Reaction of Mung Bean Plants to Infection by Isolates of *Phialophora gregata*

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ABSTRACT

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Phialophora gregata was isolated in 1991 from naturally infected Century soybean and Kiloga and Berken mung bean plants grown in Illinois and Wisconsin. Century soybean and Berken and Kiloga mung bean plants were inoculated in the greenhouse with four isolates recovered from soybeans and nine isolates recovered from mung beans. All isolates caused typical leaf disease symptoms on inoculated Century soybean plants. Leaves on inoculated mung bean plants turned brown and died without developing the interveinal chlorosis that is characteristic of this disease on soybean. The fungus was isolated from vascular tissues of stems and leaves of inoculated plants. To the best of our knowledge, this is the first report of *P. gregata* isolated from vascular tissue of soybean or mung bean leaves. Our studies demonstrate that *P. gregata* is a vascular pathogen of mung bean under natural conditions.

Brown stem rot of soybean (*Glycine max* (L.) Merr.), caused by *Phialophora gregata* (Allington & D.W. Chamberlain) W. Gams, is an important vascular disease of soybeans in the midwestern United States. The disease is favored by cool weather during the seed development stage of soybeans (1). Various yield losses have been associated with the disease (2,5,6). Mung bean (*Vigna radiata* (L.) R. Wilcz.) was reported originally as a host of *P. gregata* (1); however, to the best of our knowledge, additional work has not determined the extent of damage caused by *P. gregata* on mung bean. Our objectives were the following: to determine whether *P. gregata* infects mung bean plants under field conditions; to discover the range of symptoms produced on infected mung bean plants in the field; and to determine whether isolates of *P. gregata* recovered from soybeans infect mung bean.

MATERIALS AND METHODS

Field plots. Soybean (cv. Century) and mung bean (cvs. Berken and Kiloga) were planted 15 May 1991 and 1992 at Urbana, Illinois, and 18 May 1991 at Clinton, Wisconsin. Each experimental unit was a single row 3 m long with 76-cm spacing between rows. There were four replicates of each cultivar at each location. The field at Urbana was in continuous soybean production for 15 yr, and the field at Clinton was in corn the previous season. Plants at both locations were evaluated for foliar symptoms of brown stem rot in early September. Stems of symptomatic plants were split and observed for internal vascular browning.

Fungal isolates and inoculum production. Isolates of *P. gregata* were obtained from vascular tissues of stems by previously reported procedures (11). Pieces of stem tissue were treated with 0.6% NaOCl for 3 min, blotted onto sterile paper towels, and placed on soybean stem extract agar containing tetracycline at 50 μg/ml (11). Isolates of *P. gregata* recovered from mung bean and soybean plants at both locations were maintained on stem extract agar at 20 C (1). Inoculum was prepared by growing each fungal isolate in tubes of sterile soybean stem extract broth (10 ml per tube). Replicated tubes of media were prepared for each *P. gregata* isolate evaluated. Isolates were incubated at 20 C for 10 days, after which the contents of an individual tube were placed in a glass homogenizer and the resulting fragmented mycelia were used to inoculate individual pots. One tube of inoculum was used for each pot.

Pathogenicity tests. Six isolates of *P. gregata* recovered from mung beans and a reference strain G3 that was originally isolated from soybean in Illinois were used to inoculate Century soybeans and Berken and Kiloga mung beans. Three plants were grown for 3 wk in each pot in a steam-pasteurized mixture of soil and sand (1:1 v/v). At 3 wk, soil was removed from around the crown of each plant. A hypodermic needle was used to force a drop of ground mycelia into two wounds made in the upper taproot below the soil line. Additionally, 3 ml of inoculum was allowed to run down the taproot of each plant. The inoculation site was covered with steam-pasteurized soil. After inoculation, plants were grown in a greenhouse under previously reported conditions for 6 wk (9,11). The treatment design was an 8 × 3 factorial of seven isolates and an uninoculated control and the three cultivars (Century, Berker, and Kiloga) arranged in a completely random design with three replicates. The experiment was done twice and denoted as experiment 1. Plants were observed periodically for 6 wk until symptom expression was distinct. Inoculated plants were rated for symptoms 6 wk after inoculation.

In experiment 2, two isolates of *P. gregata* from mung bean and three from soybean from the Wisconsin field, and two isolates from soybean and six from mung bean from the Illinois field were compared for pathogenicity in the greenhouse on Century soybean and Berken mung bean. The treatment design was a 14 × 2 factorial of 13 fungal isolates and an uninoculated control and two cultivars arranged in a completely random design with three replicates. The inoculation procedure and experimental units were the same as in experiment 1. The experiment was done twice. After inoculation, plants were grown in a greenhouse under similar conditions to experiment 1. Plants were rated for symptoms 6 wk after inoculation.

