Occurrence of Chlorotic Spots on Corn Seedlings Infected with *Sphacelotheca reiliana* and Their Use in Evaluation of Head Smut Resistance

C. A. MATYAC and T. KOMMEDAHL, Department of Plant Pathology, University of Minnesota, St. Paul 55108

ABSTRACT

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Chlorotic spots containing hyphae develop on the fourth or fifth emerged leaf of corn seedlings infected with *Sphacelotheca reiliana*. Analysis of data in multidimensional contingency tables showed that symptomatic seedlings are likely to produce sori in inflorescences. Significant rank correlations between greenhouse and field trials indicated that evaluations in the greenhouse using chlorotic spots on seedlings as an indicator of infection aid in predicting relative genotypic differences in smut resistance in the field. Greenhouse tests should include 50 plants for inbreds and 100 plants for hybrids and be grown at 22C, allowing soil to dry to −1.5 bar before resaturation.

Additional key words: chlorotic fleck, head smut

Head smut is a systemic disease of corn (*Zea mays* L.) caused by *Sphacelotheca reiliana* (Kühn) Clint (*Sporisorium reiliana* (Kühn) Langdon & Fullerton). Plants are infected by soilborne teliospores during emergence or at the seedling stage. In some infected seedlings, chlorotic spots (often called flecks) occur along the midrib and the leaf blade of the fourth and fifth emerged leaves. Flecks are round to oblong (1–2 mm in diameter) and vary from three or four to several hundred per leaf (Fig. 1). Infected plants continue normal vegetative growth but are sometimes stunted. The first definitive indication of infection occurs when tassels and ears emerge and have been partially or totally replaced by sori filled with teliospores. Thus, evaluating genotypes for disease resistance requires that plants be grown to maturity (90–100 days) to determine if infection has occurred. Foster and Frederiksen (3,4) stated that this process can be shortened by evaluating plants in the greenhouse at the seedling stage (about 42 days), using flecks as an indicator of infection.

Fleck symptoms on corn infected with head smut were first reported by Tyler and Shumway (9) in 1935. They indicated that flecking was a common symptom on sorghum plants that had been injected with sexually compatible monosporidial lines of *Sorosporium reiliana* and *Sphacelotheca sorghi*. Sori, however, developed in only a few of the plants with leaf flecks. Mankin (6) reported that flecking could not be considered a reliable criterion of infection. In that study, the same compatible combinations of monosporidial lines of *S. reiliana* were injected into six corn genotypes. Flecking was observed in four genotypes but sori developed in only three. When a different compatible monosporidial combination was injected into the same six genotypes, five had flecks but sori developed in only two genotypes. Foster and Frederiksen (3,4) evaluated numerous hybrids, inbreds, and single crosses for resistance to head smut in Texas and reported that in the greenhouse, 96.7% of the plants that developed chlorotic flecks eventually developed sori. However, they reported a poor correlation between the relative frequency of the disease in the greenhouse and in the field when hybrids were evaluated and a good correlation when inbreds and single crosses were evaluated.

After the outbreak of the disease in Minnesota in 1980 (7), corn hybrids commonly grown in the area were evaluated for resistance. Further inconsistencies concerning the validity of screening seedlings arose as a result of conflicting data collected by the Minnesota State Department of Agriculture (R. M. Sushak, *unpublished*), where seedlings were screened in a greenhouse and by Stromberg et al (8) and all materials were evaluated in the field. Spearman's rank correlation coefficient of the disease frequency between the hybrids common to both tests was $r_s = -0.065$ (24 df).

The purpose of this study was to determine the cause of chlorotic spots on leaves of infected plants, to determine the statistical relationship between seedling symptoms and sorus development, and to determine the accuracy of greenhouse screening tests for evaluating relative genotypic field resistance.

