Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in Sudden Death Syndrome of Soybean

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ABSTRACT

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Sudden death syndrome (SDS) of soybean is caused by the soilborne *Fusarium solani* f. sp. *glycines* (synonym *F. virguliforme*). In a sequential approach, two multifactor factorial-design microplot experiments were conducted to investigate the effects of fungal infestation levels and soil moisture on both root necrosis and foliar SDS severity, and the interaction between *F. solani* f. sp. *glycines* and *Heterodera glycines* in fumigated versus nonfumigated soil. In 2003, soybean cv. Spencer was grown in non-fumigated or methyl bromide-fumigated soil and infested with increasing levels of *F. solani* f. sp. *glycines*, either under rainfall or irrigated after growth stage V6/R1. In 2004, interactions between *F. solani* f. sp. *glycines* and *H. glycines* were explored in a factorial inoculation design in fumigated or nonfumigated soil, planted to Williams 82 or Cyst-X20-18. In both years, higher levels of foliar SDS severity and root necrosis were found in *F. solani* f. sp. *glycines*-infested soils with *H. glycines* than in

soils without the nematode on the soybean cultivars susceptible to both pathogens. Both natural infestations of *H. glycines* in 2003 and artificially amended populations of *H. glycines* in 2004 contributed to higher foliar SDS severity. More severe foliar SDS symptoms always were associated with more root necrosis, but elevated levels of root necrosis did not predict severe leaf symptoms. In contrast to the critical role of *H. glycines*, increasing fungal infestation levels had no significant effects on increasing either foliar SDS symptoms or root necrosis. Effects of moisture regime and fungal infestation levels also were examined in factorial greenhouse and growth chamber experiments. High soil moisture resulted in higher levels of SDS root necrosis. In the greenhouse, root necrosis increased at a higher rate in low soil moisture than the rate in high soil moisture. The two pathogens acted as a complex and the disease development was strongly dependent on high soil moisture.

Additional keywords: co-inoculation, disease complex, *Glycine max*, soybean cyst nematode.

Sudden death syndrome (SDS) is an important disease in most soybean-growing areas of the United States and Canada (50). The soilborne fungus *Fusarium solani* f. sp. *glycines* (synonym: *F. virguliforme*) (2) is the causal agent of SDS (30). The pathogen first was identified as *F. solani* "form A" or a blue isolate of *F. solani* in 1989 (32,34). The pathogen infects soybean (*Glycine max* (L.) Merr.) roots early in the season (12,19,27) and, when high soil moisture is present at the beginning of the reproductive stages of soybean, the pathogen produces toxins that are translocated into aboveground parts of the plant where they cause foliar symptoms (16). In severe cases, SDS results in flower and seed pod abortion, reduced pod and seed fill, premature defoliation, and yield losses (31). The importance of root colonization, discoloration, and necrosis in identifying resistant germplasm, disease development, and the effects of differential infection on the root system have been reported earlier in greenhouse and field studies (9,19,25,27,28,34). It is surmised that resistance traits to root rot and SDS foliar symptoms are conferred independently (9,19,27). However, it is not clear how root necrosis relates to foliar symptoms of SDS under field conditions.

Management of SDS has been difficult. Fungicides have not provided effective suppression of SDS, and crop rotation is not always effective and does not provide a reliable management alternative (12,39). Selecting for host-plant resistance has been successful in identifying some resistant cultivars, but introgression remains difficult because resistance relies on several minor

genes (14). In addition, screening for resistance is difficult in the field and greenhouse (10,23,25) because disease onset and expression are strongly dependent on environmental factors (36,44). Information is limited on the potential of biological control of this disease as an alternative, environmentally friendly approach. Only limited tests have been done to evaluate the potential of biological control agents against *F. solani* f. sp. *glycines* (4,35,42,54). Perhaps most importantly, integrated approaches to management of SDS suffer from the lack of understanding of the ecology of the pathogen and factors that favor development of the disease.

In the Midwest of the United States, SDS tends to be most severe in soybean plants with high yield potential (11,31,36,40) and in soil with high levels of fertility (P, Mg, and organic matter) (40). SDS is favored by high soil moisture levels during the reproductive stages and by unseasonably low temperatures prior to or during flowering and pod set (31,36,43). The disease typically occurs first in low, wet, and compacted areas in a given field and is prevalent in irrigated fields or in areas of the field where the soybean cyst nematode (SCN), *Heterodera glycines*, has been a problem (31). *F. solani* f. sp. *glycines* alone causes SDS, but SDS often is found in association with *H. glycines* (31,32,38). Although neither *H. glycines* nor other root infections are required for SDS, they all share similar environmental factors for disease development (38). The most severe outbreaks of the disease have been observed in fields that are infested with *H. glycines* (11,31,40). Previous studies on the interaction between *F. solani* f. sp. *glycines* and *H. glycines* and their effects on SDS development were conducted under both greenhouse and field microplot conditions only in fumigated soil with continuous or periodic irrigation (20,24,32). These experiments were conducted to examine

