Effects of Cultivar, Tillage, and Cropping System on Infection of Soybean by *Diaporthe phaseolorum* var. *caulivora* and Southern Stem Canker Symptom Development

Craig S. Rothrock, Daniel V. Phillips, and Thomas W. Hobbs

Department of Plant Pathology, University of Georgia, Georgia Experiment Station, Experiment 30212.
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ABSTRACT

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The influence of cultivar, tillage, and cropping system on infection of soybean plants by *Diaporthe phaseolorum* var. *caulivora* and on incidence and severity of southern stem canker was examined. Three weeks after planting, 34% of all the plants were infected in 1985 compared with 4% in 1986. The percentage of plants infected increased throughout the growing season in both years and was lower under conventional tillage than under no tillage. Infection levels were similar for the two cultivars, but symptom expression was much more severe for Hutton than Coker 368. Disease incidence and severity for the susceptible cultivar Hutton were significantly lower for conventional tillage than no tillage in 1985 and 1986. Smaller but significant reductions in disease were found for Coker 368 under conventional tillage in both years. Although tillage did not significantly affect yield for the moderately resistant cultivar Coker 368 in 1985, the susceptible cultivar Hutton yielded 47% more under conventional tillage than no tillage. Lower infection and disease severity in 1986 resulted in similar yield by these two cultivars under the different tillage practices. Disease incidence and severity in 1985 for Coker 368 and disease incidence and severity for Hutton in 1986 were slightly greater under soybean/wheat double-cropping compared with a soybean/fallow cropping system.

Additional key words: conservation tillage, *Glycine max*.

Stem canker of soybean (*Glycine max* (L.) Merr.), caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell, was prevalent in the north central United States and Canada in the late 1940s and early 1950s (1,3,7,20). In recent years, a stem canker disease has become a major threat to soybean in the southeastern United States (2). Recent studies have shown that southern stem canker differs in symptomatology from stem canker in the North and that the two pathogens differ in several cultural and morphological characteristics (2,8,9). To avoid confusion with the disease in the North, this disease will be referred to as southern stem canker.

Cultural practices have been shown to affect the incidence of southern stem canker. Soybean cultivars vary greatly in susceptibility to this disease (6,19). Decreased use of susceptible cultivars has been associated with lower incidence of southern stem canker (13). Reduced tillage has been associated with increased disease incidence and severity (14,16,18) and the increase in no tillage or conservation tillage may have contributed to the increased prevalence of southern stem canker. Soybean/wheat double-cropping, the most common cropping system in Georgia, has also resulted in increased disease severity compared with soybean monoculture (14).

The principal objective of the present study was to examine how cultivar susceptibility, tillage practices, and cropping systems influence infection by *D. p.* var. *caulivora* and disease incidence and severity. A second objective was to determine if using cultivar resistance for control of southern stem canker was practical under a no-tillage system. A preliminary report has been published (15).

MATERIALS AND METHODS

Experimental design and plot care. The study was conducted in 1985 and 1986 at the Bledsoe Research Farm (Pike County, GA) on a Cecil sandy loam (Typic Hapludult) soil. The land has been planted with soybean annually since 1978, and southern stem canker has been present at this site since 1983 (14).

The experiment was arranged in a randomized complete block split-split plot design. The tillage treatments were the main plots and were replicated six times. Conventional-tillage plots were plowed (approximately 250 mm deep) with a moldboard plow, followed by discing twice in the fall. Before soybean was planted, conventional tillage plots were rotavated once (approximately 100 mm deep). No-tillage plots were not cultivated. Tillage plots were split into cropping system subplots, soybean/wheat double-cropping, and soybean/fallow monoculture. The cropping system subplots were planted with two soybean cultivars, making sub-subplots, which were 2.3 × 6.1 m. Coker 368 and Hutton, which are moderately resistant and susceptible to southern stem canker, respectively, were selected because they are similar in development and yield potential under Georgia conditions.