Disease assessment. Diseased and symptomless foliage from each plant was removed separately and weighed. Percent diseased foliage was determined by dividing the fresh weight of diseased foliage per plant by the total fresh weight of foliage (diseased and symptomless) of each plant. Fresh weight of leaves (top weight) also was determined as a percentage by dividing the total fresh weight of leaves from infected plants by the total fresh weight of leaves from the noninfected control plants. Data for individual plants were combined to give a pot total for each experimental unit. Stems of inoculated plants were split longitudinally, and the number of nodes with vascular browning was recorded for each plant. The percentage of stem nodes with vascular browning was determined by dividing the number of nodes with vascular browning by the total stem nodes of each plant. In experiment 1, four 2-mm cross sections of stem tissue from each plant and three 1-cm-long samples from the main veins of the third trifoliolate leaf were placed onto isolation medium (11). Data from the three plants in each pot were combined and

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analyzed as an experimental unit. Data from the two trials of experiment 1 were compared in a combined analysis, as were data from the two trials of experiment 2. Treatments were compared by mean separation tests, Bayesian Least Significant Difference (BLSD), and orthogonal contrasts. For variables that were calculated as a percentage of control treatments, the controls were not included in the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Field plots. Disease symptoms were different on mung bean plants than on soybean plants. Leaves of infected mung bean were yellow, turning brown during advanced stages of disease development before dying. Leaf symptoms on mung bean were nondescript and could have been confused easily with symptoms due to other causes, such as drought, root rot, or disorders that result in browning and necrosis of leaf tissue. In contrast, leaf symptoms with the characteristic interveinal yellowing developed on diseased Century plants at both locations in 1991 and were also evident on diseased Century plants in Illinois in 1992. When field-grown mung bean plants were evaluated in Illinois in September 1991 and 1992, all plants were dead. All mung bean and Century plants at both field locations had internal stem vascular browning. *P. gregata* was isolated from the vascular tissue of mung bean and soybean plants collected at both locations in 1991 and from all plants collected in Illinois in 1992 (Table 1).

Pathogenicity tests. *P. gregata* isolates recovered from infected mung bean plants in Illinois and Wisconsin caused disease on inoculated mung bean and soybean in the greenhouse (Tables 2 and 3). Main effects of fungal isolates and cultivars were significant for both percent top weight and percent diseased foliage (Table 2), but the interactions were not significant. Top weight of foliage was lower for soybean or mung bean plants inoculated with any of the isolates of *P. gregata* than for the noninoculated controls (Table 2). In trial 1, top weight was lowest for isolate G3, which was significantly different from two mung bean isolates, MBU8 and MBW8. In trial 2, top weight of a different mung bean isolate, MBW4, was greater than G3 and three other isolates

Table 1. Location and host plant from which *Phialophora gregata* isolates were recovered in 1991

Isolate[a]	Location	Host
G3	Illinois	Soybean
CU13	Illinois	Soybean
CW2	Wisconsin	Soybean
CW3	Wisconsin	Soybean
MBU2	Illinois	Mung bean
MBU3	Illinois	Mung bean
MBU4	Illinois	Mung bean
MBU5	Illinois	Mung bean
MBU6	Illinois	Mung bean
MBU8	Illinois	Mung bean
MBW4	Wisconsin	Mung bean
MBW6	Wisconsin	Mung bean
MBW8	Wisconsin	Mung bean

[a] Isolates are named after source cultivar (C = Century soybean, MB = mung bean) and location (U = Urbana, Illinois, W = Clinton, Wisconsin). Reference isolate G3 was isolated from a diseased Hardin soybean plant from Illinois.

Table 2. Top weight of foliage as a percentage of the control and percent diseased foliage for soybean and mung bean inoculated with seven isolates of *Phialophora gregata* (experiment 1)

	Foliage weight (%)[a]		Diseased foliage (%)[b]	
Isolates, cultivars, and contrasts	Trial 1	Trial 2	Trial 1	Trial 2
Fungal isolate[c]				
Control	100	100	0	0
MBU4	28	36	94	85
MBU6	39	47	91	80
MBU8	46	38	82	96
MBW4	24	63	93	70
MBW6	27	39	91	92
MBW8	48	49	83	86
G3	20	43	94	89
BLSD (k = 100)	20.3	19.1	NS[d]	19.5
Cultivar				
Century soybean	39	57	79	76
Berken mung bean	47	55	99	93
Kiloga mung bean	14	22	91	87
BLSD (k = 100)	10.9	9.7	7.0	10.6
Contrast[e]				
WI vs. IL isolates	*	*	NS[d]	NS[d]

[a] Top weight as a percentage of noninoculated control.
[b] Percentage of foliar tissue (by weight) that was diseased.
[c] Isolates are named after source cultivar (C = Century soybean, MB = mung bean) and location (U = Urbana, Illinois, W = Clinton, Wisconsin). Reference isolate G3 was isolated from a diseased Hardin soybean plant from Illinois.
[d] NS = nonsignificant, * = significant at $P \leq 0.05$.
[e] Contrast of three Illinois and Wisconsin isolates different at $P \leq 0.05$. Trial 1, IL = 38%, WI = 33%. Trial 2, IL = 41%, WI = 50%.