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fewer factors than those that possibly are involved in progression of foliar symptoms under field conditions. Therefore, further research with factorial investigation of both the abiotic factor (water) and biotic factors (*F. solani* f. sp. *glycines*, *H. glycines*, and other soil microorganisms) in a systematic approach is urgently needed. Such study will determine whether the presence of indigenous microorganisms in the soil alters the relationship between *F. solani* f. sp. *glycines* and *H. glycines* and SDS development in irrigated or nonirrigated field conditions. Although foliar SDS severity was higher and yields were more suppressed with *H. glycines* infestation than *F. solani* f. sp. *glycines* infestation alone (20,21,24,32), the correlation between foliar SDS severity and SCN density in soil, and soybean yield, was inconsistent (11,17,20,40,45), and more information is needed to explain the variation in such studies. In summary, the nature of this potential relationship and the putative interaction of both pathogens under field conditions still remain unclear.

Our objectives were to investigate the ecological aspects of *F. solani* f. sp. *glycines* and to identify the interactions among *F. solani* f. sp. *glycines*, *H. glycines*, or other soil microorganisms in controlled field microplot environments. In this study, in multifactor factorial design, we tested whether threshold levels of *F. solani* f. sp. *glycines* can be determined and how *F. solani* f. sp. *glycines* and *H. glycines* interact in nonfumigated versus preseason-fumigated field soil under simulated field conditions. Determining the effects of these soil treatments on the correlation between foliar SDS severity and external root necrosis was part of this study. In both 2003 and 2004, SDS-susceptible soybean was planted into fumigated and nonfumigated soil amended with inoculum of *F. solani* f. sp. *glycines*. In 2003, different soil moisture levels and infestation levels of *F. solani* f. sp. *glycines* in soil were investigated on SDS development and disease severity on soybean cv. Spencer in fumigated or nonfumigated soil. In 2004, potential interactions between *F. solani* f. sp. *glycines* and *H. glycines* were explored when they were inoculated into plots in a factorial design with fumigated or nonfumigated soil. The disease response of the *H. glycines*-resistant soybean line Cyst-X20-18 (U.S. patent #6,096,944) was compared with the SDS- and SCN-susceptible soybean cv. Williams 82. A greenhouse assay was developed to determine effects of inoculum levels of *F. solani* f. sp. *glycines* and to investigate the role of soil moisture on root rot severity. Preliminary results were reported previously (51–53).

MATERIALS AND METHODS

Production of *F. solani* f. sp. *glycines* and *H. glycines* inoculum. Two isolates of *F. solani* f. sp. *glycines* (Francesville and Lafayette) were used in this study, both isolated from diseased soybean roots in fields naturally infested with *F. solani* f. sp. *glycines* (Scott Abney, United States Department of Agriculture–Agricultural Research Service, Purdue University). The isolates were stored in 50% glycerol solution at -80°C . Active cultures were obtained on potato dextrose agar (PDA) by incubation for 2 to 3 weeks at $22 \pm 3^{\circ}\text{C}$. Each isolate was grown separately on sterile sand-cornmeal medium (SC) (1 liter of sand, 56 ml of food-grade cornmeal, and 325 ml of H_2O) for 1 month in glass mason jars at $22 \pm 3^{\circ}\text{C}$.

Populations of *H. glycines* were grown on soybean (cv. Lee 74) inoculated with second-stage juveniles in pots with autoclaved sand:soil mixture (2:1, vol/vol) (SS) under greenhouse conditions with a 12-h photoperiod of fluorescent light at $25 \pm 2^{\circ}\text{C}$ for 3 months. At the same time, non-nematode-inoculated pots were prepared for controls. After incubation, roots and plant debris were removed, and the SS was dried for 2 weeks under paper to avoid direct sun exposure. Such SS with or without cysts of *H. glycines* was sieved (8-mm aperture sieve) and used as nematode inoculum or control treatment.

Field microplots. The microplot tests of 2003 and 2004 were conducted in 45-cm-diameter, corrugated polyethylene drainage tubing with a smooth inside surface, inserted perpendicularly 55 cm deep into the ground at the Agronomy Center for Research and Education, Purdue University, West Lafayette, IN. In both years, the multifactor factorial experiments were arranged in randomized complete block designs with four replications. Methyl bromide was applied through a dry-connect system at a 25-cm depth. Plots were covered immediately with 0.1-mm thick, black polyethylene sheets and sealed. After incubation for 6 days, the plots were aerated sufficiently before the other factors were applied.

