Eyespot (*Kabatiella zeae* Narita and Hiratsuka) Disease Progression Curves in Ten Maize Hybrids[1]

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ABSTRACT

Ten commercial maize hybrids were planted in isolation plots at two different locations. The development of eyespot disease caused by *Kabatiella zeae* was followed throughout the growing season following artificial inoculation with fungus in mid-June. The number of lesions on a lower (5th or 6th), a middle (10th) and an upper (15th) leaf of ten plants per plot was estimated weekly and converted to a proportion of diseased tissue per leaf (X). The data were transformed using the Gompertz equation, and the k values (slopes) obtained by the regression analysis of $-\ln(-\ln x)$ over time were compared for the ten hybrids. Lesion diameter and number of conidia produced per lesion were determined also. The rate of increase of eyespot (k) differed among genotypes and among leaves. The middle leaves (older) were more susceptible than the upper leaves (younger) during most of the growing season. Larger k values were found for the more susceptible hybrids. The k value for leaf 10 varied from 0.008 (for H99 x A632) to 0.031 (for W64A x W117). The disease developed slowly on the upper leaves early in the season, but late in August, it developed rapidly in some hybrids. For example, B84 x B73 had an initial k value of 0.007 that increased to 0.129. However, W153 x A632 increased from k = 0.0116 to 0.04. This change in disease severity late in the season was not closely related with environmental conditions and occurred when plants had reached their physiological maturity. The k values, lesion diameter and sporulation per lesion were related; the more susceptible genotypes had the largest lesions and produced most conidia per lesion. Early maturity and eyespot susceptibility were also related.

COMPENDIO

Diez híbridos comerciales (B84 x B73, B84 x Mo17, A662 x CM7, B73 x Mo17, W64A x W117, A619 x A632, W153R x A632, W64A x B14A, W64A x CM105, H99 x A632) de maíz fueron plantados en parcelas aisladas en dos sitios. El desarrollo de la enfermedad se siguió a través de todo el ciclo del cultivo, luego de inocular artificialmente todos los genotipos con *K. zeae*. Se estimó semanalmente el porcentaje de tejido enfermo (x) en diez plantas por parcela y se transformó usando la ecuación de Gompertz. Los valores de k (tasa de incremento de la enfermedad) se obtuvieron mediante el análisis de regresión de $-\ln(-\ln(x))$ en el tiempo y fueron comparados para los diez híbridos.

Los valores de k variaron estadísticamente entre los diez híbridos y de acuerdo a la posición de la hoja en la planta. Las hojas más viejas fueron más susceptibles que las hojas superiores más jóvenes durante la mayor parte del desarrollo de las plantas. Los valores superiores de k en las hojas intermedias correspondieron a los híbridos más susceptibles, estos valores variaron de 0,008 (H99 x A632) hasta 0,031 (W64A x W117). En la parte superior de las plantas, la enfermedad se desarrolló lentamente al inicio, sin embargo, en algunos genotipos aumentó explosivamente al final del ciclo del cultivo. Por ejemplo, en el híbrido B84 x B73, k aumentó desde un valor inicial de 0,007 hasta k = 0,129. Este cambio en la severidad de la enfermedad no estuvo claramente relacionado con el cambio en los factores ambientales. Los valores de k, el diámetro de las lesiones y la esporulación/lesión estuvieron correlacionados: los híbridos más susceptibles tenían las lesiones de mayor tamaño y permitieron una mayor esporulación/lesión. Los híbridos de madurez tardía fueron más resistentes a la enfermedad que los de madurez temprana.

INTRODUCTION

Eyespot of maize caused by *Kabatiella zeae* has become a potentially serious problem to maize culture since report for the first time in Japan 1956 (1, 3, 13, 14). Favorable conditions for the disease, such as cool, humid weather and the presence of susceptible genotypes, are common to many of the maize producing areas where the disease appears to have become endemic (1, 3, 5, 11, 14).

Most of the information available on eyespot consists of reports of the disease in several countries; epidemiological data is scarce, particularly on the development of the epidemic over time in different host genotypes (1, 3, 4, 5, 11, 13, 14). This information is important because it may lead to a better understanding of the disease and to the development of more appropriate control measures.

