Effect of Soybean Growth Stage at the Time of Inoculation with *Diaporthe phaseolorum* var. *meridionales* on Stem Canker Development and Yield

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ABSTRACT

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Infections at early stages of plant development followed by a long incubation period before symptoms appear during reproductive stages is characteristic of the disease cycle of stem canker of soybean, caused by *Diaporthe phaseolorum* var. *meridionales*. To determine the effect of plant growth stage at the time of infection on symptom development and yield components, soybean plants (cv. Walters) were grown in microplots and inoculated with a suspension of ascospores (10^6 /ml) at the V₁, V₄, V₆, V₁₀, or R₂ growth stage. Noninoculated plants served as controls. Development of foliar symptoms was quantified weekly after flowering (R₂), and yields and seed weights were determined. The experiment was conducted twice, in 1992 and 1994. Foliar symptoms of stem canker developed for all treatments except the noninoculated control. Foliar symptoms appeared at the R₂ to R₅ growth stage and reached 90 to 100% incidence for most treatments. Disease development was delayed for the R₂ inoculation, suggesting that a minimum incubation period of 34 to 41 days is necessary before foliar symptoms develop. Disease (area under the disease progress curve) was greater and yield, seed number, and seed weight were less in 1994 than in 1992. Quadratic equations significantly related these variables to time of inoculation and reached maximum or minimum values between 40.9 and 43.3 days after planting. This corresponded to the V₆ growth stage. Yield and seed number were affected by disease development during pod formation (R₂ to R₅) in both years, while yield and seed weight were affected by disease development during seed formation (R₅ to R₆) in 1992.

Southern stem canker, caused by *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *meridionales* F.A. Fernandez (6), causes severe yield losses of soybean (*Glycine max* (L.) Merr.) in the southern United States and South America (11). This disease produces an elongated, brown canker on the stem that is centered around a lower node. The canker rarely girdles the stem, but it appears to damage the plant by producing a toxin that results in distinctive foliar symptoms (8). These symptoms begin as interveinal chlorotic streaks that become necrotic, killing the leaf, which remains attached to the stem. Diseased plants soon die, and yields are significantly reduced. While these symptoms of stem canker appear during reproductive development, they are the result of infections that occurred during early vegetative growth (1,10,16). The importance of early

infection was demonstrated by the disease control achieved with fungicides applied during vegetative stages of growth (1,2,16) and in field evaluations of soybean cultivars that used inoculations during early vegetative growth stages to initiate stem canker epidemics (10,13,14,17). Smith and Backman found that the greatest disease incidence occurred following application of infested debris at V₃. Disease incidence decreased rapidly thereafter for later applications (16). Only the most susceptible cultivars developed symptoms following applications of infested debris after V₁₀, and no symptoms developed in cultivars exposed to infested debris after the onset of reproductive development (R₁). The early application corresponded to the observed ascospore release which they reported to occur from late April to June in the southern United States (16).

Different results have been reported when plants were inoculated. Ploetz and Shokes inoculated plants from V₁ to V₁₁ in the greenhouse with either ascospores or conidia and found that symptoms developed no sooner than the R₂ stage and following an incubation period of 50 to 80 days (12). Pacumbaba inoculated soybeans at R₁ with a mycelial suspension and observed symptoms at R₅ (9). The incubation period was not given. Damicone et al. reported that 55 to 105 days were required

for symptoms to appear on plants inoculated at V₅ in the greenhouse following exposure to different surface wetness regimes (3). Plants in the study were first evaluated for disease at the R₄ growth stage. In a field study using naturally infested soybean debris and artificially infested oat grains, cankers first appeared at R₁ in 1986, but at V₁₀ in 1987 (4). These growth stages occurred 49 and 56 days after planting, respectively. Disease progress in this test fit the monomolecular model of disease progress, suggesting a monocyclic disease typical of diseases occurring from a fixed source of inoculum such as infested debris. They did not evaluate the development of foliar symptoms. Conversely, Subbarao et al. found that disease progress curves fit a logistic equation, suggesting a polycyclic disease with inoculum increasing during the season (19). They observed pycnidia and perithecia on plant parts after R₁, which may have been the source of secondary inoculum. They also reported that greenhouse inoculations from R₂ to R₅ resulted in disease development, but they did not report on the incubation periods.

The objectives of this study were to determine the relationship of time of infection to development of foliar symptoms of stem canker, and the effect of disease on soybean yield. An initial report has been published (20).

MATERIALS AND METHODS

The test was conducted at the University of Arkansas experiment farm, Fayetteville, Arkansas, in 1992 and 1994. Seeds of the stem canker susceptible cultivar Walters (7) were sown in pasteurized soil in 208-liter plastic barrel microplots (diameter 60 cm, height 100 cm). After emergence, the microplots were thinned to 10 seedlings per microplot.

