Influence of Tillage, Planting Date, Inoculum Survival, and Mixed Populations on Epidemiology of Southern Corn Leaf Blight

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ABSTRACT

Wholeplots of naturally infected corn residues were disk-harrowed, rotary-chopped, or plowed in November, 1970. In 1971, corn with Texas male sterile cytoplasm (Tcms) and normal cytoplasm (Nc) was planted 18 March and 15 April. There were significantly fewer southern corn leaf blight (SCLB) lesions on Tcms plants in plowed plots through 31 May when the early plants were beginning to tassel. Corn planted 15 April had a greater number of lesions than that planted 18 March, regardless of treatment. There was a highly significant negative correlation of number of SCLB lesions/subplot 21 May in both plantings and yield (r = –.46). In other plots planted to blends of Tcms and Nc, yield losses were directly proportional to the percentage Tcms plants and the distance from inoculated Tcms plants. Race T of *Helminthosporium maydis* survived in all residues from blends of Tcms and Nc, but it survived more abundantly in residues from Tcms plants. Tcms and Nc were planted separately in either disked or plowed subplots of each mixture of residues in 1972. There were significantly more lesions on Tcms plants in disked than in plowed subplots, but no significant differences in yields occurred.

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Additional key words: Phyllosticta, Colletotrichum graminicola, chlamydospores, dew period.

Southern leaf blight (SCLB) of corn (*Zea mays* L.) caused by *Helminthosporium maydis* Nisikado & Miyake race O is endemic in the subtropical corn belt of the southeastern USA. In 1970, a new biotype designated as race T caused a severe epidemic in corn with Texas male sterile cytoplasm (Tcms) in most corn-growing areas of the USA (19). Corn with normal cytoplasm (Nc) was resistant to the pathogen (9). It is not known whether race T was present in Georgia in 1969, because a severe drought reduced yields and in some areas caused a total crop failure. It is reported that rainfall, relative humidity (RH), and temp are critical factors in the spread of *H. maydis* (10, 16, 17). The survival of the pathogen on corn debris might have influenced the development of the epidemic in 1970 (6, 12, 18). Studies with *H. turcicum* Pass. indicated that survival on corn residue is instrumental in the development of epidemics of northern corn leaf blight (NCLB) in the Midwest (5). In Nebraska studies, overwintering conidia of *H. maydis* and *H. carbonum* were pathogenic on corn in greenhouse tests (4).

This study was undertaken to determine (i) the interactions of tillage practices and date of planting on the spread of SCLB from naturally infected corn debris, and (ii) the epidemiology and survival of *H. maydis* race T in blends of Tcms-Nc corn. A preliminary report has been made (13).

MATERIALS AND METHODS.—All field plots were at the Coastal Plain Station on Dothan, Tifton, or Leefield loamy sand. Cultural methods recommended by the Coastal Plain Station agronomists were followed except for the factors being studied.

Experimental plants were observed every 1-4 days after emergence until lesions were too numerous to count, and every 5-10 days thereafter. Lesions of all types were frequently observed through a microscope in order to ascertain that only lesions caused by *H. maydis* race T were included in the data. In addition, fungi from numerous lesions were isolated and identified throughout the experiments. The number of SCLB lesions seen on all plants in the center of each subplot during primary infection and in the early stages of secondary spread of the disease were recorded. When lesions were too numerous to count, a leaf blight rating scale of 1 to 5 was used (15) in which 1 = only a trace of SCLB, and 5 = all plants prematurely killed by blight.

After harvest, grain was dried with forced warm air and yields adjusted to equivalent kg/hectare (ha) at 15.5% moisture. Test weight was then determined by weighing a 500-ml sample randomly selected from each lot. Percentage of damaged kernels was determined in 1971 by randomly selecting a 50-g sample from each lot and grading it according to USDA official grain standards (20).

