Survival of *Kabatiella zeae* Narita and Hiratsuka in Maize Residues[1]

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ABSTRACT

The eyespot disease appeared later and at a lower level in plots where infected residues from the previous season were removed before planting or early in the season, than in plots where residues were present on the soil during the full season. Infected residues collected from non-till plots and spread in a disease-free field were effective sources of inoculum only when collected early in the season (May-June). In another experiment, the disease appeared later and the initial level was lower in plots that had the lowest amount of disease the previous season.

Infected leaf residues and laboratory-cultivated stromatic hyphae of *K. zeae* rapidly lost their potential for conidial production after being exposed to consecutive periods of wetting and drying in the laboratory.

COMPENDIO

Bajo el sistema de labranza mínima, la enfermedad causada por *K. zeae* apareció más tardíamente y con menor severidad, en parcelas en donde los residuos de maíz de la cosecha anterior fueron removidos antes de la siembra o bien poco después. En parcelas en donde los residuos no fueron removidos, la enfermedad apareció más temprano y fue más severa. Residuos colocados en parcelas libres de estos sirvieron como fuentes de inóculo solamente cuando fueron colocados durante las primeras etapas del desarrollo. En otro experimento, la enfermedad apareció en forma tardía y menos severa en parcelas en donde el grado de ataque había sido menor durante la siembra del año anterior. Tanto los residuos infectados, como el estroma del hongo cultivado en el laboratorio perdieron rápidamente su capacidad para producir conidios cuando fueron expuestos a períodos consecutivos de secado y humedecimiento.

INTRODUCTION

The eyespot disease of maize caused by *Kabatiella zeae* has the potential to decrease yields greatly and reduce the quality of seed (2, 6, 7, 9, 10, 12). The rapidly increasing popularity of reduced tillage systems in many countries has made the eyespot disease a real threat because the causal pathogen can overwinter as stromatic hyphae in maize residue on the soil surface (1, 3, 4, 5, 8). Observations by Martinson (6) seem to indicate that the severity of the disease is directly related to the amount of infected residue present on the ground. Therefore, the continuous planting of susceptible varieties with minimum tillage practices could lead to a steady increase in the amount of inoculum. According to Cassini (3), the survival of the eyespot organism is assured in plant parts with a low decomposition rate, such as stalks and husks. Longevity of the fungus in the infected tissue is unknown. A determination of the period within which the survival structures of the fungus are still able to produce effective inoculum is important for an understanding of the roles of the primary and secundary inocula in the development of an epidemic.

In this study, the capacity of *Kabatiella zeae* to produce effective inoculum through the cropping season from overwintering crop residues was evaluated.

MATERIALS AND METHODS

Experiments were conducted during the growing seasons of 1983 and 1984 at three sites near Ames, Iowa.

Three approaches were used. All surface residues were removed at differents intervals from plots in a field cropped in a susceptible maize variety; samples of the residues were spread in isolated plots of a susceptible variety that was grown in a field not cropped with maize for at least the two previous seasons. Finally, in 1984 an experiment was set up to study the effect of the amount of inoculum of *K. zeae* generated in the previous season on the severity of the attack on the new crop.

Residue removal experiments

A field that had been used for eyespot experiments since 1979 was used for these experiments, in 1983

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and 1984. In 1982, the field was planted to W64A x W117 with no tillage of the previous maize crop; the eyespot disease developed heavily and uniformily over the entire area, and no tillage was done after harvest.

The field had been arranged in three contiguous blocks (24.4 m x 122 m), where the long axis of each block was parallel to the adjacent block. Blocks were separated by 9.1 m alleyways of fallow soil.

In 1983, the blocks were divided into plots that were eight rows wide by 24.4 m long, and the plots were separated by eight rows of a resistant border variety (Pioneer 3713). Plant debris was removed with a stalk chopper from the border rows and the land was deeply disked before planting on May 9. The experimental plots were planted on the same date as the LH39 x A632 hybrid. Rows were 76 cm apart and the distance between plants in a row was about 20 cm.

Five treatments were used: 1) removal of residues before planting; 2) removal 45 days after planting; 3) removal 67 days after planting; 4) removal 93 days after planting and; 5) no removal of residues. During removal, the residues were gathered with a garden rake and carried from the plots so that only a few small fragments remained on the plots. Treatments were ramdomly assigned to the plots and there were six replications, two in each one of the blocks.

