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Localized Influence of *Heterodera glycines* on Sudden Death Syndrome of Soybean[1]

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Abstract: Half-root tests were established to examine the association between *Heterodera glycines* and the blue strain of *Fusarium solani*, the causal agent of sudden death syndrome (SDS) of soybean. Two independent root systems were established for soybean 'Coker 156' and inoculated (half root/half root) with *F. solani*, *H. glycines*, both organisms on opposite root halves, both organisms on one root half, or neither one. Foliar symptoms were more severe for plants inoculated with both organisms on one root half than on opposite root halves or *F. solani* alone. Root necrosis ratings were more severe when both pathogens were combined on one root half than on opposite root halves. *Heterodera glycines* population development was reduced by the combination of both pathogens on one root half compared to opposite root halves or *H. glycines* alone, regardless of inoculation time.

Key words: Fusarium solani, Heterodera glycines, interaction, nematode, sudden death syndrome, split-root.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.), is caused by a blue-pigmented, macroconidium-producing strain of *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans (18,20). The soybean cyst nematode, *Heterodera glycines* Ichinohe, is often associated with SDS (8,10,11,18). *Heterodera glycines* may enhance symptom expression of SDS (11,18) or cause SDS symptoms to appear earlier than those induced by *F. solani* alone (11). In addition, soybean cultivars resistant to *H. glycines* are less affected by SDS than *H. glycines*-susceptible cultivars (8,21). In field evaluations, however, population densities of *H. glycines* at the end of the growing season are not highly correlated with SDS disease severity or soybean yields (10,21).

Mechanisms of nematode–fungus interactions are not well defined (12,13). Some complexes occur only when both pathogens are in concomitant association on the same root system (9,15,17). An increase in fungal disease severity may be due to greater root penetration by the fungus because of root injury by the nematode (17,23). The nematode may cause a systemic biochemical change, making the plant a more suitable host (3,17,24).

The objective of this research was to determine the localized or systemic effect of *H. glycines* in the SDS soybean disease complex.

Materials and Methods

Two experiments were conducted utilizing a split root technique. Soybean 'Coker 156' seeds were surface disinfested for 10 seconds in 100% ethyl alcohol, washed for 5 minutes in 1.0% sodium hypochlorite, and placed on 26-cm × 39-cm sheets of sterile germination paper. Seedlings with radicles 1–2 cm long were split longitudinally with a sterile razor blade. Each half root was planted into steamed soil (1:1, v/v) mixture of sand and Freestone fine sandy loam soil (72% sand, 16% clay, 12% silt; 0.6% OM, 14.9 CEC, pH = 6.1) in separate plastic containers, commercially called conetainers (Stuewe & Sons, Corvallis, OR) with a volume of 150 cm^3.

Two independent root systems were established for Coker 156 soybean and inoculated (half root/half root) with *F. solani*, *H. glycines*, both organisms on opposite root halves, both organisms on one root half, or neither one. The fungus, isolated from soybean in Mississippi, was initially cultured on a modified potato dextrose agar amended with streptomycin sulfate (100 mg/liter) and aureomycin (2 mg/liter)

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(PDA/SA), was subcultured in a modified potato dextrose broth, and finally was subcultured on sterile white grain sorghum. The soil in the conetainers was inoculated with the fungus-infected sorghum at 0.05% w/w (G. L. Sciumbato, pers. comm.). Cysts of *H. glycines* race 3, cultured on Coker 156 soybean, were crushed with a modified Seinhorst cyst crusher (22). Inoculum per conetainer of 2,000 eggs and J2 was incorporated into the top 2.5 cm of soil in the appropriate treatments and covered with sterile, uninfested soil. The inoculations were done simultaneously in the first experiment (Table 1) and simultaneously and sequentially in the second experiment (Table 3).