Table 3. Percentage of leaf sections colonized, stem nodes with vascular browning, and stem sections colonized for Century soybean and Kiloga and Berken mung bean plants inoculated with seven isolates of *Phialophora gregata* (experiment 1)

	Leaf sections colonized (%)[b]			Brown stem nodes (%)[c]			Stem sections colonized (%)[d]		
Fungal isolates[a]	Century	Kiloga	Berken	Century	Kiloga	Berken	Century	Kiloga	Berken
MBU4	94	75	86	81	100	96	89	100	100
MBU6	94	97	80	84	95	94	100	100	92
MBU8	93	97	83	60	97	98	100	100	100
MBW4	100	89	100	77	100	100	100	89	100
MBW6	95	92	97	72	100	97	100	86	92
MBW8	92	94	86	84	95	92	100	94	89
G3	100	89	89	90	100	98	94	92	100
Control	0	0	0	0	0	0	0	0	0
Source of variation[e]									
Fungal isolate		NS			NS			NS	
Cultivar		NS			**			NS	
Fungal isolate × cultivar		NS			NS			NS	

[a] Isolates are named after source cultivar (C = Century soybean, MB = mung bean) and location (U = Urbana, Illinois, W = Clinton, Wisconsin). Reference isolate G3 was isolated from a diseased Hardin soybean plant from Illinois.
[b] Percentage of leaf tissue samples from which *P. gregata* was recovered.
[c] Percentage of the internal stem nodes with vascular browning.
[d] Percentage of the stem vascular tissue samples from which *P. gregata* was recovered.
[e] NS = nonsignificant, ** = significant at $P \leq 0.01$.

(MBU4, MBU8, and MBW6) with low percent top weights. In both trials, percent top weight of Kiloga mung bean was significantly lower than Berken mung bean and Century soybean, which were not different from each other. For percent diseased foliage, isolates did not differ in trial 1, but MBW4 had less diseased foliage than MBW6 and MBU8 in trial 2. In both trials, diseased foliage was less on Century soybeans than on either mung bean cultivar. Data for the two experiments were not combined because of a significant fungal isolate by experiment interaction. Three isolates, MBU8, MBW4, and MBW8, were the primary sources of the interaction among trials.

P. gregata was recovered from stem and leaf vascular tissues of inoculated plants (Table 3). In previous work, *P. gregata* was isolated from internal vascular tissues of stems of diseased plants (1,4–6), but colonization of the leaf vascular tissue has not been reported. All isolates colonized the leaf vascular tissue of both mung bean cultivars and soybean. There was a significant effect of host cultivar on the percentage of brown stem nodes, as both mung bean cultivars had a higher percentage of discolored nodes than did Century soybean (Table 3).

In 1992, when isolates of *P. gregata* recovered from mung beans and soybeans were used to inoculate plants in the greenhouse (experiment 2), all isolates caused disease on soybean and mung bean (Tables 4 and 5). As in experiment 1, main effects of fungal isolates and cultivars were significant for percent top weight. The fungal isolate by cultivar interaction also was significant for percent top weight. There also was a significant experiment by fungal isolate interaction in the combined ANOVA for experiment 2. Interactions among isolates and cultivars were typified by the reaction of isolates CW3 and MBW8 on Century and Berken in trial 1 (Table 4). The isolate-by-experiment interaction is typified by the reaction of isolates MBU4 and CU13 in trials 1 and 2. In some instances, ranks changed depending on the reactions of an isolate in a trial, such as reactions to MBU3.

For percent diseased foliage and percentage of stem nodes brown, there were no significant interactions among the two trials of experiment 2; therefore the combined ANOVA was interpreted. For diseased foliage, the main effects and the interaction of isolates and cultivars were significant. For percentage of stem nodes brown, the interaction and the isolate main effect were significant. Two isolates, CW3 and MBU2, appeared to be less aggressive than others. Other significant differences were of lesser magnitude.

