Controlled-Environment Studies of the Epidemiology of Yellow Leaf Blight of Corn

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ABSTRACT

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The effects of environmental factors on the development of *Phyllosticta maydis* on corn *(Zea mays)* leaves were determined in controlled-environment tests. Optimum temperature for conidial germination ranged from 12 to 18 C for times of 4 to 5 hours. The average number of infections was greater at temperatures from 16 to 20 C with 9- to 12-hour dew periods. Colonization, as determined by lesion size, was greatest at 21 to 27 C after 14 to 16 days. Largest numbers of pycnidia were produced at 18 to 27 C after 4 to 5 days; however, the optimum temperature for pycnidia formation was 18 C from 7 through 11 days. Greatest spore release (via oozing from pycnidia) occurred at 24 to 27 C from 4 through 9 days. More pycnidia were produced at all temperatures and times from lesions which developed at 18 C than from those which developed at 24 C.

Additional key words: Phyllosticta maydis, Mycosphaerella zeae-maydis, maize.

Yellow leaf blight of corn (*Zea mays* L.), which is caused by *Phyllosticta maydis* Arny and Nelson (1) [perfect stage, *Mycosphaerella zeae-maydis* Mukunya and Boothroyd (10)], first was reported in Wisconsin and Ontario, in 1967 (7, 14). In the following years the disease was discovered in much of the northeastern and north central corn-growing regions of the United States and in Southeastern Canada.

Corn inbreds and hybrids exhibit various degrees of resistance to the yellow leaf blight organism. Lines with Texas cytoplasmic male sterility (Tcms) and other male-sterile cytoplasms of the T Group (4) generally are much more susceptible than are these same lines in normal cytoplasm (2, 3, 12).

Dissemination of the pathogen primarily is by rainsplash and wind. Disease increase is greatest during the seedling stage and after tasseling. In general, these periods coincide with the cooler and wetter portions of the growing season. This information, along with its common occurrence in the cooler corn-growing areas of North America suggest that yellow leaf blight is a "cool weather" disease. Observations indicate that minimum tillage may increase disease severity, especially in instances where corn follows corn, since debris from infected plants may furnish large quantities of inoculum which can be readily splashed and blown to plants growing near the debris. Other than these general observations, little is known about the specific effects of environment on the development of *P. maydis*.

Accurate knowledge of the effects of environmental factors on disease development is essential for devising effective control measures. The research reported here was designed to determine the effects of certain environmental factors on infection, colonization, and sporulation under controlled-environment conditions.

MATERIALS AND METHODS

A commercial corn hybrid, Doebler's 57X Tcms, susceptible to *P. maydis,* was used in all of the experiments. Plants were grown in 11-cm diameter plastic pots in a greenhouse in a soil-peat-perlite (1:1:1, v/v) soil mix. Radiant intensity was measured with an Eppley Precision Pyranometer with a 281 nm cut-off filter. Isolate 73-2 of *P. maydis,* originally collected in central Pennsylvania, was cultured on potato-dextrose agar at 21 C for 7 to 10 days prior to use. Cultures were grown 40 cm below two 120-cm General Electric (GE) cool-white fluorescent lamps. Radiant intensity ranged from 1.4 to 1.6 mW cm^{-2}. Inoculum was prepared by flooding cultures with distilled water and filtering the spore suspension through cheesecloth.

Spore germination.—Experiments were conducted at three-degree intervals from 12 to 27 C. Water droplets containing 10^3 spores per milliliter were placed on thin sections of water agar on microscope slides. Slides were placed in petri plates to maintain a high relative humidity and incubated in unlighted, controlled-temperature

cabinets. Water-agar sections, glass slides, and petri plates were placed in controlled-temperature cabinets 15 minutes prior to placing the spore suspension on the agar surface. Spores were considered as germinated when the germ tubes were twice as long as the width of the spores. Percentage of germination was determined at 30-minute intervals from the first through the fifth hour. Three microscope fields, chosen at random, were used to measure percentage germination for each treatment. The entire experiment was repeated three times.

