Reactions of Perennial Wild Species of Genus *Glycine* to *Septoria glycines*

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ABSTRACT

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A total of 186 accessions from six perennial wild species of *Glycine* and a soybean cultivar (Williams) were evaluated for their reactions to *Septoria glycines* in the field after inoculation. Brown spot severity rated as the percentage of the total leaf area diseased ranged from 3 to 37.5% among the accessions at the R6 growth stage of Williams. Twenty-nine accessions with less than 3% severity were selected and evaluated in the greenhouse. Number and rate of formation of *S. glycines* pycnidia also were determined on infected leaves of these accessions. Four weeks after inoculation at V2–3 growth stages, brown spot severity ranged from 4 to 80% among the accessions, and the number of pycnidia on infected leaves after 7 days of incubation ranged from one to 75. Of 29 accessions, one of *G. clandestina* (PI 255745) and two of *G. tabacina* (PI 319697 and PI 321392) had less than 10% severity and fewer than five pycnidia per leaf (about 10 mm^2), suggesting that these accessions can be useful as sources of resistance to *S. glycines* in intersubgeneric hybridizations with *G. max*.

Brown spot of soybeans (*Glycine max* (L.) Merr.) caused by *Septoria glycines* Hemmi (3) is one of the most prevalent foliar diseases in Illinois and other soybean-growing areas (9,15). The disease is common wherever soybeans are grown in the midwestern United States. Brown spot first occurs early in the growing season and increases as soybeans mature. Severe infection can cause premature defoliation (3,15). Soybean yield reductions of 8–34% have resulted after inoculations with *S. glycines* (6,14,16). Yield reductions in naturally infected experimental plots have been 8–10% (6). More than 7,000 soybean plant introductions (PI) from the USDA-ARS Northern Soybean Germplasm Collection and 400 wild annual soybean (*G. soja* Sieb & Zucc.) PIs have been evaluated for resistance to *S. glycines* after field inoculations, but no source of resistance has been found (5; S. M. Lim, *unpublished*). Fifteen isolates of *S. glycines* from different states in the United States were evaluated for their pathogenic variability in the field (4). Pathogenic variability among the isolates was not detectable by quantifying the development of brown spot.

The wild perennial species of the subgenus *Glycine* have been evaluated for resistance to several soybean pathogens and for many morphological and chemical traits (13). They have not been screened for resistance to S. glycines. The objective of this study was to evaluate the reactions of perennial *Glycine* species to *S. glycines* by quantifying brown spot severity and pycnidium production on inoculated plants.

MATERIALS AND METHODS

In 1984, 186 accessions of wild *Glycine species* (23 *G. canescens* F. J. Herm., 44 *G. clandestina* Wendl., 2 *G. falcata* Benth., 10 *G. latifolia* (Benth.) Newell & Hymowitz, 74 *G. tabacina* (Labill.) Benth., and 33 *G. tomentella* Hayata) maintained in the Department of Agronomy, University of Illinois, Urbana, were evaluated for reactions to *S. glycines*. A susceptible soybean cultivar, Williams, was included. Seed of the wild perennial accessions were scarified mechanically with a razor blade to break dormancy and were incubated on moist filter paper in petri dishes. Four germinated seeds of each accession and four Williams seeds were planted in a 10-cm-diameter peat moss pot in the greenhouse. Plants at V3–4 growth stages (2) were transplanted to the field by placing pots in hills spaced 1.5 m apart at the Agronomy-Plant Pathology Farm, Urbana, on 2 June 1984. Plants in each hill were inoculated twice with a conidial suspension of *S. glycines* (ATCC 38699).

Inoculum was produced by culturing the isolate on acidified potato-dextrose agar (pH 4.0) at 22–26 C for 2 wk. Cultures were comminuted in tap water and filtered through several layers of cheesecloth. Inoculum concentration was adjusted to about 10^6 conidia per milliliter. Inoculations were made with a pressurized sprayer (5.6 kg/cm^2) on 16 and 23 June; plants were sprayed until runoff. Brown spot severity was rated on 14 July and 4 and 25 August as the percentage of the total leaf area diseased, using a modified Horsfall-Barratt rating system (8).

Seeds from 29 accessions (3 *G. canescens*, 5 *G. clandestina*, 2 *G. falcata*, 3 *G. latifolia*, 11 *G. tabacina*, and 5 *G. tomentella*) that had less than 3% brown spot at the 25 August rating were hand-harvested. In the winter of 1984, seeds of these accessions were scarified, germinated, and planted in 10-cm-diameter pots. Four germinated seeds of each of 29 accessions and four seeds of Williams were planted in each of four pots in the greenhouse. A total of 120 pots were arranged in a completely randomized design. Plants at V2–3 growth stages were moved into two dew chambers (I-35DL, Percival Manufacturing Co., Boone, IA) and inoculated with a conidial suspension of *S. glycines* (ATCC 38699) in a manner similar to the field inoculations. Inoculated plants were kept in the dew chambers for 72 hr with 16-hr (dark) dew periods at 18 C and 8-hr (light) dry periods at 24 C. Plants were then moved to a bench in the greenhouse. The experiment was repeated once under similar conditions. Brown spot severity was rated four times at weekly intervals after inoculation.