In 1984, another experiment was carried out in those plots where the maize residue remained on the soil surface throughout the previous season (treatment five in 1983). These plots were divided equally to accomodate three replications of four treatments. Each plot was eight rows wide by 6.1 m and the distance between plants in a row was about 20 cm. The treatments were 1): removal of residues before planting; 2) removal 33 days after planting; 3) removal 57 days after planting and 4) no removal of residues. The test variety was W64A x W117. Borders were sown with H99 x A632.

Disease severity as related to the amount of disease in the previous crop

Four treatments with three replications were planted in the plots where maize residues were removed at different times during the previous season. Residues were not removed in 1984.

Treatment one was corn planted in plots where the residues were not removed in 1983. Treatments two, three and four were corn planted in plots where residues were removed 45, 67 and 93 days after planting in 1983.

Both experiments in 1984 were planted on May 9. Borders were planted with H99 x A632 and the experimental plots with W64A x W117.

Data on the severity of the disease were taken on ten plants in each plot by visual estimation of the percentage of diseased tissue per leaf. During 1983, only the ear leaf was evaluated. In both 1984 experiments, data were taken on three leaves: a lower (6th), a middle (10th), and an upper (3rd below the tassel) leaf. The experiment was analized as a completely randomized block for each reading date.

Residue application experiments

Experiments were conducted at two farms in 1983 and 1984 in fields that had been cropped to soybeans and oats during the two previous seasons, and so could be considered as free of eyespot inoculum (1, 3, 6).

In 1983, isolation plots of W64A x W117 that were 3 m long by 4 rows wide were established within a border planting of Pionner 3713. Planting was done on May 26. The rows were 0.76 apart and plants within a row were about 20 cm apart. The isolation plots were separared by at least 9.1 m of the resistant hybrid.

Five treatments were used: 1) placement of infected residues over the plots at the time of plant emergence; 2) placement 37 days after emergence; 3) placement 50 days after emergence; 4) placement 76 days after emergence; and 5) no residue placement. Each plot received a sample of maize residues (about 6 kg) collected from a 10 m^2 subplot located within each one of the treatment plots at the residue removal site.

In 1984, another experiment consisted of isolation plots of :W64A x W117 established within a field planted to Ames Best AB113A. This was the border variety planted on May 15, and it separated the plots by about 10 m. The isolation plots were planted on May 21 and were two rows wide and 3 m long. Rows were 0.76 cm apart and the distance between plants in a row was about 10 cm.

There were three treatments: 1) residues placed before emergence; 2) residues placed 20 days after planting; and 3) residues placed 44 days after planting. Residues brought from the residue removal site were spread between the two rows of the isolation plots.

Disease severity was evaluated by counting the number of lesions per leaf on the 8th leaf of 10 plants per plot. When lesions were not found on the leaves, evidence for the presence of the disease was sought in the lower leaves of all plants in the plots. No statistical analysis was performed on these data.

RESULTS

Residue removal experiments

The removal of harvest residues from the previous season in a field planted to a susceptible hybrid had a marked effect in the initial and final amount of eyespot disease that developed on these plots. Plots where residues were removed before planting usually had significantly less disease than the rest of the treatments (Tables 1, 2). Removal of residue after the fifth to seventh leaf stage of growth resulted in less disease than with no debris removal, but more disease than with full-season removal; however, these differences were not significant. In general, the disease severity was very low in 1983 and 1984 in the middle and upper canopy of the plants, and it reached only 1.8% and 0.60% of diseased tissue in the middle leaf during 1983 and 1984 respectively in the plots with full-season residue exposure. In the lower leaves evaluated in 1984, the disease severity reached 45% and 34% of diseased tissue in plots where residues remained longer on the ground (treatments 3 and 4). The plots with full-season removal had only about 7% of diseased tissue on the lower leaves in 1984 (Table 2).

Residue application experiments

A sample of infected residues taken from the plots at the residue removal site was taken to another field to study its potential as a source of inoculum. Eyespot

Table 1. Proportion of eyespot disease tissue on the ear leaf on four assessment dates for a hybrid grown in 1983 in a field where maize residues from the previous year were removed on four dates during the growing season.

Removal of residues		Disease assessment date			
Plant growth stage	Date	July 10	July 20	July 30	August 20
Before planting	May 8	0.002 a[1]	0.002 a	0.003 a	0.09 a
6–7th leaf stage	Jun 23	0.15 a	0.45 ab	0.59 b	1.18 b
Early tasseling	Jul. 15	0.23 b	0.45 ab	0.57 b	1.33 b
Milk stage	Aug 10	0.23 b	0.46 ab	0.87 b	1.80 b
No removal	–	0.25 b	0.60 b	0.89 b	1.80 b

a) Means of the average of 10 plants in each of 6 replications; means in a column with the same letter are not statistically different according to Duncan's Multiple Range Test (P = 0.05)

Table 2. Proportion of eyespot disease tissue on three leaves of a hybrid grown in 1984 in a field where maize residues from the previous year were removed three times during the growing season.