Rainfall was collected 100-400 m from test sites. The number of hr of wetness (dew or rain) on electrical resistance sensors (7) was continuously recorded by National Weather Service Office instruments 3-7 km from the plots. The number of hr each day that the corn was wet was also estimated from visual observations of plots and was similar to the weather service data, but correlation coefficients were not determined. During dry weather, plants were irrigated with overhead sprinklers during the daytime every 7-10 days. However, the foliage was wet only a few hr, and irrigation did not noticeably influence the spread of SCLB. In loamy sands of the Coastal Plain, corn suffers drought stress if water is not provided every 7-10 days.

Data were analyzed by the least squares analysis of variance and simple correlation. Significant and highly significant were used to indicate 0.05 and 0.01 levels of probability, respectively.

Tillage.—The development of leaf blight was studied on both Nc and Tcms in 1971 at two different planting dates (18 March or 15 April) in a split-split-plot experiment in a randomized complete block design with three replications. Naturally infected corn residues from the 1970 crop in wholeplots 13.1 × 19.4 m were (i) plowed

15-20 cm deep, (ii) chopped with a Rite-O-Way rotary cutter, or (iii) disk-harrowed 10 November 1970. Each subplot of eight rows had four rows of inbred B37 as either Nc or Tcms lengthwise within the wholeplot. Rows were 70 cm apart, and plants were 15-30 cm apart within the row. All wholeplots were disk-harrowed 18 March across subplots. Some residue was moved laterally a few feet on the borders of each wholeplot, but leaf blight ratings were made only on 75-120 plants in the center two rows of each sub-subplot. In 1972, a similar split-split-plot experiment in a randomized complete block design was used again. The hybrid, Funks 4949G, was planted as Tcms or Nc on wholeplots of residue from 1971 mixtures of 0, 20, 40, 60, 80, or 100% Nc of the same hybrid. All residues were chopped with a rotary cutter in September, 1971. On 20 March, 1972, one-half of each plot was plowed 15-20 cm deep. Each subplot was tilled 5-10 cm deep with a disk harrow 27 March and 4 April. Four rows each of Nc and Tcms Funks 4949G were planted 11-12 April.

Mixed populations.—Funks 4949G Tcms was mechanically mixed with 4949G Nc in seed mixtures of 0, 20, 40, 60, 80, or 100% Tcms (v/v). Each lot was planted in a randomized complete block design on land not in corn in 1970. Three replications 200-300 m apart were used. Plots were 16 rows 70 cm apart and 12.8 m long. The first two replications were planted 1 April and the third 12 April 1971.

Five Tcms plants in the center of each plot were used as infection centers. When plants were 10-35 cm high (5 May), they were inoculated in the whorls with 2-5 ml of dry leaf residue collected in November, 1970, from Tcms plants naturally infected with *H. maydis* race T. The residue was stored in burlap bags in an open shed and passed through 1-2 mm screen just prior to use.

Lesions were counted on only noninoculated plants 24 May, 1 June, and 15 June. Weekly leaf blight ratings were made from 25 June through 6 August. Stalk rot was determined 10 August by sampling 50 plants in each of three rows in each plot. Plants were arbitrarily considered rotted if they collapsed when squeezed by hand. Each wholeplot was divided into a grid of nine equal subplots 3.7 × 4.2 m and each subplot separately harvested.

The residue from the mechanical mixture of Tcms and Nc was rotary-chopped 20 to 22 September, 1971, and left on the soil surface. Samples were collected at random from each plot 29 October to 11 November, 1971, and 24 February and 24 April, 1972. Samples were processed as previously described (12) and assayed for *H. maydis* race T.

RESULTS.—*Tillage in 1971.*—The amount of corn residue on the surface in the harrowed, chopped, and plowed plots declined from an average of 2,589, 1,518, and 307 kg/ha, respectively, to 2,099, 1,353, and 0 from January to March, 1971. Because of abnormally cold and wet weather, the early corn grew very slowly. From 18 March to 9 April, soil temperatures 10 cm deep varied from 10-25 C and 12 cm of rain fell.