The purpose of this study was to determine the pattern of disease development over time in ten commercial maize hybrids after artificial inoculations with *Kabatiella zeae*.

MATERIALS AND METHODS

The experiments were carried out during 1981 at two sites near Ames, Iowa. Ten corn hybrids were planted in isolation plots that were arranged in a completely randomized block design with three replications. Each plot consisted of four rows that were 10 m long and 76 cm apart.

1 Received for publication on May 19, 1987.
Portion of a thesis submitted to the Graduate College of Iowa State University as a partial fulfillment of the requirements for the M.Sc. degree. The author is grateful to Dr. C.A. Martinson for his help and advice in the realization of this work.

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The plants in each row were separated by about 25 cm. Plots were bordered by 3 m and 12 m of the resistant hybrid Mo17 x B73 (11).

Inoculum production and inoculation

Inoculum of *K. zeae* was increased using a procedure developed by C.A. Martinson (Eyespot inoculum production, Iowa State University, Ames, Iowa, 1981. Personal communication). Inoculum was produced on potato-dextrose-broth that was poured into 500 ml flasks and autoclaved. The flasks were seeded with a plug of a young PDA grown culture of *K. zeae* and shaken for two weeks on a New Brunswick RB-25 reciprocal shaker at room temperature (23-25°C).

The contents of each flask (mostly stroma) was centrifuged at 16.300 x g for three minutes and the residue was blended and diluted to about 23 000 pieces of stromatic hyphae/ml (about 8-50 cells each).

Plants at the fourth leaf stage were inoculated by spraying about 20 ml of inoculum into the whorl. One week later, the plants were reinoculated with stromatic hyphae prepared as before, but dried and mixed with a fine quartz sand (Martin Marietta Grade 37). The contents of each flask was mixed with 2 kg of sand. The sand-inoculum was poured into the whorl of the plants (1 g/plant)

Disease progression curves

The number of lesions/leaf was estimated weekly (Table 1) on three leaves (a lower (6th-7th), middle (10th) and upper (15th) leaf) on ten plants in the two central rows of each plot. The same leaves on the same plants were sampled throughout the experiment. The amount of disease was standarized as number of lesions/100 cm^2 of leaf area. The proportion of diseased tissue was estimated as the ratio of number of lesions/100 cm^2 of leaf area and the maximum possible number of lesions in 100 cm^2. The maximum possible number of lesions was established to be about 315 lesions/100 cm^2 for the lower leaves and 1286 for the middle or upper leaves. The leaf area was estimated using the equation described by Mulamba and Mock (14).

Data analysis

The logistic equation can be used to describe the progress over time of many foliar diseases and is commonly used in epidemiological studies (2, , 8, 9, 10, 15). However, there has been considerable criticism of the use of this equation to describe all polycycle-type diseases (2, 7, 8, 9, 10, 15). Gompertz developed a function that was introduced into the plant pathology literature by Berger R (2). The function derives from the differential equation $dy/dt = ky(\ln 1 - \ln y)$, where Y is the proportion of diseased tissue, t is time and k represents the rate of disease

Table 1. Rating scale used to estimate lesions of eyespot disease of maize per leaf.

Class	Lesions/leaf		
	Class interval	Class midpoint	Increment of increase
0	0	0	0
1	1 – 10	5	5
2	11 – 25	18	13
3	26 – 50	38	20
4	51 – 100	75	37
5	100 – 200	150	75
6	200 – 400	300	150
7	400 – 700	550	250
8	700 – 1 100	900	350
9	1 100 – 1 600	1 350	450
10	1 600 – 2 200	1 900	550
11	2 200 – 3 000	2 600	700
12	3 000 – 4 600	3 800	1 200
13	4 600 – 7 000	5 800	2 000
14	7 000 – 9 000	8 000	2 200

increase over time and is equivalent to the apparent infection rate *sensu* Van der Plank (2, 8, 9). The equation can be integrated to yield $Y = \exp(-B \times \exp(= kt))$, which can be linearized to $-\ln(-\ln Y) = -\ln B + kt$, where the term $-\ln B$ describes the amount of diseased tissue at time zero.