After the last disease rating, eight leaves of similar size (6–10 mm wide) from the second to fourth node of each accession were collected (two leaves from each of four pots containing four plants). Leaves larger than 10 mm wide from some of the perennial accessions and

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Williams were excised with a no. 5 cork borer (10 mm diameter). Four leaves or four excised leaf disks from each accession were placed on moist filter paper in two 9-cm-diameter petri dishes and kept on the laboratory bench under fluorescent light at 24 C. A total of 60 petri dishes were arranged in a completely randomized design. Leaves were observed daily for pycnidium production for 7 days. Pycnidia per leaf were counted at 60×.

RESULTS AND DISCUSSION

At the first rating of field-grown plants (14 July), only a few brown spots had developed on the primary leaves of Williams and no brown spot was observed on any of the accessions. At the second rating (4 August), brown spot severity on the accessions ranged from 0 to 9.5% and was 8% on Williams, which had reached the R3 growth stage. At the last rating (25 August), severity ranged from 1.5 to 37.5% among the accessions and was 31% on Williams, which was at the early R6 stage (Table 1).

Weather conditions during the middle of June 1984 through the middle of July were dry. Total precipitation in June was 30.5 mm (70.2 mm below normal), and most occurred in early June. In July, total precipitation was 116 mm (28 mm above normal); however, most occurred late in the month. Mean temperatures were 24 C in June, 22 C in July, and 23 C in August. The temperature at the Agronomy-Plant Pathology Farm was favorable for brown spot development, but lack of moisture after inoculation delayed development of brown spot. A leaf wetness period of at least 72 hr is necessary for leaf infection; longer periods of leaf wetness accelerate disease development (10,11).

In a previous study (5), differences in brown spot severity among soybean germ plasms were observed during the early growing season; however, the differences became insignificant as the season progressed, and all germ plasms were severely diseased at maturity. In this study, however, differences in brown spot severity were observed among the accessions of perennial wild species of *Glycines* at the pod-filling stages. Twenty-nine accessions had less than 3% brown spot severity (Table 1). No differences in lesion types were observed among the accessions. Nonchlorotic lesions developed on the infected leaves of the accessions, similar to those that developed on soybeans grown from green seeds (5). Leaves of most accessions were small (<10 mm wide), and nonchlorotic lesions were smaller than those on soybean leaves. Hence, nonchlorotic lesions on the infected leaves of the accessions were not as visible as brown spots on soybean leaves, and some ratings of severity on accessions may have been lower than ratings on Williams for that reason. The lack of moisture after inoculation also may have prevented the development of brown spot and resulted in the lower severity ratings on small leaves.

In the greenhouse, results of brown spot severity between two experiments were consistent ($r = 0.81$) and data were combined. No brown spots were observed on any of the 29 accessions or Williams at the first rating, 1 wk after inoculation. At the second rating, brown spot severity ranged from 0 to 18.7% among the accessions and was 4.5% on Williams. At the third rating, severity ranged from 0 to 37.5% among the accessions and was 9.5% on Williams. At the fourth rating, severity ranged from 4.5 to 80.1% among the accessions and was 18.7% on Williams. Although brown spot severity on all 29 accessions had been less than 3% at their late growth stages in the field, postinoculation moist periods in the dew chamber favored the development of brown spot in the greenhouse. However, brown spot did not develop rapidly, and severity was low at all ratings for three accessions, PI 255745, PI 319697, and PI 321392 (Table 2). Because most accessions grew very slowly in the greenhouse, the increase in percentage of leaf area diseased was greater than on Williams. Williams plants grew rapidly and leaves expanded greatly, which probably resulted in the small increase in leaf area diseased. Williams plants had reached the V3 growth stage at inoculation and V8 at the last rating.

The average number of pycnidia on infected leaves 7 days after incubation ranged from 1 to 75 among the accessions and was 34 on Williams. Number and rate of formation of pycnidia on infected leaves of the three accessions (Table 2) were very low compared with those of the other accessions, which had greater severity.

Success has been achieved in crossing wild perennial *Glycine* species with the soybean (1,7,11,12). The reduction in brown spot severity and pycnidium production on the three accessions indicated that these can be used as sources of resistance for crosses with soybeans and study of the inheritance of resistance in subsequent progeny. However, quantification of brown spot severity and pycnidium production on infected leaves of wild *Glycine* species are difficult because of small leaves, and improved techniques for identifying sources of resistance are needed.