In both tests, plants were allowed to grow for 60 days. Foliar disease index severity ratings were made daily using a scale of 0 to 7 where 0 = no symptoms, 1 = mosaic mottling, 2 = chlorotic mottling, 3 = interveinal chlorosis, 4 = interveinal chlorosis with leaf edge necrosis, 5 = interveinal necrosis, 6 = defoliation with the leaflets separating from the petiole but the petiole remaining attached to the plant, and 7 = plant death.

At harvest on day 60, root necrosis index severity ratings, numbers of *H. glycines* cysts, females, males, eggs, and juveniles, and number of cysts colonized by *F. solani* were made. The necrosis scale was 1 to 5 (1 = no necrosis, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%). Shoot and root fresh and dry weights and plant heights were also recorded. *Heterodera glycines* cysts were extracted from the soil using gravity screening (2). Second-stage juveniles were extracted from the soil using gravity screening and centrifugal flotation (sucrose specific gravity = 1.13) (6). To determine the percentage of *H. glycines* cysts colonized by *F. solani,* 100 light brown to tan cysts were surface disinfested for 4 minutes in 0.525% sodium hypochlorite, washed three times for 1 minute each in 1.0% streptomycin sulfate, aseptically transferred to PDA/SA plates, and incubated in the dark for 7 days at 25 C.

The experimental design for both tests was a randomized complete block with four replications and seven subsamples per treatment. Each experiment was run three times. Data were subjected to analysis of variance. Means were compared using Fisher's protected least significance difference test.

Results

Symptoms of sudden death syndrome developed only in the treatments that received *F. solani* alone or in combination with *H. glycines*. The foliar disease indices were significantly higher in treatments with *F. solani* and *H. glycines* on the same root-half than with *F. solani* on one half and *H. glycines* on the other half or *F. solani* alone on a root half (Table 1). Root and shoot data were negatively associated with disease indices, although some differences were not statistically different.

Numbers of *H. glycines* were less (P = 0.05) if the nematode and fungus were on the same half-root than in any other combinations (Table 2). There was a tendency toward a lower population density when *F. solani* and *H. glycines* were on opposite root halves compared to *H. glycines* alone.

Fusarium solani colonized the cysts only from the half-root system where both organisms were present. Fourteen percent of the cysts were colonized. Eggs from these cysts were also infected by the fungus.

Delaying inoculation with the fungus 2 weeks after *H. glycines* inoculation did not alter disease expression or plant growth (Table 3). Overall, trends were similar to the first experiment, with the exception in the second experiment that root necrosis did not differ on plants inoculated with the nematode and fungus on the same half root or separate half roots. In the first experiment, the nematode on a separate half root did not significantly alter the disease effects from that of the fungus alone. Effects of the fungus on nematode population densities were similar to that in experiment 1 (Table 2). Colonization of cysts and eggs by the fungus was also similar between experiments.

TABLE 1. Foliar disease index (FD), root necrosis index (RD), plant height (cm), and shoot and root weight (g) of 'Coker 156' soybean as influenced by *Heterodera glycines* (N) race 3 and *Fusarium solani* (F) on sudden death syndrome of soybean.

Treatment					Shoot weight		Root weight	
Root-half1	Root-half2	FD†	RD‡	Plant height	Fresh	Dry	Fresh	Dry
—	—	0.00	0.00	22.3	3.36	1.00	5.68	1.47
F	—	1.46	3.37	21.2	3.18	0.95	5.15	1.44
N	—	0.00	2.56	22.3	3.77	1.00	4.00	1.23
F	N	1.66	3.49	21.1	3.11	0.86	3.51	1.20
FN	—	2.10	4.20	20.0	2.88	0.80	3.42	1.11
FLSD ($P = 0.05$)		0.40	0.79	2.6	0.33	0.20	1.38	0.50

Data are means of three runs and four replications of each treatment containing seven subsamples. Means compared using Fisher's protected least significant difference test.

† Foliar disease index: 0 = no symptoms, 1 = mosaic mottling, 2 = chlorotic mottling, 3 = interveinal chlorosis, 4 = interveinal chlorosis with leaf edge necrosis, 5 = interveinal necrosis, 6 = defoliation of leaflets, and 7 = plant death.

