Heterodera glycines Infection Increases Incidence and Severity of Brown Stem Rot in Both Resistant and Susceptible Soybean

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ABSTRACT

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Growth chamber experiments were conducted to investigate whether parasitism by *Heterodera glycines*, the soybean cyst nematode, increases incidence and severity of brown stem rot (BSR) of soybean, caused by *Phialophora gregata*, in both resistant and susceptible soybean cultivars. Soybean genotypes with various combinations of resistance and susceptibility to both pathogens were inoculated with *P. gregata* alone or *P. gregata* plus *H. glycines*. In most tests of *H. glycines*-susceptible genotypes, incidence and severity of internal stem discoloration, characteristic of BSR, was greater in the presence than in the absence of *H. glycines*, regardless of susceptibility or resistance to BSR. There was less of an increasing effect of *H. glycines* on stem symptoms in genotypes resistant to both BSR and *H. glycines*; however, *P. gregata* colonization of these genotypes was increased. Stems of both a BSR-resistant and a BSR-susceptible genotype were colonized earlier by *P. gregata* in the presence than in the absence of *H. glycines*. Our findings indicate that *H. glycines* can increase the incidence and severity of BSR in soybean regardless of resistance or susceptibility to either pathogen.

Additional keywords: BSR, pathogen interaction, *Phialophora gregata,* SCN

Phialophora gregata (Allington & D. W. Chamberlain) W. Gams, the causal agent of brown stem rot (BSR) of soybean (*Glycine max* L.) (1), and *Heterodera glycines* Ichinohe, the soybean cyst nematode, are widely distributed throughout the midwestern United States (32). The current recommended management strategy for both pathogens is the use of resistant soybean cultivars combined with rotation to nonhost crops (26,33). BSR resistance is believed to be controlled by three nonallelic *Rbs* genes (2,4,21,31) and one or more unknown genes derived from PI 88788 (6), a soybean line that also is a source of *H. glycines* resistance (20,27).

Both *H. glycines* and *P. gregata* are common and both infect soybean; therefore, they may interact. In 1984, Negishi and Kobayashi (12) reported that *H. glycines* infection increased symptoms of BSR of adzuki bean, *Vigna angularis* (Willd.). In the early 1990s, researchers in Iowa noticed increased symptoms of BSR in both BSR-susceptible and BSR-resistant soybean cultivars grown in *H. glycines*-infested fields (H. Tachibana, *personal communication*). Preliminary experiments based on these observations also suggested increased BSR symptoms when soybean plants were grown in *H. glycines*-infested soil in a greenhouse (25). In 1997, Sugawara et al. (22) reported that, in a greenhouse experiment, *H. glycines* increased both percent stem length and percent node discolored in a soybean genotype susceptible to both *P. gregata* and *H. glycines*. They, however, did not find an increase in symptoms in two other genotypes, one resistant to BSR and another resistant to *H. glycines*. Given the limited numbers of genotypes that have been tested in controlled experiments and the conflicting data available, it is necessary to further study the effects of *H. glycines* on BSR symptoms.

Data also are needed on the effect of *H. glycines* on the colonization of stems by *P. gregata*. To date, the only information available on whether *H. glycines* affects BSR is based on observation of stem symptoms. To conclusively test whether *H. glycines* increases susceptibility of soybean to *P. gregata*, the extent of stem colonization by the fungus needs to be assessed. It is possible, for example, that simultaneous infection of soybean by *P. gregata* and *H. glycines* could result in changes in the host that could lead to increased severity of BSR symptoms without a corresponding increase in colonization by the fungus. In addition, the relationship between colonization and the presence of

BSR symptoms is not clear, even in the absence of *H. glycines*. Development of internal stem discoloration is greatly affected by the environment (19,28), but whether this is due to an effect on the fungus or on stem symptoms is not clear. In addition, Tabor et al. (23) reported that soybean plants could be infected by *P. gregata* without expressing stem symptoms. For example, 12 weeks after inoculation and incubation in a growth chamber, the majority of stems of both the BSR-susceptible genotype Sturdy and the BSR-resistant BSR101 were extensively colonized but did not exhibit visible internal stem discoloration (23). Thus, any effect of *H. glycines* on the susceptibility of soybean to BSR needs to be confirmed by assessing colonization of the host by the fungus.

Another aspect of the *H. glycines–P. gregata* interaction that needs to be examined is the effect of *H. glycines* on the progression of infection of the host by *P. gregata*. Weber et al. (29) reported that yield loss from BSR largely was due to a reduction in seed number rather than seed size. Thus, the timing of infection by *P. gregata* relative to seed set may be important in determining yield loss. Early season colonization by the fungus might result in more yield loss than late-season colonization. The difference in the timing of colonization by the fungus also appears to be an important difference between resistant and susceptible soybean genotypes. For instance, in growth chamber experiments, Tabor et al. (23) showed that stems of the BSR-susceptible genotype Sturdy were colonized earlier than stems of the BSR-resistant genotype BSR101. Thus, the potential of *H. glycines* to affect the progression of colonization by *P. gregata* should be determined in both resistant and susceptible soybean genotypes.

The objectives of this research were to determine if *H. glycines* (i) increases the incidence and severity of internal stem discoloration in soybean genotypes with various combinations of resistance and susceptibility to both diseases, (ii) increases incidence and severity of *P. gregata* colonization of the soybean stem; and (iii) affects the progression of colonization of stems by *P. gregata* over time.

