Influence of Environment and Plant Maturity on Gray Leaf Spot of Corn Caused by *Cercospora zeae-maydis*

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ABSTRACT

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This study was undertaken to determine why gray leaf spot commonly affects corn grown in eastern, but not in central Kentucky. Weather conditions in locations where gray leaf spot was present were compared with conditions in locations where the disease was not present. The disease was restricted to locations (in eastern Kentucky) having long daily periods of high relative humidity (RH) and leaf wetness (LW). During the months of disease development (August and September) temperatures were the same at sites with and without the disease, but there were more days with 12–13 hr of RH >90% and 11–13 hr of LW at sites with the disease. Disease progress was monitored in five plantings of corn made at 3-wk intervals. Disease onset and progress were influenced by the physiologic age of the plant. Initial symptoms appeared at or near anthesis on lower leaves and on progressively higher leaves later in the season. Daily aerial spore counts were highest in early afternoon and occurred when there was a rise in temperature, a drop in RH, and drying of leaves. Germination of spores following drying and rewetting was reduced significantly on glass slides and on live tissue in the laboratory. The data suggest that gray leaf spot disease is a problem in eastern Kentucky because there is a high frequency of days having long, uninterrupted periods of LW and high RH. These moist periods may influence spore germination and hence infection.

Gray leaf spot of corn (*Zea mays* L.) caused by *Cercospora zeae-maydis* Tehon & Daniels, is a late-season, foliar disease found predominantly in river valley fields of the southern Appalachian region (4,6,8). Long grayish brown, rectangular lesions first appear on the lower leaves in late July to mid-August (4,8) and later on the upper leaves. Gray leaf spot reaches maximum severity in early September. Although gray leaf spot has not been shown to affect yields directly, severely infected plants may be predisposed to stalk rot, which can lead to increased lodging (3,8). Up to 100% lodging occurred in 1977 in some Kentucky fields severely affected by gray leaf spot (3).

The purpose of this study was to determine the influence of environment and plant maturity on disease development and of environment on production, dissemination, and survival of spores.

MATERIALS AND METHODS

Relative humidity (RH), temperature, and leaf wetness (LW) were recorded at two locations where gray leaf spot was usually present (University of Kentucky's Robinson Agricultural Research Substation at Quicksand, KY, and the C. Rose farm near Hazel Green, KY) and at one location where the disease was usually absent (University of Kentucky's South Farm near Lexington). RH and temperature were measured with a recording hygrothermograph (Bendix, Baltimore, MD 21204) placed in a standard (76×76×51 cm) weather shelter 20 cm above the ground. LW was recorded with a DeWitt LW recorder (Valley Stream Farm, Orono, Ontario, Canada) mounted on a wood post 107–120 cm above the ground. The instruments were located within the canopy of the corn plots. Rainfall was recorded at Quicksand in 1978 and 1979 and at South Farm in 1978 with a recording rain gauge (Belfort Instrument Co., Baltimore, MD 21224) located near the corn. In addition, temperature and RH data were obtained for 1977 from standard weather stations located in open grassy areas at Quicksand and at the University of Kentucky's Spindletop farm near Lexington, KY (also an area not having gray leaf spot).

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The daily number of spores of *C. zeae-maydis* in the air was estimated with a Burkhart spore trap (Burkhart Scientific Sales Ltd., Rickmansworth, Hertfordshire, England) at Quicksand from 29 August to 30 September 1979. The spore trap was set up between plots in a 1.5-m-wide alleyway 9 m from the weather station and with the orifice 75 cm above the ground. Spores were trapped on cellophane tapes coated with silicone grease. The tapes were changed weekly, cut in 24-hr segments, stained with lactophenol cotton blue, and the spores deposited each hour were counted by using a compound microscope (×400).

To study the effect of plant maturity on disease development, corn (PAG Hybrid SX17A) was planted five times at Quicksand in a randomized block design at 3-wk intervals from 9 May to 2 August 1979. Disease progress and plant maturity were followed on 10 randomly selected plants from the center two rows of each plot at weekly intervals from 5 July to 29 August and then biweekly until 24 October. On each plant, the number of lesions on three leaves were counted: the third or fourth leaf below the ear; the ear leaf or the leaf below the ear; and the fifth or sixth leaf above the ear leaf. Disease progress was followed by counting the number of lesions on each of these leaves. Standard growth stages were used to record development (5).