MATERIALS AND METHODS

Evaluation of fleck symptom. Sori were collected from a cornfield at the Staples Area Vocational Technical Institute, Staples, MN, at the end of the 1981 growing season and stored in an unheated building during the winter. Immediately before planting, the teliospores were removed from the sori, filtered through a No. 16, 1.18-mm soil sieve, and mixed with soil at a ratio of 1:200 (v/v), which resulted in 1.8×10^6 spores per gram of soil. The soil mix consisted of seven parts silt loam, two

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parts manure, three parts sand, and one part peat. The genotypes selected and listed in Table 1 represent a wide range of susceptibility based on the previous season's field evaluations (8). Two seeds per pot were planted 4 cm deep in 54, 8-cm peat pots (Jiffy Pot Ltd., Shippega, Canada). Peat pots were placed in plastic trays (16 pots per tray), set on a greenhouse bench in a completely randomized design, and watered to saturation. Ten gypsum blocks that had been calibrated with a soil psychrometer (Wescor, Inc., Logan, UT) were installed in randomly selected pots. Gypsum blocks were read with a KSl moisture meter (Delmhorst Instrument Co., Boonton, NJ). Water potential was allowed to drop to about −1.5 bar before soil was watered to saturation. This watering cycle was followed for the first 4 wk, then seedlings were watered daily. Plants were thinned to one per pot after emergence. Supplemental light consisted of 2.4-m-long banks of fluorescent lamps on a 18-hr/day schedule. The temperature was maintained at 22 ± 5 C. After 4 wk of growth, all plants were fertilized with a 20-20-20 (NPK) solution.

Table 1. Percentage of flecking in greenhouse and head smut field tests in 15 hybrids and five inbreds

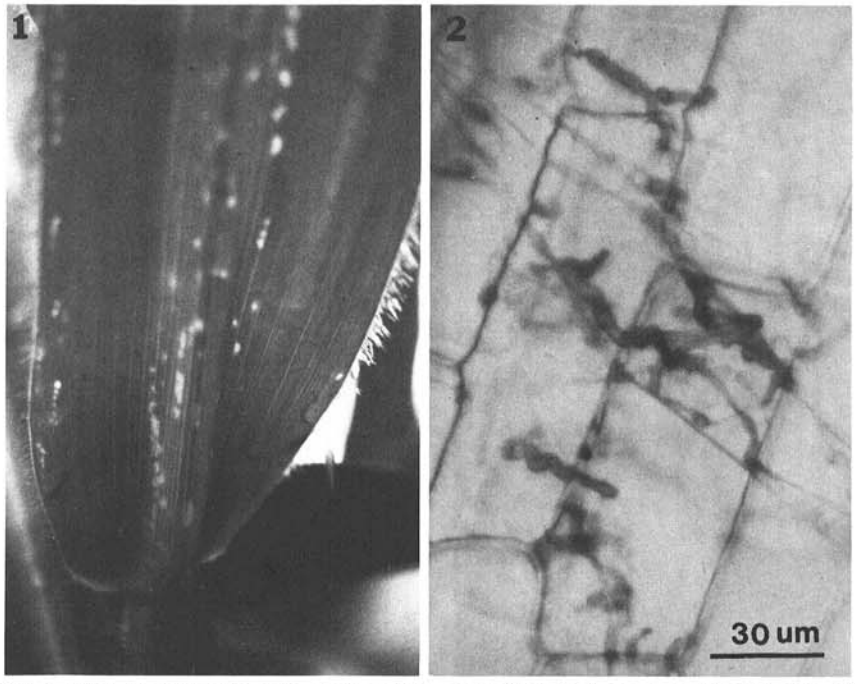
Genotypes	Plants infected (%)[a]	
	Greenhouse[b]	Field[c]
Hybrids		
Minn. 5301	47.2	28.9
Pioneer 3978	49.0	28.7
Minn. 4201	58.3	25.3
Cenex 2155	47.7	23.0
Northrup King PX24	43.7	22.4
Funks G5048	18.7	18.1
Payco SX599	56.4	10.5
Northrup King PX11	10.3	8.3
Minn. 7301	31.9	7.7
Minn. 6305	34.1	6.0
Northrup King X6668	6.6	4.8
Minn. 8301	46.7	4.7
Minn. 4202	11.7	0.4
Minn. 5202	17.1	0.4
Inbreds		
Va26	71.8	56.0
B68	62.0	34.0
B37	53.2	24.0
W593	59.5	16.0
Holden LH39	13.7	10.3
B73	20.0	6.0

[a] Spearman's rank correlation coefficient between the greenhouse and field test was $r_s = 0.74$ (18 df), significant at $P = 0.001$ for hybrids and inbreds combined.
[b] Percentage of flecking based on three trials, with three replicates per trial and 18 plants per replicate, in the greenhouse at 22C.
[c] Percentage of head smut based on three trials, with three replicates per trial and 30 plants per replicate, in fields at Staples, MN.