MATERIALS AND METHODS

Ten growth-chamber experiments were conducted to determine the effect of *H.*

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glycines on the incidence and severity of BSR in a range of soybean genotypes. Eight of these experiments were conducted to determine the effect of *H. glycines* on BSR stem symptom development. Experiments 1, 2, and 4 each were repeated (1A and 1B, 2A and 2B, 4A and 4B). Experiments 3 and 5 were conducted once each. A total of 17 soybean genotypes were tested in these eight experiments. In addition, experiments 4A, 4B, and 5 determined the effect of *H. glycines* on colonization of soybean stems by *P. gregata*; a total of seven soybean genotypes were tested in these three experiments. Two experiments (6A and 6B) were conducted to determine the effect of *H. glycines* on colonization of stems by *P. gregata* over time in a BSR-resistant and a BSR-susceptible genotype. All soybean genotypes used in the experiments described in this article and their sources of resistance or susceptibility to both *H. glycines* and BSR are indicated in Table 1.

H. glycines inoculum production. *H. glycines* was increased on *H. glycines*-susceptible Corsoy 79 or Kenwood 94 soybean in a greenhouse. Eggs were obtained by dislodging female nematodes and cysts from roots of infected plants with a stream of water, and wet-sieving and decanting (3,13) *H. glycines*-infested soil. Females and cysts were recovered on a 250-μm-pore sieve nested below an 850-μm-pore sieve. Eggs were released from females and cysts by crushing them in water with a motorized pestle (13) and then collected on a 25-μm-pore sieve nested under a 75-μm-pore sieve. Eggs were separated from plant debris and soil particles by centrifugal flotation (7) and counted by direct microscopic observation. Inoculum population densities used in the experiments are listed in the tables and figure legends.

***P. gregata* inoculum production.** Our experiments were conducted over several years and the methods of *P. gregata* inoculum production were modified over time. Thus, three inoculum production methods were used; identical sources of inoculum were used between repeats of the same experiments.

Artificially infested straw (experiments 1A and B). The *P. gregata* isolate used to prepare this artificially infested straw was isolated from soybean genotype Kenwood 94, grown at the Iowa State University Curtiss Research Farm. Two-week-old cultures grown on soybean stem agar (1) were cut into cubes (approximately 0.5 cm^3) and transferred to flasks containing 60 ml of sterile soybean stem broth. The broth was made from 25 g of greenhouse-grown soybean stems blended using a motorized blender in approximately 300 ml of deionized water, strained through gauze cloth, and diluted to 1 liter with deionized water. The fungal cultures were incubated on a rotary shaker for 12 days at room temperature (approximately 22°C). The conidia produced were counted, and approximately 2.4×10^8 *P. gregata* conidia in 600 ml of water were sprayed onto each of two 1,200-g lots of twice-autoclaved (1 h at 126°C, 1.4 kg/cm^2), shredded, field-grown soybean straw collected after harvest. *P. gregata* population densities (conidia per gram of straw) were determined after 15 days of incubation at room temperature by agitating a known weight of straw inoculum in deionized water and counting conidia by direct microscopic observation.

Naturally infested straw (experiments 2A and B, and 3). Soybean straw naturally infested with *P. gregata* was collected from fields for use as inoculum. The straw was stored at 5°C and ground through a screen with 0.5-mm-diameter openings. Inoculum density was assessed by serial dilution and plating on a *P. gregata* semiselective medium (11). Inoculum density was approximately 1×10^4 CFU of *P. gregata* per gram of infested straw.

Straw artificially infested with isolate OH2-3 (experiments 4A, 4B, 5, 6A, and 6B). The *P. gregata* strain OH2-3 (8) used in these experiments was a single-spore isolate of strain Oh$_2$ (2) provided by C. Nickell at the University of Illinois. Cultures were grown on green bean extract (GBE) medium (ground frozen *Phaseolus vulgaris* L. green pods at 35 g/liter and agar at 20 g/liter) supplemented with ampicillin at 50 mg/liter and were incubated at room temperature (21 to 23°C) in the dark until abundant sporulation was evident. Conidia were suspended in sterile deionized water, mixed with twice-autoclaved, shredded soybean straw, and incubated for 20 to 38 days at room temperature in the dark. The number of *P. gregata* CFU per gram of straw inoculum was determined by serial dilution and plating on GBE agar.

Inoculation and incubation. In all experiments, inoculum was incorporated into an autoclaved mix of equal volumes of sand and soil. The soil and inoculum mix was placed in either 1.75-liter capacity clay pots (experiments 1A, 1B, 2A, 2B, and 3) or 4-by-21-cm plastic, cone-shaped containers (experiments 4A, 4B, 5, 6A, and 6B) before the planting of soybean seed. In experiments 1A, 1B, 2A, 2B, 3, 4A, 4B, and 5, in which internal stem discoloration was assessed, pathogen treatments were *P. gregata*, *P. gregata* + *H. glycines*, *H.*

