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Interrelationship of *Heterodera glycines* and *Fusarium solani* in Sudden Death Syndrome of Soybean[1]

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Abstract: Experiments were established in field microplots to examine the association between *Heterodera glycines* and the blue form of *Fusarium solani* in sudden death syndrome of soybean (SDS). Foliar disease symptoms occurred on more plants per plot, appeared 3 to 7 days earlier, and were more severe on plants grown in plots infested with *F. solani* + *H. glycines* than on those inoculated with *F. solani* only. Yields were suppressed only in treatments that included the nematode. Numbers of *H. glycines* cysts and second-stage juveniles were significantly lower in plots infested with *F. solani* + *H. glycines* than with the nematode alone. *Fusarium solani* was able to infect cysts and eggs.

Key words: Disease complex, *Fusarium solani, Heterodera glycines,* interaction, microplot, nematode, soybean cyst nematode, sudden death syndrome.

Sudden death syndrome (SDS) of soybean (*Glycine max* (L.) Merr.), is caused by a blue-pigmented, macroconidium-producing form of *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans (17,18). Symptoms of SDS include root rot, crown necrosis, vascular discoloration of roots and lower stems, interveinal chlorosis and necrosis of leaflets, defoliation, and pod abortion (6,17,18,20). SDS occurs in Alabama, Arkansas, Illinois, Indiana, Kentucky, Louisiana, Mississippi, Missouri, and Tennessee (7,17,18). The disease tends to occur on well-managed soybeans growing under conditions where high yields are likely (5,18). Soybean yield losses up to 80% have been reported (7).

The soybean cyst nematode, *Heterodera glycines* Ichinohe, has often been associated with SDS (5,10,17,18). In greenhouse studies, inoculation of soybean seedlings with the blue-pigmented *F. solani* and *H. glycines* produced more severe foliar symptoms of SDS (10,17) and they occurred 3–5 days earlier (10) than those caused by *F. solani* alone. The role of *H. glycines* in SDS under field conditions has not been resolved, although soybean cultivars resistant to *H. glycines* have been less affected by SDS than have *H. glycines*-susceptible cultivars (5,19).

Interactions between *H. glycines* and fungi that result in increased crop damage are well documented (15). Within 5 years after the first identification of *H. glycines* in the United States, the nematode was shown to increase the incidence and severity of Fusarium wilt on soybeans (16). *Heterodera glycines* race 3 and *Phytophthora megasperma* Drechs. var. *sojae* Hild. were shown to additively increase seedling disease severity on soybean (1). Variable results have been shown between *Macrophomina phaseolina* (Tassi) Goid. and *H. glycines*. In one test, crop losses were increased by *M. phaseolina* in the presence of *H. glycines* (22); however, in a separate study, no interaction between the two pathogens was observed (4). *Calonectria crotalariae* (Loos) Bell & Sobers enhanced *H. glycines* penetration in both susceptible and resistant soybeans (14). The objective of this research was to determine the influence of *H. glycines* on the development of SDS of soybeans under field conditions.

MATERIALS AND METHODS

Tests were conducted in field microplots located on the North Plant Science Research Farm at Mississippi State University. Microplots consisted of 76-cm-d fiberglass cylinders placed 45 cm deep in a Stough fine sandy loam soil (68.4% sand, 11.6% clay, 20.0% silt; 0.3% OM, 8.0 CEC, pH 6.4). The soil in the microplots was fumigated with 680 g methyl bromide per

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30.5 m^2 soil. These plots were covered with a 114-μm (4.5-mil) thick polyethylene tarp for 72 hours. The tarp was removed and plots were planted 45 days later. Treatments were *F. solani, H. glycines, F. solani* + *H. glycines* combination, and an untreated control (no nematode or fungus). Four treatments were arranged in a randomized complete block design with six replications in 1990 and four replications in 1991.

Fusarium solani was isolated from soybean roots. Roots were washed in running tap water for 5 minutes and cut into 3–5 mm sections. The cortical tissue was separated from vascular tissue using sterile forceps. The tissues were surface-disinfected for 5 seconds in 100% ethyl alcohol and 1 minute in 1.0% sodium hypochlorite, placed on potato dextrose agar (Difco Laboratories, Detroit, MI) amended with streptomycin sulfate (100 μg/ml) and aureomycin (2 μg/ml) (PDA/SA), and cultured for 5 to 7 days. Agar disks 5-mm-d containing *F. solani* were aseptically placed into sterile cornmeal–sand cultures (250 cm^3 dry sand, 14 cm^3 cornmeal, and 100 ml distilled water) and maintained in the dark for 21 days at 24 C (17). Cultures were shaken periodically to distribute the fungus. All the cornmeal–sand inoculum was removed from the flasks, combined, mixed by hand, and weighed. The appropriate microplots were then infested by incorporating the cornmeal–sand inoculum into the upper 15 cm of soil (0.05% w/w) by mixing with a garden hoe.