Removal of residues		Disease assessment date							
		Lower Leaf			Middle leaf			Upper leaf	
Plant growth stage	Date	June 22[1]	July 6	July 18	July 6	July 18	August 5	August 5	August 17
Before planting	May. 9	2 a[2]	14 a	7 a	0.0 a	0.01 a	0.02 a	0.0 a	0.002 a
5–6th leaf stage	June 11	46 ab	4 a	20 ab	0.01 b	0.06 a	0.06 a	0.001 ab	0.02 b
9–10th leaf stage	July 5	65 b	15 b	45 b	0.04 c	0.20 a	0.35 a b	0.01 a	0.56 b
No removal		61 b	18 b	34 b	0.03 c	0.10 a	0.60 b	0.005 ab	0.38 b

1. Mean of the number of lesions per leaf. Data in all other columns in the table are proportion of diseased tissue

2. Mean of the average of 10 plants in each one of three replications; means in a column with the same letter are not statistically different according to Duncan's Multiple Range Test (P = 0.05).

disease developed only in the plots where debris was placed immediately after planting in the 1983 experiments (Table 3).

Traces of the disease (1-5 lesions per plant) eventually appeared in the other treatments, including those where no residues were placed. Inoculum for these infections may have come from another eyespot experiment planted nearby. During 1984, only the residues placed in May and June were effective as sources of inoculum (Table 3).

Disease severity as related to the level of disease the previous season

During 1984, an experiment was conducted in a field managed under no-till practices and where the plots had a known level of eyespot in 1983. The appearance of the disease was delayed and the initial severity was significantly lower in those plots that had the lowest amount of disease the previous season, as a consequence of the removal of infected residues before planting in 1983. The proportion of diseased tissue on the lower leaves in these plants reached only 1.3% compared to an average of 15% in the other treatments (Table 4).

Sporulation of *Kabatiella zeae* stromatic hyphae under laboratory conditions

Stromatic hyphae that were exposed to consecutive periods of wetting and drying rapidly lost their potential to produce conidia. This rapid loss of sporulation capacity was observed in both laboratory-produced inoculum and in naturally infected leaves (Table 5). About one fourth of the initial potential for conidial production was lost after one cycle of wetting and drying in the cultured stromatic hyphae. A similar loss in sporulation capacity was observed in naturally infected leaves. Three cycles of wetting and drying seemed to exhaust the inoculum potential of the leaves to a level below detection. The stromatic hyphae were functional for sporulation after five cycles, but at a level about 0.01% less than the original inoculum.

DISCUSSION

The present research confirmed that *K. zeae* can overwinter in infected corn residues; it apparently does this by forming resistant stromatic hyphae in the infected tissue.

Table 3. Number of eyespot lesions on the 10th leaf of a hybrid grown in isolation plots with eyespot infected maize residues added at different times during the growing season.

Site 1 (1983)

Placement of residues		Disease assessment date		
Plant growth stage	Date	July 11	July 31	August 25
Emergence	June 4	54	100	150
6–7th leaf stage	July 2	0	0	Trace
Early tasseling	July 15	0	0	Trace
Dough stage	Aug. 10	0	0	Trace
None		0	0	Trace

Site 2 (1984)

		July 2	July 17	Aug. 14	Aug. 17
Emergence	May 31	43	19	50	100
4–5th leaf stage	June 11	23	15	67	87
8–9th leaf stage	July 5	0	0	0	0
None		0	0	0	0

Table 4. Proportion of eyespot disease in 1984 in a maize hybrid grown in plots that had a known amount of eyespot disease in 1983 and managed with no-till practices.

		Disease in 1984							
Removal of harvest residues during 1983	Disease on August 20, 1983[1]	Lower leaf			Middle leaf			Upper leaf	
		June 22[3]	July 6	July 18	July 6	July 18	August 5	August 5	August 17
Before planting	0.09 a[2]	7 a	0.40 a	1.3 a	0.0 a	0.03 a	0.10 a	0.0 a	0.001 a
6–7th leaf stage	1.18 b	15 b	8 b	13 b	0.001 a	0.07 ab	0.65 b	0.005 a	0.01 b
Early tasseling	1.33 b	24 c	8 b	17 b	0.005 a	0.16 b	1.2 c	0.05 a	0.23 b
Milk stage	1.60 b	27 c	7 b	14 b	0.005 a	0.09 ab	0.90 bc	0.06 a	0.31 b

1. Data from Table 3.

2. Means in a column followed by the same letter are not statistically different according to Duncan's Multiple Range Test ($P = 0.05$).