Table 1. Soybean genotypes used in experiments

Soybean genotypes	Parental cross	Reference or source	Proposed BSR resistance gene[s]	*Heterodera glycines* resistance source
Harosoy	Mandarin × (Mandarin X AK)	30	None	None
LN92-12054[t]	Century 84 × PI 437833	14	None	None
Sturdy	M70-127 × Century	17	None	None
Newton	BSR101 × CN210	10	None	Peking
BSR101	L69U40-16-4 × A76-304020	24	*Rbs*1, *Rbs*3	None
IA1006[u]	IA2008 × Kenwood	Cianzio[v]	*Rbs*1 and/or *Rbs*3	None
IA2008R[u]	IA2008 × Archer	Cianzio[v]	*Rbs*1 and/or *Rbs*3	None
L67-6301[u]	Clark × PI 84946-2	Nickell[w]	*Rbs*1 and/or *Rbs*3	None
L68-0107[u]	Harosoy × PI 84946-2	Nickell[w]	*Rbs*1 and/or *Rbs*3	None
LN92-12033[t]	Century 84 × PI 437833	14	*Rbs*2	None
PI 84946-2	Plant introduction	NPGS[x]	*Rbs*1	None
PI 437833	Plant introduction	NPGS	*Rbs*2	None
PI 437970	Plant introduction	NPGS	*Rbs*3	None
PS2465N	Unavailable	Profiseed[y]	Unknown[z]	PI 88788
Bell	Fayette × LN80-10398	15	Unknown[z]	PI 88788
Freeborn	Ozzie × Fayette	18	Unknown[z]	PI 88788
Jack	Fayette × Hardin	16	Unknown[z]	PI 88788

[s] BSR = brown stem rot.
[t] Genotypes are near isolines for *Rbs*2.
[u] Genotypes for which resistance is derived from PI 84.946-2 or BSR101.
[v] Experimental genotypes obtained from S. Cianzio, Iowa State University, Ames.
[w] Seed obtained from C. Nickell, University of Illinois, Urbana-Champaign.
[x] National Plant Germplasm System, Soybean Germplasm Collection, 180 EASB, 1101 West Peabody Dr. Urbana, Illinois 61801.
[y] Profiseed Inc., 1691 Highway 65 North, Hampton, IA 50441.
[z] BSR resistance derived from unknown source.

glycines, and no pathogens. The *H. glycines* and the no-pathogen treatments received equal amounts of twice-auto-claved, *P. gregata*-free shredded straw. In experiment 6A and B, in which internal stem discoloration was not assessed, the no-pathogens treatment was not included.

Plants were grown in a growth chamber at a constant temperature of 22°C and 16 h of light until border plants began to show symptoms. This occurred at 9 weeks in experiments 1A and 1B, 8 weeks in experiments 2A, 2B, and 3, and 10 weeks in experiments 4A, 4B, and 5. In experiments 6A and 6B, in which only colonization of stems by *P. gregata* was monitored over time, plants were sampled repeatedly from 4 to 13 weeks. The pathogen inoculum population densities used in each of the experiments are listed in the tables and figure legends.

Experimental design. In all experiments, containers were arranged in randomized complete block design, and blocks were locations in the growth chamber. In experiments 1A, 1B, 2A, 2B, and 3, there were four blocks, and a treatment combination occurred once per block and comprised two plants in a clay pot. In experiments 4A and 4B, there were three blocks and each treatment combination occurred once per block. Each treatment combination comprised five and seven plants (one plant per container) in a row in experiments 4A and 4B, respectively. In experiments 5, 6A, and 6B there were three blocks, and a treatment combination occurred once in each block and comprised five plants (one plant per container) in a row.

Incidence and severity assays. In experiments 1A, 1B, 2A, 2B, 3, 4A, 4B, and 5, incidence and severity of internal stem discoloration were assessed. Plants were severed at the soil surface, their stems were split longitudinally, and the height of internal stem discoloration characteristic of *P. gregata* infection from the soil line was measured. Incidence (presence or absence) and severity of stem discoloration [(highest point of stem discoloration/total plant height) × 100] were calculated for each plant. Stem discoloration data for each experiment were collected by one trained observer without knowledge of the treatments. Foliar symptoms did not occur consistently and reliably in plants in the *P. gregata* treatments. Consequently, foliar symptoms were not recorded.

In experiments 4A, 4B, 5, 6A, and 6B, stem colonization was assessed. To assess percentage of stems colonized and severity of stem colonization, stems were severed at the soil line and immersed for 3 min in 70% ethanol, followed by 5 min in 10% sodium hypochlorite (Clorox) and a final rinse in sterile, deionized water. Stem length was measured, and the stems were cut into 2-cm-long pieces. In experiments 4A, 4B, and 5, in which both discoloration and colonization data were collected, the stem pieces were split in half and discoloration was assessed prior to plating. The pieces were plated on GBE agar supplemented with ampicillin (50 mg/liter) and incubated at 15°C in the dark for 15 days. The emerging fungal mycelia were examined microscopically for conidia and conidiophores with morphology characteristic of *P. gregata*. A stem (plant) was considered colonized if *P. gregata* was recovered from any portion of the stem piece. In all instances, stems were colonized starting from the base up to the maximum height colonized without gaps. Consequently, severity of colonization was calculated by dividing the maximum height colonized by the total stem height. Colonization data were collected without knowledge of the treatments.