Southern corn leaf blight was first observed in 1971 on 17 May, when the earlier planted corn was 30-60 cm tall. Lesions were first found on lower leaves of Tcms plants in harrowed and rotary-chopped treatments. Within 3 days, 318 lesions were noted; 58% of them were on 18 plants, and 33% on only five plants. In one subplot, 33 lesions were seen on one plant and 18 and six on each of two adjacent plants. By 21 May, there were significantly more lesions on Tcms in chopped and harrowed plots than in plowed plots (Table 1).

The Tcms plants in the plowed treatment were significantly less severely damaged through 31 May in both the early (Fig. 1) and late plantings, and less leaf blight damage was evident in plowed plots through 9 July. All Tcms plants were dead in both plantings by 19 July. The early planting was almost mature, but the late planting was killed 1 to 2 wk prematurely by leaf blight. Many flecks, typical of a resistant reaction to SCLB, were observed on Nc in mid-July; and severe flecking was apparent on all leaves 26 July. Approximately 5 to 10% of the Nc plants in the April planting were also prematurely killed.

A Hirst automatic volumetric spore trap was in operation with its orifice 75 cm above the ground 1 May to 31 August in a field ca. 400 m from the plots. Conidia of the *H. maydis*-*H. setariae* type were not abundant until mid-July, when there were numerous lesions on lower leaves of all corn surrounding the trap.

Anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wilson, and lesions containing a *Phyllosticta* sp.

TABLE 1. Effect of interaction of tillage and date of planting with inbred B37 in Tcms (Texas cytoplasm, male-sterile) or normal (N) cytoplasm on development of southern leaf blight, yield, and test wt in 1971

	Lesions/plot[x]						Yield		Test wt	
	21 May		26 May		31 May		(g/plot)		(g/500 ml)	
Treatment	Tcms	N	Tcms	N	Tcms	N	Tcms	N	Tcms	N
Tillage[y]										
Chopped	36.0 a	1.3 b	131.5 a	1.3 b	168.0 a	1.0 b	966 b	5,412 a	371	387
Harrowed	48.2 a	1.7 b	136.5 a	1.0 b	152.5 a	1.0 b	802 b	5,419 a	371	380
Plowed	6.5 b	1.5 b	16.8 b	1.0 b	18.7 b	1.0 b	1,910 b	5,432 a	374	380
Date of planting[z]										
18 March	38.2 a	1.0 b	77.7 a	1.0 b	122.9 a	1.0 b	1,690 b	4,679 a	386	377
15 April	22.1 a	0.5 b	144.1 a	1.0 b	103.0 a	1.0 b	762 b	6,159 a	358	388

[x] Numbers followed by the same letter are not significantly different ($P = 0.05$); no significant difference in test wt.

[y] Average of 18 March and 15 April plantings.

[z] Average of chopped, harrowed, and plowed tillage treatments.

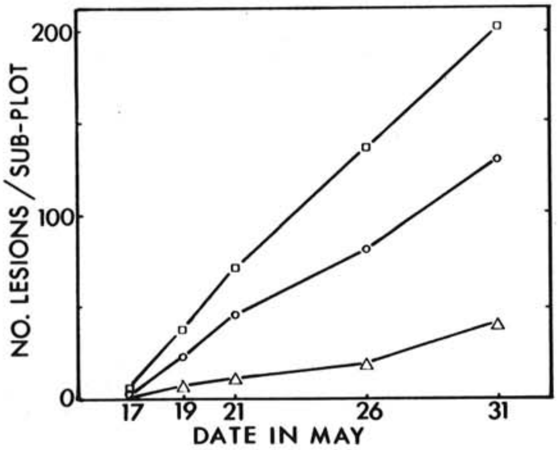

Fig. 1. Effect of tillage on development of southern corn leaf blight on Tcms (Texas cytoplasm, male-sterile) corn in May, 1971 (planting of 18 March only). □ = harrowed, O = chopped, and △ = plowed.

similar to yellow leaf blight (1) were common on lower and middle leaves of both Nc and Tcms through mid-May but spread no further..

