plants with gray or black vascular discolorations and interveinal chlorosis and necrosis of leaves in October in both 1982 and 1983 (about 3–4 wk before maturity). In 1983, isolations were made

from stem tissues 0, 5–10, and 15–25 cm above the ground in 19 plants. *F. oxysporum* was isolated from 63, 42, and 56% of the stems at the three heights, respectively. *Diaporthe phaseolorum* (Cke. & Ell.) was isolated from 11, 21, and 56% of the stems at the three heights, respectively. *Macrophomina phaseolina* and *Rhizoctonia*-like binucleate fungi CAG-2, CAG-4, and CAG-5 were isolated infrequently from stems adjacent to the ground and 5–10 cm above ground but not from stems 15–25 cm above ground. *F. solani* and unidentified *Fusarium* spp. also were isolated infrequently.

Experiment 1. Soils. More Brown Sugar Crowder plants were killed in heat-treated soil infested with *F. oxysporum* than in any other treatment (27%), but occasional plants (3–15%) of every cultivar were killed. *F. oxysporum* was isolated from dead or wilted plants of each cultivar in all soil treatments except heat-treated, noninfested soil.

There were no significant differences in the number of plants that died or showed symptoms of leaf scorch among soils, soil treatments, or cultivars. Gray to black internal stem discoloration in the vascular and surrounding tissues occurred in all cultivars except Yelnando in soils B and C (Table 1). The most severe internal discoloration was in Cobb. Most of the plants with discoloration were grown in soil from field C, where *F. oxysporum* was isolated from soybean plants with interveinal chlorosis and necrosis in 1982 and 1983. A few plants of Cobb had vascular discoloration more than 20 cm into the stem in soils B and C, but discoloration rarely extended more than 1–3 cm into the stems of other cultivars.

Nematodes. Twelve weeks after planting, only heat-treated soil from field C contained nematodes (Table 2). Non-heat-treated soil from fields B and C had moderate population levels of *B. longicaudatus*, but none were present in soil from field A. *Meloidogyne incognita* was not recovered from any soil, but very light galling occurred on plants grown in all soil treatments from field A. Light galling also occurred on plants grown in non-heat-treated soil from fields B and C. The presence of light galling on soybean roots but the absence of *M. incognita* larvae in the soil indicated a low population level that had entered the roots but had not reproduced. Other nematodes present in low population levels in non-heat-treated soil from all fields were *Paratrichodorus minor*, *Pratylenchus* spp., and *Cricone-mella* spp. *Helicotylenchus* spp. were also present in the soil from field B. Multiple regression stepwise analysis showed that *B. longicaudatus* and *Pratylenchus brachyurus* caused more plants to show internal stem discoloration ($R^2 = 0.15$, $P = 0.01$) than other nematodes while *M. incognita* populations were negatively

related to the number of plants with internal stem discoloration.

Experiment 2. Fungi isolated and cultivars. All isolates of *F. oxysporum* from Cobb soybean caused some stem discoloration in Cobb and Yelredo, and

three isolates caused slight discoloration in Groit and Brown Sugar Crowder (Table 3). A total of 10, 5, 3, and 1% of the Brown Sugar Crowder, Groit, Yelredo, and Cobb plants, respectively, died in soil infested with *F. oxysporum*.

None of the plants died in the control. Emergence was low in California Blackeye because of poor seed viability, but no wilting or stem discoloration was observed in the few plants that were grown for 12 wk. Wounding did not influence the extent of stem discoloration or plant mortality.

F. oxysporum was reisolated from the stems of 16 Cobb, Yelredo, or Groit plants with vascular discoloration. In four plants (two Cobb and two Yelredo) with extensive stem discoloration, the pathogen was reisolated from stem sections 1–2, 8–10, and 15–18 cm above the ground. The pathogen was isolated frequently from both wounded and nonwounded plants.

Plants were rarely killed in soil infested with isolates of *R. solani* AG-4, *Rhizoctonia*-like CAG-5, and *M. phaseolina*, and no internal stem discoloration was observed in Groit, Cobb, Yelredo, Bragg, or Hutton.

DISCUSSION

Because the *F. oxysporum* isolates caused seedling mortality, wilt, and internal vascular discoloration in Brown Sugar Crowder and Groit cowpea and Yelredo soybean but not in California Blackeye No. 5 cowpea or Yelnando soybean, we concluded that the pathogen causing wilt in fields of Cobb soybean was *F. o. f. sp. tracheiphilum* race 1 (1–4). The lower incidence of seedling death and wilt in our tests than reported by the Armstrongs (1,4) may have been because we infested soil before planting rather than after planting. Others have reported that the extent of vascular discoloration in the primary node of cowpea was more reliable than symptoms of wilt as a measure of disease (17).

