Field Evaluation of Pathogenic Variability in Isolates of *Septoria glycines*

T. A. KAMICKER, Graduate Research Assistant, and S. M. LIM, Professor and Research Plant Pathologist, Department of Plant Pathology, University of Illinois and USDA Agricultural Research, Urbana 61801

ABSTRACT

Kamicker, T. A., and Lim, S. M. 1985. Field evaluation of pathogenic variability in isolates of *Septoria glycines*. Plant Disease 69:744-746.

Isolates of *Septoria glycines* were evaluated for their pathogenic variability on the susceptible soybean cultivar Williams 79 in the field. Twelve isolates from different states in the United States were evaluated in 1981 and 15 isolates were evaluated in 1982. Treatments included inoculation, fungicide application, and check (natural infection) in both years. All isolates evaluated produced typical brown spot lesions (angular brown spots surrounded by a yellow area) on leaves of infected plants. In 1981, there were significant differences in brown spot severity among some isolates at the R6 growth stage. In 1982, however, there were no significant differences in brown spot severity. In both years, there were significant differences in brown spot vertical progress among isolates but no differences in defoliation. Area under the disease progress curve was similar among isolates. No significant differences in either soybean yield or 300-seed weight were found among inoculated and check plots for either year. Although there were significant differences in the severity and vertical progress of brown spot among some isolates, results were not consistent and indicate that pathogenic variability among isolates of *S. glycines* was not detectable by quantifying the development of brown spot in the field.

Additional key words: *Glycine max*

Brown spot of soybean (*Glycine max* (L.) Merr.) caused by *Septoria glycines* Hemmi (4) is one of the most common foliar diseases of soybeans in Illinois (13). The disease occurs first on unifoliate leaves, progresses from lower to upper leaves, and defoliates plants prematurely (5,17). Brown spot has caused soybean yield losses of up to 8–10% in naturally infected plots (10,11) and up to 8–34% in inoculated plots (10–12,16,18). Although more than 7,000 soybean plant introduction lines from the USDA-ARS Northern Soybean Germplasm Collection were evaluated for brown spot resistance (S. M. Lim, *unpublished*), no source of resistance was found. All lines and cultivars appeared susceptible to *S. glycines* at ratings taken late (R6 growth stage) in the season.

Understanding pathogen variability is important in programs for breeding for disease resistance. Studies describing pathogenic variability of *S. glycines*, however, have not been reported previously. The objectives of this study were to determine the pathogenic variability in isolates of *S. glycines* from different geographical regions in the United States and to determine their effects on 300-seed weight and yield of soybean in the field. Preliminary results of this research were reported earlier (7).

MATERIALS AND METHODS

The 12 isolates evaluated in 1981 were from infected leaf tissue obtained from Arkansas (AR), Georgia (GA), Illinois (IL), Kansas (KS), Minnesota (MN), Missouri (MO), Maryland (MD), North Carolina (NC), South Carolina (SC), South Dakota (SD), Tennessee (TN), and Wisconsin (WI). Fifteen isolates evaluated in 1982 included the 12 isolates from the 1981 study with the addition of three more isolates from Florida (FL), Iowa (IA), and Mt. Vernon, IL (MV-IL).

Isolates of *S. glycines* were obtained from leaves by transferring conidial masses from sporulating pycnidia to potato-dextrose agar (PDA) amended with antibiotics (0.01 g/L streptomycin and 0.01 g/L tetracycline). Cultures were maintained on PDA plates at 25 C or stored for long periods on PDA in tubes at 4 C.

Field experiments were carried out at the Agronomy and Plant Pathology Farm, Urbana, IL. The isolates were evaluated on Williams 79, a cultivar grown widely in the Midwest. Certified Williams 79 seed from plants that had received two late-season sprays of benomyl was planted on 21 May 1981 and 18 May 1982 in fields that had not been planted to soybeans previously. The field used in 1981 had been a fruit orchard for at least 15 yr and the field used in 1982 had been a peach orchard for at least 15 yr before being planted corn in 1981. A commercial formulation of *Rhizobium* (Nitragin Soil Implant Inoculant, Nitragin Co., Inc., Milwaukee, WI) was applied. Plots were six rows wide with 76-cm spacing and were 5.5 m long.