Early vs. late planting in 1971.—As shown in Table 2, Tcms plants yielded 55% less in the late planting than in the early; whereas Nc plants yielded 32% more in the late planting. The Tcms plants yielded 64% less than Nc plants in the early planting compared to 88% in the late planting. In plowed plots, yield reductions were 48 and 78% respectively, in the March and April plantings of Tcms. Test weights were not significantly different, but were 8% less in Tcms than in Nc in the April planting.

Interaction between planting date and tillage in 1971.—Date of planting and tillage were not significantly correlated with lesions/subplot, yield, percentage damaged kernels, or test wt when both Nc and Tcms plants were included in the analysis. However, the negative correlation of yield with lesions/subplot 19-20 May (r = –.46) and disease severity 2 and 9 July (r = –.78) was highly significant. There was a highly significant correlation between lesions/subplot both 26 and 31 May and percentage damaged kernels (r = .56).

TABLE 2. Effect of planting date and tillage treatment on yield losses in inbred B37 in Tcms (Texas cytoplasm, male sterile) and normal (N) cytoplasm in 1971

Date of planting	Tillage[z]	Yield (g/plot) N	Yield (g/plot) Tcms	Yield decrease (%)
	Plowed	4,898 ab	2,529 bcd	48
18 March	Chopped	4,803 ab	1,588 cd	67
	Harrowed	4,338 abc	954 d	78
	Plowed	5,967 a	1,294 cd	78
15 April	Chopped	6,021 a	345 d	94
	Harrowed	6,500 a	649 d	90

[z] Numbers followed by the same latter are not significantly different ($P = 0.05$).

1972 experiments.—SCLB was first observed in the research plots 15 May. On 27 May when plants were 50-75 cm high, nine lesions were noted on three Tcms plants on one row of a disk-harrowed subplot and five on one lower leaf. However, very few additional lesions were noted before the mid-silk stage (ca. 25 June). Lesions per subplot, counted 7 July, ranged from 5 to 2,700 on Tcms and 0 to 13 on Nc plants. There were no significant differences in the number of lesions on Tcms plants grown on residues from different mixtures of Tcms and Nc, but there were significantly more lesions on Tcms plants in the disk-harrowed than the plowed subplots (495 vs. 236). Nevertheless, leaf blight ratings were not significantly different 28 July, and there were no significant differences in yield or test wt among residue mixtures or tillage treatments. In contrast with 1971, leaf blight lesion counts and ratings were not correlated with yield. Nc plants yielded significantly more than Tcms plants (57 vs. 63 quintals/ha), and grain from Nc plants had a significantly greater test wt. Drought was severe throughout much of the season, and in spite of frequent irrigation, the crop was under drought stress at times.

In 1972, there were 6, 5, 10, and 1 days in May, June, July, and 1 to 18 August when >10 hr of dew were recorded on electrical resistance sensors in contrast to 12, 16, 22, and 14, respectively, in 1971. Rainfall during the growing season in 1972 was 36.6 cm vs. 55.2 cm in 1971.

The differences in the two growing seasons were also indicated by the absence of anthracnose and the rarity of *Phyllosticta* sp. during the dry spring of 1972 compared to their widespread occurrence in the cool, wet spring of 1971.

Effect of mixed populations on epidemiology of SCLB.—Further information on rate of spread of *H. maydis* race T in 1971 was gathered from artificial infection foci in mixed populations of Tcms and Nc corn. On 6 and 7 May, water was present in the whorls of the inoculated Tcms plants 6-12 hr each day because of dew or heavy fog. Five days after inoculation, numerous necrotic flecks 1 to 3 mm in diam with 1 to 5 mm chlorotic borders were observed on all plants. Lesions were rarely observed 24 May in plots with 0 and 20% Tcms. Plots with 40, 60, 80, and 100% Tcms averaged 66, 65, 101, and 243 lesions, respectively. Most lesions were on plants within a 1.2 m radius of the inoculated plants. A drought 15 May to 8 June reduced secondary spread, and lesion counts 1 June were similar to 24 May. Plants were wet ca. 20 hr 8 June and 13.5 hr 10 June, and the number of lesions began to increase logarithmically 11 June.