Plots were irrigated (25 mm) on 21 June 1985 and 16 June 1986 with a Rain Bird 105C irrigation gun (Rain Bird Sprinkler Corp., Glendora, CA) to facilitate tillage operations and ensure adequate moisture for emergence. Fertilizer (P at 34 kg/ha and K at 77 kg/ha) was applied before planting. Soybeans were planted on 26 June 1985 and 23 June 1986 in three-row plots, 0.76 m row spacing, using a Cole no-till planter. In 1986, plots were also irrigated (25 mm) on 10 July and 4 August to lessen the effects of an extended drought. Weeds were controlled by pulling and by the application of 2.3 L/ha of paraquat on 21 July 1985 and 19 June and 31 July 1986. The four middle rows within a cropping-system subplot, i.e., two rows of each cultivar, were harvested for yield on 31 October 1985 and 31 October 1986 with a plot combine. Plant residues were returned to their respective plots after harvest. Seed weight was adjusted to 13% moisture. Environmental data were recorded 150 m from the field study.

Recovery of southern isolates of *D. p.* var. *caulivora*. Plants from the rows that were later harvested for yield were sampled each year to determine percent infection. In 1985, four plants were sampled randomly 21 and 49 days after planting and six plants 98 days after planting from each plot, corresponding to growth stages V3, R2, and R6, respectively (4). In 1986, six plants were sampled on 24, 56, 80, and 112 days after planting from each plot, corresponding to growth stages V3, R2, R5, and R6, respectively. Leaves and petioles were removed from stems, and the stems from each plant were cut into 20-mm sections. All stem sections were plated for all sample dates, except for the final sample date each year when only the top, middle, and bottom 100 mm were plated.

Stem sections were surface-disinfested by immersion for 1 min in 70% ethanol followed by immersion for 2 min in 1.05% sodium hypochlorite. The sections were then plated on DPC medium A (D. V. Phillips, *unpublished*), which is selective for southern isolates of *D. p.* var. *caulivora* (10). Plates were incubated for 7–10 days at room temperature and the percent isolation of *D. p.* var. *caulivora* from stem sections from each plant determined. A plant was considered infected when the pathogen was isolated from one or more stem sections from that plant.

Disease assessment. Plants were examined for southern stem canker symptoms 103 and 112 days after planting at growth stage R6. All plants in 3.05 m from each of the two yield rows per plot were examined for disease symptoms. Plants were rated as: 0 = healthy (no lesions); 1 = lesions restricted to one node (minor lesions); 2 = lesions including more than one node (major lesions); or 3 = dead. Disease incidence was calculated from the total number of plants examined that had stem canker symptoms. A disease severity index for each plot was calculated by multiplying the proportion of the plants in each disease category by the rating of that category and adding the products together.

Statistical analyses. Data were analyzed by analysis of variance consistent with the experimental design using SAS (SAS Institute, Cary, NC). A significant interaction was found between the main effects cultivar and tillage for disease incidence, dead plants, and the disease severity index in both seasons and for yield in 1985 and between the main effects cultivar and cropping system for disease incidence, dead plants, and the disease severity index in 1986. Because of the large difference in cultivar susceptibility and interaction of cultivar with other main effects all subsequent analyses on disease and yield were done by cultivar. When data were combined over years data were analyzed with year as the main plot.

RESULTS

D. p. var. *caulivora* was isolated from 34, 80, and 97% of the plants 21, 49, and 98 days after planting in 1985, respectively. Infection was not significantly different between Coker 368 and Hutton at any of the sample dates during 1985 (Fig. 1C). Cropping system also did not affect plant infection during this season (Fig. 1B). However, infection was significantly lower under conventional tillage until the final sample date, 98 days after planting (Fig. 1A).

The percentage of plants infected in 1986 was much lower than in 1985. Mean infection for both cultivars was 4, 9, 64, and 84% infection 24, 56, 80, and 112 days after planting, respectively. Although the first, second, and final samples were taken 3, 7, and 14 days later in 1986 than for corresponding samples in 1985, the percentage of plants infected was significantly lower in each 1986 sample than in the equivalent sample in 1985 ($P = 0.05$). In 1986, plant infection was similar for both cultivars with the exception of the third sample date, when a lower percentage of Coker 368 plants was infected (Fig. 2C). Cropping system significantly influenced infection only on the final sample date (Fig. 2B). Significantly less plant infection was found for the conventional-tillage treatment 56 and 112 days after planting (Fig. 2A).

D. p. var. *caulivora* was isolated more frequently from the bottom 10 cm of the stem in 1985 than from the stem section at either the middle or top of the plant (Table 1). In 1986, isolation was lowest in the bottom section of the plant and was highest in the top section of the plant. The ratio of isolation in the bottom stem section to top stem section was 1.1 in 1985 and was significantly greater than 0.2 in 1986 ($P = 0.05$).

