Epidemiology of Soybean Stem Canker in the Southeastern United States: Relationship Between Time of Exposure to Inoculum and Disease Severity

E. F. SMITH, Research Associate, and P. A. BACKMAN, Professor, Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Auburn 36849

ABSTRACT

Smith, E. F., and Backman, P. A. 1989. Epidemiology of soybean stem canker in the southeastern United States: Relationship between time of exposure to inoculum and disease severity. Plant Disease 73:464-468.

Experiments to determine the influence of the timing of exposure to inoculum and the influence of planting date to the development of soybean stem canker in the southeastern United States indicated that disease severity was primarily dependent upon plant growth stage at the time of exposure and was independent of planting date. The relationship between disease severity and the time of inoculum application was best described by Cauchy distribution models. These models predict exponentially increasing disease incidence in plants exposed to inoculum from emergence to a maximum for those exposed to inoculum at 22 and 24 days (V3 stage) postplanting. Plants exposed from the V3 stage through the V10 stage develop progressively less disease. Even a highly susceptible cultivar would not develop disease when exposed after the vegetative stages.

Stem canker disease of soybean (*Glycine max* (L.) Merr.) has recently become a problem in several areas of the southeastern United States, especially in Texas, Alabama, Georgia, Mississippi, Louisiana, and parts of Florida (1,2,4,10). The causal organism of soybean stem canker in the Southeast is *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. f. sp. *meridionalis* (7). Stem canker can be a very destructive disease, killing soybean plants well before harvest. Infection of soybean by *D. phaseolorum* f. sp. *meridionalis* occurs throughout the growing season (12,13). However, plants infected during vegetative growth remain asymptomatic until they enter the reproductive phase of growth, around 60–70 days after planting (5,9,10). The first symptoms of stem canker are small, reddish-brown lesions that originate at leaf scars and/or nodes. Lesions progress acropetally, becoming sunken necrotic cankers that can completely girdle stems by reproductive stage R5, pod fill (2,9). Cankers are usually unilateral and delimited by healthy tissue. In 1983, yield losses attributed to stem canker in the Southeast were estimated at $37 million (2). However, with the increased planting of resistant cultivars, much lower levels of disease and losses have been reported in recent years, even in areas with a prior history of serious losses to stem canker (10).

At present, recommendations for control are based on preliminary test results, with little understanding of the basic epidemiological factors involved. Current recommendations for controlling stem canker in Alabama include 1) planting resistant cultivars, 2) rotation to a nonsusceptible crop, 3) planting after 15 June, and 4) planting clean or treated seed (4). The recommendation for planting after 15 June is based solely on observational data that indicated that disease levels were lower if planting dates were delayed until late June.

The objectives of the present study were to 1) determine if infection occurring at different developmental stages ultimately produced different levels of disease and 2) examine the influence on disease levels of planting date and soybean growth stage at time of exposure to inoculum.

This research was supported by Grant No. 85053 from the American Soybean Association.

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

Alabama Agricultural Experiment Station Journal Series No. 18-881592P.

Accepted for publication 21 November 1988.

MATERIALS AND METHODS

Inoculum conditioning. The inoculum used in each test was soybean debris collected in the fall of 1984 from a field infested with *D. phaseolorum* f. sp. *meridionalis* at the Black Belt Substation, Marion Junction, AL. The debris was conditioned before use in our experiments by continuously applying water with a sprinkler irrigator for a period of 7–10 days to induce perithecium production.

Headland experiment. A field test was performed at the Wiregrass Substation, Headland, AL, to determine the effect of time of infection by *D. phaseolorum* f. sp. *meridionalis* on stem canker incidence in the moderately susceptible soybean cultivar Kirby. Inoculum was applied to plots at several vegetative and reproductive growth stages in order to relate time of exposure to inoculum to disease reaction. Vegetative growth stages (V1–V12) refer to the average number of trifoliates on growing soybean plants; reproductive stages refer to a range from early bloom (R1) to harvest maturity (R8), as described by Fehr et al (3).