In this experiment the data on disease proportion for randomly-picked replications of each genotype were analyzed initially using both the logistic (15) and the Gompertz (2) functions. Both equations seemed to fit the data reasonably well, but in most cases the initial data point fit the regression line much better with the Gompertz transformation than with the logistic transformation. Further analysis of residuals from the predicted regression lines showed a better fit of data to the Gompertz equation. Therefore this equation was used to transform the data on proportion of disease on the different leaves of the ten hybrids. Individual regression analysis was done for each hybrid by replication. The k values obtained as the regression coefficient of $-1n(-1n(y))$ over time were compared to determine differences among genotype reactions to the eyespot disease.

Determination of lesion diameter and sporulation potential

Data on diameters of the necrotic area of the lesions were taken on the tenth leaf of five plants per plot eleven weeks after the first inoculation at one location.

The number of conidia produced per lesion was estimated twelve weeks after the first inoculation and again nine days later. For the first reading, only five hybrids were sampled. Sufficient pieces of leaves were cut from the tenth or eleventh leaf to yield 100 young lesions per sample (one sample per plant and five plants per plot). The pieces were washed with a 0.1% solution of polyoxyethylene sorbitan monolaurate (Tween 20) and gently brushed with a camel hair brush to remove spores already formed. Each sample was placed on moist filter paper in a petri dish at room temperature for 48 hours. The spores produced were then washed with a known volume of water containing five drops to one of Tween 20. The concentration of spores was calculated with an haemacytometer and expressed as the number of spores per lesion. The second set of samples was taken from the twelfth to thirteenth leaf of all ten hybrids but from only one replication in the field.

RESULTS

Disease progression curves

Symptoms of eyespot appeared nine days after the first inoculation in all ten hybrids. Despite the uniformity in the incubation period, the severity of the disease varied by genotype. Hybrids A662 x CM7 and W64A x W117 were the most affected, while H99 x A632 and B84 x B73 had only a few lesions per leaf (Table 2). The presence of necrosis due to causes other than eyespot (physiological aging and inadequate soil moisture early in the season) made the estimation of disease severity in the lower leaves difficult and not totally reliable. Clear differences among genotypes, however, appeared early in the season (Table 2).

The disease increased very slowly in the middle leaves of H99 x A632 and at an intermediate rate in B84 x B73, A619 x A632 and B73 Mo17. The hybrid

Table 2. Number of lesions on the 6th or 7th leaf nine days after inoculation of ten corn hybrids with stromatic hyphae of *Kabatiella zeae* (actual lesions counts) on two locations.

Hybrid	Site 1	Site 2	$\bar{X}$
W64A x W117	83	97	90 a[2]
A662 x CM7	87	84	86 a
W64A x B14A	55	50	53 b
W153R x A632	49	41	45 bc
W64A x CM105	40	42	41 bc
B84 x Mo17	43	36	40 bc
A619 x A632	21	50	36 bcd
B73 x Mo17	27	39	33 bcd
H99 x A632	35	25	30 cd
B84 x B73	20	26	23 d

1. Mean of three replications
2. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

H99 x A632 ended the season with only about 7% of diseased tissue. Conversely W64A x W117 and A662 x CM7 ended the season with a high percentage of diseased tissue, and much of the middle canopy died prematurely, presumably due to the severity of the attack (Fig 1). The development of the disease followed similar patterns at the two planting sites, and there was no statistical evidence of a location/hybrid interaction. The ranking of the ten hybrids based on the rate of disease increase over time (k values) on the middle leaf closely followed the susceptibility of these varieties during much of the growing season (Table 3).