Most lesions were on lower leaves of plants within 3-6 m of the inoculated plants 15 June when plants were 1.5 to 2.1 m tall and beginning to tassel. Increase in percentage Tcms plants in a plot significantly increased the number of susceptible-type lesions observed. No resistant flecking was then noted in 100% Nc plots.

Plants were continuously wet from rain 10 to 17 hr each day 13 to 22 June. Plants were 80% silked by 25 June, and resistant plants were lightly flecked in all plots. The proportion of plants with susceptible-type lesions in each plot was similar to the proportion with Tcms, and lesions were primarily confined to the lower six-to-eight leaves. In 100% Tcms plots, the lower three-to-four leaves were dead or dying, and scattered lesions were evident on

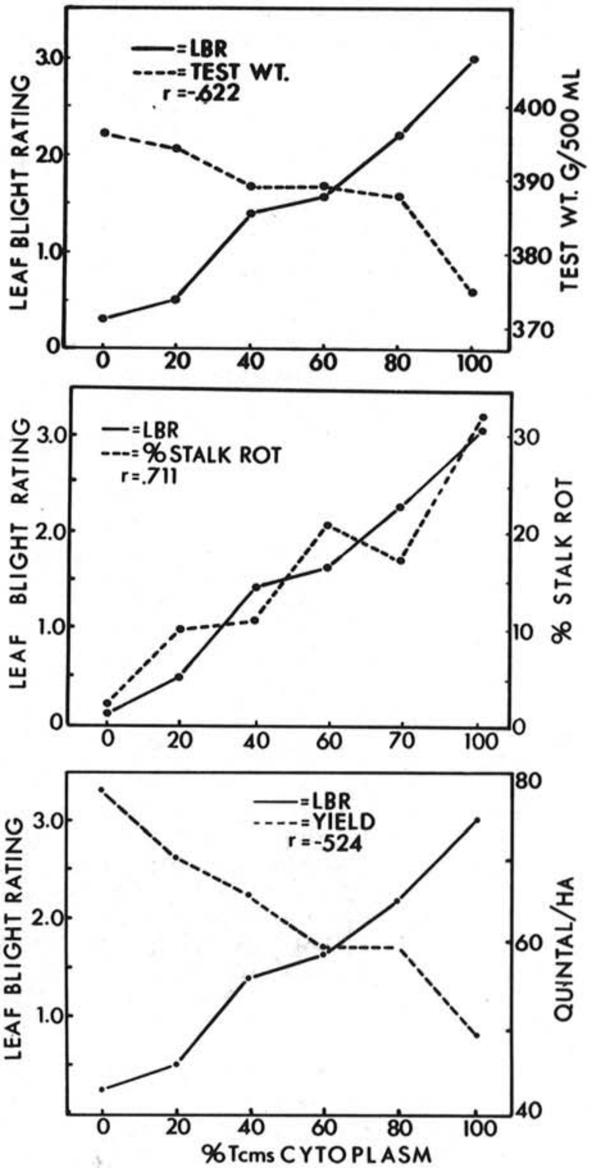

Fig. 2-4. Correlation of southern corn leaf blight severity 2 July, 1971 and **2)** test weight, **3)** percent stalk rot 10 August, and **4)** yield in mechanical mixtures of Tcms and normal cytoplasm. LBR = leaf blight rating, 0 = none, and 5 = plants prematurely killed.

upper leaves, stalks, shanks, and husks in the oldest corn. The leaf blight incidence in each treatment was significantly different from all other treatments 2 July.