Additional disease development and plant maturity data were obtained in 1979 from Hazel Green, KY, on one planting of corn, cultivar Pioneer Hybrid 3184. Disease and plant maturity ratings were begun on 22 August when the corn was silking and already had lesions on the lower and middle leaves. These ratings followed the same schedule as those at Quicksand, but since a different hybrid was used at Hazel Green, the lower leaf at that location was three leaves below the ear leaf, the middle leaf was the ear leaf, and the upper leaf was three leaves above the ear leaf.

During the 1978 growing season, the effect of introduced inoculum on disease development was determined at the South Farm location. Corn was grown in a field that had been treated with corn stubble and debris collected from a corn field (near Hazel Green) that had been severely infected with gray leaf spot disease the previous season. The debris, which consisted of corn stalks, leaves, and root material, was collected and applied to the field plots early in the spring and lightly disked into the soil prior to planting.

The effect of temperature on spore germination was determined with conidia obtained from cultures of single-spore isolates of *C.*

zeae-maydis grown in the dark on V-8 juice agar medium (10) for 5–7 days at 25 C. Spores were removed by flooding each plate with 10 ml of deionized water and rubbing the surface of the agar with a glass rod. The spore suspension was filtered through four layers of cheesecloth to remove mycelial fragments. Two or three drops of a spore suspension were spread over the surface of 2% water agar so that no more than 20 spores were seen in the viewing field of a compound microscope at ×100. The longest germ tube of each of the first 10 germinated spores observed was measured with an ocular micrometer to determine germ tube growth.

To study the effect of desiccation on spore germination, a conidial suspension of 7.6×10^4 spores per milliliter from a 5-day-old culture was spread over the surface of clean glass slides. The slides were either allowed to dry at room temperature, which took approximately 0.5 hr, or were immediately placed in a dark moist chamber for 14 hr at 25 C and then allowed to dry overnight at 4 C. Dry slides were rewetted immediately after drying or after a 24- or 48-hr dry period. Spores were stained with lactophenol-cotton blue, and percent germination and germ tube length were determined.

In additional studies on the effect of desiccation on germ tube growth, spore suspensions spread over the surface of clean glass slides were incubated in moist chambers at 25 C in the dark. Control slides remained in the moist chamber for 15 hr and the other slides were removed after 9 hr and allowed to dry at room temperature. Some of the dried slides were immediately rewetted and placed in the moist chamber for another 6 hr. All slides were allowed to dry overnight at 4 C, spores were stained, and germ tube lengths were measured.

The effect of desiccation on spores on the leaf surfaces was studied on corn hybrid PAG SX17A in the late silking growth stage at South Farm. The cut ends of harvested leaves were placed in water, with two leaves in each 600-ml beaker separated with a strip of Parafilm® (American Can Co., Greenwich, CT 06830). The next day a spore suspension (10^4 spores per milliliter) was spread by means of a glass rod over the surface of the leaves. Depending on the treatment, leaves were either allowed to dry at room temperature, requiring about 0.5 hr, or were immediately placed in clear plastic bags thoroughly misted on the inside with a fine spray of deionized water. The bags were held tight against the beakers with rubber bands. Wet paper towels inside the bags helped maintain high RH. The leaves were sampled periodically by removing them from the moist chambers and placing them in a refrigerator at 4 C overnight to dry. Spores were removed from leaves with clear cellophane tape, placed on a slide, stained, and percent germination and germ tube lengths were measured. During this experiment, temperatures in the laboratory ranged from 22.2 to 23.3 C and RH ranged from 44 to 62%.

In a second experiment a more concentrated conidial suspension (1.8×10^5 spores per milliliter) was used; spores on the leaves were either dried immediately and rewetted after 1, 6, or 12 hr, or dried after 9 hr of incubation and then rewetted after 1 or 12 hr.