3. Mean of the number of lesions/leaf in 10 plants in each one of three replications. The rest of the data in the table correspond to the mean of proportion of diseased tissue.

The initial severity of the disease was related to the level of disease in the field during the previous season. Several factors may help to explain the importance of initial inoculum in this disease. The plant debris provided a prolonged source of inoculum that appeared to be effective for several weeks; this was a source for repeated spore production. The relatively dry years decreased the number of secondary cycles, the production of secondary inoculum, and or the number of successful infections. Finally, the pathogen spread upward on the plant and the number of initial lesions on the lower leaves greatly affected the inoculum potential for the upper leaves. If this phenomenon is of real importance in the epidemiology of eyespot, knowledge of the amount of disease the previous year, survival of *K. zeae* in the residues, amount of residue burial by tillage, and host genotype resistance will all be important for crop management decisions in an integrated disease control program.

The removal of residues from the previous crop had a significant effect on the initial and sometimes the final amount of disease. The initial level of disease was lower in plots with residue removal before planting than in plots with residue during the juvenile stages of plant growth. The initial amount of inoculum again had a significant effect on the epidemic. In plots where residues were removed after plant emergence, the early differences in amount of disease tended to disappear as the season progressed. This indicated that the inoculum in the debris rapidly lost effectiveness as a source of inoculum.

Because conditions in 1983 and 1984 were highly unfavorable for an eyespot epidemic, it is imposible to determine from these data the epidemiological im-

Table 5. Conidial production by *Kabatiella zeae* in infected leaf residue and from laboratory-cultured stromatic hyphae after exposure of these structures to consecutive cycles of wetting and drying in the laboratory.

Cycles of wetting[1]	Leaf residues[2]	Stromatic hyphae[3]
1	14 000	9 650 000
2	3 000	2 279 000
3	600	1 005 000
4	0	682 000
5		25 000
6		7 500
7		0
Control[4]	13 000	8 300 000

1. Infected residues were exposed to consecutive cycles of two days of wetting and four days of drying. For the stromatic hyphae mixed with sand, these periods were two days of wetting and ten days of drying.

2. Mean of the number of conidial/100 lesions.

3. Mean of the number of conidia produced by 1 g of the stromatic hyphae-sand inoculum mixture.

4. Inoculum kept dry until the end of the experiment and then wetted and assayed.

portance of primary inoculum potential (duration and amount) under a more conducive environment for disease development. The results, however, indicate that under unfavorable environmental conditions for an epidemic any reduction in the initial amount of inoculum will delay and reduce the epidemic significantly. C.A. Martinson (Personal communication) found that early season eyespot severity differences were soon masked by an epidemic of the disease, but they still resulted in a significant yield reduction. Yield data were taken in this experiment, but the severity of the disease was too mild to result in any significant yield reduction.

The loss in the ability of weathered maize residues to initiate new lesions on a susceptible hybrid correlated very well with the response of the fungus in the laboratory where diseased leaves and stromatic hyphae rapidly lost their ability to produce conidia after being wetted and dried several times. There are probably other factors that have an effect on the reduction of the inoculum potential of *K. zeae* in infected maize residues, and different kinds of microbial antagonism are without a doubt of primary importance (6). The fact that the disease is more prevalent in fields managed with reduced tillage practices also indicates that microbial antagonism in its various forms may play an important role in the erradication of *K. zeae* in a field. An excellent control of the disease is achieved by plowing under the harvest residues, which are then expored to microbial degradation (1, 2, 3, 6).

An epidemic of eyespot in the field, therefore, begins almost entirely from the inoculum generated from the maize residues; however, the survival structures of the fungus in these residues have a limited capacity for repetitive inoculum (conidia) prodution.

Further development of the epidemic depends mostly on secondary disease cycles. The lower plant canopy was originally infected by primary inoculum. Secondary inoculum was dispersed by splashing rain (11), and this resulted in an upward sequential pattern of disease development.

Little is known about the epidemiology and etiology of the eyespot disease on maize. Resistant genotypes have been identified. This is probably enough to make fairly intelligent disease control recommendations, but we are still ignorant of many of the important epidemiological parameters surrounding this disease. Eyespot of maize would be an excellent system for disease modeling and for the development of an integrated pest management program.

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