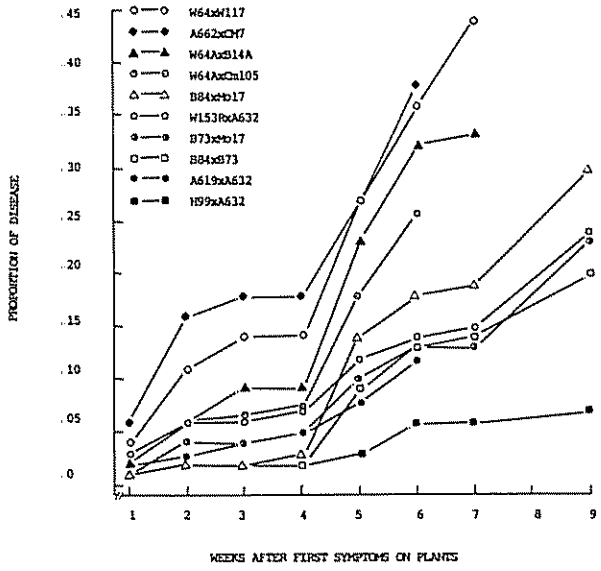

Fig 1. Proportion of eyespot disease at various reading dates on the middle leaf of 10 maize hybrids (data for one planting site).

Eyespot disease progressed very slowly in the upper leaves of the plants during the first weeks of the epidemic. When plants reached maturity, the disease spread rapidly, although not at the same rate in all genotypes (Fig. 2). The level of disease changed drastically after the seventh week of observations on B84 x B73, B84 x Mo17 and W64A x W117. The first two hybrids reacted strongly, even though they had looked moderately resistant up to that time. A single k value was considered inappropriate to describe the development of the disease in the upper leaves, so one value was calculated for each of the two parts of the disease progress curve (DPC). The seventh week was considered to be the inflection point of the curve, separating two distinct phases of the epidemic (Fig 2.) The two slopes were calculated using a regression analysis described by Draper and Smith (6).

The k values for the second phase of the DPC were much higher than those for the first phase (Table 3). Hybrids B84 x Mo17 and B84 x B73 had the largest change in the k values. For two hybrids, W153R x A632 and H99 x A632, the k value increased very little late in the season. The hybrid A662 x CM7 matured very early, due perhaps to a severe attack by the disease, and therefore it was not possible to gather data for the second phase of the epidemic.

Sporulation and lesion size

During the first reading on spore production, the number of conidia per lesion estimated for W64A x W117 was five times greater than for H99 x A632.

Table 3. Mean values for k (slopes) for increase of eyespot disease on the middle and upper leaves of the maize hybrids (combined analysis with data from two locations).

Hybrid	Middle leaf	Upper leaf, through week 7	Upper leaf; after week 7
W64A x W117	0.0307 a[1]	0.0252 ab	0.1018 b
W64A x B14A	0.0296 a	0.0209 bcd	0.0630 cd
A662 x CM7	0.0289 ab	0.0294 a	–
W64A x CM105	0.0275 ab	0.0246 ab	0.0502 d
B84 x Mo17	0.0254 b	0.0188 cd	0.1275 a
B84 x B73	0.0196 c	0.0072 f	0.1294 a
B73 x Mo17	0.0178 c	0.0124 e	0.0833 bc
A619 x A632	0.0173 c	0.0223 bc	0.0745 c
W153R x A632	0.0155 c	0.0166 de	0.433 d
H99 x A632	0.0081 d	0.0073 f	0.0457 d

1 Mean of six observations. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

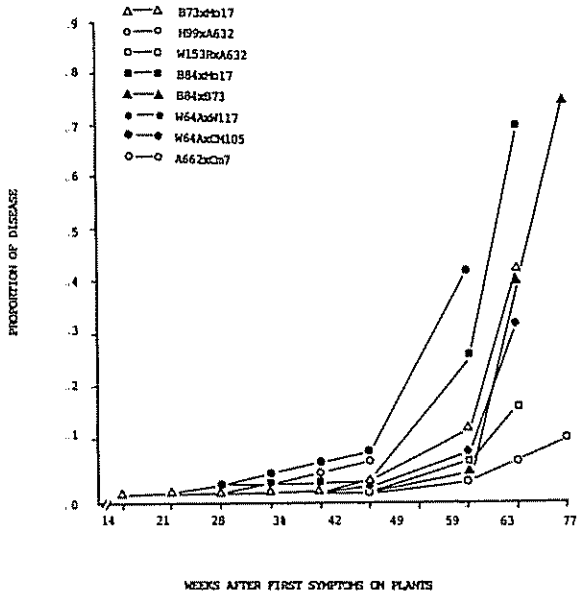

Fig. 2. Proportion of eyespot disease at various readings dates on the upper leaves of 10 maize hybrids (data for W64AxB14A and A619 xA632 were similar to W64xM105).