In spring 2003, soil (22% sand, 56% silt, 22% clay, pH 7) from a field naturally infested with *H. glycines* was mixed and placed in the plots. In a three-factor factorial design, the following treatments were applied: (i) nonfumigated or methyl bromide-fumigated at 450 kg/ha; (ii) natural precipitation or 15 liters of water added once per week after R1 (8); and (iii) four *F. solani* f. sp. *glycines* infestation levels. The SC inocula of the two *F. solani* f. sp. *glycines* isolates were mixed with autoclaved, previously infested SC to establish an infestation series of 0, 6.3×10^3 , 3.2×10^4 , or 1.7×10^5 macrospores/ml of soil by amending 400 g of such mixes to each plot and incorporating into the top 15 cm of soil, because the pathogen was shown to be most frequent in this depth layer in naturally occurring SDS fields (38). Noninfested plots received autoclaved, previously infested SC in the same way. Soybean cv. Spencer (susceptible to both *F. solani* f. sp. *glycines* and *H. glycines*) was planted on 6 May 2003 and thinned to 20 plants per plot in three parallel rows at 12.5-cm spacing after emergence.

In spring 2004, field-derived soil without a documented history of SDS (52% sand, 30% silt, 18% clay, pH 6.9) was mechanically broken with a soil shredder (Model 112; Royer Foundry and Machine Co., Kingston, PA), mixed, and placed in the plots. The following treatments were applied in a four-factor factorial design: (i) nonfumigated or methyl bromide-fumigated at 450 kg/ha; (ii) noninoculated or inoculated with 400 g of *F. solani* f. sp. *glycines* SC inoculum resulting in a rate of 3.9×10^5 macrospores/ml of soil; (iii) noninfested or infested with 400 g of *H. glycines* inoculum, adding 9.4×10^4 eggs in 1,207 cysts per plot, resulting in 390 eggs per 100 ml of soil and 5 cysts per 100 ml of soil; and (iv) planted to Williams 82 (susceptible to both *F. solani* f. sp. *glycines* and *H. glycines*) or Cyst-X20-18 (resistant to *H. glycines* but with unknown resistance status to *F. solani* f. sp. *glycines*). Williams 82 was chosen because it is one of the parents of the original cross to generate Cyst-X20-18 (7). The SC inocula of the two *F. solani* f. sp. *glycines* isolates or the SS with or without cysts of *H. glycines* were mixed into the upper 15 cm of soil on 12 May 2004. Autoclaved, previously infested SC was applied in the same way to the proper treatments; noninfested greenhouse-culture soil was amended to non-*H. glycines*-infested treatments. Soybean cvs. Williams 82 or Cyst-X20-18 were planted in three parallel rows at 12.5-cm spacing on 15 May 2004 and thinned to 20 plants per plot after emergence.

In both years, beginning at the V6/R1 growth stage of soybean, the appropriate plots (in 2003) or all plots (in 2004) received 15 liters of water once or twice per week to keep soil moisture levels ≤ 50 mbar as determined with tensiometers in two randomly chosen plots. All plots were monitored for SDS occurrence. Disease reactions were assessed by the severity of SDS foliar symptoms. Population densities of *H. glycines* were determined by taking soil samples from the plots before fumigation in 2003, at planting in 2004, and after harvest in both years. Populations of *H. glycines* were extracted by elutriation (3) and the cysts were counted. Cysts were crushed with a modified cyst crusher (6) and then eggs were counted. At R6 on 29 August 2003, R5-R6 on 3 September 2004, foliar SDS severity was rated on a scale of 0 to 9 (19). Percent defoliation was rated on 19 September 2003 and

on 13, 17, 22, and 25 September 2004. On 19 September 2003 (R6) and 22 September 2004 (R6 to R8), soybean plants of the two side rows in each plot were removed for assessment of external root necrosis (Table 1) and presence of *Diaporthe* stem rot was recorded. The middle row was harvested for seed yield on 15 October 2003 and 12 October 2004, respectively. Yields were converted to 13% moisture before analysis.

Greenhouse and growth chamber experiments. *F. solani* f. sp. *glycines* inoculum (Francesville isolate) raised in SC was used in this assay. After 1 month's growth, fungal inocula were mixed with SS to obtain five serial infestation levels of 0.08, 0.4, 2, 10, and 50% (vol/vol). Final macrospore density per milliliter of potting soil was adjusted to increase exponentially at $11,700 \times 5^I$, where the exponent $I = 0$ to 4 (equivalent to 1.2×10^4 to 7.3×10^6). Inoculum-SS mixtures were placed in 120-ml plastic cones (Stuewe & Sons, Inc., Corvallis, OR) in modification of the method described by Gray and Achenbach (9). A nonamended SS (C1) and a mix of autoclaved SC inoculum and SS (1:1, vol/vol) (C2) were controls. Soybean cv. Williams 82 was grown in the cones in a three-factor factorial experiment. Factors were (i) five infestation levels; (ii) watered from the bottom (cone tip submerged 5 cm deep into a 150-cm³ water-filled plastic cup) or watered from the top; and (iii) planted to 10-day-old seedlings or direct-seeded. The experiments were arranged in randomized complete block designs with four replications and conducted twice for each of the following environments: (i) in a heated greenhouse at $25 \pm 3^\circ\text{C}$, 16-h day and 8-h night cycle; or (ii) in an environmental growth chamber at the same temperature and day-night cycle, with 70% relative humidity and light intensity of 300 $\mu\text{mol}/\text{m}^2/\text{s}$. During the growth period, each cone received 30 ml of nutrient solution (15% N, 13% P, 12% K, and various micronutrients) once per week via a commercial fertilizer at label rates (Miracle Gro; Scotts Miracle-Gro Products Inc., Marysville, OH). After 8 weeks, soybean plants were harvested, roots washed free of soil, and root dry weights determined. The percentage of root necrosis was estimated visually on a scale of 0 to 100%.