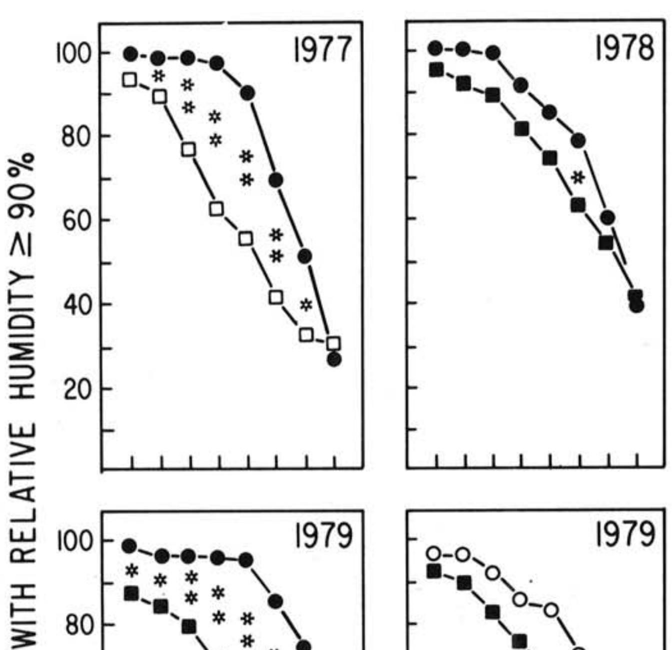

Fig. 1. Percent of days from 12 July to 30 September with periods (hours) of relative humidity (RH) above 90%. Data are plotted for Quicksand (●——●) and Spindletop (□——□), KY, in 1977; Quicksand and South Farm (■——■) in 1978 and 1979; and Hazel Green (o——o) and South Farm in 1979 (8 August to 30 September). Asterisks indicate RH >90% durations at which significant differences between locations occurred; *, $P = 0.05$; **, $P = 0.01$.

RESULTS

The average daily high and low temperatures during July, August, and September 1977–1979 at locations with corn gray leaf spot disease (Quicksand and Hazel Green) and at locations without the disease (South Farm and Spindletop) were compared. High temperatures ranged from 25 to 30 C and low temperatures from 15 to 20 C at all locations and were not significantly different, as indicated by an analysis of variance ($P = 0.05$) among locations. Rainfall amounts also did not differ significantly among locations.

The percentage of the days with at least a certain number of hours of RH above 90% for the mid-July to September period were compared for the various test sites (Fig. 1). These comparisons were made using a chi-square test in 2 × 2 contingency tables. The percentage of days at each of two sites with at least a certain duration of RH above 90% were compared for each hour from 10 to 17 hr. Longer periods of RH above 90% occurred more frequently in areas where the disease was present than in areas where it was absent. For example, 13 hr of high RH occurred on more than 90% of days in areas with gray leaf spot vs less than 70% of days in areas without the disease. In three of the four comparisons significant ($P = 0.05$) differences were detected.

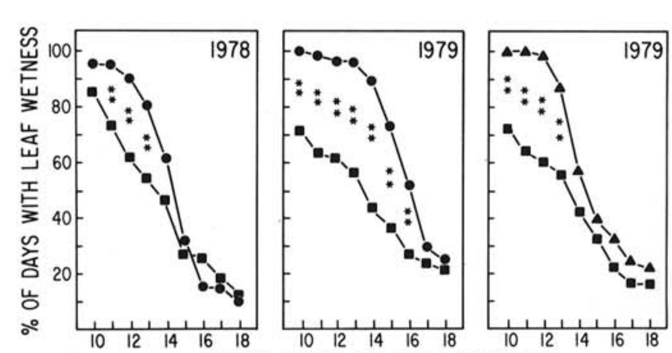

Fig. 2. Percent of days from 12 July to 30 September with periods (hours) of leaf wetness (LW). Data are plotted for Quicksand (●——●) and South Farm (■——■) in 1978 and 1979 and between Hazel Green (▲——▲) and South Farm in 1979 (8 August to 30 September). LW was recorded with a DeWitt LW recorder. Asterisks indicate LW durations at which significant differences between locations occurred; **, $P = 0.01$.