After 6 wk, all plants were examined for chlorotic spots on leaves. Plants with spots were tagged with a 30-cm piece of 3-cm-wide plastic tagging material. The frequency of flecking of each genotype was noted and compared with the disease frequency in the field (8) using Spearman's rank, nonparametric correlation procedure. After evaluation, all leaves were removed from plants to reduce wind resistance and transpiration stress in the field. Plants were transplanted to furrows 15 cm deep in a field on the Staples Area Vocational Technical Institute, then irrigated and fertilized. At maturity, the presence or absence of sori was noted.

Results for each genotype were entered into one of four categories in a 2 × 2 contingency table, where each variable represented a dimension of the table. The categories were as follows: tagged and smutted, tagged and not smutted, not tagged and smutted, and not tagged and not smutted. The contingency tables were combined for hybrids (2 × 2 × 18), inbreds (2 × 2 × 6), and both (2 × 2 × 24) (Table 2). The interactions between flecking (variable A), sorus development (variable B), and genotype (variable C) were examined with a series of hierarchical log linear models generated by CTAB (S. P. Yen, University Computer Center), a program for analysis of multidimensional contingency tables on the Cyber 730 computer at the University of Minnesota. The various models represented a range from total independence of each variable to interactions between all combinations of the variables. Pearson's chi-square (X^2) and likelihood-ratio chi-square (G^2) values were calculated for each model by comparing expected counts with observed counts. X^2 and G^2 statistics were used as criteria for selection of the model that best represented the data.

Etiology. Ten leaves with and without chlorotic spots were collected from two hybrids (Pioneer 3978 and Funks G5048) and two inbreds (Holden LH39 and Va26) at the time of evaluation. Thirty chlorotic spots were dissected from the symptomatic leaves, and 30 tissue pieces were randomly selected from the asymptomatic leaves of each genotype. Twenty tissue pieces from each group were cleared in boiling ethanol for 20 min, transferred to warm lactophenol containing aniline blue for 60 min, stained further with toluide blue in lactophenol for 60 min, mounted in glycerol on glass slides, and examined for the presence of hyphae. Ten tissue pieces of each genotype were rinsed in running tap water for 15 min, dipped in 0.05% NaOCl for 45 sec, rinsed in sterile distilled water three times, placed on potato-dextrose agar acidified with 50% lactic acid, and incubated at 25 C for 10–14 days. Sporidia growing from the leaves were transferred to potato-dextrose broth and incubated at 25 C for 7 days. Several liquid cultures from each genotype were bulked and used for hypodermic inoculation of the shoot apices of healthy corn seedlings (1) of the genotypes from which they were isolated. Plants were allowed to flower, then the presence or absence of sori was noted.



Figs. 1 and 2. (1) Chlorotic spots on the midrib and leaf blade of a corn seedling infected with *Sphacelotheca reiliana*. **(2)** Intercellular hyphae of *S. reiliana* observed within the zone of chlorosis on leaves of corn seedlings.

RESULTS

Etiology. Intracellular and intercellular hyphae were consistently observed in tissue of leaves with chlorotic spots (Fig. 2). No hyphae were observed in corn leaves that did not have chlorotic spots.

Sporidia were cultured from 20% of the tissue pieces with chlorotic spots. Chlorotic spots developed on leaf midribs of seedlings injected with sporidial cultures but not on seedlings injected with

sterile distilled water. Sori developed in 87% of plants that had been injected with sporidia. Teliospores were collected from these sori and identified as *S. reiliana*.

Interactions between flecking, sorus development, and genotype. Sori were produced in 78.5% of plants that developed fleck symptoms as seedlings, whereas no sori occurred on 83.4% of symptomless seedlings. Analysis of the data in multidimensional contingency tables (2) provides statistical evidence for the apparent interaction between seedling symptoms and sorus development in adult plants and is demonstrated by the abbreviated form of the model developed by CTAB: (AB) (AC) (BC). This model indicates that sorus development (variable B) is a response of the seedling symptom (variable A) and that these variables differ from genotype to genotype (variable C) by no more than random variation. The same model was chosen for hybrids, inbreds, and both combined. X^2 and G^2 values for combined data were 35.5 and 37.7 (23 df), respectively.