Data analysis. Analysis of variance (ANOVA) was conducted with the general linear model (GLM) in SAS (SAS Institute, Cary, NC). The microplot data were entered into a randomized complete block design after testing for homogeneity of error variances. Data were transformed before entering into ANOVA: top rating, arcsine (square root of [top rating/9]); root rating, arcsine (square root of [root rate/5]); and nematode numbers, $\log_{10}(x + 1)$. The appropriate least significant differences were generated at $P = 0.05$ and 0.10 to compare the treatments. In the greenhouse and growth chamber experiments, data were transformed prior to ANOVA: root necrosis, arcsine (square root of [percent root necrosis/100]); and root dry weights, square root of root dry weight. When ANOVA error variances were homogeneous, experiments were combined. For the root necrosis, inoculum effects and interactions of the factors were partitioned into orthogonal polynomial contrasts. Each control was compared with the five individual inoculum levels by t tests ($P = 0.05$) within each environment, each plant stage at planting, and each watering method. Regression models were developed for significant inoculum effects.

RESULTS

Field microplots. In 2003, SDS foliar symptoms were most severe in the irrigated/nonfumigated soil, less in the nonirrigated/nonfumigated soil, limited in the irrigated/fumigated soil, and barely detectable in the nonirrigated/fumigated soil ($P \leq 0.05$) (Fig. 1A). The progression of defoliation followed a pattern similar to the SDS foliar symptoms (Table 2). Although, in spring before fumigation, the nonfumigated plots had medium egg population densities of *H. glycines* (400 eggs/100 g of soil), the soybean plants under natural rainfall supported higher egg and

Fig. 1. Effects of soil treatments on foliar sudden death syndrome (SDS) severity and population density of *Heterodera glycines* in microplots in 2003; original means are shown; analysis of variance was conducted on transformed data. **A**, Foliar SDS severity at R6 (scale: 0 to 9) (arcsine [square root of (top rating/9)]). **B**, Population densities of *H. glycines* at harvest, ($\log_{10}(x + 1)$). Bars with the same letter are not significantly different at $P = 0.05$; (–) factors not applied and (+) factors applied.

TABLE 1. External root necrosis rating scheme for soybean in microplots during 2003 and 2004

Rating	2003	2004
0	Tap root healthy, no necrosis, copious secondary and fibrous roots	...
1	Some necrosis on tap root, copious secondary and fibrous roots	Tap root healthy, no necrosis, copious secondary and fibrous roots
2	Tap root with more necrosis and somewhat reduced secondary roots	Healthy tap root, <50% necrotic fibrous and secondary roots
3	Tap root with necrosis, reduced number of fibrous and secondary roots	Healthy tap root, >50% fibrous roots lost or necrotic, necrosis on secondary roots
4	Fibrous roots only on the top of the intact tap root, secondary roots necrotic	<50% necrotic tap root, no healthy fibrous or secondary roots
5	Severely necrotic tap root, sometimes tap root broken	>50% necrotic tap roots, no healthy fibrous or secondary roots

cyst population densities of *H. glycines* (6,643 eggs/100 g of soil) than those under irrigation (4,094 eggs/100 g of soil) at harvest ($P \leq 0.05$) (Fig. 1B; Table 2). All these nonfumigated plots with high population densities of *H. glycines* had high levels of foliar SDS severity at harvest (Fig. 1A and B). On average, external root necrosis was higher in the nonfumigated plots compared with pre-season-fumigated plots and in irrigated compared with nonirrigated, but the interaction of these two factors was not significant (Table 2). Effects of the infestation levels of the fungal pathogen were not significant (data not shown). Treatments that resulted in increased foliar SDS severity also resulted in increased incidence of Diaporthe stem rot but no correlation between Diaporthe stem rot and SDS was detected (data not shown).