Comparisons of LW were made in the same manner (Fig. 2). Corn grown at Quicksand and Hazel Green had significantly longer periods of LW than did corn grown at South Farm. These differences were significant ($P = 0.01$) for 11, 12, and 13 hr in all three comparisons. In addition, restricted or shorter periods of LW did occur more frequently at South Farm than at the farms in eastern KY. For example, an average of 25 and 1.5% of the days (1978 and 1979) at South Farm and Quicksand, respectively, had less than 10 hr of LW.

No disease developed at the South Farm location even when a potential inoculum source, as debris and stubble, was introduced equivalent to the amount found in conventionally tilled farm fields at Quicksand or Hazel Green. This further supports the hypothesis that environmental factors such as LW and RH are important in restricting the development of gray leaf spot disease.

Delaying corn planting resulted in more rapid development and maturation of later plantings (Table 1). However, plantings one through four entered the last maturity stages, dent and mature, after about the same number of days. The 2 August planting did not grow normally. Many plants died and those that lived were stunted, grew no taller than 1.2 m, and never formed a canopy. Despite this, some of these plants formed tassels and produced small ears. Because of the poor growth, it was impossible to establish maturity ratings. Thus, for the 2 August planting, disease was rated on the entire plant and not on leaf groups.

Time of initial appearance of gray leaf spot lesions on the lower, middle, and upper leaves was influenced by both the time of year and the maturity of the corn (Table 2). In all plantings, the first lesions were seen on the lower leaves no earlier than at the whorl-to-tassel growth stages.

In the first four plantings, the disease increased slowly after the initial infection of the middle and upper leaves until 5 September and then rapidly increased (Fig. 3). Leaves were dead in most plantings by 25 September. The disease progress curves for leaf groups of one planting lagged 4–7 days behind those for the same leaf groups of the next earlier planting. The same lag was seen between the middle and upper leaves of the same planting.

Temperatures were fairly stable from 19 August to 4 September with lows of 20–22 C and highs of 27–31 C. Hours of RH >90% and hours of LW ranged from 14 to 19 and 15 to 19 hr, respectively. A cooling trend, reflected especially in the daily lows, occurred after 30 September and coincided with the delayed disease progress on the 2 August planting and on the upper leaves of the 11 July planting (not shown in Fig. 3). At Hazel Green, disease ratings were begun with gray leaf spot already present on the lower and middle, but not the upper leaves of the corn. As at Quicksand, the major increase in gray leaf spot at Hazel Green occurred from 1 to 22 September (Fig. 4). The disease first increased rapidly on the lower leaves and similar rapid increases occurred 4 days later on the middle leaves and 11 days later on the upper leaves. The weather at Hazel Green from 20 August to 6 September was similar to that at Quicksand with lows ranging from 19 to 21 C and highs from 25 to 30 C. Hours of continuous LW ranged from 11 to 23 hr and hours of RH >90% ranged from 13 to 44 hr.

Average hourly airborne spore content was compared to the average hourly temperature, RH, and LW from 29 August to 30 September 1979 (Fig. 5). The average number of trapped spores was positively correlated with temperature and negatively correlated with RH and LW. All correlations were significant ($P = 0.01$). Aerial spore content was usually greatest between 1300 and 1400 hours each day. Although wind velocity was not measured in this study, it was generally greatest during the middle of the day and may be an important factor in spore release. Also, short periods of rainfall not exceeding 1 cm often resulted in the release of large

TABLE 1. The effect of planting date on the growth of corn at Quicksand, KY, in 1979

Planting date	Time (days) required to reach stage: Seedling	Knee high	Whorl[a]	Tasseling	Silks green	Doughs	Dent	Mature
9 May	···	56	63	70	77	81	105	116
31 May	35	42	49	···	70	84	105	112
20 June	14	28	49	56	70	80	101	108
11 July	7	28	49	55	58	72	100	···
2 August	6	41	···	51	83	···	···	···

[a] Whorl = collar of the eighth leaf visible.