The experimental design was a randomized complete block with four replicates both years and 14 treatments in 1981 and 17 treatments in 1982. In 1981, treatments consisted of the 12 isolates, a check of natural infection, and benomyl protection. In 1982, the 17 treatments were the 14 treatments from 1981 and three additional isolates.

One inoculation was made at the V4 (3) soybean growth stage in 1981 on 18 June. In 1982, two inoculations were made at the V3 growth stage on 11 June and at the V4 growth stage on 23 June. Inoculum was prepared from 2- to 3-wk-old cultures on PDA, and concentration ranged from 2×10^6 to 7×10^6 conidia per milliliter. Conidial suspensions were applied with plastic hand-pump plant misters. In each plot, plants in the two center rows were inoculated 2–3 hr before sunset when conditions were favorable for dew formation.

Fungicide was applied to protected treatments at 2-wk intervals throughout the growing season to prevent natural infection by *S. glycines*. Benomyl (Benlate 50WP) was applied at a rate of 1.1 kg/ha (10) with a tractor-mounted sprayer equipped with hand-held nozzle guns. Five applications were made each year.

Disease was assessed in the two center rows of each plot nine times at 7- to 10-day intervals in 1981 and seven times at 10- to 14-day intervals in 1982. Disease severity ratings were made using a Horsfall-Barratt rating scale (6) and were converted to percent severity with the Elanco Conversion Tables (Elanco Products Co., Indianapolis, IN). Disease severity ratings were based on the proportion of diseased leaf tissue and did

Present address of first author: Biochemical Data Specialist, PPG Industries, Inc., Pittsburgh, PA 15272.

Portion of a thesis submitted by the first author in partial fulfillment of the requirements for the M.S. degree.

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Accepted for publication 6 March 1985 (submitted for electronic processing).

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not account for defoliation. Soybean growth stage (3), vertical progress (12), and nodes defoliated were also recorded for each rating date. Area under the disease progress curve (AUDPC) (15) values were calculated from the severity data.

Precautions were taken to prevent spread of brown spot between plots. Brown spot ratings were done in the afternoon when leaves were dry. Fields were cultivated before inoculation. The tractor and cultivator were rinsed with water to remove soil before entering the field.

The two center rows of each plot were trimmed at harvest to 4.6 m. Seed harvested from the center rows was dried at 38 C for 72 hr to 7% moisture and adjusted to 13% moisture for yield. Yield was estimated by converting grams per plot to quintals per hectare. The 300-seed weights were measured from seed dried to 7% moisture.

RESULTS

Before inoculation, no brown spot symptoms were observed. In 1981, symptoms developed about 10 days after inoculation and the naturally infected plots remained relatively disease-free (severity $\leq 5\%$) for 7 wk. In 1982, symptoms developed 3 wk after the first inoculation and 1 wk after the second inoculation. Naturally infected plots remained relatively disease-free for 5 wk. No symptomatic differences were observed on plants inoculated with the various isolates. Typical brown spot symptoms (angular brown spots surrounded by a yellow area) developed on leaves of all infected plants.

Brown spot severity, vertical progress, and defoliation at the R6 growth stage are presented in Tables 1 and 2. Severity ranged from 0 to 25% and from 0 to 13.2% in 1981 and 1982, respectively. In 1981, severity was greatest on plants inoculated with the WI isolate. Brown spot severity resulting from the WI isolate was significantly different from the AR, MN, TN, naturally infected, and protected treatments. There were no significant differences among isolates other than WI. Severity in naturally infected plots was significantly less than that of four of the isolates. In 1982, there were no significant differences in brown spot severity among any of the isolates or naturally infected treatments. In both years, the protected plots had the lowest severity, since few brown spot lesions appeared on senescencing leaves at the physiological maturity (R7).

Vertical progress of the NC isolate in 1981 was higher than GA, IL, MN, MO, SD, TN, naturally infected, and protected treatments. In 1982, vertical progress was highest in plots inoculated with the FL isolate and was significantly higher than the AR, MO, SD, naturally infected, and protected treatments. Vertical progress was from 0 to 11 nodes in 1981 and from 0 to 10 nodes in 1982. During both years, there were no significant differences in defoliation among the treatments at R6, although there was a significant difference in defoliation between inoculated and protected treatments at R7. In the protected plots, no leaves had brown spot lesions at R6 but six or seven nodes had defoliated, probably because of natural soybean leaf senescence.