Plant infection.—Experiments were carried out in Percival Dew Chambers Model DC-20 and ISCO Growth Chambers Models E2 and E3A (Percival Refrigeration and Manufacturing Company, Boone, Iowa, and Instrumentation Specialties Company, Inc., Lincoln, Nebraska, respectively). Experiments were conducted at two-degree intervals from 12 to 28 C and at dew periods ranging from 3 to 12 hours. Plants at the three- to four-leaf stage were inoculated with a spore concentration of 2.5×10^3 spores per milliliter of water. Plants were placed in dew chambers for 15 minutes prior to inoculation to obtain a thin film of moisture on the leaf surfaces and were inoculated by spraying approximately 40 ml of inoculum over all leaves. Inoculated plants were placed in unlighted dew chambers and removed hourly from the third through twelfth hour. Plants then were placed in unlighted growth chambers where the leaves dried in approximately 10 minutes. After the 12th hour all plants were incubated at 20 C for 2 to 5 days in a growth chamber operating on a 12-hour, day-night cycle. The growth chambers were equipped with 12 120-cm GE cool-white, Power-Groove fluorescent lamps and three GE 100 W, 120 V incandescent lamps. Radiant intensity at 45 to 55 cm from the lamps ranged from 17.8 to 19.8 mW cm^{-2}. Infection was determined by removing the upper two leaves from each plant and counting all infection points in a microscope field 1 cm in diameter midway between the base and tip of the leaf. The resulting counts were averaged to give an overall treatment mean. All treatments were repeated three times.

Colonization.—Experiments to determine lesion size were conducted at three-degree intervals from 15 to 27 C in Uni-Therm growth cabinets (Puffer-Hubbard Refrigeration Division, Grand Haven, Michigan). Plants at the two- to three-leaf stage were inoculated with a spore concentration of approximately 200 spores per milliliter of water. Inoculated plants were placed in dew chambers at 20 C for 12 hours and then in growth chambers operating on a 12-hour, day-night cycle. The cabinets

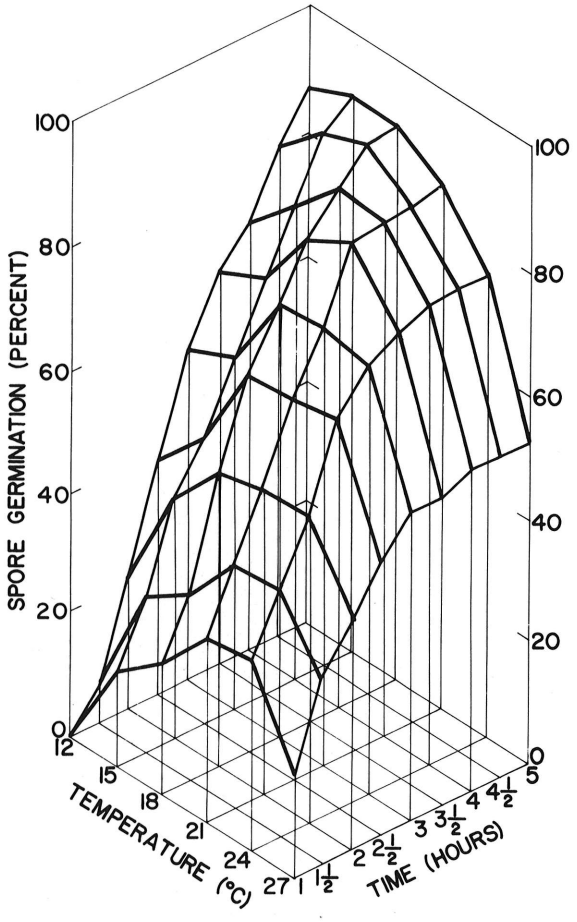

Fig. 1. Germination (percent) of *Phyllosticta maydis* conidia in moist chambers at several combinations of temperature (C) and time (hours).