Soybeans were planted on 22 May 1985 in a Dothan sandy loam soil using a Latin square design with seven treatments replicated seven times. Conditioned inoculum was applied at growth stages V1, V3, V6, V9, V12, and R2 as treatments to whole plots. Plots were four 10-m rows with 0.9-m spacing between rows. Inoculum was applied to the center two rows at the rate of 1.1 kg (dry wt) per plot. After each inoculum application, the entire test area was irrigated for 24 hr with a center pivot irrigation system calibrated to deliver approximately 0.2 cm/hr. Plots were irrigated to enhance ascospore release and dispersal and to maintain an environment conducive to infection (13,14). To determine if *D. phaseolorum* f. sp. *meridionalis* infections had occurred as a result of inoculum application, five plants were collected on 26 June and 9 July from control plots and plots previously exposed to inoculum. On 30 July, an additional sample consisting of two plants was collected as before from plots when plants were at reproductive stage R2 (full bloom). To determine infection, all nodes were excised from sample plants by cutting through the stem and attached petiole 1.5 cm from each side of the node. Nodes were surface-disinfested for 2 min in 0.75% NaOCl and 95% ethanol, successively, and were plated on Phillip's selective medium (8). Tween 20 (1 ml) was added to the NaOCl solution to improve wetting. After 14 days, plates were examined for *D. phaseolorum* f. sp. *meridionalis* colonies.

Cankered plants in the center two rows (10 m/row) of each plot were counted and the mean number per treatment was calculated. Stem canker incidence, rated on 25 September 1985 when soybeans were at R6, was related to time of inoculum application by using standard methods for regression analysis (11) and procedures for curve fitting (6).

Auburn experiment. A field test was also conducted using the susceptible cultivar Hutton in a randomized split plot design with four replicates. Subplots (one 5-m row) within whole plants were planted at weekly intervals starting 27 May 1985 for 6 wk. Inoculum (debris) was applied to whole plots 1, 2, and 3 on 3 June, 14 June, or 1 July at the rate of 0.23 kg (dry wt) per subplot. In whole plots 1 and 2, *D. phaseolorum* f. sp. *meridionalis* inoculum was applied before the planting of many subplots. Inoculum collection, conditioning, and application were the same as for the Headland experiment. The entire plot area was irrigated with a sprinkler system for 8 hr after each planting and inoculation to induce both seed germination and infection.

Two plants were collected from subplots on 26 June and 12 July, to determine if infection had occurred. Nodes were excised from plants collected on 26 June and were surface-disinfested for 2 min in 1.0% NaOCl and 70% ethanol, successively. Nodes from plants collected 12 July were surface-disinfested for the same length of time, but in 0.75% NaOCl and 95% ethanol, successively. In both cases, 1 ml of Tween 20 was added to the NaOCl solution to improve wetting. Nodes were plated on petri dishes containing Phillip's selective medium to detect *D. phaseolorum* f. sp. *meridionalis*, and were examined after 14 days incubation at 25 C.

Stem canker ratings were made at the R5 stage on 17 September and again at R6 on 1 October. The percentage of dead or dying plants was estimated using a transformed 0–5 scale. The pretransformed arc sine scale is similar to a system that Hills et al developed, which is based on one-fifth the angular transformation from 0 to 90 (15). A plot with no dead or dying plants was rated 0.0, one with 10% dead or dying plants was rated 1.0, 35% was rated 2.0, 65% was rated 3.0, 90% was rated 4.0, and 100% received a rating of 5.0. All plots were rated to the nearest tenth of a point. The pretransformed arc sine scale was chosen to evaluate disease because it allows assessment of visible symptoms in a linear fashion and is sensitive to both low and high levels of disease (2). Data relating stem canker severity to time of inoculum application were analyzed using standard methods for regression analysis and procedures for curve fitting (6,11).

RESULTS

Headland experiment. Infection could only be detected in plants exposed to inoculum for 21 or more days. Infection in control plots not directly exposed to inoculum was not detected at any of the collection dates (Table 1).