An ascospore suspension of *D. phaseolorum* var. *meridionales* was produced from a single-spore culture isolated from a diseased plant in southwestern Arkansas. Senescent soybean stem segments (3 to 4 cm long) were soaked in deionized water for 30 min and sterilized by autoclaving for 45 min on 2 successive days. Two to four of these sterilized stem segments were placed in 9-cm plastic petri dishes, and potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) was added. A 0.5-cm-diameter agar disk plus mycelium was

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placed in the center of each plate, which were sealed (Parafilm, American National Can, Greenwich, CT) and incubated at room temperature (22°C) and ambient light for 6 to 8 weeks to produce ascospores. Ascospores were collected by flooding the plate with deionized water and rubbing the surface of the stem segments with a metal spatula. The resulting suspension was filtered through four layers of cheesecloth. The plates were reflooded, and the procedure was repeated. Inoculum concentration was adjusted to 10^6 ascospores per ml using a hemacytometer, and 1 ml of Tween 20 per liter was added as a surfactant.

To determine the effect of inoculation timing on disease development, plants were inoculated at the V₁, V₄, V₆, V₁₀, or R₂ growth stage or not inoculated (5). Soybean growth stages are divided into vegetative (designated by V with a numbered subscript indicating the number of nodes above the unifoliate node) and reproductive (designated by R with a numbered subscript). The reproductive stages were R₁ (beginning bloom), R₂ (full bloom), R₃ (beginning pod, pod 5 mm long), R₄ (full pod, pod 2 cm long), R₅ (beginning seed, seed 3 mm long), R₆ (full seed, seed fills locule), R₇ (beginning maturity, physiological maturity), and R₈ (harvest maturity). Plants were sprayed with inoculum until runoff, and large white plastic bags were placed over each inoculated microplot and secured with adhesive tape. The bags were removed after 3 days. Dates and days after planting (DAP) of each treatment in each year are presented in Table 1.

Beginning at full flower (R₂), disease incidence and plant growth stage were assessed weekly. A plant was considered diseased if foliar symptoms of stem canker appeared on one or more leaves. Ratings were taken until the plants reached physiological maturity (R₇). Yields and weight of 100 seed were taken from each microplot and expressed as dry weight (0% moisture). The total seed per plot was calculated from the yield and 100 seed weight.

The experiment was conducted as a randomized complete block design with growth stage at inoculation as the treatment. There were five replicates, and the experiment was conducted twice, in 1992 and 1994.

Analysis. Area under the disease progress curve (AUDPC) was calculated for each treatment (15). AUDPC, yield, seed weight, and seed number were individually related to time of inoculation with quadratic equations. Results were analyzed over years to allow statistical comparisons between years.

To further relate disease development to yield, disease progress was divided into three sections: the proportion of plants that became diseased from R₂ to R₅ (the period from flowering to the beginning of seed development); the proportion of plants that

became diseased from R₅ to R₆ (the period of major seed development); and the proportion of plants that became diseased from R₆ to R₇ (the period after the seed fills the seed locule until physiological maturity). Disease was related to yield,

seed weight, and seed number with multiple regression. Yields of the uninoculated control plants were not used in any analysis.

All statistical analyses were carried out using SAS Version 6.12 (SAS Inc., Cary,

Table 1. Dates, growth stage, and days after planting (DAP) of soybean (cv. Walters) grown in microplots in 1992 and 1994

Growth stage ^a	1992		1994	
	Date	DAP	Date	DAP
Planting	11 May	0	2 May	0
V ₁	26 May	15	19 May	17
V ₄	11 June	31	2 June	31
V ₆	18 June	38	10 June	39
V ₁₀	8 July	58	27 June	56
R ₂	28 July	78	19 July	78
R ₃	14 August	95	27 July	86
R ₄	24 August	105	15 August	105
R ₅	1 September	113	22 August	112
R ₆	22 September	134	5 September	126
R ₇	29 September	141	12 September	133

^a Soybean growth stages as described by Fehr et al. (5).

Fig. 1. Proportion of plants with stem canker foliar symptoms resulting from inoculations with *Diaporthe phaseolorum* var. *meridionales* at either the V₁, V₄, V₆, V₁₀, or R₂ growth stage in microplots in 1992 and 1994. Flowering (R₂) date is signified by an asterisk (*).

NC). All differences that were designated significant were statistically significant at $P < 0.05$.