In 2004, SDS foliar symptoms first were observed in the fumigated plots planted to Williams 82 inoculated with both *F. solani* f. sp. *glycines* and *H. glycines* (data not shown). Six days later, SDS foliar symptoms began to develop in the nonfumigated plots planted to Williams 82 infested with both pathogens, but SDS severity in the nonfumigated plots developed faster than in the fumigated plots. Thereafter, the plants in other treatments, including some plots inoculated only with *H. glycines* cysts, began

to show traces of SDS foliar symptoms. In the combined analysis of nonfumigated and pre-season-fumigated soil, co-inoculation with both pathogens consistently resulted in the most severe SDS foliar symptoms in Williams 82; only traces of SDS foliar symptoms (not significantly different from 0) were observed in Cyst-X20-18 ($P \leq 0.01$) (Fig. 2). At harvest, cyst and egg population densities under Williams 82 were higher after inoculation with *H. glycines* than in noninoculated soil in both nonfumigated and fumigated soil (Fig. 3; Table 3); egg population densities under Cyst-X20-18 were significantly increased after inoculation compared with the noninoculated treatment in the fumigated soil only (Fig. 3). Egg population densities were higher in each soil treatment with Williams 82 than with Cyst-X20-18, with the exception of the fumigated and noninfested treatment, in which almost no eggs were detected under both cultivars (Fig. 3). External root necrosis was increased in the presence of *H. glycines* on Williams 82 but not on Cyst-X20-18 (Table 3). Seed yields were reduced in *H. glycines*-infested treatment with Williams 82 but not with Cyst-X20-18 (Table 3). On average, defoliation of both cultivars increased beginning at the second monitoring time (from 23 to 33, 56, and 91%). Defoliation was highest and occurred earliest in

TABLE 2. Effects of soil fumigation and watering regimes on sudden death syndrome in microplots in 2003^v

Treatment ^w					
Watering	Fumigation	Defoliation (%) ^x	External root necrosis (0–5) ^y	Cysts per 100 g of soil ^z	Seed yield (g/plot)
–	–	62 ± 5 b	3.5 ± 0.9	63 ± 7 a	309 ± 12
+	–	83 ± 5 a	4.1 ± 1.0	47 ± 6 b	312 ± 15
–	+	13 ± 4 c	1.2 ± 0.3	1 ± 0 c	310 ± 15
+	+	18 ± 5 c	1.3 ± 0.3	1 ± 0 c	345 ± 25
<i>P</i> for					
Watering	...	<0.01	<0.01	ns	ns
Fumigation	...	<0.01	0.0295	<0.01	ns
Watering × fumigation	...	0.0512	ns	0.0383	ns

^v Summary of a three-factor factorial experiment: (i) natural precipitation or 15 liters of water added once a week, (ii) nonfumigated or pre-season-fumigated with methyl bromide at 450 kg/ha, and (iii) inoculated with two isolates of *Fusarium solani* f. sp. *glycines* at 0, 6.3 × 10³, 3.2 × 10⁴, or 1.7 × 10⁵ macrospores/ml of infestation soil. Means across inoculum levels were not presented because such effects were mostly nonsignificant. Numbers followed by the same letter within one column were not significantly different at $P = 0.05$ when the watering × fumigation effects were significant; ns = nonsignificant.

^w Treatments were not applied (–) or applied (+).

^x Rating data of defoliation were arcsine-transformed (arcsine[square root of defoliation/100]) before analysis of variance (ANOVA); original means were presented; although there were significant infestation level effects ($P = 0.0426$), these were not presented because there were no consistent trends associated with infestation levels.

^y Rating data of external root necrosis were arcsine-transformed (arcsine[square root of root rating/5]) before ANOVA; original means were presented.

^z Nematode cyst numbers at harvest were log-transformed (log₁₀[x + 1]) before ANOVA; original means were presented.

Fig. 2. Effects of inoculation with *Heterodera glycines* and *Fusarium solani* f. sp. *glycines* on severity of sudden death syndrome (SDS) foliar symptoms on Williams 82 and Cyst-X20-18 in microplots on 3 September 2004. Original means are presented. Mean separation of the arcsine-transformed data for cultivar × *H. glycines* × *F. solani* f. sp. *glycines*, averaged across fumigation treatments, is significant at $P \leq 0.01$. Bars with the same letter are not significantly different at $P = 0.05$; (–) factors not applied and (+) factors applied; SCN = *H. glycines*; FSG = *F. solani* f. sp. *glycines*.

Fig. 3. Soil population densities of *Heterodera glycines* in *Fusarium solani* f. sp. *glycines*-infested microplots under Williams 82 and Cyst-X20-18 after harvest in 2004. Original means are presented. Mean separation of the log-transformed data for cultivar × fumigation × *H. glycines*, averaged across *F. solani* f. sp. *glycines* infestation, are significant at $P \leq 0.05$. Bars with the same letter are not significantly different at $P = 0.05$; (–) factors not applied and (+) factors applied; SCN = *H. glycines*.