Treatment means were plotted against time of inoculum application using planting date 22 May as a reference (Fig. 1). Plots that received inoculum at different developmental stages had significantly different ($P < 0.05$) amounts of cankered plants per 20 m of row when disease was assessed at reproductive stage R6. The mean number of cankered plants per treatment ranged from 1.7 in plots infested 69 days after planting (reproductive stage R2) to a mean of 16.2 in plots infested 22 days after planting (vegetative stage V3) (Fig. 1). The relationship between stem canker inci-

Table 1. Effect of time of application of soybean debris naturally colonized by *Diaporthe phaseolorum* f. sp. *meridionalis* on its isolation from cv. Kirby soybean stems at Headland, AL

Debris application date[a]	Approximate growth stage at application	Infected plants by isolation date (%)[b]		
		26 June	9 July	30 July
3 June	V1	0	17	I[c]
13 June	V3	0	14	I
26 June	V6	NP[d]	0	64
9 July	V9	NP	NP	43
23 July	V12	NP	NP	0
30 July	R2	NP	NP	NP
No debris	···	0	0	0

[a] All plots were planted on 22 May 1985.
[b] Plants infected by *D. phaseolorum* f. sp. *meridionalis*. Data are based on five plants from each plot in each replicate on 26 June and 9 July, and on two plants from each plot in each replicate on 30 July. All nodes were excised from plants by cutting through stems and petioles 1.5 cm from the node. Nodes were surface-disinfested for 2 min in 0.15% NaOCl and 95% ethanol, successively, and plated on Phillip's selective medium (8).
[c] Plots already known to be infected from previous isolation; no plants sampled.
[d] Plots were treated after isolation date; no plants sampled.

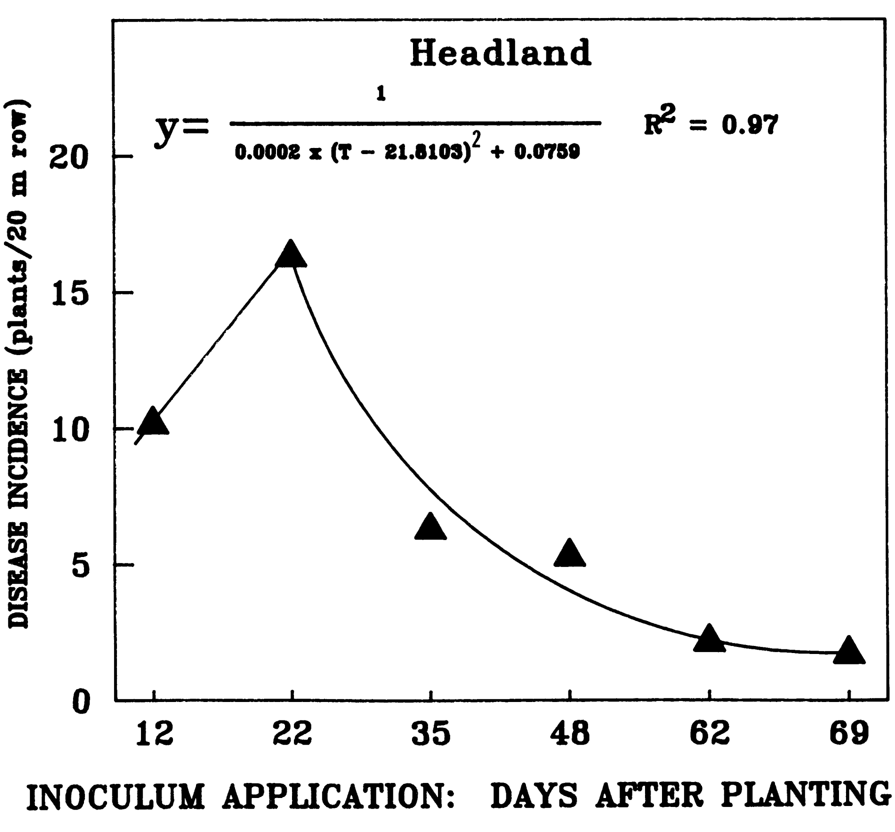

Fig. 1. Relationship of soybean stem canker incidence at late reproductive stage R6 to the time of application of *Diaporthe phaseolorum* f. sp. *meridionalis* inoculu n after planting cultivar Kirby soybeans.

dence at late R6 and time of exposure to inoculum relative to plant growth stage was best described by the Cauchy distribution model. The Cauchy distribution was represented by the exponential equation (corrected coefficient of determination for six pairs, $R^2 = 0.97$): $SCS = 1/0.0002(T - 21.8103)^2 + 0.0759$, where SCS = stem canker severity measured as mean number of cankered plants per 18 m of row at R6, and T = time of inoculum application in days after planting.