RESULTS

Foliar symptoms of stem canker first appeared after flowering (R_2) in both years (Fig. 1). In 1992, foliar symptoms first appeared between 88 and 95 DAP for inoculation at V_1 , between 78 and 88 DAP for inoculations at V_4 and V_6 , between 105 and 113 DAP for inoculation at V_{10} , and between 113 and 127 DAP for inoculation at R_2 (Table 1). In 1994, symptoms first appeared between 86 and 95 DAP for inoculations at V_1 and V_4 , between 95 and 105 DAP for inoculations at V_6 and V_{10} , and between 112 and 119 DAP for inoculation at R_2 . Inoculations at V_1 had the longest incubation period in both years, between 73 and 80 and between 69 and 78 days in 1992 and 1994, respectively. Inoculations at R_2 had the shortest incubation period, between 35 and 49 and between 34 and 41 days in 1992 and 1994, respectively. Stem canker did not develop in the uninoculated treatment in either year.

For inoculations during the vegetative stages of growth, stem canker progressed fastest following the V_6 inoculation and slowest following the V_1 inoculation (Fig. 1). Disease onset following the inoculation at R_2 was delayed in both years until R_5

(Fig. 1, Table 1). Excluding the inoculation at R_2 , disease incidence at R_5 ranged from 8 to 50% in 1992 and from 44 to 74% in 1994. There was little increase in disease after R_6 for the inoculations at V_4 through V_{10} in 1992 and at V_1 through V_{10} in 1994.

At each inoculation time, AUDPC was higher and yield, seed weight, and seed number were lower in 1994 than in 1992 (Fig. 2). These data were significantly related to the time of inoculation with quadratic equations ($P = 0.0004, 0.0002, 0.0005$, and 0.0248 for AUDPC, yield, seed weight, and seed number, respectively). For all four dependent variables, the quadratic equations had the same coefficients for DAP and DAP² but significantly different intercepts for the 2 years (Table 2). Based on these equations, the estimated maximum or minimum values for all of these factors ranged from 40.9 to 43.3 DAP.

To further understand the effect of disease development on yield, the effect of disease development at different reproductive stages was determined using multiple regression on data from both years separately. In 1992, disease that developed from R_2 to R_5 (period from flowering to the beginning of seed formation) and disease that developed between R_5 and R_6 (major period of seed development) were significantly related to both yield and seed

weight (Table 3). Seed number was significantly related to disease that developed from R_2 to R_5 only. In 1994, yield and seed number were significantly related to disease that developed from R_2 to R_5 only. Yield, seed weight, and seed number were not related in either year to disease that developed between R_6 and R_7 (the period after the seed fills the locule until physiological maturity).

DISCUSSION

Development of foliar symptoms of stem canker depends on two things: soybean reproductive development and a minimum incubation period. Regardless of growth stage at the time of inoculation, foliar symptoms did not develop until the onset of flowering (R_2). This agrees with previous reports (1,9,12) except for that of Damicone et al. (4), who reported symptoms at V_{10} . In the latter report, they measured only canker formation, which probably occurred before foliar symptoms. However, canker appearance was still associated with flowering, since cankers appeared 56 days after planting in the second year of the study and 49 days after planting in the first year, when the plants were at R_1 .

Besides being in reproductive development, there appears to be a minimum incubation period before foliar symptoms de-

Fig. 2. Relationship of the number of days after planting that plants were inoculated with ascospores of *Diaporthe phaseolorum* var. *meridionales* and development of stem canker (area under the disease progress curve [AUDPC]), yield, seed weight, and seed number in 1992 (solid line and solid circles) and 1994 (dashed line and open circles). Circles represent means of each treatment, and lines represent estimated values from fitted quadratic equations.

velop. In our study, the earliest that symptoms occurred was between 34 and 41 days after inoculation when inoculated at R₂. Because of the need for plants to be in reproductive development, symptoms appeared in the other inoculated treatments after longer periods of time following inoculation, with the V₁ inoculation taking the longest. In other studies, a minimum of 50 to 55 days after inoculation were required to develop symptoms in greenhouse inoculations (3,12). The length of this incubation period may be influenced by the amount of infection. Damicone et al. (3) reported that the appearance of first symptoms was related to the duration of surface moisture at the time of infection: the longer the surface moisture period and, presumably, the greater the infection, the shorter the incubation period. This may explain differences in disease progress between the 2 years. Greater infection in 1994 may have resulted in more rapid disease development for all treatments after

disease onset than in 1992. This difference between years may also reflect different environments. Although temperatures were similar both years, rainfall was 6.4 to 9.5 cm less in 1994 than in 1992 during May, June, July, and September (data not shown) and may have resulted in increased drought stress in 1994. The role of the environment in foliar symptom development after infection has not been determined.