Williams 82 co-inoculated with *F. solani* f. sp. *glycines* and *H. glycines* compared with all other treatments at each evaluation (Fig. 4A and B). In Williams 82, treatments that included at least one pathogen defoliated more slowly than the co-inoculated treatments, but faster than the noninoculated plots (Fig. 4A). In contrast, the patterns of defoliation in Cyst-X20-18 were more similar among infestation treatments (Fig. 4B). In 2003, relationship between foliar SDS severity and external root necrosis appeared grouped independent of infestation levels, but grouped according to fumigation and irrigation treatments: highest root necrosis ratings and foliar SDS severity in irrigated, nonfumigated plots; second-highest root necrosis ratings and medium foliar SDS severity in nonirrigated and nonfumigated plots; and lower root necrosis ratings and only traces of foliar SDS symptoms in fumigated soil, independent of irrigation treatment (Fig. 5A). In 2004, only co-inoculated plots had high foliar SDS severity together with severe root necrosis. Most other treatments had low levels of foliar SDS severity, although severity of root necrosis varied from low to high levels. Although Cyst-X20-18 in the plots with infestation of both pathogens developed high levels of external root necrosis, it did not develop high levels of foliar SDS severity (Fig. 5B). In Williams 82, different levels of root necrosis existed but only the co-inoculated treatment had severe foliar SDS symptoms; in Cyst-X20-18, only traces of foliar SDS symptoms were detected in the co-inoculated treatment in nonfumigated soil, although varying levels of root necrosis similar to those in Williams 82 were observed (Fig. 5B).

Greenhouse and growth chamber experiments. In both environments, arcsine root necrosis (RN) was greater in infested soil than in the controls; RN was more severe when cone tips were

submerged in cups filled with water than when they were watered from the top (Fig. 6A and B). In all tests, roots were more necrotic when inoculum levels increased exponentially ($11,700 \times 5^I$, where exponent $I = 0$ to 4); the greatest incremental increase in RN was in the top-watered greenhouse condition (Fig. 6B). A significant correlation between infestation levels and RN was observed in both environments after the combined regression analysis ($R^2 = 0.9944$ at $P < 0.0001$ in the growth chamber experiments and $R^2 = 0.9180$ at $P = 0.0123$ in the greenhouse experiments). The square root of root dry weight (DW) of soybean was affected by the soybean growth stage at planting (seed or transplant), watering method, and inoculum densities (data not shown).

Although significant differences in root DWs were limited among the inoculation treatments, regression relationships between the root DWs and infestation levels were detected. In the growth chamber, the DW of the infested, direct-seeded soybean plants showed no response to varying inoculum levels, but the transplants had a strong negative response to inoculum levels ($DW = 46.98 - 2.48 I$; data not shown). In the greenhouse, increasing inoculum levels resulted in reduced root DW ($DW = 34.55 - 2.11 I$), with the top-watered transplants having the greatest overall root DW (data not shown). However, the slopes were similar between the growth chamber-infested transplants and all of the greenhouse-infested soybean (-2.48 and -2.11 , respectively; data not shown).

DISCUSSION

In this study, both root necrosis and foliar SDS severity in *F. solani* f. sp. *glycines*-infested soil were greatly increased by the

TABLE 3. Effects of cultivar and *Heterodera glycines* infestation on sudden death syndrome in microplots in 2004^x

Cultivar	<i>H. glycines</i>	External root necrosis (1–5) ^y	Cysts per 100 g of soil ^z	Seed yield (g/plot)
Williams 82	–	2.9 ± 0.3 b	9.4 ± 5.4 b	109 ± 8 a
Williams 82	+	4.0 ± 0.2 a	68.0 ± 0.1 a	71 ± 7 b
Cyst-X20-18	–	3.0 ± 0.2 b	0.3 ± 6.9 d	95 ± 7 a
Cyst-X20-18	+	3.3 ± 0.3 b	4.7 ± 0.6 c	102 ± 10 a
<i>P</i> for				
Cultivar	...	<0.01	<0.01	ns
<i>H. glycines</i>	...	<0.01	<0.01	<0.01
Cultivar × <i>H. glycines</i>	...	<0.01	<0.01	<0.01

^x Data of the complete model are presented, means are across fumigation treatments and *Fusarium solani* f. sp. *glycines* infestation pattern. Analysis of variance (ANOVA) was done for the appropriately transformed data. Numbers followed by the same letter within each column were not significantly different when tested for cultivar × *H. glycines* at $P = 0.05$; ns = nonsignificant.

^y Data were arcsine transformed before ANOVA and mean separation test.

^z Nematode population density data at harvest were log-transformed ($\log_{10}[x + 1]$) before ANOVA and mean separation test.