Disease incidence increased exponentially from a moderate level for plants exposed to inoculum 12 days after planting (vegetative stage V1) to a maximum for those exposed 22 days after planting (vegetative stage V3). Disease levels were progressively lower in plants exposed to inoculum from stages V6 through R2. Plants exposed to inoculum in late vegetative stage V12 and early reproductive stage R2 did not have significantly different levels of disease than noninfested control plants.

Table 2. Effect of time of application of soybean debris naturally colonized by *Diaporthe phaseolorum* f. sp. *meridionalis* on its isolation from cv. Hutton soybean stems at Auburn, AL

Planting date	Debris application date	Infected plants by isolation date (%)[a] 26 June	12 July
27 May	3 June	13	63
3 June		38	75
10 June		0	25
17 June		NP[b]	0
24 June		NP	0
1 July		NP	NP
27 May	14 June	0	38
3 June		13	50
10 June		0	63
17 June		NP	38
24 June		NP	0
1 July		NP	NP
27 May	1 July	NP	38
3 June		NP	38
10 June		NP	38
17 June		NP	0
24 June		NP	0
1 July		NP	NP

[a] Plants infected by *D. phaseolorum* f. sp. *meridionalis*. Data are based on two plants from each subplot in each replicate on 26 June and 12 July. All nodes were excised from plants by cutting through stems and petioles 1.5 cm from the node. Nodes were surface-disinfested for 2 min in 0.15% NaOCl and 95% ethanol, successively, and plated on Phillip's selective medium (8).

[b] Subplots had not been planted or plants had not emerged; no plants sampled.

Auburn experiment. *D. phaseolorum* f. sp. *meridionalis* was not isolated from plants less than 23 days old or exposed to inoculum for less than 11 days (Table 2).

Disease severity data, collected on each of two dates, were analyzed separately using SAS procedures for orthogonal, quadratic, and linear contrasts (11). Data collected on 17 September and 1 October were statistically similar overall, but differences were more pronounced for data collected 1 October, with a higher degree of significance detected ($P < 0.0001$). Therefore, only data for 1 October were used for curve fitting procedures.

Factorial analysis of data for the second rating indicated that disease severity was significantly ($P < 0.05$) affected by both planting date and time of inoculum application (Table 3). Significant differences ($P < 0.05$) in disease also occurred as a result of an interaction between planting date and time of inoculum application.

Disease levels for each subplot were plotted against planting date relative to time of inoculum application. Inoculum application dates for the first two whole plots, 3 or 14 June, were chosen as the reference dates for establishing the length of time inoculum was in the field at the time each subplot was planted.

Disease severity in each of the six subplots planted in the first whole plot decreased in a linear fashion ($P < 0.0001$) as the time interval between inoculum introduction and subsequent planting date increased (Table 3). Disease levels ranged from a mean of 3.48 (78% dead) for plants emerging at the time of inoculum application (3 June) to 1.07 (10% dead) for plants planted 4 wk after inoculum application. In the second whole plot, all plants in the first subplot had emerged at the time of inoculum application; plants in the five remaining subplots had not emerged. Disease levels ranged from a mean of 3.82 (85% dead) to 1.03 (10% dead) as the time interval between inoculum application and emergence increased. Disease severity was highest in subplots emerging at the same time as inoculum introduction and decreased in a linear fashion as the planting date was delayed beyond that date (Fig. 2).

Disease levels for each subplot in the third whole plot were plotted against time using inoculum application date (1 July) as the reference date for establishing the age of the plants at the time of inoculum application.