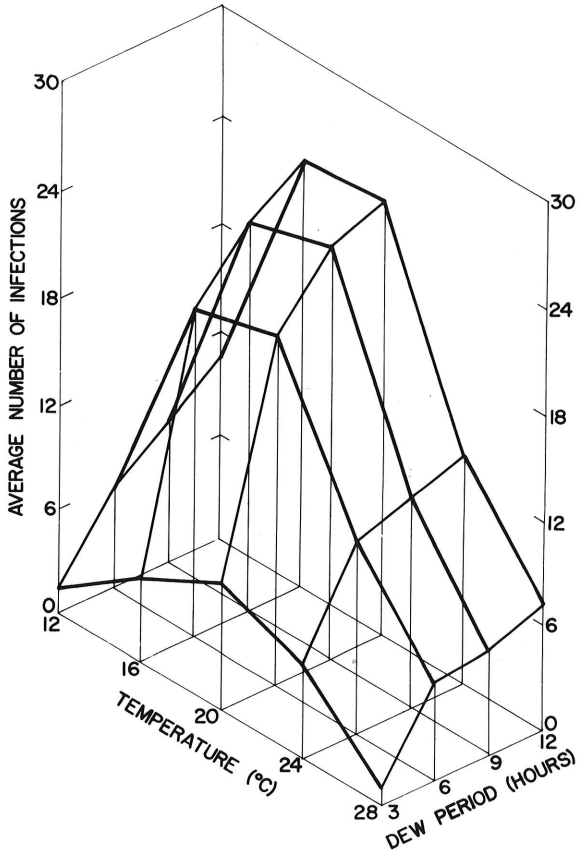

Fig. 2. Average number of infections produced per 0.8 cm^2 of leaf surface on seedling Texas cytoplasmic male-sterile corn by a given number of *Phyllosticta maydis* conidia at several combinations of temperature (C) and dew period (hours).

were equipped with two 60-cm GE cool-white fluorescent lamps. Radiant intensity at 30 to 40 cm from the lamps ranged from 0.9 to 1.1 mW cm^{-2}. The length and width of 25 lesions were measured 4 days after inoculation and measurements on the same lesions were repeated every 2 days for a period of 12 days. A relative measure of lesion size was calculated by multiplying the length times width. All treatments were repeated twice.

Sporulation.—Plants were inoculated as described above for the colonization experiments and placed in lighted growth cabinets at 18 and 24 C for 10 days. Individual lesions were excised, placed on moistened filter paper in petri plates, and incubated in unlighted controlled temperature cabinets at three-degree intervals from 12 to 27 C. The number of mature pycnidia, based on size and color, and the number of pycnidia with oozing spores were recorded at 1- to 2-day intervals from the 4th through 11th days. The entire experiment was repeated four times.

Analysis of variance techniques were used to determine if means were significantly different. Standard data transformations were used when necessary to increase the homogeneity of variance (13). Spore germination and spore-release data were transformed to arcsine values, plant-infection data were transformed to square roots, and colonization data were transformed to natural logarithms. Analysis of variance techniques were performed on the transformed data. Means were separated using Duncan's Modified (Bayesian) Least Significant Difference Test (DLSD) (6).