Fig. 4. Defoliation of soybean canopies in microplots over the period from 13 to 25 September 2004: **A**, Williams 82; **B**, Cyst-X20-18. Differences tested with the least significant difference at 0.05 ($LSD_{0.05} = 11.9$ for comparing cultivar × *Heterodera glycines* × *Fusarium solani* f. sp. *glycines* × time ($P \leq 0.01$)). The treatments were control, no inoculum (●); inoculated with *F. solani* f. sp. *glycines* only (■); inoculated with *H. glycines* only (○); and inoculated with both *F. solani* f. sp. *glycines* and *H. glycines* (□).

presence of *H. glycines*. The correlation between *F. solani* f. sp. *glycines* and *H. glycines* was evident from two different perspectives. In both years, independent of the source of *H. glycines*—natural infestation or artificial inoculation—the nematode was the key factor for increased foliar SDS severity. The fungal pathogen and *H. glycines* damaged the plant synergistically, resulting in severe SDS. In 2003, plots with natural populations of *H. glycines* had high levels of SDS, indicating the important role of the nematode in increasing SDS. In 2004, only plots planted to Williams 82 that were co-inoculated with *H. glycines* and *F. solani* f. sp. *glycines* developed severe foliar SDS symptoms. Documentation of *H. glycines* population densities is indicated when comparing experiments conducted in fields with infestations of both pathogens. Our observation under field conditions further confirmed previous reports (11,20,24,32,36) of a positive correlation between severity of SDS foliar symptoms and populations of *H. glycines* in *H. glycines*-susceptible soybean cultivars. The presence of *H. glycines* hastened and intensified the development and severity of foliar symptoms of SDS in greenhouse or field microplot tests (18,20,21,24,32). In one such test, foliar symptoms of SDS-diseased soybean plants inoculated with both *F. solani* f. sp.

glycines and *H. glycines* occurred 3 or 9 days earlier than those caused by *F. solani* f. sp. *glycines* alone (20). The relationship between *F. solani* f. sp. *glycines* and *H. glycines* is crucially important, particularly in the Midwest, where *H. glycines* was present in 47 to 83% of the fields surveyed from 1995 to 1996 (49). Similar disease complexes are known for several plant pathogens when nematodes modify the rhizosphere in concomitant infection with soilborne fungi (13). For example, *H. glycines* damages soybean plants synergistically with *F. oxysporum* in Fusarium wilt (29) or in combination with *Phytophthora megasperma* var. *sojae* (1).

The second critical factor for SDS development in this study was soil moisture. In microplots, plants grown in the irrigated plots exhibited more severe foliar SDS symptoms than those in the nonirrigated plots. Higher soil moisture treatments also always were associated with higher levels of root necrosis in experiments in the greenhouse and growth chamber. Such interrelation had been found previously for severity of foliar SDS symptoms (24,32,43), which increased with increasing soil moisture in controlled growth chamber and field conditions; however, in those studies, such effects were not related to root

Fig. 5. Scattergram of foliar sudden death syndrome (SDS) severity over external root necrosis of soybean: **A**, In 2003, each of the four infestation levels in soil was indicated the following ways: fumigated and nonirrigated (Δ); fumigated and watered ($\diamond$); nonfumigated and nonirrigated ($\square$); and nonfumigated and irrigated ($\circ$); symbol fills: hollow = no infestation; cross hatch = lowest level of inoculum; vertical hatch = second-highest level of inoculum; and filled = highest level of inoculum of *Fusarium solani* f. sp. *glycines*. **B**, In 2004, soybean cultivars at the four infestation patterns were: Cyst-X20-18 in fumigated soil ($\diamond$); Cyst-X20-18 in nonfumigated soil ($\circ$); Williams 82 in fumigated soil (Δ); and Williams 82 in nonfumigated soil ($\square$); symbol fills: hollow = no infestation, cross hatch = inoculated with *F. solani* f. sp. *glycines*; vertical hatch = inoculated with *Heterodera glycines*; and filled = inoculated with both *H. glycines* and *F. solani* f. sp. *glycines*.

Fig. 6. Root necrosis (RN): arcsine (square root of [percent necrosis/100]) on soybean cv. Williams 82 in plastic cones filled with sand:soil mix infested with increasing levels of *Fusarium solani* f. sp. *glycines* expressed as $11,700 \times 5^I$, where the exponent $I = 0$ to 4, with two controls: C1 (noninoculated mixture of autoclaved sand:soil [2:1, vol/vol]) and C2 (mix [1:1, vol/vol] of autoclaved sand:soil mixture and autoclaved spent sand-cornmeal media). **A**, Growth chamber; for infestation level, the mutual regression was $R^2 = 0.9944$ at $P < 0.0001$. **B**, Greenhouse; for infestation level, the mutual regression was $R^2 = 0.9180$ at $P = 0.0123$. The treatments were top watered ($\bullet$) and cone tip submerged in water-filled cup ($\blacksquare$).

necrosis of SDS-diseased soybean under greenhouse and field conditions.