AUDPC values were not significantly different among isolates in either year, except for the MN isolate, which had a significantly lower AUDPC value than five isolates in 1981. The naturally infected plot had a significantly lower AUDPC value than all isolates in 1981 but was similar to all isolates in 1982. Protected plots had the lowest AUDPC value and were significantly different from all the other treatments for both years. The range for AUDPC values was 45–1,250 in 1981 and 18–837 in 1982.

Yield or 300-seed weight did not differ significantly among treatments in either year (Table 3). Combined analysis of the 14 treatments common in both years indicated that yield and 300-seed weight were higher in 1981 than in 1982. Brown spot severity, vertical progress, and defoliation at R6 were highly correlated with AUDPC values in both years (Table 4).

DISCUSSION

Results showed that pathogenicity of *S. glycines* isolates was similar on Williams 79 soybeans in the field. Consequently, the results of several

Table 1. Severity (SEV), vertical progress (VP), and defoliation (DFL) at the R6 growth stage and area under the disease progress curve (AUDPC) of brown spot on Williams 79 soybeans inoculated with isolates of *Septoria glycines* in 1981

Treatment	SEV (%)	VP (nodes)	DFL (nodes)	AUDPC
AR[a]	15.5[b]	10.3	7.5	1,034
GA	19.1	9.5	7.0	1,029
IL	12.1	9.5	7.3	1,081
KS	17.5	10.0	7.3	1,149
MN	14.4	9.8	7.8	924
MO	20.3	9.8	7.5	1,230
MD	19.5	10.0	8.0	1,054
NC	20.3	11.0	8.5	1,158
SC	18.3	10.3	8.0	1,169
SD	20.3	9.5	7.3	1,164
TN	16.7	9.5	7.3	1,111
WI	25.0	10.8	8.0	1,250
NI[c]	12.1	8.8	7.0	672
PT[d]	0.0	0.0	6.8	45
FLSD ($P = 0.05$)	7.6	1.2	NS	277

[a] State origin of isolates of *S. glycines*.
[b] Each value is the mean of four replicates.
[c] Natural infection.
[d] Protected with benomyl spray (1.1 kg/ha at 14-day intervals).

Table 2. Severity (SEV), vertical progress (VP), and defoliation (DFL) at the R6 growth stage and area under the disease progress curve (AUDPC) of brown spot on Williams 79 soybeans inoculated with isolates of *Septoria glycines* in 1982

Treatment	SEV (%)	VP (nodes)	DFL (nodes)	AUDPC
AR[a]	10.8[b]	9.3	7.3	756
FL	13.2	10.0	8.0	800
GA	12.0	9.8	7.3	831
IL	12.0	9.5	7.8	827
IA	13.2	9.5	7.5	786
KS	12.0	9.5	7.5	804
MN	13.2	9.8	7.5	779
MO	10.8	9.0	7.0	743
MD	12.0	9.5	7.3	735
MV-IL	13.2	9.5	7.5	837
NC	12.7	9.5	7.5	771
SC	12.0	9.5	7.5	814
SD	12.0	9.3	7.3	774
TN	12.0	9.5	7.8	798
WI	10.8	9.5	7.5	786
NI[c]	12.0	9.3	7.3	722
PT[d]	0.0	0.0	6.5	18
FLSD ($P = 0.05$)	2.9	0.7	NS	105

[a] State origin of isolates of *Septoria glycines*.
[b] Each value is the mean of four replicates.
[c] Natural infection.
[d] Protected with benomyl spray (1.1 kg/ha at 14-day intervals).

studies on brown spot in which various isolates of *S. glycines* were used are widely applicable with regard to the pathogen.