Disease severity within the whole plot receiving inoculum on 1 July was significantly lower ($P < 0.0001$) than for those inoculated on either 3 or 14 June. Disease severity within whole plot 3 ranged from 0.60 (10% dead) in subplots planted 1 July to 1.48 (20% dead) in subplots planted 27 May. The relationship between stem canker severity at late R6 and the time of inoculation, relative to developmental stage of the plant, was best described by the Cauchy distribution model (corrected coefficient of determination for six pairs, $R^2 = 0.96$). The Cauchy distribution was represented by the exponential equation: $SCS = 1/0.0022(T - 23.7845)^2 + 0.4515$, where SCS = stem canker severity measured by the pretransformed arc sine scale, and T = time of inoculum application for each subplot in days after planting.

Disease severity increased exponentially from a mean of 0.60 in subplots planted at the time of inoculum application to a mean of 2.18 in subplots receiving inoculum at vegetative stage V3 planted 21 days earlier. A maximum level of disease, according to the Cauchy distribution model, would occur if infection developed at approximately vegetative stage V3, 24 days after planting. In terms of the predictive model and observed disease levels, severity dropped sharply for plants that were at vegetative stages V5 or V6, planted 28 or 35 days before inoculum application, respectively (Fig. 3).

Table 3. Effect of debris naturally colonized by *Diaporthe phaseolorum* f. sp. *meridionalis* on incidence of stem canker in cv. Hutton soybeans at Auburn, AL

Planting date	Stem canker rating[a] by debris application date 3 June	14 June	1 July	Planting date $\overline{X}$
27 May	3.48	3.15	1.48	2.70
3 June	3.55	3.82	1.70	3.02
10 June	2.55	3.55	2.18	2.76
17 June	2.28	3.07	1.50	2.28
24 June	2.38	1.38	1.02	1.59
1 July	1.07	1.03	0.60	0.90
Debris date $\overline{X}$	2.55	2.66	1.41	

[a] Disease ratings made on 1 October 1985, and expressed on a 0–5 pretransformed arc sine scale.

DISCUSSION

Soybean plants were found to be susceptible to infection by *D. phaseolorum* f. sp. *meridionalis* at all growth stages and times during the growing season, but remained asymptomatic until the reproductive stages. However, plants infected during late vegetative or reproductive stages rarely developed symptoms. The determination of a disease progress curve was not possible because symptom development was not a reliable indicator of infection.

D. phaseolorum f. sp. *meridionalis*

could not be isolated from surface-disinfested asymptomatic plant tissue until approximately 3 wk after infestation. However, infection almost certainly occurred earlier than this because *D. phaseolorum* f. sp. *meridionalis* was probably removed from newly infected tissue by the stringent disinfestation regime.

Soybeans planted at one date, to which inoculum was added at various growth stages (Headland experiment), or soybeans planted at different dates inoculated at a single date (Auburn experiment, whole plot 3), generated similar mathematical relationships between disease incidence and time of infection. For both the Auburn and Headland experiments, the relationship between stem canker severity (measured by symptom development at late R6) and time of inoculation was best described by the Cauchy distribution model (see above equations). Maximum disease levels developed when inoculum was applied 21 or 22 days after planting at vegetative stage V3. The Cauchy distribution models for these two experiments predicted that maximum disease would theoretically occur if inoculum was applied and infections occurred 22 and 24 days after planting, respectively. Similarity of results from both experiments, despite the different approaches taken, differing cultivar susceptibilities, and different environmental conditions, suggests that disease severity in large part depends on the time that infection occurs relative to plant growth stage. Plants infected at vegetative stage V3 (21–24 days after planting) were most severely diseased at R6.

Another aspect of stem canker epidemiology was found to influence disease severity in the Auburn test. The relationship between stem canker severity at R6 and the time of inoculum application relative to planting date was consistently linear when the inoculum was applied before plant emergence. Because plants in all subplots were at vegetative stage V3 at some point and equally susceptible to infection by *D. phaseolorum* f. sp. *meridionalis*, the decrease in disease severity over time presumably was due to a decline in numbers of viable ascospores and their dissemination. Disease levels were found at their lowest 2 wk earlier when inoculum was applied in mid-June, suggesting that ascospore viability was affected more rapidly in late June. Fluctuations in environmental conditions probably influence ascospore production and longevity. The relationship between disease severity at R6 and time of inoculum application (infection) relative to planting date would still be linear when inoculum was applied before planting.