Southern stem canker symptoms developed at the experimental site in both years of the study. In 1985, 60% of the Hutton plants examined had symptoms of southern stem canker, and 37% of the plants died prematurely. In contrast, only 4% of the Coker 368 plants had symptoms and 2% of the plants died prematurely. The disease severity index for Hutton was 1.49 and for Coker 368 was 0.09 in 1985. In 1986, 30% of the Hutton plants had symptoms, including 6% dead plants. Coker 368 had 9% of the plants with symptoms and 1% of the plants were dead. The disease severity

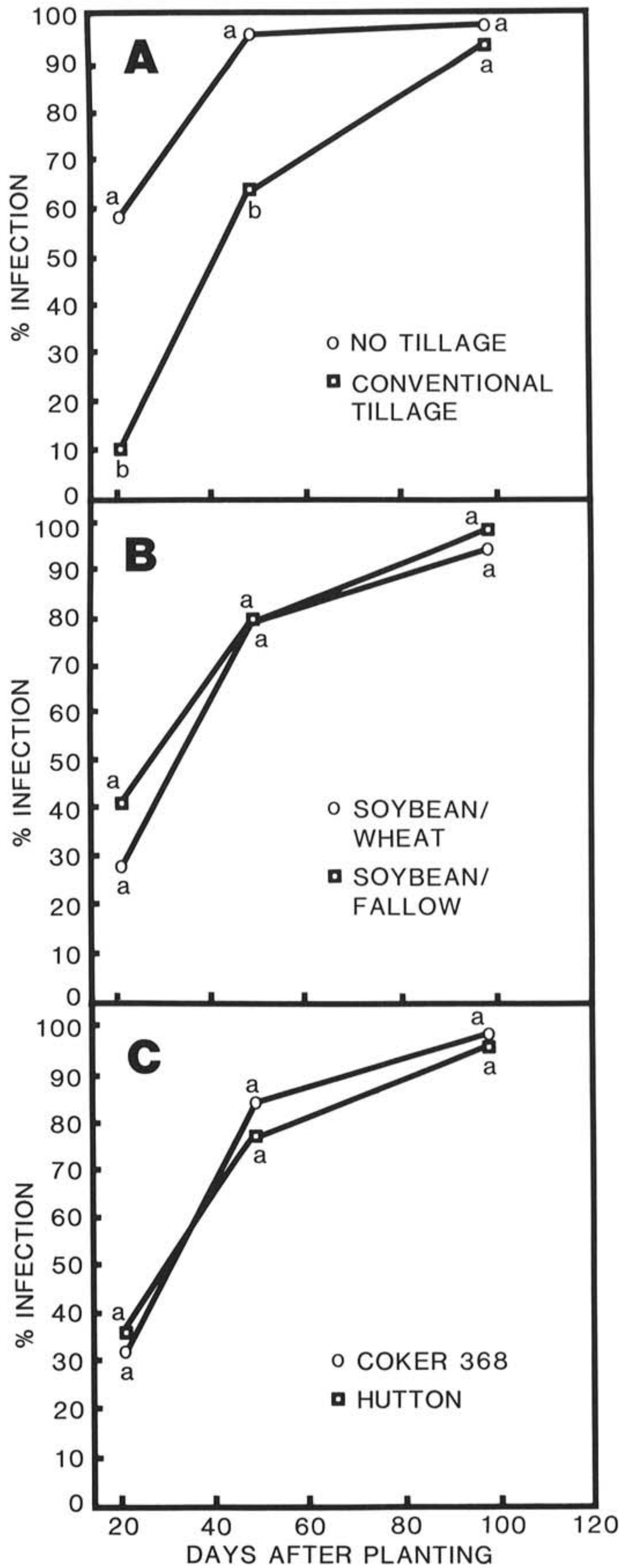

Fig. 1. Infection of soybean by *Diaporthe phaseolorum* var. *caulivora* in 1985 as influenced by tillage **A,** cropping system **B,** or cultivar **C.** Means for the same sample time with a common letter are not significantly different ($P = 0.05$).

index in 1986 was 0.50 for Hutton and 0.12 for Coker 368.

A dramatic reduction in disease incidence, disease severity index, and dead plants was found in 1985 for the susceptible cultivar Hutton under the conventional-tillage treatment compared with the no-tillage treatment (Table 2). In 1986, all three measures of disease were again lower for Hutton under conventional tillage than under no tillage. The effects of tillage were similar for the moderately resistant Coker 368, but the differences between treatments were much smaller.