Although there were significant differences in brown spot vertical progress and AUDPC values among some of isolates, the differences were not consistent between 1981 and 1982. Differences in weather in the 1981 and 1982 growing seasons affected brown spot development and are reflected in the lower AUDPC values for 1982. Latent period was twice as long in 1982 as in 1981, probably because of dry, cool weather early in the season. Rainfall was more frequent during the 2 wk after inoculation in 1981, whereas it did not occur until 4 and 5 days after the first and second inoculations, respectively, in 1982. Mean temperature for June was 22.8 C in 1981 and 19.5 C in 1982. Brown spot is favored by warm, moist weather (4).

In this study, benomyl application did not increase yield or 300-seed weight. This is in contrast to other studies where yield increases in benomyl-sprayed plots were described (1,10–12), with greatest yield responses in high-yielding environments (1,12). In this study, yields of benomyl-sprayed plots and inoculated plots were probably similar because brown spot developed late in the season. Symptoms developed in inoculated plots 3–4 wk after brown spot appeared in other nearby fields that had been in continuous soybeans or in soybean-corn rotation. Brown spot severities observed in this study were less than those reported elsewhere (10–12). Apparently, the delayed epidemic development and the lower severity of brown spot did not reduce yield in inoculated plots significantly. Also, other leaf, pod, and stem diseases were either absent or at very low levels. Yields in this study were 3.3–4.0 q/ha higher and 300-seed weights were 6–9 g higher than in other fields that were planted to Williams 79 during the same years in Urbana. Possibly, this land was so conducive to soybean growth that vigorous plants were not stressed by brown spot, resulting in no yield reductions. Young and Ross (18) suggested that lack of yield reduction in *S. glycines*-inoculated plots compared with uninoculated plots was a result of limited environmental stress.

Pathogenic variability among isolates of other *Septoria* spp., particulary those on wheat, have been noted. Differences in pathogenicity were described for *S. nodorum* Berk. (*Leptosphaeria nodorum* Muller) (8,9,14) and physiologic specialization for *S. tritici* Rob. ex Desm. (2), yet in either case, designation of races is not common. Environmental factors cause variable responses of *S. nodorum* isolates on wheat (9,14). The pathogenic variability in *S. glycines* should be investigated further with collections of isolates from a wide range of geographical regions and soybean cultivars representing various genetic backgrounds.

Table 3. Yield and 300-seed weight of Williams 79 soybeans inoculated with isolates of *Septoria glycines* in 1981 and 1982

	1981		1982	
Treatment	Yield (q/ha)	300-Seed (g)	Yield (q/ha)	300-Seed (g)
AR[a]	39.5[b]	61.83	36.6	56.41
FL	...	...	36.3	56.20
GA	40.3	60.56	35.2	55.20
IL	40.2	62.51	35.7	55.57
LA	...	...	33.8	54.29
KS	41.1	62.03	36.2	56.09
MN	43.6	61.98	35.9	55.94
MO	42.7	66.06	38.8	58.37
MD	41.7	61.38	36.7	56.84
MV	...	...	34.1	55.06
NC	43.3	62.61	36.5	56.41
SC	40.4	62.56	35.3	53.94
SD	42.2	62.43	35.2	56.46
TN	42.0	62.46	37.6	55.99
WI	41.9	61.89	36.0	55.86
NI[c]	41.7	62.01	35.7	55.54
PT[d]	44.4	62.25	37.0	57.77
FLSD ($P = 0.05$)	NS	NS	NS	NS

[a] State origin of isolates of *Septoria glycines*.
[b] Each value is the mean of four replicates.
[c] Natural infection.
[d] Protected with benomyl spray (1.1 kg/ha at 14-day intervals).

Table 4. Correlation coefficients (r) between severity (SEV), vertical progress (VP), and defoliation (DFL) at the R6 growth stage and area under the disease progress curve (AUDPC) in isolate studies of *Septoria glycines* on Williams 79 soybeans in 1981 and 1982[a]

	SEV	VP	DFL	AUDPC
SEV	...	0.73**	0.33**	0.82**
VP	0.77**	...	0.50**	0.86**
DFL	0.28*	0.56**	...	0.39**
AUDPC	0.83**	0.90**	0.40**	...

[a] Values above the diagonal are for 1981 and below the diagonal for 1982; * = significant at $P = 0.05$ and ** = significant at $P = 0.01$.

ACKNOWLEDGMENT

We thank R. L. Warsaw for assistance in the field experiments.

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