These results indicated that the sporadic nature of stem canker occurrence in southern soybeans is due to epidemiological factors, some of which have been described here. When environmental conditions are favorable for ascospore development and dispersal, infection is likely to occur. Generally, the earlier that infections occur the more severe the disease will be at R6. Year-to-year variations in disease severity, and to some extent differences among localities, probably reflect stage of plant development when infection occurs.

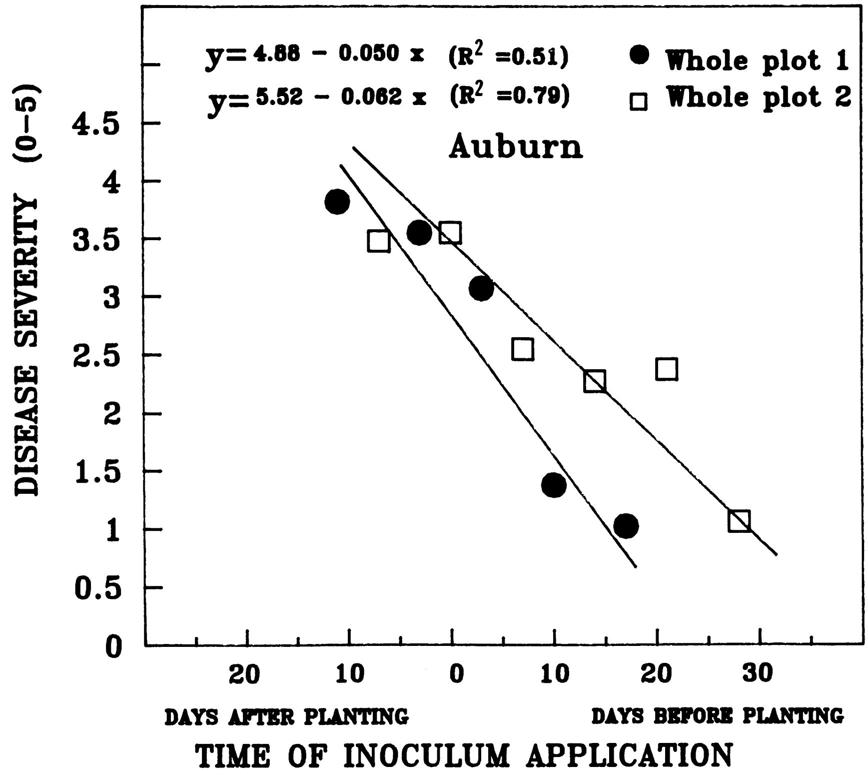

Fig. 2. Relationship of soybean stem canker severity at late reproductive stage R6 to planting date relative to inoculum application.