By 30 July, 4-5 wk past mid-silk, the leaf blight rating was 4.85 in 100% Tcms plots and 4.0 - 5.0 on susceptible plants in the other plots. All Nc plants were severely flecked 7 August, and some were prematurely killed. Test wt was significantly reduced by leaf blight (Fig. 2). In the plots with 100% Tcms, 1-2% of the stalks were lodged and 5-10% of the ears were dropped, indicating weak shanks. On 10 August, stalk rot was directly related to the proportion of Tcms plants in each plot (Fig. 3) and was significantly greater in the center rows than in the outside rows.

Yield was significantly reduced by leaf blight (Fig. 4). As the proportion of Tcms increased, the percentage of damaged kernels significantly increased. Yields were lower in the center than in the other eight subplots, but there were no significant differences in yield, percentage damaged kernels, or test wt. However, the center subplot had significantly more lesions than the other subplots 1 and 15 June, and a significantly higher leaf blight rating 2 July. The center three subplots parallel with the direction of the rows had significantly greater leaf blight 2 July than rows on the left and right. The plants in the plot margins were not severely blighted until 17 days after tasseling when plants in the center plots were nearly dead. The average yield in the center subplot was 18% less than those in the corner subplots 3-7 m from the focus of primary infection.

There was a significant negative correlation of yield with lesions/subplot 1 June (r = –.17) and a highly significant negative correlation of yield with lesions/subplot 15 June (r = –.34), leaf blight rating 2 July (r = –.52), and percentage stalk rot (r = –.43), respectively.

Survival of Helminthosporium maydis race T in mixtures of Tcms and Nc.—Susceptible-type lesions were produced on all plants of Tcms with filtrates from all residue samples collected in October-November, 1971 and February, 1972 (Table 3). In contrast, lesions were produced only by filtrates from three of six samples collected 24 April 1972. The average number of lesions produced with the filtrates from 1.0 g of residue was 5.6, 2.2, and 0.05 from residue collected 29 October to 11 November, 24 February, and 24 April, respectively. Race T appeared to survive better in residue from plots with a higher percentage Tcms plants in 1971. On 24 February, there were significantly more lesions produced with filtrates from residues from 100% Tcms than with filtrates from other residues.

Leaf residues from both Tcms and Nc from the 1971 crop were collected 24 September and separately placed in two 1.0-cm mesh wire cages on the ground and two similar cages 25 cm above the ground in a fallow field. In addition, leaves were buried 6-10 cm deep in a field of Dothan loamy sand. Other leaves were placed in burlap bags and suspended 2-3 m from the ground in an open shed. Residue was assayed 4 October 1971, and 14 February and 7 June 1972. The average number of susceptible-type lesions produced with the filtrate from 1.0 g of Tcms leaf residue in cages was 33.1, 2.8, and 0.05, respectively. Results were similar with residue from Nc. The fungus survived better above the ground than on or under the ground in residue from both cytoplasms, but it survived in all residues. On 7 June, the filtrate from a gram of Tcms residue stored in the shed produced 33.2 susceptible-type lesions.

The survival pattern of the fungus in 1971-1972 was similar to 1970-1971. Nevertheless, consistently fewer susceptible-type lesions were produced with filtrates from residue collected February to June, 1972 than during a similar period in 1971.

Chlamydospores were evident in some *Helminthosporium* spp. conidia on most residue samples collected October to June in both seasons, but chlamydospores were more frequently observed in

TABLE 3. Survival of *Helminthosporium* spp. conidia on rotary-chopped residue from mixtures of Tcms (Texas cytoplasm, male sterile) and normal (N) cytoplasm and pathogenicity of filtrates from those residues on Tcms plants

Composition of residue (%)		Total conidia of *Helminthosporium* spp./g of air-dry residue[z]			Susceptible-type lesions on Tcms cytoplasm/g of air-dry residue		
		1971	1972		1971	1972	
N	Tcms	29 Oct. to 11 Nov.	24 Feb.	24 April	29 Oct. to 11 Nov.	24 Feb.	24 April
100	0	2,565 bc	2,019 b	879	1.07	0.34 b	0
80	20	1,657 c	2,037 b	699	4.30	0.73 b	0.03
60	40	3,686 c	2,222 b	625	7.05	1.67 b	0.04
40	60	4,621 a	1,926 b	663	5.50	1.74 b	0
20	80	4,342 ab	3,185 b	514	9.53	1.60 b	0
0	100	4,213 ab	7,426 a	1,192	5.97	6.93 a	0.04