A race 3 population of *H. glycines* was increased on soybean (Coker 156) in a greenhouse. Light brown to tan colored cysts were dislodged from the roots with a strong water spray and collected on nested sieves with pore sizes of 850 and 250 μm. Cysts were placed into 20-ml glass test tubes and crushed with a modified Seinhorst cyst crusher (21). The resultant suspension was passed through a 75-μm-pore sieve nested on a 28-μm-pore sieve to remove broken cysts and debris. The inoculum was incorporated in the appropriate treatments by pipetting the nematode suspension into 10 depressions 5 cm deep and 2 cm wide. Soil was then mixed with a garden hoe to a depth of 15 cm, obtaining an inoculum level of 2,500 eggs and J2/250 cm^3 soil.

Coker 156 soybean seed were surface sterilized for 10 seconds in 100% ethyl alcohol, washed for 5 minutes in 1.0% sodium hypochlorite, and placed on sheets of 26-cm × 39-cm sterile germination paper (Anchor Paper, St Paul, MN) for 36 hours. Seedlings with radicles of 1–2 cm in length were plated in a single row in each plot. Plots were watered manually in 1990 and with drip irrigation in 1991. Maximum and minimum weekly temperatures and amount of rainfall was recorded for both years.

Foliar disease severity ratings were made at 3-day intervals beginning 10 weeks after planting using a 0 to 7 scale: 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 leaving the petiole attached to the plant, and 7 = plant death. The incidence of symptom expression, plant height, number of pods per plant, seed yield, and weight of 100 seed were also recorded.

The number of *Heterodera glycines* was determined at soybean maturity. Six soil cores 2.25-cm-d × 15-cm deep were collected from the soybean root zone in each microplot. *Heterodera glycines* cysts were extracted from 250 cm^3 soil by sieving as described for inoculum production. J2 passing through the sieves used to collect cysts were extracted from the suspension using gravity-screening (2). Final separation of J2 in the fraction collected on the 28-μm-pore sieve was by sucrose centrifugal flotation (sucrose specific gravity = 1.13) (8).

The percentage of *H. glycines* cysts colonized by *F. solani* was determined. One hundred light brown to tan cysts were placed in 0.525% sodium hypochlorite for 4 minutes, 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.

Soybean root tissue was collected when

SDS symptoms appeared in the plots (soybean R3 to R5 growth stage) (3) from each treatment. Samples were fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer, postfixed in 2% osmium tetroxide, dehydrated in a graded ethanol series, and critical-point dried in carbon dioxide. Specimens were mounted on aluminum stubs and sputter coated with gold palladium. Mounted samples were observed with a JEOL U.S.A. JSM-35CF scanning electron microscope at 15 kV, and images were recorded on Polaroid Type SSP/N film.

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 plots inoculated with *F. solani* alone or in combination with *H. glycines*. Foliar leaf symptoms, incidence, and severity of SDS were greater (P = 0.05) on plants in the *F. solani* + *H. glycines* treatment than on plants grown in the *F. solani* treatment (Fig. 1). Symptoms of SDS developed in the *F. solani* + *H. glycines* combination treatment plots 85 (R3—beginning pod formation) and 98 (R5—beginning seed formation) days after planting in 1990 and 1991, respectively. SDS symptoms developed in the *F. solani* treatment 7 and 3 days later in each of these years. The combination treatment produced 35% more symptomatic plants in 1990 and 18% more in 1991 than in the *F. solani* treatment.

Percentage soybean plant survival, plant height, and weight per 100 seed were affected by treatment (Table 1). Soybean seed yields were similarly suppressed by *F. solani* + *H. glycines* and *H. glycines*. Pod production was also reduced by these two treatments.

Heterodera glycines population numbers at the end of the season were lower in the presence of *F. solani* than with the nematode by itself (Table 2). The presence of the fungus resulted in 19, 57, and 40% fewer cysts, J2, and eggs, respectively. Total number of cysts, eggs, and J2 at harvest were correlated with SDS foliar ratings (r = 0.51, P = 0.05). *Fusarium solani* colonized 17% (1990) and 14% (1991) of the cysts recovered at harvest from the *F. solani* + *H. glycines* treatment. Eggs were also colonized with the fungus, but this colonization was not quantified. *F. solani* was not isolated from *H. glycines* cysts or eggs from the *H. glycines* treatment.