Disease incidence was not significantly greater in soybean/wheat double-cropping compared with the soybean/fallow cropping system in 1985 for the susceptible cultivar Hutton (Table 2). In 1986, disease incidence, dead plants, and disease severity index were all greater under soybean/wheat double-cropping for Hutton. Southern stem canker was slightly lower after winter fallow than after wheat for Coker 368 in 1985, but Coker 368 was not significantly affected by cropping system in 1986.

Yield for Hutton was significantly greater with conventional tillage in 1985, but yield for Coker 368 was not affected by tillage (Table 2). In 1986 yield was not significantly affected by tillage treatment for either cultivar (Table 2). Cropping system did not significantly influence yield for either cultivar in either year.

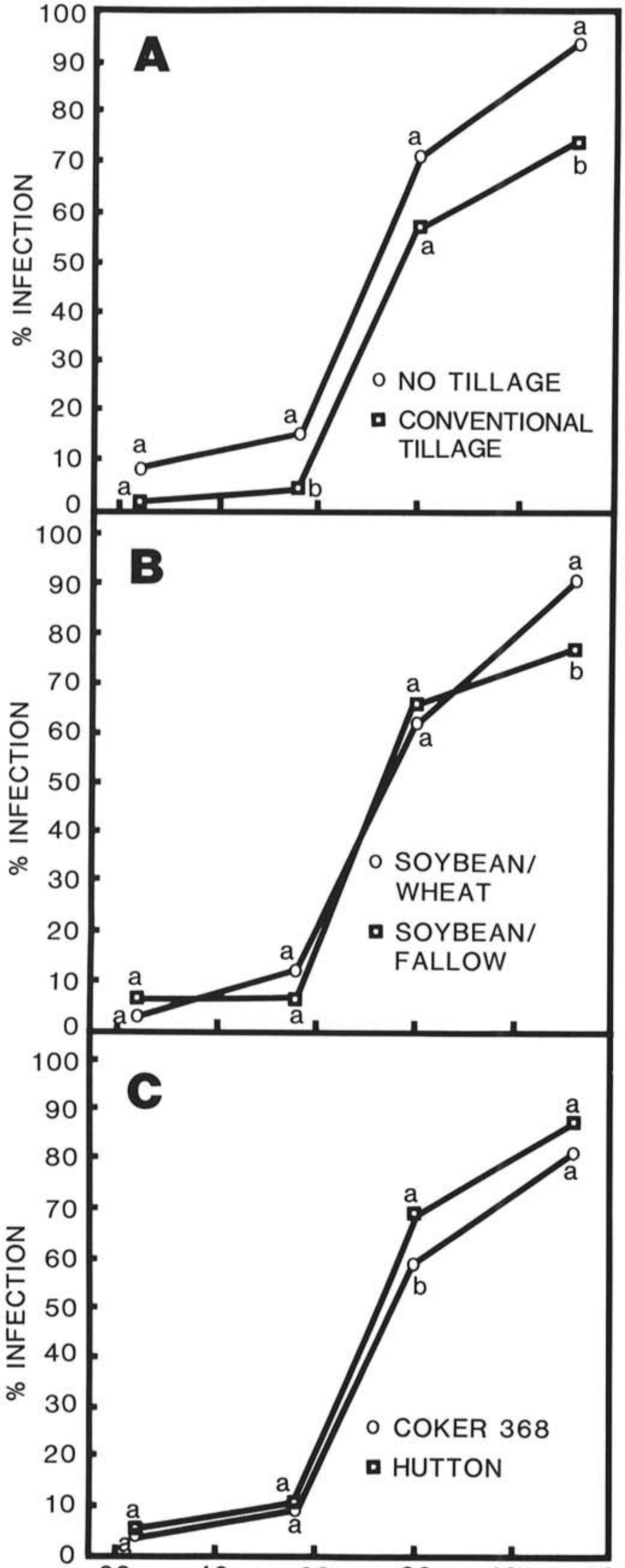

Fig. 2. Infection of soybean by *Diaporthe phaseolorum* var. *caulivora* in 1986 as influenced by tillage **A,** cropping system **B,** or cultivar **C.** Means for the same sample time with a common letter are not significantly different ($P = 0.05$).

DISCUSSION

Although soybean cultivars have been reported to vary greatly in susceptibility to southern stem canker (6,19), plant infection did not differ significantly between Hutton and Coker 368 in this study. Similar incidences of infection among susceptible and resistant cultivars have been reported in controlled environment (11,12) and field studies (17). These results with the susceptible Hutton and moderately resistant Coker 368 corroborate these previous findings.