TABLE 2. Time of appearance of initial gray leaf spot symptoms on upper, middle, and lower leaves in each of five corn plantings at Quicksand, KY, in 1979[a]

Planting date	Leaf: Lower Days[b]	Lower Stage[c]	Middle Days	Middle Stage	Upper Days	Upper Stage
9 May	70	Whorl-tassel	91	Dough	106	Dough
31 May	70	Silking	70	Silking	82	Dough
20 June	49	Whorl	56	Whorl-tassel	77	Silking
11 July	49	Whorl	56	Tassel	73	Silking
2 August[d]	51	Kneehigh-tassel	···	···	···	···

[a] The lower leaf was three to four leaves below the ear leaf, the middle leaf was the ear leaf or the leaf below it, and the upper leaf was five to six leaves above the ear leaf.

[b] Number of days after planting that gray leaf spot symptoms were first observed in that leaf group in each of the plantings.

[c] Stage of growth in which lesions were first seen in that leaf group in each of the plantings.

[d] Initial infection date corresponds to the first lesions seen on any leaf in the fifth planting.

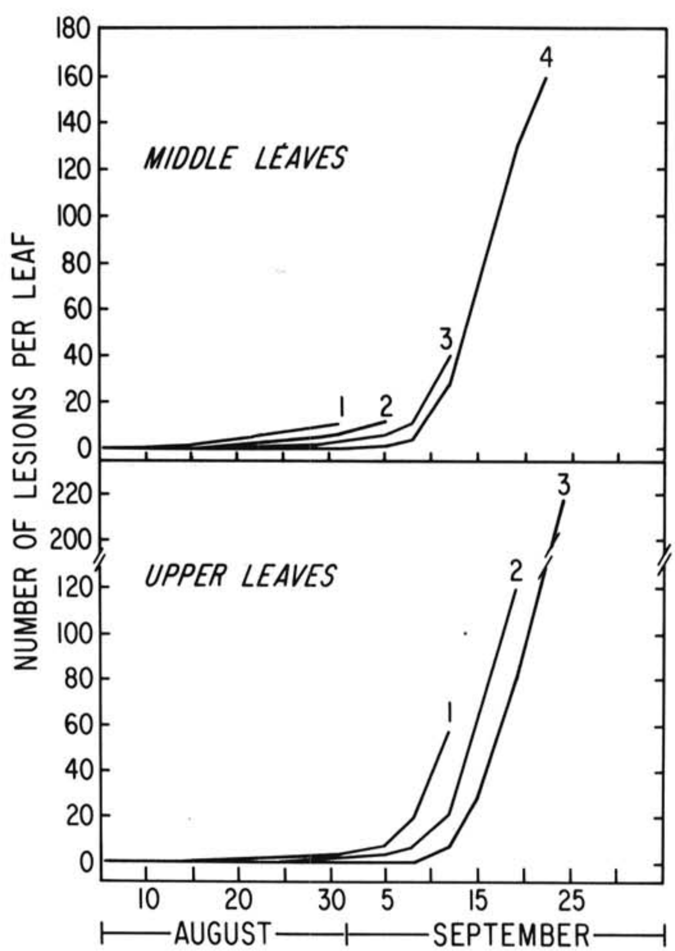

Fig. 3. Number of gray leaf spot lesions per leaf on middle and upper leaves on the corn hybrid PAG SX17A from 2 August to 24 October 1979 at Quicksand, KY. Middle leaves were either the ear leaf or the leaf below the ear. Upper leaves were five or six leaves above the ear leaf. Numbers above the end of each line signify the planting date with 1 = 9 May, 2 = 31 May, 3 = 20 June; and 4 = 11 July. Each line ends when the corresponding leaf died.

numbers of spores, but extended periods of rain reduced spore numbers. This pattern of spore release resembles that of other *Cercospora* species (2,7,9).

Spores germinated on water agar from 10 to 35 C with an optimum temperature between 18 and 25 C (Table 3). Within the optimal temperature range, over 70% of the spores had germinated after 6 hr and 90% had germinated after 15 hr. The optimal temperature for germ tube growth was 25 C.