Considerably fewer spores were produced on A619 x A632 than on the more susceptible genotypes; these differences, however, were not significant (Table 4).

When spore production was evaluated for the second time, a striking change had occurred in the severity of the disease in some genotypes; these same hybrids also supported the strongest spore production.

Hybrids H99 x A632, W64A x CM105, W64A x B14A and W153R x A632 revealed the lowest production of conidia per lesion (Table 4).

The diameter of the necrotic center portion of the lesions was measured during mid-season and reflects the response of the hybrids before the disease changed in severity late in the season. The smallest lesions were found on the leaves of B84 x B73 and B84 x Mo17 and those hybrids containing A632 germplasm (Table 4).

DISCUSSION

The rate of increase of a disease over time is a good indicator of the degree of resistance of a

Table 4. Conidia produced per lesion *in vitro* and diameters of the central necrotic portion of lesions of eyespot disease in 10 maize hybrids.

Hybrid	Rating[1]	Conidia/lesion		Lesion diameter[2] Aug. 17
		Aug. 20[3]	Aug. 29[4]	
B84 x B73	R	484 a	2 200 a	1 02 c
B84 x Mo17			1 712 ab	1 08 c
A662 x CM7			1 688 ab	1.37 ab
B73 x Mo17			1 463 b	1.26 b
W64A x W117	S	2 068 b	1 425 b	1.44 a
A619 x A632	I	965 b	1 400 b	1 11 c
W153R x A632			1 138 bc	1.11 c
W64A x B14A	S	1 787 b	1 138 bc	1.35 ab
W64A x CM105			488 cd	1.35 ab
H99 x A632	R	412 a	450 d	1 10 c

1. Disease rating prior to the August 20 samplin
R = resistant, S = susceptible, and I = intermediate.

2. Means of 3 replications; 50 lesions/replication; values in mm.

3. Means of 3 replications; 4 samples of 100 lesions/replication.

4. Means of 4 replications; each replication consisted of 100 lesions.

5. Means in the same column followed by the same letter are not significantly different, according to Duncan's Multiple Range Test (P = 0 05).

particular host genotype (2, 7, 8, 9, 10, 15). The development of eyespot disease during the growing season differed greatly among the ten hybrids studied; these differences were reflected in the rate of disease increase (k values), calculated using a regression analysis of the Gompertz transformed values of proportion of disease over time.

In general, it seemed that the middle (older) leaves of the plants were more susceptible to the disease than the upper (younger) leaves, at least during most of the growing season. The most resistant hybrid was H99 x A632. Its foliage remained green until very late in the season, because of its late maturity and a very low level of disease. The hybrid A662 x CM7 and the genotypes with W64A germplasm were the most susceptible. The inbred W64A has been considered very susceptible to eyespot disease (2, 11, 14). The other hybrids with A632 and B84 germplasm and B37 x Mo17 were less resistant than H99 x A632, but more resistant than the remaining five genotypes when judged on disease reaction in the middle leaves

The diseases developed very slowly on the upper leaves during the first weeks in all ten hybrids, but after the plants reached maturity, it became very pronounced in some hybrids. This behavior made it necessary to analyze the DPC in two separate phases. Differences among genotypes during the first phase were significant but small when compared with differences found after plants matured. The two hybrids with B84 germplasm and B73 x Mo17 had very little diseased tissue during most of the season but showed a very rapid increase after mid-August. The inbred B73 is related to B84, and this change in disease reaction late in the season may indicate a real genetic response in these hybrids. The hybrid W64A x W117, which suffered a more severe attack early in the season, also exhibited a rapid increase of the disease during the second phase of the DPC in the upper leaves. The hybrid A662 x CM7 matured very early, due perhaps to a severe attack by the eyespot disease, and therefore it was not possible to gather data for the second phase of the epidemic. Lesions showed a marked tendency to coalesce in this genotype.