[z] Numbers in the same column followed by the same letter are not significantly different ($P = 0.05$). Data columns without letters have no significant differences.

conidia from leaf residue from Tcms than Nc. Conidia with chlamydospores were seen in 19 of 24 Tcms and 4 of 19 Nc residue samples collected 4 October 1971 through 7 July 1972. Chlamydospores were more commonly seen in conidia on leaf than on stalk tissue in both years.

DISCUSSION.—Minimal tillage practices leaving residues on the surface as compared to burying the residues by plowing caused significant increases in early development of SCLB in both 1971 and 1972. Nevertheless, yield losses in Tcms were only observed in 1971 when rainfall and high RH were conducive to leaf blight development during most of the growing season. In our study, secondary spread of leaf blight was not apparent in dry weather when only 0-10 hr dew was recorded per day. Others have reported that leaves must be wet 7 or more hr at 15 C for *H. maydis* (10, 16, 17) and *H. turcicum* (2) to cause infection. Maximum infection with *H. maydis* race T occurs in controlled conditions with 12 hr of dew temp at 18 to 29 C (17), and post-dew temp of 28 to 31 C (16).

Early planting in 1971 led to increased yields in Tcms because the grain was nearly ripe before the plants were killed. In the late planting, the Tcms corn was killed 2 to 4 wk prematurely. In 1972, it is doubtful that differences in planting dates would have caused significant differences in injury by leaf blight since the entire growing season was unfavorable for an epidemic.

The local sources of inoculum were greatly reduced by burying the residue, corroborating the findings of other researchers (6, 18). The epidemic in 1971 was delayed 1 or 2 wk by clean tillage, even in our relatively small research plots, resulting in increases in yields of Tcms. In commercial fields it is possible that an epidemic might be delayed longer and yield losses reduced even further. The residues steadily deteriorated during the winter months with all tillage practices, but only deep plowing eliminated surface sources of inoculum. Nevertheless, *H. maydis* was recovered from residue unearthed during planting operations in plowed plots in 1971 (12) and from buried leaf residue in June 1972.

In Pennsylvania, race O survived better on residue from Nc than did race T (3), but we saw no susceptible-type SCLB lesions on Nc plants inoculated with residue filtrates or on Nc plants in the field. In our studies, race T survived slightly better on residue from Tcms than from Nc. Chlamydospores were also more commonly observed on Tcms. Other investigators have suggested that the formation of chlamydospores may provide a mechanism for survival for *H. turcicum* (5) and *H. maydis* (6).

Our research suggests that the primary source of inoculum originating SCLB epidemics in the Georgia Coastal Plain during the years under study was overwintering conidia on residue. Few *H. maydis*-type conidia were caught in a Hirst spore trap until several wk after the epidemic started in minimal tillage plots. Also, several primary lesions were usually found on lower leaves of only a few adjacent plants each spring before SCLB became general throughout a plot. The spread of SCLB from foci in mixtures of Tcms and Nc plants provides additional support for the importance of local sources of inoculum. The highly significant negative correlation of lesions/subplot prior to tasseling and yield in 1971 suggests early infection from local inoculum may greatly increase the severity of an epidemic. Research in Nebraska indicated that NCLB primary inoculum also came from conidia on overwintering residue, rather than from "spore clouds" or "spore showers" (4, 5, 14).

Our findings give additional support to other reports that mixing of Tcms and Nc only reduces the severity of SCLB to the extent that Tcms is replaced with Nc (11). We found no indication of any protection of Tcms plants by Nc plants in any of the mixed populations we studied. In addition, the severity of stalk rot in the mixed populations was directly related to the percentage Tcms plants in the population. Previous reports indicate that injury by SCLB increases the incidence of stalk rot (8, 11).

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