Spore germination was significantly reduced on leaves having less than 9 hr of continuous LW (Table 4). Germ tube lengths were not significantly affected by drying and rewetting. Desiccation of spores on glass slides had a significant effect on both spore germination and germ tube length. Only 5% of the spores, which were dried and immediately rewetted, germinated on the slides. In contrast, spores exposed to 9 hr of continuous wetness had 91% germination and an average germ tube length of 66 μm. When these spores were dried and immediately rewetted for another 6 hr there was no change in germ tube length. Spores exposed to 15 hr of continuous wetness had an average germ tube length of 134 μm.

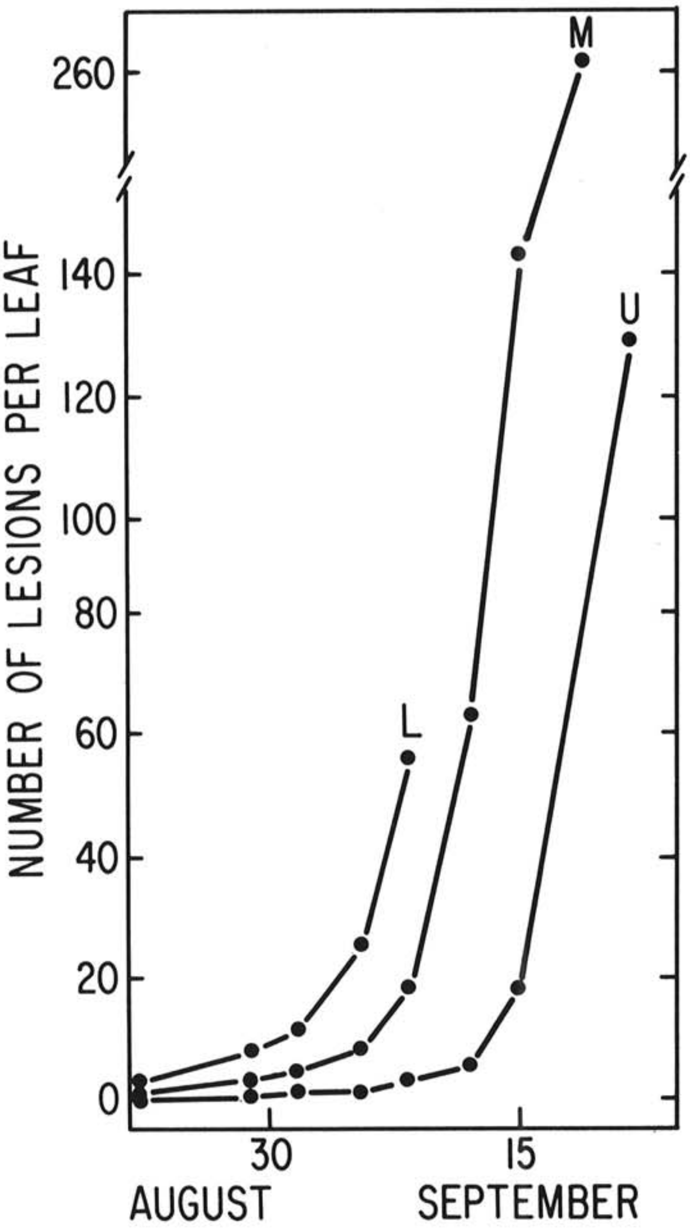

Fig. 4. Average number of gray leaf spot lesions per leaf on the lower (L), middle (M), and upper (U) leaves of corn hybrid Pioneer 3184 at Hazel Green, KY, from 22 August to 22 September 1979. Lower leaves were three leaves below the ear leaf, middle leaves were the ear leaf, and upper leaves were three leaves above the ear leaf. Each line ends when the corresponding leaf group died.