Data analyses. All statistical analyses were conducted using the SAS software package (version 8.2; SAS Institute Inc., Cary, NC). Data from experiments 1A, 1B, 2A, 2B, 3, 4A, 4B, and 5 were analyzed using the GLM procedure. Data from ex-

Table 2. Incidence of internal stem discoloration in soybean genotypes inoculated with various combinations of *Phialophora gregata* (*P.g.*) and *Heterodera glycines* (*H.g.*)

| Soybean genotype | Pathogen reaction | | Pathogen treatment[u] | | | | | | | | | | | |
| | P.g. | H.g. | Pooled exp. 1A and 1B[v] | | Pooled exp. 2A and 2B[w] | | Experiment 3[w] | | Pooled exp. 4A and 4B[x] | | Experiment 5[y] | | Pooled across repeated exp.[z] | |
			P.g.	P.g. + H.g.	P.g.	P.g. + H.g.	P.g.	P.g. + H.g.	P.g.	P.g. + H.g.	P.g.	P.g. + H.g.	P.g.	P.g. + H.g.
Harosoy	S	S	…	…	…	…	6 b	94 a	…	…	…	…	…	…
LN92-12054	S	S	…	…	…	…	13 b	94 a	…	…	…	…	…	…
Sturdy	S	S	50 b	94 a	38 b	88 a	69 a	94 a	57 b	98 a	0 b	13 a	48 b	92 a
Newton	S	R	13 b	94 a	…	…	…	…	…	…	…	…	13 b	94 a
BSR101	R	S	13 b	75 a	0 b	81 a	6 b	100 a	17 a	32 a	0 a	7 a	10 b	63 a
IA1006	R	S	…	…	19 b	69 a	…	…	…	…	…	…	19 b	69 a
IA2008R	R	S	…	…	0 b	81 a	…	…	…	…	…	…	0 b	81 a
L67-6301	R	S	…	…	…	…	25 b	88 a	…	…	…	…	…	…
L68-0107	R	S	…	…	…	…	13 b	100 a	…	…	…	…	…	…
LN92-12033	R	S	…	…	…	…	13 b	100 a	…	…	…	…	…	…
PI 84946-2	R	S	…	…	…	…	…	…	…	…	0 a	11 a	…	…
PI 437833	R	S	…	…	…	…	…	…	…	…	0 a	0 a	…	…
PI 437970	R	S	…	…	…	…	…	…	…	…	0 a	0 a	…	…
PS2465N	R	R	0 a	13 a	…	…	…	…	17 a	36 a	…	…	8 a	24 a
Bell	R	R	6 a	13 a	…	…	…	…	…	…	…	…	6 a	13 a
Freeborn	R	R	0 a	6 a	0 b	44 a	…	…	23 a	45 a	…	…	8 b	32 a
Jack	R	R	…	…	6 b	38 a	…	…	…	…	…	…	6 b	38 a

[u] Values are percentages of plants with internal stem discoloration and are means of 8 plants in experiments 1A, 1B, and 3; 16 plants for experiment 2 (pooled 2A and 2B); 36 plants for experiment 4 (pooled 4A and 4B); and 15 plants for experiment 5. For each experiment or pooled experiments, values followed by different letters within a row are significantly different at $P < 0.05$. Treatments were compared using orthogonal comparisons. S = susceptible, R = resistant, … = not determined.

[v] Inocula were 1.6×10^8 CFU of *P. gregata* on artificially colonized straw and 1,200 *H. glycines* eggs per 100 cm^3 of soil mix in 1.75-liter capacity clay pots, and incidence of discoloration was assessed 9 weeks after inoculation.

[w] Inocula were 3×10^4 CFU of *P. gregata* from naturally colonized straw and 1,200 *H. glycines* eggs per 100 cm^3 of soil mix in 1.75-liter capacity clay pots, and incidence of discoloration was assessed 8 weeks after inoculation.

[x] Inocula were 7.3×10^8 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers, and incidence of discoloration was assessed 10 weeks after inoculation.

[y] Inocula were 2.9×10^9 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers and incidence of discoloration was assessed 10 weeks after inoculation.

[z] Pooled data across several repeated experiments were analyzed by analysis of variance using experiment as a random effect. Data from single experiments were not included in the analysis. Treatments were compared using orthogonal comparisons.

periments 1A and 1B were pooled; despite a significant ($P < 0.05$) experiment–treatment interaction, results between the two experiments were similar. Data from experiments 2A and 2B were pooled because combined analysis of data did not show significant ($P > 0.05$) experiment–treatment interaction. Similar pooling of data was done for experiments 4A and 4B for the same reason. To summarize results for genotypes tested over several repeated experiments, data were pooled across experiments. The pooled data were analyzed by a mixed model of analysis of variance (9) treating experiment as a random effect. For each experiment and pooled experiments, orthogonal comparisons were used to compare pathogen treatments within a soybean genotype. Data on incidence and severity of *P. gregata* colonization over time (experiments 6A and 6B) were ana-

Table 3. Severity of internal stem discoloration of soybean genotypes inoculated with various combinations of *Phialophora gregata* (*P.g.*) and *Heterodera glycines* (*H.g.*)