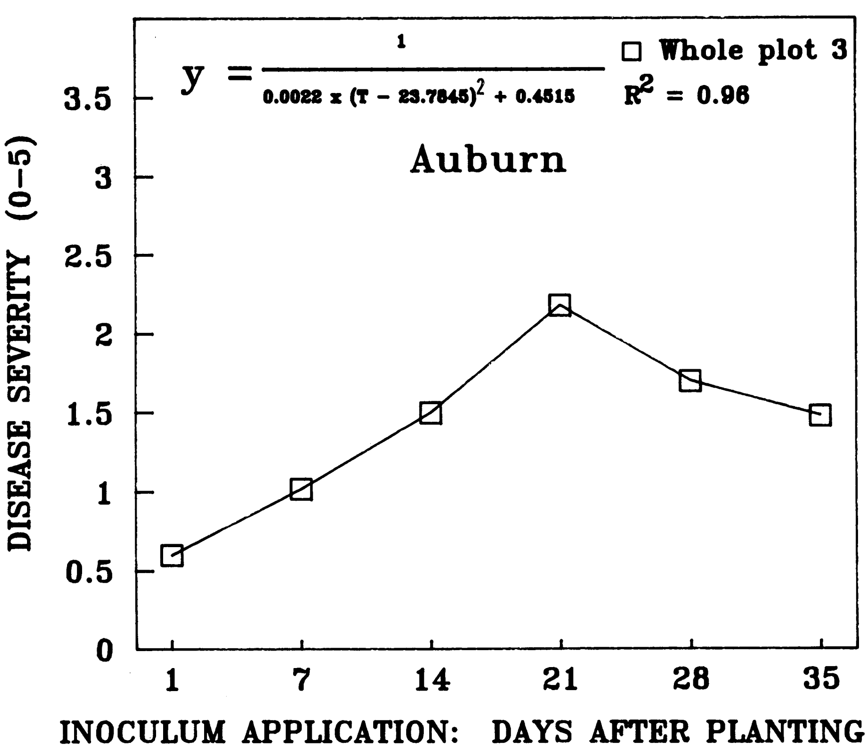

Fig. 3. Effect of debris naturally colonized by *Diaporthe phaseolorum* f. sp. *meridionalis* on incidence of stem canker in cultivar Hutton soybean.

Because June typically is one of the driest months of the year in the Southeast and *D. phaseolorum* f. sp. *meridionalis* infections have been linked to rainy periods (14), the probability of infection is reduced if planting is delayed into June. However, late planting by no means guarantees escape from infection by *D. phaseolorum* f. sp. *meridionalis*, because even soybeans planted in late June or July can be infected and become diseased if inoculum is available and weather conditions are appropriate for spore development and infection during the early vegetative stages.

ACKNOWLEDGMENTS

We wish to acknowledge the important contributions of Linda Carter and Mark Crawford in the development of this research.

LITERATURE CITED

1. Backman, P. A., Crawford, M. A., White, J., Thurlow, D. L., and Smith, L. A. 1981. Soybean stem canker: A serious disease in Alabama. Auburn Univ. Agric. Exp. Stn. Highlights Agric. Res. 28(4):6.
2. Backman, P. A., Weaver, D. B., and Morgan-Jones, G. 1985. Soybean stem canker: An emerging disease problem. Plant Dis. 69:641-647.
3. Fehr, W. R., Caviness, C. E., Burmond, D. L., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. Crop Sci. 11:929-931.
4. Gazaway, W. S., and Henderson, J. B. 1983. Stem canker disease in soybeans. Ala. Coop. Ext. Serv. Timely Inf. Sheet. 3 pp.
5. Hobbs, T. W., Schmitthenner, A. F., Ellett, C. W., and Hite, R. E. 1981. Top dieback of soybean caused by *Diaporthe phaseolorum* var. *caulivora*. Plant Dis. 65:618-620.
6. Kolb, W. M. 1983. Curve Fitting for Programmable Calculators. IMTEC, Bowie, MD. 146 pp.
7. Morgan-Jones, G. 1989. The *Diaporthe/Phomopsis* complex: Taxonomic considerations. Pages 1699-1706 in: World Soybean Research Conference IV: Proceedings. A. Pascale, ed. Buenos Aires.
8. Phillips, D. V. 1984. A selective medium for *Diaporthe phaseolorum* var. *caulivora*. (Abstr.) Phytopathology 74:815.
9. Ploetz, R. C., and Shokes, F. M. 1985. Soybean stem canker incited by ascospores and conidia of the fungus causing the disease in the southeastern United States. Plant Dis. 69:990-992.
10. Ploetz, R. C., Sprenkel, R. K., and Shokes, F. M. 1986. Current status of soybean stem canker in Florida. Plant Dis. 70:600-602.
11. SAS Institute. 1982. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC. 956 pp.
12. Smith, E. F. 1986. Epidemiology of *Diaporthe phaseolorum* var. *caulivora* in southern soybeans. (Abstr.) Proc. South. Soybean Dis. Workers Conf. 13:16.
13. Smith, E. F., and Backman, P. A. 1988. Soybean stem canker: An overview. Pages 47-55 in: Soybean Diseases of the North Central Region. T. D. Wyllie and D. H. Scott, eds. APS Press, St. Paul, MN.
14. Smith, E. F., Backman, P. A., and Crawford, M. A. 1986. Epidemiology of *Diaporthe phaseolorum* var. *caulivora* and stem canker development in southern soybeans. (Abstr.) Phytopathology 76:1094.
15. Weaver, D. B., Cosper, B. H., Backman, P. A., and Crawford, M. A. 1984. Cultivar resistance to field infestations of soybean stem canker. Plant Dis. 68:877-879.