DISCUSSION

Environment has considerable influence on development of gray leaf spot of corn. Beckman et al (1) reported that while high temperatures and low rainfall were not limiting factors for gray leaf spot of corn, such factors as RH might be of importance in disease development. Locations where the disease was present (Quicksand

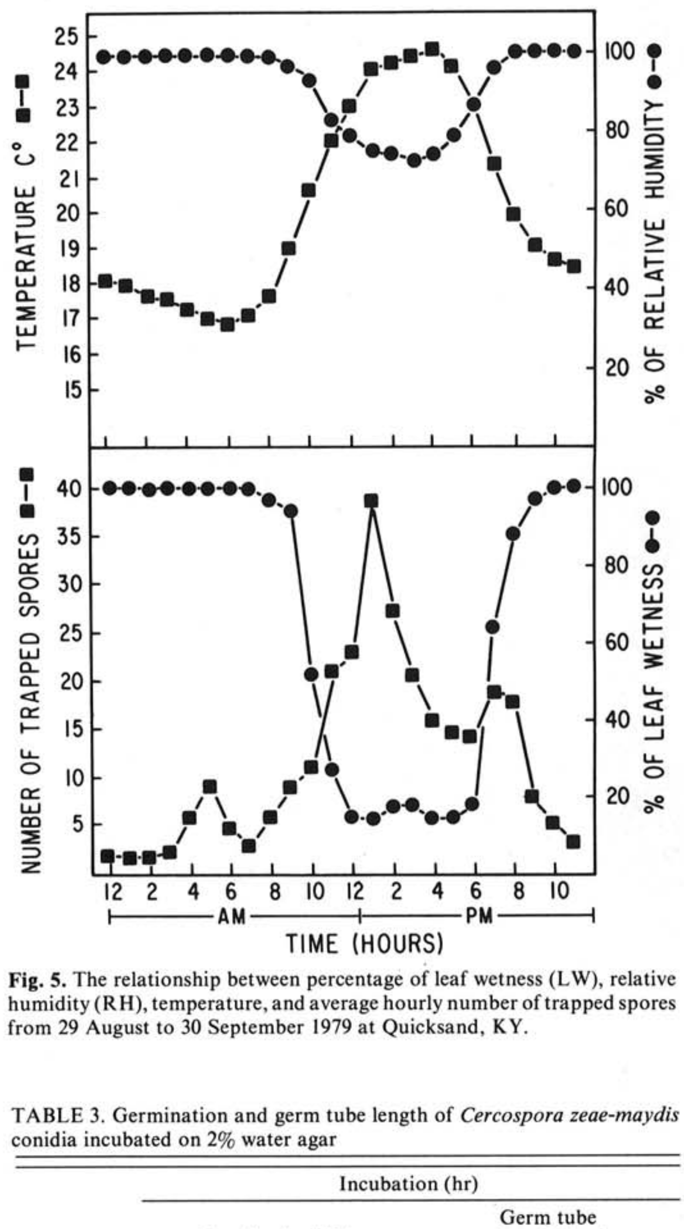

Fig. 5. The relationship between percentage of leaf wetness (LW), relative humidity (RH), temperature, and average hourly number of trapped spores from 29 August to 30 September 1979 at Quicksand, KY.

TABLE 3. Germination and germ tube length of *Cercospora zeae-maydis* conidia incubated on 2% water agar

Temperature (C)	Incubation (hr)								
	Germination[y] (%)					Germ tube length (μm)			
	2	6	10	15	24	6	10	15	24
10	1	22 c[z]	64 b	65 c	70 b	12 b	15 d	32 d	43 c
15	3	50 b	78 a	83 b	81 a	26 b	47 c	63 c	95 b
18	9	70 a	86 a	92 a	96 a	40 a	70 b	92 b	192 a
25	11	74 a	89 a	92 a	96 a	48 a	77 a	116 a	199 a
30	3	43 b	65 b	76 b	83 b	20 b	34 c	47 c	100 b
35	2	22 c	63 b	66 c	61 c	14 b	26 d	32 d	58 c

[y]A spore was considered germinated if the length of the germ tube was at least as long as the width of the spore.

[z]Numbers followed by the same letter are not significantly different, according to Duncan's multiple range test ($P = 0.05$).

and Hazel Green) almost every day from 1 July to 30 September had 12–13 hr of RH >90% and 11–13 hr of LW. At locations without gray leaf spot (South Farm and Spindletop), similar periods of high RH and LW occurred much less frequently. Since temperatures were not, but moisture was, significantly different at the different locations, gray leaf spot epidemics appear to depend upon long daily periods of LW and high RH commonly found in mountainous areas of eastern Kentucky.