Soybean genotype	Pathogen reaction		Pooled exp. 1A and 1B[v]		Pooled exp. 2A and 2B[w]		Experiment 3[w]		Pooled exp. 4A and 4B[x]		Experiment 5[y]		Pooled across repeated exp.[z]	
	P.g.	*H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*
Harosoy	S	S	…	…	…	…	1 b	87 a	…	…	…	…	…	…
LN92-12054	S	S	…	…	…	…	7b	60 a	…	…	…	…	…	…
Sturdy	S	S	9 b	43 a	9 b	49 a	20 b	85 a	34 b	75 a	0 b	8 a	17 b	57 a
Newton	S	R	4 b	49 a	…	…	…	…	…	…	…	…	4 b	49 a
BSR101	R	S	3 b	43 a	0 b	29 a	1 b	66 a	6 a	12 a	0 b	14 a	3 b	28 a
IA1006	R	S	…	…	6 a	23 a	…	…	…	…	…	…	6 b	23 a
IA2008R	R	S	…	…	0 b	29 a	…	…	…	…	…	…	0 b	29 a
L67-6301	R	S	…	…	…	…	13 b	57 a	…	…	…	…	…	…
L68-0107	R	S	…	…	…	…	3 b	87 a	…	…	…	…	…	…
LN92-12033	R	S	…	…	…	…	7 b	60 a	…	…	…	…	…	…
PI 84946-2	R	S	…	…	…	…	…	…	…	…	0 a	3 a	…	…
PI 437833	R	S	…	…	…	…	…	…	…	…	0 a	0 a	…	…
PI 437970	R	S	…	…	…	…	…	…	…	…	0 a	0 a	…	…
PS2465N	R	R	0 a	2 a	…	…	…	…	6 a	13 a	…	…	3 a	7 a
Bell	R	R	2 a	3 a	…	…	…	…	…	…	…	…	2 a	3 a
Freeborn	R	R	0 a	3 a	0 a	15 a	…	…	9 a	19 a	…	…	3 b	12 a
Jack	R	R	…	…	2 a	8 a	…	…	…	…	…	…	2 a	8 a

[u] Values are highest point of stem discoloration/total plant height × 100% and are means of 8 plants in experiments 1A, 1B, and 3; 16 plants for experiment 2 (pooled 2A and 2B); 36 plants for experiment 4 (pooled 4A and 4B); and 15 plants for experiment 5. For each experiment or pooled experiments, values followed by different letters within a row are significantly different at $P < 0.05$. Treatments were compared using orthogonal comparisons. S = susceptible, R = resistant, … = not determined.

[v] Inocula were 1.6×10^8 CFU of *P. gregata* on artificially colonized straw and 1,200 *H. glycines* eggs per 100 cm^3 of soil mix in 1.75-liter capacity clay pots, and incidence of discoloration was assessed 9 weeks after inoculation.

[w] Inocula were 3×10^4 CFU of *P. gregata* from naturally colonized straw and 1,200 *H. glycines* eggs per 100 cm^3 of soil mix in 1.75-liter capacity clay pots, and incidence of discoloration was assessed 8 weeks after inoculation.

[x] Inocula were 7.3×10^8 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers, and incidence of discoloration was assessed 10 weeks after inoculation.

[y] Inocula were 2.9×10^9 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers and incidence of discoloration was assessed 10 weeks after inoculation.

[z] Pooled data across several repeated experiments were analyzed by analysis of variance using experiment as a random effect. Data from single experiments were not included in the analysis. Treatments were compared using orthogonal comparisons.

Table 4. Incidence and severity of *Phialophora gregata* colonization of soybean genotypes ten weeks after inoculation with various combinations of *P. gregata* (*P.g.*) and *Heterodera glycines* (*H.g.*)

Genotype	Pathogen reaction		Pooled experiments 4A and 4B[w]				Experiment 5[x]			
			Incidence (% plants colonized)[y]		Severity (% colonization)[y,z]		Incidence (% plants colonized)[y]		Severity (% colonization)[y,z]	
	P.g.	*H.g.*	*P.g*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*	*P.g.*	*P.g.* + *H.g.*
Sturdy	S	S	98 a	100 a	90 a	96 a	65 b	100 a	57 b	100 a
BSR101	R	S	23 b	76 a	11 b	39 a	0 b	92 a	0 b	91 a
PI 84946-2	R	S	…	…	…	…	20 b	93 a	32 b	83 a
PI 437833	R	S	…	…	…	…	7 b	87 a	3 b	57 a
PI 437970	R	S	…	…	…	…	0 b	80 a	0 b	60 a
PS2465N	R	R	33 b	79 a	13 b	35 a	…	…	…	…
Freeborn	R	R	13 b	38 a	6 b	22 a	…	…	…	…

[v] Values are means of 36 plants for pooled experiments 4A and 4B and 15 plants for experiment 5. The corresponding discoloration data are given in Tables 2 and 3 under subtitles experiment 4 and 5. S = susceptible, R = resistant, … = not determined.

[w] Inocula were 7.3×10^8 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers, and incidence and severity of colonization was assessed 10 weeks after inoculation.

[x] Inocula were 2.9×10^9 CFU of *P. gregata* (OH2-3) on artificially colonized straw and 1,600 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers and incidence and severity of colonization was assessed 10 weeks after inoculation.

[y] Within these columns, values with different letters in a row are significantly different at $P = 0.01$. Treatments were compared using orthogonal comparisons.

[z] Maximum height of stem colonized by *P. gregata*/total stem height × 100%.

lyzed by logistic regression (5) using the GENMOD procedure; χ^2 analysis was used to test slope and intercept differences of the regression of *P. gregata* colonization over time between pathogen treatments within a soybean genotype.