Plant infection began early in the growing season and continued throughout the summer. Early infection appeared to be more closely related to final disease severity. In 1985, 34% of the plants sampled were infected 21 days after planting, and 37% of the Hutton plants died prematurely, compared with 4% plant infection 24 days after planting in 1986 when only 6% of the Hutton plants died prematurely. In Alabama, Smith et al (17) have reported a similar scenario for infection and the subsequent development of disease.

Differences in rainfall patterns between the two growing seasons could account for differences in infection and thus symptom development. In July of 1985 (5–36 days after planting), when plant infection was high, rainfall was near the normal 129 mm compared with little rainfall in July 1986 (8–39 days after planting), when little plant infection was recorded (Fig. 3). Differences in the pattern of infection between the two seasons are also evident in the ratio of isolation of the pathogen from the bottom to top of the plant. *D. p.* var. *caulivora* was isolated with similar frequency from the top and bottom of the stem in 1985. However, in 1986 the pathogen was isolated from the top of four times as many plants as the bottom. One explanation for this is that only younger plant parts are highly susceptible to infection. The lower portion of the stem would have been in the juvenile stage during the period of low rainfall in 1986 (Fig. 3), and, thus, little infection occurred. Later, when higher rainfall promoted greater infection, only the upper

TABLE 1. Relationship between portion of the soybean stem plated and isolation of *Diaporthe phaseolorum* var. *caulivora*

	Isolation (%)	
Isolation position[y]	1985	1986
Top	76.7 a[z]	69.5 a
Middle	74.3 a	56.7 b
Bottom	85.4 b	17.4 c

[y] Position of stem on soybean plant used for isolation at the final stage sampling date.

[z] Means followed by the same letter for a column are not significantly different ($P = 0.05$).

plant parts were in the juvenile, highly susceptible stage. Thus, the base of the stem, although exposed to as many or more spores, had much less infection.

Disease incidence and severity were always lower with conventional tillage for Hutton in this study. Coker 368 was also affected but to a lesser degree by tillage. This is similar to previous reports on the effect of tillage on stem canker (14,16,18). A probable cause for the decrease in disease incidence and severity with tillage is the burial of residue containing the pathogen and, thus, lower inoculum levels in close proximity to aboveground plant parts. Lower levels of plant infection particularly early in the season with conventional tillage support this view. The importance of inoculum in differences in disease is also shown in previous research (14) by large differences in disease between fumigated and nonfumigated plots, which have the same air-soil environment but differ in internal plot inoculum levels. These large differences between fumigated and nonfumigated plots, as well as adjacent plots, indicate that pathogen spread is so limited that interplot spread was of no consequence in this study.

Disease incidence and severity were significantly greater in the soybean/wheat double-cropping treatment for the moderately resistant Coker 368 in 1985 and for the susceptible Hutton in 1986. In previous field studies at this site, the increase in stem canker severity or incidence with soybean/wheat double-cropping has been observed (14). The absence of differences in plant infection under the two cropping systems with the exception of one sample date in 1986 (Fig. 2B) suggests that any cropping system effects may relate to symptom expression rather than plant infection.

Tillage alters many soil factors such as soil temperature, bulk density, soil moisture, and distribution of soil nutrients, as well as biological activity (5). Hutton and Coker 368 were used in the present study to separate the effect of southern stem canker on yield from other factors associated with tillage, which may influence yield. In 1985, no significant yield differences were found between tillage treatments for Coker 368, indicating tillage did not influence yield in the absence of losses associated with southern stem canker. However, for Hutton there was a 47% higher yield associated with 59% fewer plants with stem canker symptoms under conventional tillage compared with no tillage. Hutton yielded 29% less than Coker 368 under the conventional-tillage system and 49% less than Coker 368 under the no-tillage system. Slightly greater yields under no tillage in 1986 for both cultivars were probably associated with a more favorable water status in the dry season as a result of residue cover and lack of tillage operations. In 1986, Coker 368 yielded 10% more than Hutton over all tillage and cropping systems. This small difference could have been a result of cultivar differences or slight yield losses in Hutton caused by southern stem canker. Although substantial infection occurred during the latter portion of the 1986 season and 30% of the Hutton plants developed symptoms of southern stem canker, low infection early in the season and little premature plant death