Cyst nematodes increased Fusarium wilt in soybean (15), and root-knot nematodes increased Fusarium wilt in cowpea (18). Sting nematodes increased Fusarium wilt in cotton (9), and a root-rot complex in soybean in South Carolina was observed only in fields infested with *F. o. f. sp. glycines* and sting nematodes (3). However, the complex was not investigated in a controlled environment. In field plots of double-

Table 1. Internal stem discoloration in soybean and cowpea cultivars grown in soils from three fields infested with *Fusarium oxysporum*

Soil treatments	Fields ^a					
	A		B		C	
	Number of plants ^y	Discoloration (cm/plant)	Number of plants	Discoloration (cm/plant)	Number of plants	Discoloration (cm/plant)
Field soil	0.2	0.4	0.1 b ^z	0.2	1.0	4.6
Field soil + <i>F. oxysporum</i>	0.1	0.1	0.8 a	3.1	0.8	1.7
Heat-treated soil	0.0	0.0	0.1 b	0.3	0.1	0.3
Heat-treated soil + <i>F. oxysporum</i>	0.3	0.4	0.2 b	0.2	0.3	0.4
Cultivars						
Yelredo soybean	0.1	0.1	0.2	0.5	0.7 ab	1.3 ab
Yelnando soybean	0.1	0.2	0.0	0.0	0.0 b	0.0 b
Cobb soybean	0.2	0.3	0.5	2.5	1.2 a	6.0 a
Brown Sugar Crowder cowpea	0.4	0.6	0.2	0.5	0.6 ab	0.7 b
D78-468 soybean	0.0	0.0	0.5	1.2	0.3 ab	0.8 b

^a All soils were Tifton loamy sand. Fields A and B were adjacent to C, where Fusarium wilt was identified in soybean in 1982 and 1983.

^y Average of 4.1 plants per pot.

^z Numbers within soil treatments or cultivars followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Absence of letters indicates no significant differences.

Table 2. Populations of nematodes and root-knot indices of soybean and cowpea grown for 12 wk in soils from three fields

Soil treatments	Fields (A–C) ^w											
	<i>Belonolaimus longicaudatus</i> ^x			<i>Pratylenchus brachyurus</i> ^x			<i>Paratrichodorus minor</i> ^x			Root-knot indices ^y		
	A	B	C	A	B	C	A	B	C	A	B	C
Field soil	0	46 a ^z	17 b	0	1	21	5 a	4 b	18 a	1.1 b	1.1	1.2
Field soil + <i>F. oxysporum</i>	0	60 a	72 a	0	0	7	13 a	14 a	30 a	1.3 a	1.0	1.1
Heat-treated soil	0	0 b	1 b	0	0	0	0 b	0 b	0 b	1.1 b	1.0	1.0
Heat-treated soil + <i>F. oxysporum</i>	0	0 b	0 b	0	0	0	0 b	0 b	0 b	1.1 b	1.0	1.0

^w All soils were Tifton loamy sand. Fields A and B were adjacent to C, where Fusarium wilt was identified.

^x Larvae per 150 cm³ of soil.

^y 1 = 0, 2 = 1–25, 3 = 26–50, 4 = 51–75, and 5 = 76–100% of roots galled.

^z Numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Absence of letters indicates no significant differences.

Table 3. Internal stem discoloration in soybean and cowpea cultivars grown in soil infested with different isolates of *Fusarium oxysporum*

Cultivar	<i>F. oxysporum</i> isolates ^w										Control	
	1		2		3		4		5			
	No. ^x	cm ^y	No.	cm	No.	cm	No.	cm	No.	cm	No.	cm
Brown Sugar Crowder cowpea	0.2	0.7	0.3 b ^z	0.3 b	0.3	0.4	0.0 b	0.0 b	0.0	0.0	0	0
Groit cowpea	0.2	0.1	0.2 b	0.7 b	0.3	0.1	0.0 b	0.0 b	0.0	0.0	0	0
Cobb soybean	0.8	0.8	2.7 a	4.8 a	1.2	1.5	1.5 a	2.4 b	0.5	0.9	0	0
Yelredo soybean	1.0	1.5	2.5 a	7.8 a	0.7	0.7	0.5 a	1.0 a	0.3	0.8	0	0

^w All isolates were from Cobb soybean.

^x Number of plants with internal stem discoloration (average of 3.5 plants per cultivar).

^y Average length of internal vascular discoloration (cm/plant).

^z Numbers followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Absence of letters indicates no significant differences.

cropped corn/soybean at the Coastal Plain Station, symptoms of wilt were less severe in Cobb when nematicides were used than in controls (14). Twenty-five percent of the yield variations was attributed to *B. longicaudatus*. The nematode is very destructive, even in low numbers, and in our tests, it increased wilt severity more than other nematodes. Ross (15) found that cyst nematodes were more effective than root-knot nematodes in predisposing Lee soybean to Fusarium wilt, but we found no relationship between root-knot nematodes and severity of wilt. However, the populations of root-knot nematodes in soil and the root gall indices were very low.