RESULTS

Effect of *H. glycines* on internal stem discoloration (experiments 1A, 1B, 2A, 2B, 3, 4A, 4B, and 5). *H. glycines* increased the incidence or severity of stem symptoms in soybean genotypes with various combinations of resistance to *P. gregata* and *H. glycines* (Tables 2 and 3). Pooled data across repeated experiments suggested that *H. glycines* increased the incidence and severity of stem symptoms for all *H. glycines*-susceptible genotypes, and these effects were irrespective of whether or not the genotypes possess BSR resistance. *H. glycines* also increased internal stem discoloration for the one genotype tested that was resistant to *H. glycines* but not to BSR. *H. glycines* also increased the incidence or severity of stem symptoms in some genotypes that possess both *H. glycines* and BSR resistance. Stem discoloration in the no-pathogens and *H. glycines*-only treatments was negligible (*data not shown*) and did not change the significance of the comparisons of the *P. gregata*-only and the *P. gregata* + *H. glycines* treatments.

Data were pooled across repeated experiments, then grouped by resistance reaction to compare the effect of *H. glycines* on the group of genotypes with resistance to both pathogens versus the group of genotypes resistant only to the fungus. The pooled data were analyzed by a mixed model of analysis of variance treating experiment as a random effect. Orthogonal comparisons were used to compare the responses of the two groups of genotypes to a given pathogen treatment. *H. glycines* increased the incidence and severity of stem symptoms less ($P < 0.0001$) for the group of genotypes with resistance to both pathogens than for the group of genotypes resistant only to the fungus. There was no difference between these two groups of genotypes in the overall incidence ($P = 0.5715$) or severity ($P = 0.7277$) of stem symptoms in the absence of *H. glycines*.
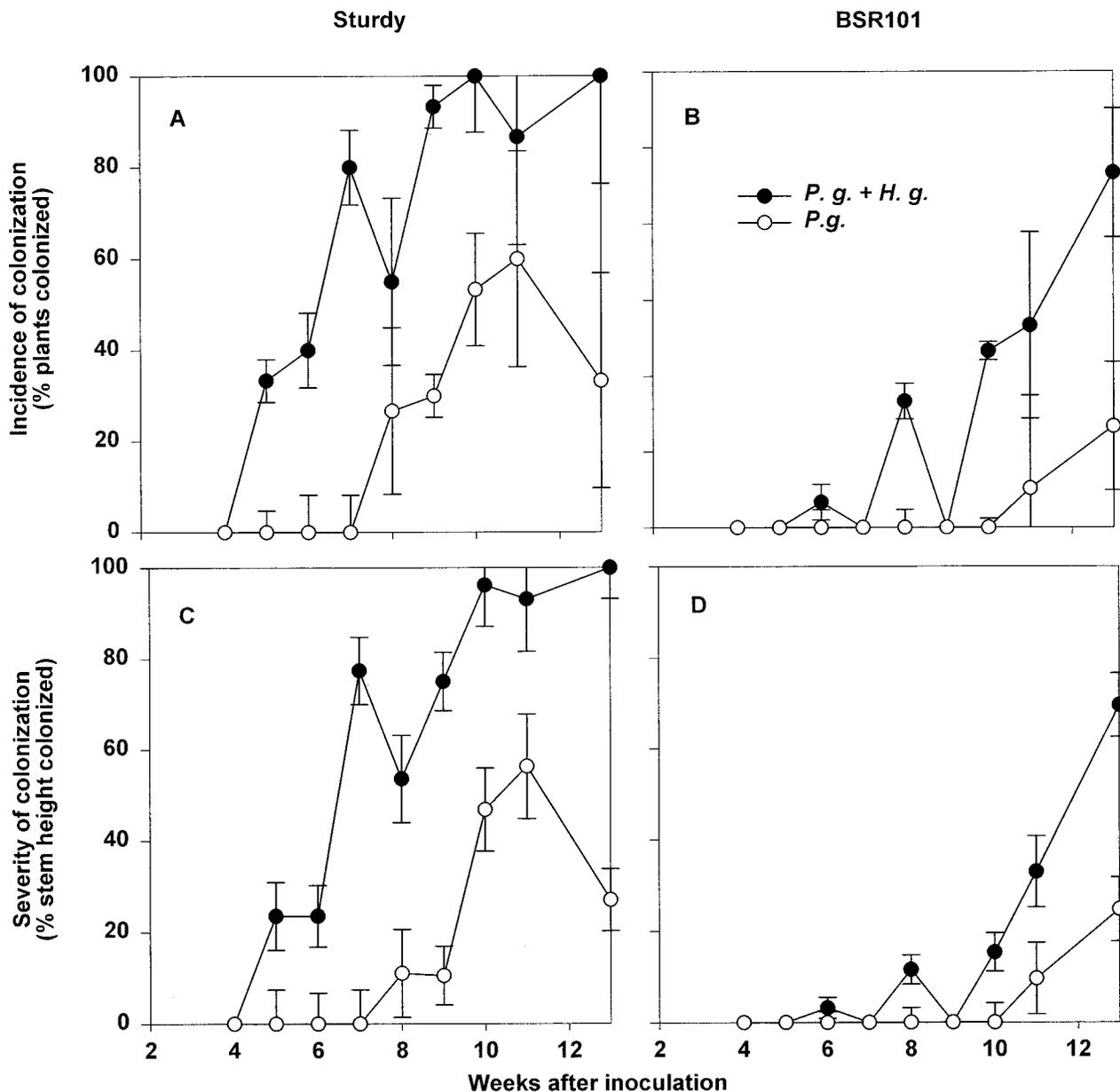
Fig. 1. *Phialophora gregata* (*P. g.*) colonization of susceptible (Sturdy) and resistant (BSR101) soybean genotypes in the presence and absence of *Heterodera glycines* (*H. g.*) in experiment 6A. Inocula were 6.25×10^8 CFU of *P. gregata* on 5 g of artificially colonized straw and 7,500 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers. Data points are means of three replicates with five plants per replicate. Error bars are standard errors.