H. glycines females not associated with *F. solani* were lemon shaped and ruptured through the root epidermis. In contrast, females in the *F. solani* + *H. glycines* treatment were flattened and associated with

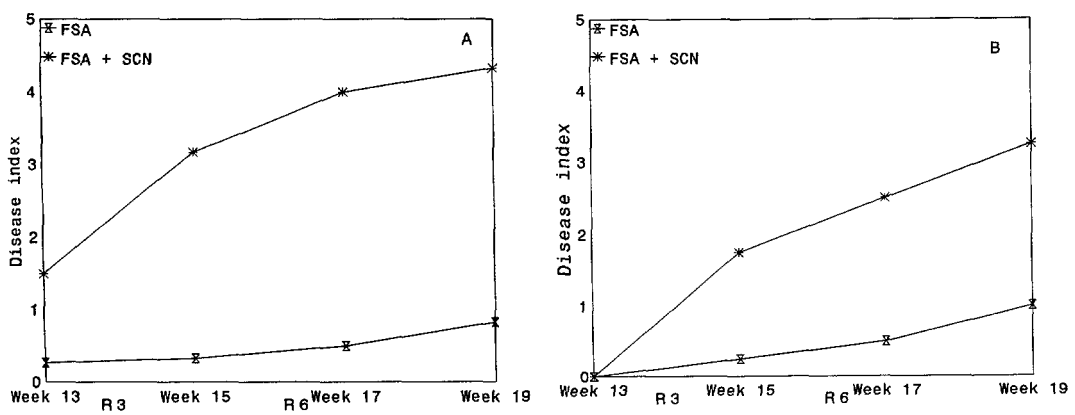

FIG. 1. Sudden death syndrome disease index ratings for soybean plants in 1990 (A) and 1991 (B) in microplots infested with *Fusarium solani* (FSA) and with or without *Heterodera glycines* (SCN). Foliar disease severity rating scale: 0 = no symptoms, 1 = mosaic mottling, 2 = chlorotic mottling, 3 = interveinal chlorosis, 4 = interveinal chlorosis with leaf edge necrosis, 5 = interveinal necrosis, 6 = defoliation with the leaflets separating from the petiole, leaving the petiole attached to the plant, and 7 = plant death.

TABLE 1. Effect of *Heterodera glycines* (SCN) race 3 and *Fusarium solani* (FSA) on height, yield, number of pods/plant, survival (% of control), weight/100 seed, and percentage of symptomatic plants of Coker 156 soybean grown in field microplots.

Treatment	Plant† height (cm)	Survival %	Seed yield (g/plot)	Pods per plant	Seed g/100
		1990			
FSA + SCN	99.3	97	340	107.9	12.4
FSA	102.6	92	467	141.1	12.6
SCN	99.1	98	359	94.7	12.2
Control	108.2	100	475	142.8	12.1
FLSD (P = 0.05)	NS	NS	118	24.5	NS
		1991			
FSA + SCN	96.5	98	264	75.8	12.8
FSA	101.0	98	334	86.8	12.4
SCN	97.8	96	271	80.7	13.2
Control	106.0	100	372	90.5	11.4
FLSD (P = 0.05)	9.4	NS	112	NS	NS

All yield parameters were measured at plant maturity. Values are averages of six replications in 1990 and four replications in 1991.

† Height of plants at R5 growth stage.

fungal hyphae. Many of the females did not rupture the root epidermis.

In 1990, the average maximum and minimum ambient air temperatures for weeks 1–3 after planting were 26.8 C and 15.9 C, respectively; for weeks 4–12, they were 31.6 C and 20.2 C, respectively (Fig. 2). Eighty percent of the rainfall occurred during the 12 weeks before symptom appearance. During week 13 when symptoms appeared, the maximum and minimum weekly temperatures decreased 2–3 C to 29.9 C and 17.1 C, respectively, then in week 14 increased 4 and 6 C to 35.5 C and 21.8 C, respectively. During weeks 14–17, maximum and minimum temperatures averaged 34.8 C and 21.6 C, respectively. In 1991, during weeks 1–3, average maximum and minimum ambient air temperatures were 29.1 C and 20.7 C, respectively, followed by 31.8 C and 20.2 C, respectively, during weeks 4–12. Fifty percent of the rainfall for the growing season occurred during week 2 after planting. The rainfall for weeks 3–13 was sporadic. Symptoms of SDS were observed when plants reached the R5 soybean growth stage late in week 14. In 1991, a reduction in temperature was not observed in week 13 or 14 prior to symptom development. Temperatures remained relatively constant for weeks 15–19 after symptom expression, and they averaged 31.9 C and 21.0 C, respectively. Although more precipitation was recorded in 1991, less occurred early in the season than in 1990. The lack of rainfall early in the season required supplemental moisture, which was provided through irrigation resulting in fluctuating levels of soil moisture.