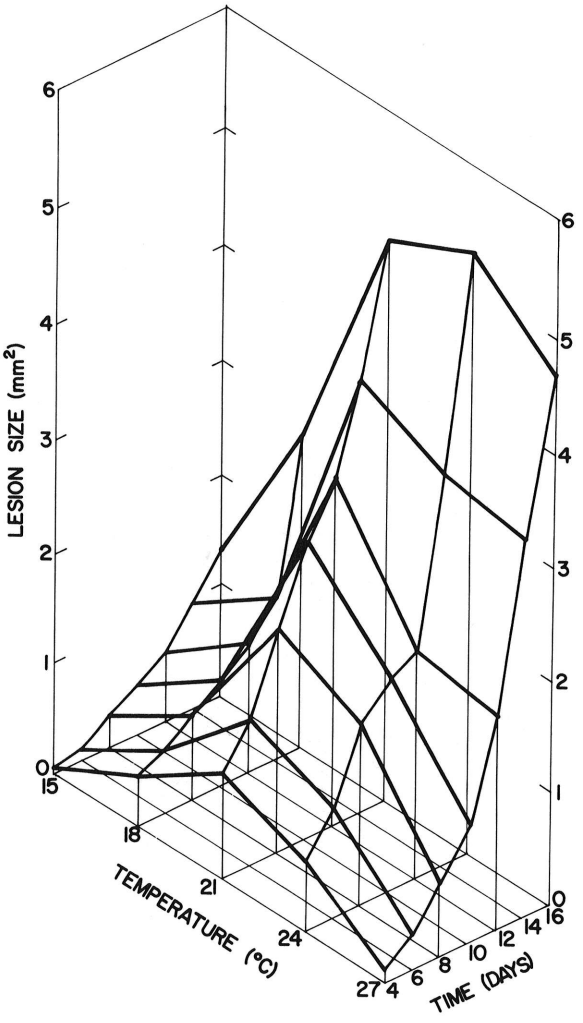

Fig. 3. Size (mm^2) of *Phyllosticta maydis* lesions on seedling Texas cytoplasmic male-sterile corn leaves at several combinations of temperature (C) and time (days).

RESULTS

Spore germination, infection, and colonization.—Ninety to 95% of the spores germinated within 5 hours at the more favorable temperatures (12, 15, and 18 C). Preliminary experiments showed that few spores germinated before 1 hour or that few additional spores germinated after 5 hours. Maximum germination was time- and temperature-dependent and ranged from 50% at 27 C to 90% at 15 and 18 C (Fig. 1). Percentage germination was significantly greater at 12 to 24 C than at 27 C with time periods of 4 hours or more. However, more spores germinated at 18 to 24 C than at lower or higher

TABLE 1. Statistical comparison at six temperatures of the average number of *Phyllosticta maydis* pycnidia produced per 0.8 cm^2 of lesion surface in a moist chamber. Lesions developed on seedling Texas cytoplasmic male-sterile corn leaves from plants growing at 18 and 24 C

Temperature for production of pycnidia (C)	Average number of pycnidia[a]		
	From lesions which developed at 18 C		From lesions which developed at 24 C
12	62.4		48.6
15	77.4		65.9
18	101.5	**[b]	84.3
21	92.2	**	76.4
24	85.4		81.4
27	83.4	**	74.6

[a]Average of five time periods.
[b]Asterisks ** indicate that the two row means are significantly different, $P = 0.01$.

TABLE 2. Statistical comparison at five times of the average number of *Phyllosticta maydis* pycnidia produced per 0.8 cm^2 of lesion surface in a moist chamber. Lesions developed on seedling Texas cytoplasmic male-sterile corn leaves from plants growing at 18 and 24 C

Time for production of pycnidia (days)	Average number of pycnidia[a]		
	From lesions which developed at 18 C		From lesions which developed at 24 C
4	46.8		46.1
5	74.7	*[b]	63.6
9	89.9	**	74.2
7	99.8	**	87.4
11	107.1	**	90.5

[a]Average of six temperatures.
[b]Asterisks * or ** indicate that the row means are significantly different, $P = 0.05$ or 0.01, respectively.

temperatures with germination periods of 3.5 hours or less.

The number of infections was greater at 16 to 20 C than at 12, 24, and 28 C (Fig. 2). (Only selected temperatures and times are shown in Fig. 2 resulting in a simpler and more understandable figure without sacrificing accuracy in the presentation of data.) Few infections occurred when dew periods were less than 3 hours and little further increase occurred after 12 hours of dew at any temperature. The number of infections was reduced significantly at dew periods of 3 hours or less.

Lesion size increased very slowly at 12 C, based on preliminary experiments, and at 15 C (Fig. 3). Significantly larger lesions developed at 21 and 24 C than at 15, 18, and 27 C from 4 through 10 days. Similarly, significantly larger lesions developed at 21 to 27 C than at 15 and 18 C after 14 and 16 days. Lesion increase followed a generally linear pattern for the first 10 days at all temperatures.

Sporulation.—Significantly greater numbers of pycnidia were produced at 18 to 27 C than at 12 and 15 C after 4 to 5 days (Fig. 4). Similarly, after 11 days more pycnidia had formed at 18 to 24 C than at 12, 15, and 27 C. The number of pycnidia produced at 18 C was significantly greater than at 12 and 15 C at all time periods. There was no significant increase in the number of pycnidia produced from 9 to 11 days at any temperature.