Effect of *H. glycines* on colonization of stems by *P. gregata* (experiments 4A, 4B, and 5). *H. glycines* increased both the incidence and severity of stem colonization by *P. gregata* in all BSR-resistant genotypes tested, regardless of the source of BSR resistance and regardless of resistance or susceptibility to *H. glycines* (Table 4). There was no significant (*P* > 0.05) increase in colonization in the genotype that is susceptible to both pathogens (Sturdy) except in experiment 5, presumably because the stems of this genotype already were nearly fully colonized in the *P. gregata*-only treatments. *P. gregata* was isolated occasionally from the no-pathogens and *H. glycines* treatments (*data not shown*); this could be due to cross contamination during watering. However, significance of the comparisons of the *P.*

gregata-only and *P. gregata* + *H. glycines* treatments was not affected.

Effect of *H. glycines* on the progress of colonization of stems by *P. gregata* over time (experiments 6A and 6B). *H. glycines* affected the progress of colonization of stems by *P. gregata* in both soybean genotypes tested. For the BSR-susceptible Sturdy, incidence of *P. gregata* colonization progressed faster (*P* = 0.0309) and occurred earlier (*P* < 0.0001) in experiments 6A and 6B, respectively, in the presence than in the absence of *H. glycines* (Figs. 1A and 2A). Severity of colonization for Sturdy progressed faster (*P* = 0.0002) and occurred earlier (*P* < 0.0001) in experiments 6A and 6B, respectively, in the presence than in the absence of *H. glycines* (Figs. 1C and 2C). For the BSR-resistant

BSR101, in both experiments, incidence of *P. gregata* colonization progressed earlier (*P* < 0.0001) in the presence than in the absence of the nematode (Figs. 1B and 2B). Severity of colonization for BSR101 progressed faster (*P* < 0.0001) and occurred earlier (*P* = 0.001) in the presence than in the absence of *H. glycines* in both experiments (Figs. 1D and 2D).

DISCUSSION

Our results demonstrate that *H. glycines* increases susceptibility to BSR in a wide range of soybean genotypes. We reached this conclusion based on results from repeated tests of nine genotypes representing all combinations of resistance and susceptibility to BSR and *H. glycines*. The *H. glycines*-induced increase in BSR appears
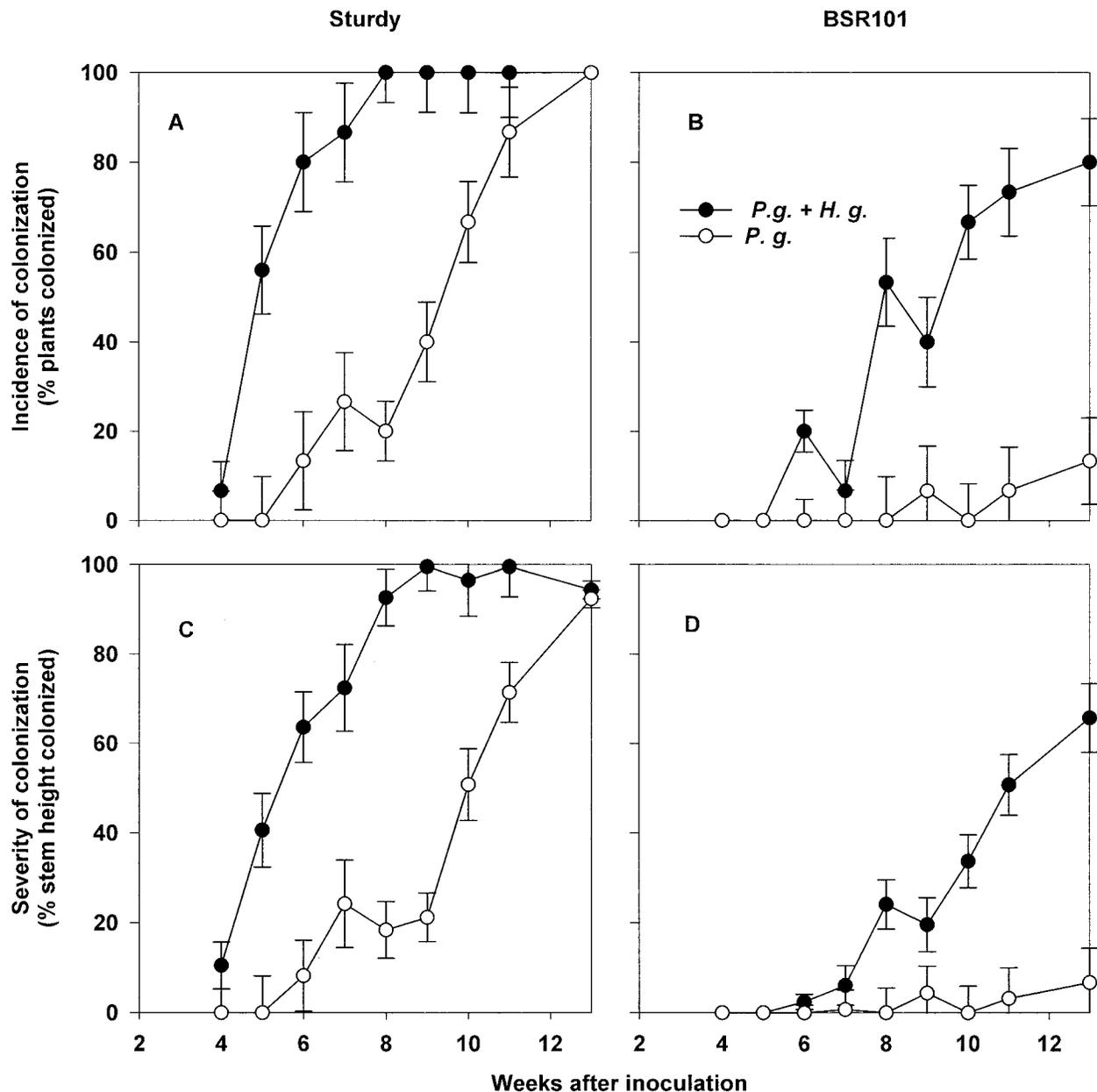
Fig. 2. *Phialophora gregata* (*P. g.*) colonization of susceptible (Sturdy) and resistant (BSR101) soybean genotypes in the presence and absence of *Heterodera glycines* (*H. g.*) in experiment 6B. Inocula were 7×10^8 CFU of *P. gregata* on 3.8 g artificially colonized straw and 7,500 *H. glycines* eggs in 4-by-21-cm plastic, cone-shaped containers. Data points are means of three replicates with five plants per replicate. Error bars are standard errors.