The lesions on the leaves of W64A x CM105 and W64A x B14A had a marked tendency to coalesce and form large necrotic areas; this presumably caused a premature death of the foliage, preventing new lesions from becoming established and inhibiting any appreciable change in the spread of the disease late in the season.

The reasons for the rapid change in disease severity on the upper leaves are not known, but this phenomenon has been observed in other studies as well (1, 3, 13). In the field, Arny *et al.* (1) observed that only a few lesions appeared on the plants during the early season, but when plants approached maturity, the infection became more widespread, particularly in the upper leaves. Cassini (3) observed that symptoms did not appear until leaves were completely developed. Arny *et al.* (1) concluded that maturing leaves were more susceptible than younger leaves.

In this study, only a few lesions were observed in young leaves. If we assume a constant incubation period of nine days, then we can expect the leaves to be almost fully expanded by the time lesions appear. The young leaves are obviously susceptible, since the method of incubation involves the placement of the inoculum in the whorl of the plants.

Attempts to relate disease development to rainfall and temperature were only partially satisfactory. The rapid increase of the disease in the upper canopy occurred during late August, after moist, cooler conditions had prevailed for about four weeks. These conditions supposedly favor the disease (1, 4). If this change in disease severity is truly a change in resistance, its mechanisms shald be studied. This may requiere a more precise definition of the DPC, particularly during the later part of the growing season. An outbreak of the eyespot disease late in the season may have a minor effect on final yields, but this assumption needs experimental support. If maize is planted continuously under minimum tillage, a late increase in the spread of the disease could augment the amount of inoculum available for the onset of an epidemic in the next crop (1, 5, 11, 14).

An important factor that may determine the degree of resistance to the eyespot disease is the tendency of the lesions to coalesce and form large necrotic areas, rather than to remain as discrete lesions. Coalescing of lesions was rare in H99 x A632 and in the hybrids with B84 germplasm, where lesions remained discrete even after the death of the tissue.

Cassini (3) observed the phenomenon of lesion coalescence and stated that real differences among genotypes can be observed at the time of tasseling. He further found that absence of "burning" of the leaves can be used to estimate practical resistance to eyespot disease.

An apparent relationship between early maturity and susceptibility to eyespot disease was observed; hybrids A662 x CM7 and W64A x W117 were the earliest genotypes and exhibited a high degree of susceptibility from early in the season. This

susceptibility was reflected in the disease response after the initial inoculation and in the rate of disease increase on both the middle and upper leaves of the plants. Conversely, H99 x A632 was the latest to mature and also the most resistant genotype. Army *et al.* (1) stated that cool, moist weather late in the season appeared to favor the disease, yet resistance was observed most often in the late maturing varieties.

This near consistent relationship between maturity and disease reaction is worthy of further study, to separate genetic resistance from physiological aging of the plant.

A good relationship seemed to exist between the rating of disease severity and the number of spores produced per lesion. Data were compared on the five hybrids for which sporulation had been determined twice, and this indicated a close correspondence between the amount of spores produced per lesion and the change in disease severity late in the season in those hybrids. For example, the number of spores per lesion in B84 x B73 changed from an average of 484 to 2 200 during the second sampling. The number of spores per lesion in H99 x A632 changed from an average of 412 to only 450, and this hybrid exhibited only a moderate increase in the level of disease late in the season.

The potential for conidia production was assessed by using areas of the leaves with discrete young lesions surrounded by green tissue. However many of the lesions may have been older than desired in some of the very susceptible hybrids. Sporulation from older lesions probably was less than from younger lesions and may have accounted for the low sporulation counts on W64A x B14A and W64A x CM105. A close relationship seemed to exist between disease ratings and diameter of the lesions: the most susceptible hybrid had larger lesions. The lesion diameter measured on August 17 related clearly with the sporulation data collected on August 20, as the larger lesions produced more spores. The lesion measurement did not relate with the sporulation estimates made on August 29; lesion size may, however, have altered with the change in disease severity.

The k values and the general susceptibility of the hybrids during the first part of the season also related to lesion diameter. The k values, lesion diameter, sporulation per lesion and lesion number are obviously related.

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VOL. XXXVI DICIEMBRE, 1986 No. 4

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