Plant growth stage at the time of inoculation influenced later disease development. As estimated by quadratic equations, the highest level of disease and the lowest yields, seed weights, and seed numbers occurred when the plants were inoculated 40 to 43 days after planting. This corresponds to the V₆ growth stage. These results contrast with those of Smith and Backman (16), who reported that the optimum growth stage for infection was V₃ and that disease declined rapidly at earlier and later growth stages. Only in the most susceptible cultivars did they report disease

development with exposure to inoculum after V₁₀ and little or no disease with exposure to inoculum after R₂. In these experiments, they used infested debris as inoculum, so differences in disease development could be due to differences in environment or changes in the amount of inoculum produced during the season. Others have reported development of stem canker symptoms with inoculations at R₂ or later (9,19).

Yield was affected by the total amount of disease and also by when disease occurred. The significant effect of disease incidence between R₂ and R₅ growth stage on yield and seed number in both years was related to plant development. Pods develop between these growth stages, so disease occurring then would affect yield by precluding seed formation. Disease incidence that developed between R₅ and R₆ significantly affected seed weight in 1992. During this period of R₅ to R₆, most of the seed mass is formed, so disease during this time would have its greatest effect on seed weight. High levels of disease at R₅ in 1994 probably reduced the effect of disease development from R₅ to R₆ on this component of yield, as shown in the multiple regression equations.

These results indicate that, with the cultivar Walters, infection that leads to disease can occur over a long period of time and that foliar symptoms develop after both the onset of flowering (R₂) and a minimum incubation period. Foliar symptoms were closely related to yield loss. Further research is needed to determine the effect of cultivar on optimum time of infection, incubation period, and resulting disease development, and the effect of the environment on production of inoculum and symptoms. A more complete understanding of the epidemiology of stem canker could improve existing control measures and help in the development of new control measures for this important disease of soybean.

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Table 2. Quadratic regression equations relating the development of stem canker and yield to time of inoculation^a (days after planting or DAP) of soybean with *Diaporthe phaseolorum* var. *meridionales* in microplots in 1992 and 1994, Fayetteville, Arkansas

Factor	Intercept		Coefficient ^b		R ²	Min./max. values (DAP)
	1992	1994	DAP	DAP ²		
AUDPC (%-days)	-208.5 (462.9) ^d	526.4 (470.5)	135.78 (22.00)	-1.66 (0.23)	0.94	40.9 (1.6)
Yield (g/plot)	161.8 (16.1)	116.6 (16.3)	-4.51 (0.16)	0.05 (0.01)	0.95	42.0 (1.6)
Seed weight (g/100seed)	14.1 (1.0)	11.9 (1.0)	-0.27 (0.05)	0.003 (0.0004)	0.94	43.3 (1.6)
Seed number ^c (number/plot)	1,410.1 (275.3)	1,063.5 (279.8)	-30.37 (13.08)	0.36 (0.13)	0.77	42.6 (4.1)

^a Plants inoculated at 15, 31, 38, 58, and 78 DAP in 1992 and 17, 31, 39, 56, and 78 DAP in 1994 with 10⁶ ascospores per ml of *D. phaseolorum* var. *meridionales*.

^b Regressions significant at *P* = 0.0004, 0.0002, 0.0005, and 0.0248 for AUDPC, yield, seed weight, and seed number, respectively.

^c Number of DAP that a minimum or maximum value was estimated to occur by the quadratic equation.

^d Number in parentheses is the standard error for the value immediately above.

^e Calculated from yield and seed weight.

Table 3. Multiple regression coefficients relating disease development from R₂ to R₅, R₅ to R₆, and R₆ to R₇ to plot yield, 100 seed weight, and seed number per plot from soybean inoculated at various growth stages in microplots in 1992 and 1994, Fayetteville, Arkansas

Component	Parameter				
	Intercept	R ₂ to R ₅	R ₅ to R ₆	R ₆ to R ₇	R ₂
1992					
Yield (g/plot)	164.97 (0.0001) ^a	-1.394 (0.0001)	-0.0814 (0.0002)	-0.2173 (0.5857)	0.77
Seed weight (g/100 seed)	14.4175 (0.0001)	-0.0641 (0.0001)	-0.0594 (0.0001)	-0.0326 (0.1614)	0.72
Seed number (number/plot)	1,204.965 (0.0001)	-7.6897 (0.0004)	-2.9295 (0.1016)	1.2782 (0.7296)	0.58
1994					
Yield (g/plot)	286.127 (0.0066)	-2.7002 (0.0096)	-1.6864 (0.0968)	-2.2589 (0.0583)	0.77
Seed weight (g/100 seed)	18.7176 (0.0158)	-0.1261 (0.0912)	-0.0875 (0.2411)	-0.1143 (0.1913)	0.50
Seed number (number/plot)	2,872.794 (0.0272)	-26.4782 (0.0400)	-12.2957 (0.3317)	-22.0429 (0.1405)	0.70

^a Number in parentheses is *P* value for *t* test of coefficient and indicates significance at *P* = 0.05 if it is equal to or less than 0.05.

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