Pratylenchus brachyurus may have increased symptoms of wilt, but the populations in soil were very low. Some nematodes may have been in the roots. *P. pratensis* was associated with wilt of resistant cotton (*Gossypium hirsutum* L.) cultivars in Georgia (16), and *P. penetrans* increased Verticillium wilt of eggplant (*Solanum melongena* L.) in Canada (12).

We were rarely able to reproduce the symptoms of interveinal chlorosis and necrosis in the greenhouse that we observed in the field. In greenhouse tests, leaf scorch and yellowing occurred occasionally in all treatments but appeared to be related to physiological stress rather than disease severity. If populations of nematodes had been greater in the soils and if the soybean plants had been grown until pods were formed, the symptoms of wilt might have been more typical of those observed in

the field.

Wounding roots with a knife in soil infested with *F. oxysporum* did not increase wilt. Nematodes may cause a physiological change in Cobb as well as mechanical injury to the roots, as has been suggested for the root-knot nematode-Fusarium wilt complex in tomato (*Lycopersicon esculentum* Mill.) (11). *Pratylenchus minyus* influenced the length of the incubation period and the severity of wilt in peppermint (*Mentha piperita* L.), even when the nematodes and the fungus were on separate root systems on the same plant (6).

ACKNOWLEDGMENTS

This research was supported by ARS, USDA, and state funds allocated to the Georgia Agricultural Experiment Station.

LITERATURE CITED

1. Armstrong, G. M., and Armstrong, J. K. 1950. Biological races of the *Fusarium* causing wilt of cowpeas and soybeans. *Phytopathology* 40:181-193.
2. Armstrong, J. K., and Armstrong, G. M. 1958. A race of the cotton-wilt *Fusarium* causing wilt of Yelredo soybean and flue-cured tobacco. *Plant Dis. Rep.* 42:147-151.
3. Armstrong, G. M., and Armstrong, J. K. 1965. A wilt of soybean caused by a new form of *Fusarium oxysporum*. *Phytopathology* 55:237-239.
4. Armstrong, G. M., and Armstrong, J. K. 1980. Cowpea wilt *Fusarium oxysporum* f. sp. *tracheiphilum* race 1 from Nigeria. *Plant Dis.* 64:954-955.
5. Cromwell, J. R. 1917. Fusarium-blight or wilt disease, of the soybean. *J. Agric. Res.* 8:421-439.
6. Faulkner, L. R., Bolander, W. J., Skotland, C. B. 1970. Interactions 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.
7. Ferrant, N. P., and Carroll, R. B. 1981. Fusarium wilt of soybean in Delaware. *Plant Dis.* 65:596-599.
8. Fisher, N. L., Burgess, L. W., Toussoun, T. A., and Nelson, P. E. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72:151-153.
9. Holdeman, Q. L., and Graham, T. W. 1954. Effect of the sting nematode on expression of Fusarium wilt in cotton. *Phytopathology* 44:683-685.
10. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.* 48:692.
11. Jenkins, W. R., and Coursen, B. W. 1957. The effect of root-knot nematodes, *Meloidogyne incognita acrita* and *M. hapla*, on Fusarium wilt of tomato. *Plant Dis. Rep.* 41:182-186.
12. McKeen, C. D., and Mountain, W. B. 1960. Synergism between *Pratylenchus penetrans* (Cobb) and *Verticillium albo-atrum* R. & B. in eggplant wilt. *Can. J. Bot.* 38:789-794.
13. Minton, N. A., Parker, M. B., and Flowers, R. A. 1975. Response of soybean cultivars to *Meloidogyne incognita* and to combined effects of *M. arenaria* and *Sclerotium rolfsii*. *Plant Dis. Rep.* 11:920-923.
14. Minton, N. A., Parker, M. B., and Sumner, D. R. 1985. Nematode control related to Fusarium wilt in soybean and root rot and zinc deficiency in corn. *J. Nematol.* 17:314-321.
15. Ross, J. P. 1965. Predisposition of soybeans to Fusarium wilt by *Heterodera glycines* and *Meloidogyne incognita*. *Phytopathology* 55:361-364.
16. Smith, A. L. 1940. Distribution and relation of meadow nematode, *Pratylenchus pratensis*, to Fusarium wilt of cotton. (Abstr.) *Phytopathology* 30:170.
17. Swanson, T. A., and Van Gundy, S. D. 1985. Influences of temperature and plant age on differentiation of races of *Fusarium oxysporum* f. sp. *tracheiphilum* on cowpea. *Plant Dis.* 69:779-781.
18. Thompson, I. J., Irwin, D. L., and Garber, M. J. 1959. The relationships of the root-knot nematode, *Meloidogyne javanica*, to Fusarium wilt of cowpea. *Phytopathology* 49:602-606.