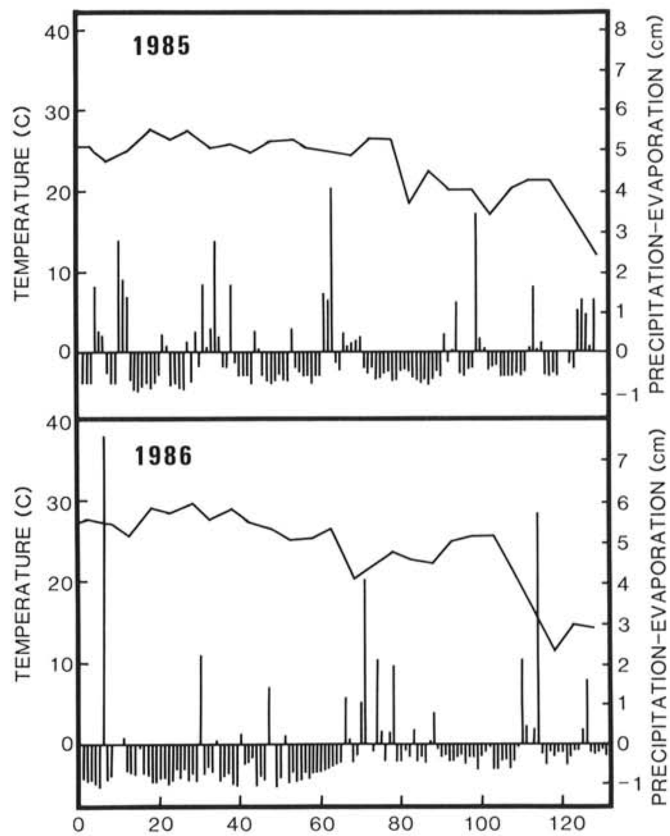

Fig. 3. Temperature and moisture environment for soybean crop. Line, 5-day mean temperatures. Bars, daily precipitation minus evaporation.

TABLE 2. Effect of cultivar, tillage, and cropping system on southern stem canker and soybean yield

Season	Cultivar	Tillage	Cropping system	Plants with symptoms (%)	Dead plants (%)	Disease severity index[x]	Yield (kg/ha)
1985	Coker 368	No	Combined	6.3 a[z]	2.9 a	0.14 a	1567 a
	Coker 368	Conventional[y]	Combined	2.5 b	1.1 b	0.05 b	1647 a
	Hutton	No	Combined	85.3 a	59.5 a	2.22 a	794 a
	Hutton	Conventional[y]	Combined	34.7 b	15.0 b	0.77 b	1166 b
	Coker 368	Combined	Soybean/wheat	6.3 a	2.8 a	0.14 a	1677 a
	Coker 368	Combined	Soybean/fallow	2.5 b	1.2 b	0.05 b	1536 a
	Hutton	Combined	Soybean/wheat	64.1 a	37.1 a	1.57 a	984 a
	Hutton	Combined	Soybean/fallow	56.0 a	37.3 a	1.42 a	977 a
1986	Coker 368	No	Combined	10.9 a	0.8 a	0.14 a	2526 a
	Coker 368	Conventional[y]	Combined	6.2 b	0.6 a	0.09 a	2278 a
	Hutton	No	Combined	41.3 a	8.0 a	0.68 a	2342 a
	Hutton	Conventional[y]	Combined	18.3 b	4.3 b	0.32 b	2010 a
	Coker 368	Combined	Soybean/wheat	8.1 a	0.6 a	0.11 a	2434 a
	Coker 368	Combined	Soybean/fallow	9.0 a	0.7 a	0.12 a	2370 a
	Hutton	Combined	Soybean/wheat	34.3 a	8.1 a	0.61 a	2272 a
	Hutton	Combined	Soybean/fallow	25.2 b	4.2 b	0.39 b	2079 a

[x] Disease severity index; 1 = minor lesion, 2 = major lesion, 3 = dead plant.
[y] Moldboard plowing and rotavating, see text for details.
[z] Means followed by the same letter for a comparison within a column are not significantly different ($P = 0.05$).

may have limited any yield losses associated with the disease. Data indicate disease incidence and severity and yield loss caused by southern stem canker can be effectively reduced by cultivar selection. In addition, the results indicate that moderately resistant cultivars, such as Coker 368, can be successfully incorporated into cultural systems that are advantageous for other reasons but may result in increased levels of infection and disease.

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