‡ Root necrosis index: 1 = no necrosis, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.

DISCUSSION

The concomitant association of *F. solani* and *H. glycines* increases the effects of sudden death syndrome. *Heterodera glycines* produced a localized effect to enhance the disease caused by *F. solani*. However, there may be conditions in which the nematode may cause a systemic effect, as occurred in the second experiment. A translocatable effect on Fusarium wilt developed with *Meloidogyne incognita* on tomato (3,24). These organismal complexes on cotton and tobacco gave only localized effects (9, 14,15). The localized effect of *H. glycines* in our study may be due, in part, to wounding. These wounds would provide a path of entry into the soybean roots for *F. solani*. Mere wounding may not be the sole answer, as noted for tobacco inoculated with *F. oxysporum* and *Meloidogyne* spp. (16) and cowpea inoculated with *M. javanica* and *F. oxysporum* f. sp. *tracheiphilum* (5). Morphological and anatomical changes occur in the susceptible host root infected by the nematode (4,17).

TABLE 2. Population densities (per 150 cm^3) of *Heterodera glycines* (N) race 3 life stages as influenced by *Fusarium solani* (F) on 'Coker 156' soybean.

Treatment							
Root-half1	Root-half2	TIF†	Cysts	Juveniles	Males	Eggs/cyst	Females
			Test 1				
N	—	0	798	988	517	289	85
F	N	0	533	764	309	263	92
FN	—	0	169	226	68	68	38
FLSD ($P = 0.05$)			518	719	297	163	82
			Test 2				
N	—	0	1220	518	495	263	184
F	N	0	870	421	576	100	203
FN	—	0	436	283	456	92	92
F	N	14	900	597	760	197	176
FN	—	14	450	422	452	118	111
FLSD ($P = 0.05$)			546	394	222	130	116

Data are means of three runs with four replications of each treatment containing seven subsamples. Means compared using Fisher's protected least significant difference test.

† Time of inoculation with *Fusarium solani*.

TABLE 3. Effect of time of inoculation (TIF) with *Fusarium solani* (F) on foliar disease index (FD), root disease index (RD), plant height (cm), and shoot and root weight (g) of 'Coker 156' soybean infected with *Heterodera glycines*.

Treatment						Shoot weight		Root weight	
Root-half1	Root-half2	TIF	FD†	RD‡	Plant height	Fresh	Dry	Fresh	Dry
—	—	0	0.00	0.00	19.8	4.52	1.27	4.89	0.93
F	—	0	1.33	1.59	19.6	4.53	1.25	4.93	0.72
N	—	0	0.00	1.22	19.6	4.00	1.21	4.28	0.74
F	N	0	1.52	3.20	20.9	4.16	1.06	4.03	0.70
FN	—	0	2.12	3.17	20.1	3.80	1.00	3.90	0.65
F	—	14	1.50	2.13	20.8	4.39	1.24	4.97	0.77
F	N	14	1.58	3.34	20.2	4.14	1.15	4.13	0.73
FN	—	14	2.05	3.20	19.7	4.16	1.14	3.62	0.66
FLSD ($P = 0.05$)			0.45	0.67	2.1	0.70	0.21	0.98	0.29

Data are means of three runs containing four replications each of treatments. Means compared using Fisher's protected least significant difference test.

† Foliar disease index: 0 = no symptoms, 1 = mosaic mottling, 2 = chlorotic mottling, 3 = interveinal chlorosis, 4 = interveinal chlorosis with leaf edge necrosis, 5 = interveinal necrosis, 6 = defoliation of leaflets, 7 = plant death.

‡ Root necrosis index: 1 = no necrosis, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%.

Population levels of sedentary forms of plant-parasitic nematodes are generally depressed as a result of interactions with fungi (1,7,17,19). This reduction is due to fewer feeding sites for the nematode. Pathogenicity of *F. solani* to *H. glycines* through colonization of the cysts and eggs further reduces *H. glycines* population levels and subsequent generations (11).

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