to be irrespective of susceptibility or resistance to BSR or *H. glycines*. However, the increase was less in genotypes with resistance to both pathogens than in genotypes with resistance to BSR or *H. glycines* only. Eight soybean genotypes described in the manuscript were tested only once. In all of these genotypes, *H. glycines* increased stem discoloration or stem colonization by *P. gregata* and, consequently, reinforced the overall conclusion that *H. glycines* increases BSR.

In our experiments, the internal stem discoloration and colonization assays differed in their sensitivity in detecting the effect of *H. glycines* on BSR. For instance, there was little or no increase in internal stem discoloration due to *H. glycines* in some soybean genotypes, but there was an increase in *P. gregata* colonization. This can be seen for PS2465N and Freeborn in experiments 4A and 4B and PI 84946-2, PI 437833, and PI 437970 in experiment 5 (Tables 2, 3, and 4). The absence of symptoms could be because symptoms did not develop by the time the data were collected or because some soybean genotypes do not readily exhibit stem symptoms regardless of the severity of colonization of the stem by *P. gregata*. It is important to note that our studies assessed severity of colonization by measuring the percentage height of the stem from which the fungus could be retrieved. It is not known how this measure relates to the actual biomass of fungus in the stem. It is possible that measures of fungal biomass would correlate better with stem symptoms than does height of colonization.

Our results also showed that infection by *H. glycines* can cause earlier colonization of soybean stems by the fungus (Figs. 1 and 2). Weber et al. (28) reported that yield loss from BSR was due largely to a reduction in seed number rather than seed size, suggesting that the onset of infection by *P. gregata* relative to seed set may be important in determining yield loss. Thus, *H. glycines*-induced earlier infection by *P. gregata* may increase yield loss caused by the fungus.

The mechanism by which *H. glycines* increases the susceptibility of soybean to *P. gregata* was not investigated in our experiments. Wounds created by nematode activities such as movement, feeding, growth, and reproduction on soybean roots may provide a direct route of entry into the root for the fungus. In addition, the disease defense mechanisms of the roots in the vicinity of these wounds might be compromised, thus facilitating colonization of the plants by the fungus. Similarly, *H. glycines* infection might compromise the general health of the plants and adversely affect resistance to colonization by the fungus.

Our results have implications for both growers and BSR researchers. The sources of BSR resistance in the soybean genotypes tested in our experiments are similar to the sources of BSR resistance in most currently available commercial cultivars. Consequently, cultivars with resistance only to BSR may be ineffective for management of the fungal disease in fields infested with *P. gregata* and *H. glycines*. Our data also indicate that the effect of *H. glycines* on BSR is widespread across soybean genotypes, suggesting that the presence of the nematode should be taken into consideration when assessing BSR under field conditions.

It is important to note that our data are from growth-chamber experiments with a limited range of pathogen populations and population densities. Thus, whether *H. glycines* induces susceptibility of soybean plants to BSR under field conditions and whether soybean genotypes with resistance to both pathogens provide effective resistance to BSR in the presence of *H. glycines* are questions yet to be tested in controlled field experiments.

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