Table 1. Frequency distribution of percentage of leaf area diseased in accessions of six perennial wild species of the genus *Glycine* subgenus *Glycine* after inoculation with *Septoria glycines* in the field and in the greenhouse

Severity (%)		Number of accessions	
		Field	Greenhouse
0[a]	(0)[b]	0[c]	0[d]
1–3	(1.5)	29	0
3–6	(3.7)	32	1
6–12	(9.5)	60	2
12–25	(18.7)	56	9
25–50	(37.5)	9	4
50–75	(62.5)	0	8
75–87	(81.0)	0	4

[a] Range of disease severity expressed as the percentage of total leaf area diseased, based on Horsfall-Barrett rating scale (8).
[b] Mean of percentage of leaf area diseased calculated by conversion of disease ratings (8).
[c] Plants were grouped based on ratings in single hills containing three or four plants at the R6 growth stage of Williams (2).
[d] Plants were grouped based on mean of eight pots (four plants per pot) rated 4 wk after inoculation at the V2–3 growth stages (2). After inoculation, plants were kept in dew chambers for 72 hr (16-hr dew [dark] periods at 18 C and 8-hr dry [light] periods at 24 C), then plants were moved into greenhouse.

Table 2. Brown spot severity on single accessions of three wild species of the genus *Glycine* and number of *Septoria glycines* pycnidia produced at daily intervals on infected leaves

Species	Plant introduction number	Severity (%)[a]		Pycnidia produced per incubation interval (days)						
		Field	Greenhouse	1	2	3	4	5	6	7
G. clandestina	255745	0[b]	9.5[c]	0[d]	0	0	0	1	1	1
G. tabacina	319697	<3	9.5	0	0	0	1	4	4	4
G. tabacina	321392	<3	4.5	0	1	2	2	2	2	2
G. max (Williams)		31	18.7	0	0	2	10	20	34	34

[a] Severity expressed as the percentage of the total leaf area diseased (8).
[b] Each value is from a single hill containing three or four plants rated at the R6 growth stage of Williams (2).
[c] Each value is the mean of eight pots (four plants per pot) rated 4 wk after inoculation at the V2–3 growth stages (2).
[d] Each value is the mean of eight infected leaves (about 10 mm^2).

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LITERATURE CITED

1. Broue, P., Douglas, J., Grace, J. P., and Marshall, D. R. 1982. Interspecific hybridization of soybeans and perennial *Glycine* species indigenous to Australia via embryo culture. Euphytica 31:715-724.
2. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11:929-931.
3. Hemmi, T. 1915. A new brown-spot disease of the leaf of *Glycine hispida* Maxim. caused by *Septoria glycines* sp. n. Trans. Sapporo Nat.

Hist. Soc. 6:12-17.
4. Kamicker, T. A., and Lim, S. M. 1985. Field evaluation of pathogenic variability in isoates of *Septoria glycines*. Plant Dis. 69:744-746.
5. Lim, S. M. 1979. Evaluation of soybean for resistance to Septoria brown spot. Plant Dis. Rep. 63:242-245.
6. Lim, S. M. 1980. Brown spot severity and yield reduction in soybean. Phytopathology 70:974-977.
7. Newell, C. A., and Hymowitz, T. 1982. Successful wide hybridization between the soybean and a wild perennial relative, *G. tomentella* Hayata. Crop Sci. 22:1062-1065.
8. Park, E. W., and Lim, S. M. 1985. Empirical estimation of the asymptotes of disease progress curves and the use of the Richards generalized rate parameters for describing disease progress. Phytopathology 75:786-791.
9. Pataky, J. D., Lim, S. M., Jordan, E. G., and Warsaw, R. L. 1979. Monitoring soybeans for foliar diseases. Ill. Res. 21(3):3-4.
10. Peterson, D. J., and Edwards, H. H. 1982. Effects of temperature and leaf wetness period on brown spot disease of soybeans. Plant Dis. 66:995-998.
11. Ross, J. P. 1982. Effect of simulated dew and postinoculation moist periods on infection of soybean by *Septoria glycines*. Phytopathology 72:236-238.
12. Singh, R. J., and Hymowitz, T. 1985. An intersubgeneric hybrid between *Glycine tomentella* Hayata and the soybean, *G. max* (L.) Merr. Euphytica 34:187-192.
13. Vaughan, D. A., and Hymowitz, T. 1983. Progress in characterization of the wild perennial relatives of the soybean. Plant Genet. Resour. Newsl. 56:7-12.
14. Williams, D. J., and Nyvall, R. F. 1980. Leaf infection and yield losses caused by brown spot and bacterial blight diseases of soybeans. Phytopathology 70:900-902.
15. Wolf, F. A., and Lehman, S. G. 1926. Brown spot disease of soybean. J. Agric. Res. 33:365-374.
16. Young, L. D., and Ross, J. P. 1979. Brown spot development and yield response of soybeans inoculated with *Septoria glycines* at various growth stages. Phytopathology 69:8-11.