Periods of prolonged LW and high RH were frequent during 2 wk prior to the sharp increase in disease at both Quicksand and Hazel Green. During this time, daily low temperatures remained near 20 C and highs near 30 C. Spore germination data indicate that these conditions would allow maximum germination and germ tube growth. The data, therefore, suggest that these are ideal conditions for gray leaf spot epidemics.

The need for long daily periods of high RH and LW may relate to spore survival. In the desiccation experiments, even short periods of dryness on a glass slide killed almost all of the spores. This suggests that the spores must germinate and establish infection during a single period of wetness. When these experiments were repeated on live leaf tissue in the laboratory, spore survival increased; nevertheless, significantly fewer spores germinated when the moist period was interrupted or shortened. Restricted moist periods were shown to occur more frequently at South Farm and Spindletop.

In addition to environmental conditions, plant maturity influenced development of gray leaf spot. Hilty (4) has reported that while symptoms were induced in the greenhouse on 2- to 3-wk-old corn seedlings, initial symptom appearance in the field did not occur until anthesis. In our study, on all five plantings the initial symptoms also did not appear until plants were near anthesis. The disease first appeared on the lower, then the middle, and finally the upper leaves. For most plantings, a 3-wk delay in planting resulted in a 3-wk delay in symptom appearance in all three leaf groups.

Although there was not a 3-wk delay in initial symptom appearance between the second and third plantings, plant maturity still played a role in initial symptom appearance. Later plantings developed faster in the early stages than did earlier plantings; however, maturation slowed down during the later stages. This may explain the absence of a lag between the time of appearance of initial symptoms in plantings 2 and 3 on the lower leaves, but a 1- and 2-wk lag on the middle and upper leaves, respectively.

Alternate hypotheses to plant maturity as influencing the late-season development of gray leaf spot disease are a delay in the appearance of initial inoculum or the effect of plant canopy on the microclimate. While the formation of a closed canopy would have increased RH and LW for the lower leaves, it would have had much less effect on the upper leaves. However, the upper leaves not only showed symptoms, but disease progress was the same as on the middle leaves (Figs. 3 and 4). Also, in the 2 August planting the plants did not form a complete canopy; nevertheless, symptoms appeared and the disease progressed.

Delayed appearance of initial inoculum did not seem to delay disease development. Symptoms had already appeared on the lower, middle, and upper leaves of the 9 and 31 May plantings before symptoms appeared on the lower leaves of the 11 July planting. Also, lesions were already present on plants when the 2 August planting was established. However, symptoms did not develop on these plants until 51 days later.

TABLE 4. Germination and germ tube length of *Cercospora zeae-maydis* conidia incubated on corn leaf surfaces

	Experiment 1[w]		Experiment 2[x]	
Treatment[y]	Germination (%)	Germ tube length (μm)	Germination (%)	Germ tube length (μm)
Wet 15 hr	86 a[z]	81 a	58 a	68 a
Dry 1 hr wet 15 hr	41 b	...	24 b	67 a
Dry 6 hr wet 15 hr	...	...	23 b	52 a
Dry 12 hr wet 15 hr	13 c	...	30 b	70 a
Dry 24 hr wet 15 hr	9 c	...	...	...
Wet 9 hr	66 ab	39 a	52 a	62 a
Wet 9 hr dry 1 hr wet 6 hr	56 b	48 a	61 a	55 a
Wet 9 hr dry 12 hr wet 6 hr	...	...	36 b	56 a

[w] Inoculum = 10^4 conidia per milliliter.
[x] Inoculum = 1.8×10^5 conidia per milliliter.
[y] Following application of inoculum, leaves were allowed to remain wet and/or to dry at room temperature for the times indicated.
[z] Numbers in a column followed by the same letter are not significantly different, according to Duncan's multiple range test ($P = 0.05$).

The data presented in this study strongly suggest that plant maturity is an important factor in the late season development of gray leaf spot of corn. Environmental conditions are also very important for the development of this disease, restricting it to areas where long daily periods of high RH and LW occur, as in the southern Appalachian region. Gray leaf spot may be expected to be a major corn disease problem wherever these conditions of long daily periods of LW and high RH occur.

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