DISCUSSION

Fusarium solani (blue form) causes sudden death syndrome, but the disease is en-

TABLE 2. End-of-season numbers of *Heterodera glycines* (SCN) race 3 per 250 cm^3 soil as affected by *Fusarium solani* (FSA).

Treatment	1990 Cyst	1990 J2	1990 Eggs/cyst	1991 Cyst	1991 J2	1991 Eggs/cyst
FSA + SCN	1179	146	152	332	83	64
SCN	1458	343	255	729	186	135
FLSD (P = 0.05)	221	74	NS	170	NS	NS

Values are means of six replications in 1990 and four replications in 1991.

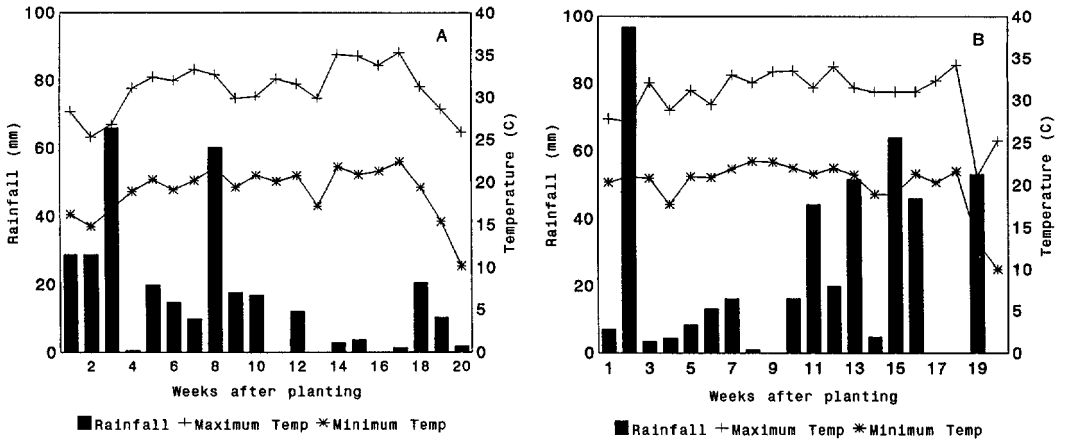

FIG. 2. Weather data for field microplot experiments involving sudden death syndrome in 1990 (A) and 1991 (B). Rainfall (mm) is indicated by bars; maximum and minimum temperatures are represented by solid lines.

hanced by *Heterodera glycines*. The large increase in SDS and the earlier expression of symptoms by *H. glycines* in microplots provides evidence that the nematode is an important factor in the disease as found in greenhouse research (10,12,13,17). The correlations between population levels of *H. glycines* at harvest and the ratings of severity of SDS foliar disease symptoms is large enough to add support that the nematode enhances the disease. In addition, soybean cultivars resistant to *H. glycines* are less affected by SDS than nematode-susceptible cultivars (5).

The reduction of the numbers of *H. glycines* may be a response to at least two factors. The fungus causes a root rot, which would limit feeding sites and interferes with nematode development. The colonization of *H. glycines* cysts and eggs by *F. solani* probably accounts for some reduction in the population density of the nematode as found previously (10,12,13). Egg production by *H. glycines* females was also reduced.

Suppression of soybean seed yield has been associated with SDS (23). Yield was affected only by *H. glycines* in our experiment. The relationship between SDS disease ratings and yields has not been consistent (5,17,20). This relationship is likely influenced by the soybean growth stage when infection occurs and the conditions under which symptoms occur (9). If symptoms develop before pods are fully formed, then yields tend to be suppressed (5).

The difference in foliar disease indexes of the *F. solani* + *H. glycines* treatment between years may be due to variation in environmental factors. The growing season in 1990 was cool and wet during May, moist in June and July, and hot and dry in August and September. The 1991 growing season was wet in May, hot and dry in June and July, and mild and wet in August and September. Irrigation was required in early June and resulted in fluctuations in soil moisture. We speculate that a growing season that begins cool and stays moist followed by relatively high temperatures creates optimal environmental conditions for SDS symptom expression. Cool, wet conditions when soybean plants are in the seedling and early vegetative growth stages are conducive to SDS disease development (9). Early and late planted soybean plants have been reported to develop SDS symptoms during cool, wet growing seasons (5,6,9); however, severe disease has occurred during hot and dry weather conditions (18). A cool period with rain in early August is also associated with SDS appearance (R. D. Riggs, pers. comm.). The variations in appearance of SDS symptoms, and their severity, stress the need for additional infor-

mation on the environmental conditions that influence SDS symptom expression. Temperature and moisture influences on presymptomatic stages of SDS foliar symptom expression should be examined further to explain adequately the variation observed.

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