The number of pycnidia produced in lesions was influenced by the temperature during lesion development and by the temperature during pycnidia formation (Table 1). Significantly greater numbers of pycnidia were produced at 18, 21, and 27 C from lesions that developed at 18 C than from lesions that developed at 24 C. There were no significant differences in the numbers of pycnidia produced at 12, 15, and 24 C from lesions which developed at 18 and 24 C. Significantly larger number of pycnidia were produced from lesions that developed at 18 C than at 24 C over time periods of 5 to 11 days (Table 2).

Percentage spore release (via oozing from pycnidia) was significantly greater at 24 and 27 C than at 12 to 21 C from 4 through 9 days (Fig. 5). There were no significant differences in the percentage of oozing pycnidia which occurred at 15, 18, 24, and 27 C after 11 days. Not more than 65% of the pycnidia released spores under the conditions of this study.

The temperature of lesion development was not an important influence on spore release. Spore release was similar from pycnidia produced at 12, 15, 18, 24, and 27 C whether lesions developed at 18 or 24 C; release at 21 C was significantly higher for pycnidia from lesions which developed at 18 than at 24 C. Significantly greater spore release occurred from lesions which developed at 24 C as opposed to 18 C at 4 days and from lesions which developed at 18 C as opposed to 24 C at 11 days. The

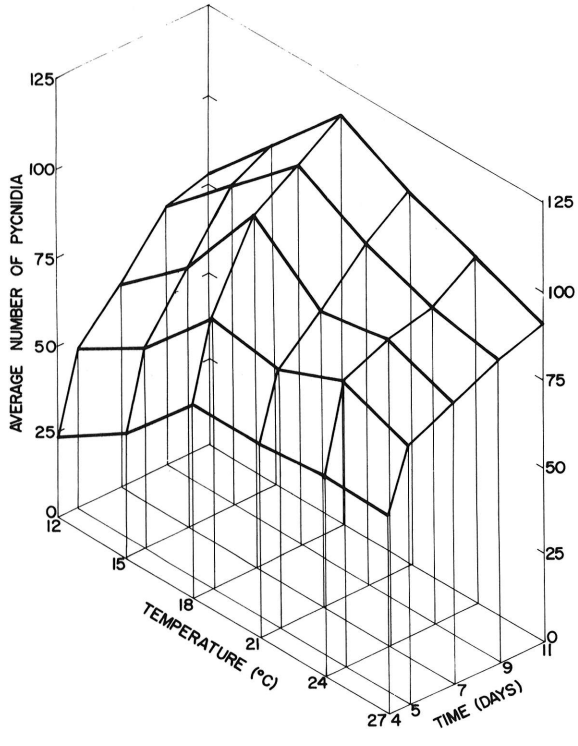

Fig. 4. Average number of *Phyllosticta maydis* pycnidia produced per 0.8 cm^2 of lesion surface in moist chambers at several combinations of temperature (C) and time (days) from lesions which developed on seedling Texas cytoplasmic male-sterile corn leaves at 18 and 24 C.

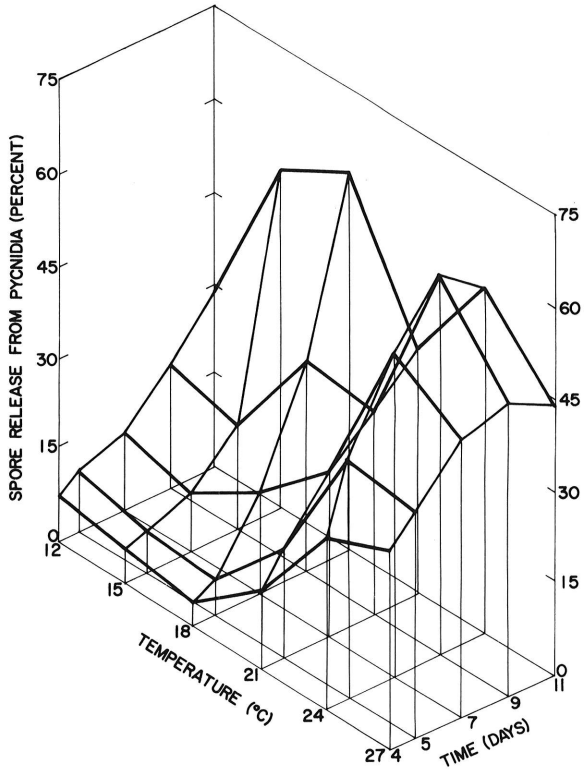

Fig. 5. Spore release (pycnidial oozing) (percent) from *Phyllosticta maydis* pycnidia produced in moist chambers at several combinations of temperature (C) and time (days) from lesions which developed on seedling Texas cytoplasmic male-sterile corn leaves at 18 and 24 C.