The isolate-by-experiment interaction that we observed in both experiments has been reported previously (10). It indicated that fungal isolates differ among greenhouse experiments. Severity of symptoms resulting from fungal isolates appears to be affected by greenhouse environmental conditions. For example, reference strain G3 from soybean is an aggressive isolate that can kill plants, but the amount of disease caused by this isolate on soybean varies from experiment to experiment (L. E. Gray, *unpublished*). Strain G3 resulted in the death of plants inoculated in fall experiments, but symptoms were less severe in spring trials. This type of variability should be taken into consideration when disease reactions of numerous genotypes are evaluated over

Table 4. Top weight foliage[a] as a percentage of controls for Century soybean and Berken mung bean plants inoculated with 13 isolates of *Phialophora gregata* (experiment 2)

	Trial 1		Trial 2	
Isolates[b] and contrasts	**Century**	**Berken**	**Century**	**Berken**
Fungal isolates				
Control	100[a]	100[a]	100[a]	100[a]
G3	28	27	27	35
CU13	36	18	38	35
CW2	26	25	38	44
CW3	41	130	54	67
CW4	59	67	39	52
MBU2	67	72	77	66
MBU3	23	20	39	65
MBU4	32	38	65	49
MBU5	19	40	29	32
MBU6	31	27	33	76
MBU8	32	66	26	33
MBW6	24	58	34	53
MBW8	28	90	31	71
BLSD ($k = 100$)	22.8		15.8	
Contrasts[c]				
MB vs. SB	NS[d]		NS	
WI vs. IL-SB	**		**	
WI vs. IL-MB	NS		NS	

[a]Leaf weight as a percentage of noninoculated control.
[b]Isolates are named after source cultivar (C = Century soybean, MB = mung bean) and location (U = Urbana, Illinois, W = Clinton, Wisconsin). Reference isolate G3 was isolated from a diseased Hardin soybean plant from Illinois.
[c]Contrasts of mung bean vs. soybean isolates, Wisconsin vs. Illinois soybean isolates, and Wisconsin vs. Illinois mung bean isolates.
[d]NS = nonsignificant, ** = significant at $P \leq 0.01$.

Table 5. Percent diseased foliage and percentage of stem nodes with vascular browning for Century soybean and Berken mung bean plants inoculated with 13 isolates of *Phialophora gregata* (experiment 2)

	Diseased foliage (%)[b]		Brown stem nodes (%)[c]	
Isolates[a] and contrasts	**Century**	**Berken**	**Century**	**Berken**
Fungal isolates				
Control	0	0	0	0
G3	100	98	96	93
CU13	100	98	82	96
CW2	100	93	93	90
CW3	69	23	74	58
CW4	94	73	90	84
MBU2	64	73	63	74
MBU3	97	89	91	90
MBU4	88	86	83	89
MBU5	100	99	97	95
MBU6	100	91	94	92
MBU8	100	87	98	89
MBW6	99	90	92	88
MBW8	100	66	98	72
BLSD ($k = 100$)	19.1		12.8	
Contrasts				
MB vs. SB	NS[d]		NS	
WI vs. IL-SB	**		**	
WI vs. IL-MB	NS		NS	

[a]Isolates are named after source cultivar (C = Century soybean, MB = mung bean) and location (U = Urbana, Illinois, W = Clinton, Wisconsin). Reference isolate G3 was isolated from a diseased Hardin soybean plant from Illinois.
[b]Percentage of foliar tissue (by weight) that was diseased.
[c]Percentage of internal stem nodes with vascular browning.
[d]NS = nonsignificant, ** = significant at the $P \leq 0.01$.

several winter months. A set of standard cultivars and isolates needs to be evaluated over several greenhouse environments to determine how to best compare results from various trials.

Although considerable attention has been given to the development of *P. gregata* in stem vascular tissue (3,5–8), there has been little work on the development of *P. gregata* in leaf vascular tissue. From the present study, it appears that *P. gregata* readily spreads to the leaf vascular tissue of infected mung bean and soybean plants. Additional research needs to be done to determine how environmental factors influence sporulation in stems and the spread of the fungus in leaf vascular tissue. Vascular stem tissue turns brown when field-grown plant stems are infected over a wide range of soil moistures and air temperatures (5–9), but leaf symptoms appear only under periods of cool, wet weather (1,5, 6). Possibly, there are two distinct phases of brown stem rot in soybean that are regulated by temperature. Symptoms developing from leaf colonization may be very sensitive to high ambient air temperatures, but development of *P. gregata* in the stem vascular tissue appears to be less sensitive to high temperature.

In this study, *P. gregata* was demonstrated to be a vascular pathogen of mung bean. Isolates of *P. gregata* from soybean and mung bean infected mung bean plants under greenhouse conditions. The fungus was reisolated from the stem and leaf vascular tissue of inoculated soybean and mung bean plants. Although the acreage of mung bean is limited in the midwestern United States, *P. gregata* could be a serious problem when mung beans are grown in the cooler areas of the Midwest, especially if soybeans and mung beans are grown in rotation, causing populations of *P. gregata* to increase because of continued cropping of susceptible hosts.

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