SDS onset was accelerated by *H. glycines* in nonfumigated and fumigated plots, resulting in earlier and more severe foliar SDS symptoms than in other plots. Similar observations were made under natural or controlled field conditions (11,20,24,40,45). In 2004, SDS foliar symptoms developed earlier in the fumigated plots than in the nonfumigated plots with infestation of both *F. solani* f. sp. *glycines* and *H. glycines*. The natural microflora in the otherwise equivalent nonfumigated soil may have delayed disease onset at the aboveground level. However, the epidemics of foliar SDS symptoms developed faster in the nonfumigated soil compared with the fumigated soil, possibly because a number of other soil-inhabiting fungi and oomycetes were impacting the root health of soybean in addition to the inoculum amendments. This potentially complex interactive system may explain the common complication in SDS research: “the disease may be present in a given field one year and be totally absent or too mild in one or more succeeding years to allow for screening” (23). Although this sporadic disease occurrence probably is due to many factors, the interaction of the nematode and the fungus may be a contributing factor. Possibly, this interaction has more dimensions than promotion of SDS; there seems to be a detriment to the nematode in this interaction because severe SDS resulted in lower *H. glycines* population densities than mild SDS, as reported previously (22, 40,45) and in this study. In 2003, pathogen populations in soils were in two known groups: group 1, amendment of *F. solani* f. sp. *glycines* inoculum with natural *H. glycines* populations in the nonfumigated plots, or group 2, amendment of *F. solani* f. sp. *glycines* inoculum without *H. glycines* in the fumigated plots. Root necrosis and foliar SDS severity were high in group 1 and were low in group 2. In 2004, when the two pathogen populations were modified more drastically by amending them into nonfumigated or fumigated plots, varying levels of root necrosis were found in treatments that received at least one pathogen, but severe root necrosis was coupled with severe SDS foliar symptoms only in the co-inoculated treatments of Williams 82. We hypothesize that root necrosis caused by the infection of *F. solani* f. sp. *glycines* reduced availability of feeding sites for *H. glycines*, thus decreasing nematode population densities. The notoriously scattered distribution of population densities of *H. glycines* may cause inconsistencies in results of field experiments to screen germplasm for resistance and in analyses of the interaction of both pathogens. On the other hand, *F. solani* f. sp. *glycines* not only can colonize, survive as thick-walled chlamydospores in, and be isolated from *H. glycines* cysts (22,24,33), but also can affect population development of *H. glycines* (22). Cysts of *H. schachtii*, a close relative to *H. glycines*, have been shown to be effective transmitters for microorganisms (48), which can be beneficial in transferring nematode biocontrol organisms or detrimental by transmitting plant diseases.

In this study, both biotic (SCN) and abiotic (soil moisture) factors exacerbated the severity of SDS foliar symptoms. Effects of population levels of amended *F. solani* f. sp. *glycines* on SDS development were minor in both fumigated and nonfumigated soil. Although soil suppressiveness probably was reduced in fumigated soil, as is the case for many soilborne pathogens (47), disease severity was not increased at any infestation level of *F. solani* f. sp. *glycines* after biocidal treatments in 2003. Moreover, the effects of amended *F. solani* f. sp. *glycines* on SDS development were not significantly different from each other at different inoculum levels in the nonfumigated soil, but foliar SDS severity was increased by *H. glycines* across all infestation levels. Effective management of SDS requires consideration of several factors, such as soil moisture level and the temperature at planting (36,43), field managing practice (e.g., irrigation) (24,32), the physical and chemical composition of soil (38,40,41,45,46), the genetics of soybean resistance to SDS (5,10), the pathological and

physiological variability among different isolates of *F. solani* f. sp. *glycines* (17,31,43), and the interaction of *F. solani* f. sp. *glycines* with *H. glycines* and other soil microorganisms (20,24,32).

Understanding the interaction between *F. solani* f. sp. *glycines* and *H. glycines* is mandatory for management of SDS in fields with a history of *H. glycines* infestation. Management practices to reduce *H. glycines* populations are thought to delay the onset of SDS. Potentially, avoiding build-up of population densities of *F. solani* f. sp. *glycines* and *H. glycines* will be beneficial in reducing the risk for SDS (45). A strategy to accomplish the limitation of *H. glycines* build-up could include the use of *H. glycines*-resistant soybean cultivars. However, information is limited on the influence of *H. glycines* on SDS development in nematode-resistant soybean lines grown in *F. solani* f. sp. *glycines*-infested soil (15). Some *H. glycines*-resistant cultivars are more tolerant to SDS (11,26,37), but some *H. glycines*-resistant soybean cultivars are particularly susceptible to SDS (26,27). In general, SDS severity is greater on *H. glycines*-susceptible cultivars grown in *H. glycines*-infested fields (11,36,37). For the future, it is imperative to determine *H. glycines* population densities when evaluating SDS tests in natural soils.

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