percentage of spore release from lesions which developed at 18 and 24 C was similar at time periods from 5 through 9 days.

DISCUSSION

Although *P. maydis* can infect susceptible corn lines after short periods of dew at 12 to 28 C, more infections (and thus the potential for more disease) will occur at 16 to 20 C if the dew period is not limiting. In general, with longer time periods spore germination and infection were favored by lower temperatures (12 to 20 C), and colonization and sporulation were favored by higher temperatures (20 to 27 C). Disease still could occur under less favorable temperatures and times, although in smaller amounts.

The number of pycnidia produced and the percentage of pycnidia that release spores are influenced by the temperature at which the lesions developed. In general, more pycnidia were produced from lesions which developed at 18 C than from lesions which developed at 24 C. This phenomenon may reflect the greater accumulation of soluble carbohydrates in tissues of corn plants grown at lower temperatures (5). Plants grown at lower temperatures could furnish more nutrients for fungal growth if the soluble carbohydrates could be utilized by the fungus. In addition, pycnidia produced at 27 C were slightly smaller and exuded conidia in a smaller droplet. Based on this observation, fewer spores may be produced and released per given number of pycnidia produced at 27 C than at lower temperatures. Thus, sporulation could be increased at lower temperatures if these phenomena operated under field conditions.

Pycnidia required several days to form, even at higher temperatures, and may function over extended periods of time. Only about 65% of the pycnidia released spores, even though all formed spores. This may be a unique feature of the pathogen which enables it to survive periods of unfavorable weather and to extend the time that mature spores are ready for release. Pycnidia also may retain conidia through the winter and release them in the spring when conditions are favorable, a phenomenon that occurs with *Physalospora obtusa* which causes frogeye leaf spot of apple (8).

The early part of the growing season typically is cool and rainy in the northern corn-producing areas of the U.S. These conditions appear optimum for sporulation, spore dispersal, and infection. Warm and dry conditions during the middle of the growing season would reduce infection and spore dispersal resulting in a greatly reduced rate of disease increase even though conditions are favorable for colonization. Environmental conditions are cool again in the latter part of the growing season, but may be dryer than in the early spring. Spore dispersal would be limited but the infection efficiency would be high. The plants would be senescing at this time and colonization could be rapid despite the cooler temperatures.

The early part of the growing season is the critical period for disease development. The amount of initial inoculum and the environmental conditions determine the amount of disease which develops during this period. Large amounts of inoculum and a high infection efficiency resulting from a cool, rainy environment would increase the levels of disease. Thus, any attempt to control the disease would involve measures which reduced or eliminated the initial inoculum, such as plowing under debris from infected plants.

Yellow leaf blight and southern corn leaf blight *(Helminthosporium maydis* Nisikado and Miyake race T) clearly illustrate the influence of changing technology on the occurrence and severity of plant diseases. The increased use of susceptible Tcms lines, coupled with favorable environmental conditions, allowed yellow leaf blight to become an important disease during the late 1960's. Fortunately, the rapid return to resistant lines containing normal cytoplasm was effective in controlling yellow leaf blight although susceptible inbreds are still being used in the production of commercial hybrids. Most commercial lines presently available in the northern U.S. have excellent resistance to *P. maydis*. However, a recent report has shown that the future use of Tcms may be feasible with sufficient nuclear-genic resistance to race T of *H. maydis* (9). Suitable screening for resistance of lines to *P. maydis* and the incorporation of nuclear genes for resistance (11) when necessary should be given the utmost priority if the use of Tcms is attempted.

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