Correlation between greenhouse and field trials. The relative degree of resistance of the genotypes tested in the greenhouse was similar to that observed in the field (Table 1). When hybrids and inbreds were examined together, statistically significant correlation coefficients of $r_s = 0.66$, 0.73, and 0.74 (18 df) were obtained when the rank of disease frequency of one, two, or three greenhouse planting dates, respectively, was compared with the rank of the average disease frequency of three planting dates for field evaluations. When examined individually, significant r_s values of 0.93, 0.94, and 0.87 (4 df) were obtained for the data from the first, second, and third planting dates, respectively, of inbreds. Only two of the three planting dates of hybrids resulted in significant r_s values of 0.69 and 0.54 (12 df). When the percent disease from two greenhouse plantings of hybrids were averaged, significant r_s values of 0.70, 0.62, and 0.65 were obtained and when all three were averaged, $r_s = 0.69$. Correlation coefficients for inbreds were always higher than those for hybrids, and these values increased as the number of planting dates increased.

Table 2. Number of corn plants in each of four possible categories based on the presence or absence of flecks in seedlings and sorus development in adult plants

	Flecked		No flecking	
Genotypes	**Smutted**	**No smut**	**Smutted**	**No smut**
Hybrids				
CB 596	23	0	40	27
Cenex 2155	77	4	34	30
Funk 5048	19	1	14	82
Minn. 4201	30	4	5	11
Minn. 4202	11	3	2	83
Minn. 5202	21	5	7	99
Minn. 5301	59	3	20	44
Minn. 6305	45	1	24	55
Minn. 7301	44	12	6	83
Minn. 8301	40	3	11	36
Northrup King PX11	18	3	20	85
Northrup King PX24	57	4	22	71
Northrup King PX403	59	2	27	54
Northrup King X6668	8	2	4	109
Northrup King X6393	26	2	3	82
Payco SX599	59	2	19	25
Pioneer 3978	46	1	14	77
Inbreds				
B37	48	2	16	28
B68	41	17	9	26
B73	14	4	8	111
Va26	81	3	13	20
W117	69	4	19	30
W593	21	3	2	15
Holden LH39	4	0	12	23

DISCUSSION

Several workers have observed chlorotic flecks on leaves of corn seedlings infected with *S. reiliana* (3–6,9), but the association between this symptom and sorus development has not been clearly established. Foster and Frederiksen (3,4) were the first to associate flecking with sorus formation on a large number of genotypes. They observed populations of corn plants at the seedling stage and at maturity, then calculated the ratio of the number of corn plants producing sori to the number of seedlings that had developed symptoms earlier. Their experiment examined the outcome of two events (seedlings with or without flecks and mature plants with or without sori) and correlated the resulting frequencies. In our study, individual plants were followed throughout their development, and four possible outcomes were recorded. These kinds of categorical data are best analyzed by chi-square statistical tests. Multidimensional contingency tables are used when several genotypes are evaluated at one time. Our results indicate that in any investigation of the various aspects of this disease, seedling symptoms can be used as an indicator of infection, thus eliminating the extensive time and cost required to grow plants to maturity for disease assessment. Although the association between flecking and sorus development is not absolute, it is statistically significant ($P = 0.05$).

Previous reports (3,4; R. M. Sushak, *unpublished*) indicate that corn genotypes have been evaluated in the greenhouse using the fleck symptom as an indicator of infection. Foster and Frederiksen's data (3,4) indicate that this technique could not be used to predict reactions of hybrids in the field but could be used to predict the reactions of inbred lines. Although plant breeders are often interested in disease reactions of inbred lines, hybrids are of considerable interest because of their widespread use. Analysis of data collected independently by two sources on several Minnesota hybrids further indicated a potential problem with this technique.

In our study, when greenhouse and field tests of hybrids and inbreds were compared, statistically significant correlations were obtained. These correlations were probably due to 1) uniform distribution of inoculum and adequate replications in the field, 2) controlled temperature and soil water potential in the greenhouse, and 3) adequate populations of individual genotypes in the greenhouse and field. Under the controlled conditions described, screening of genotypes in the greenhouse using the chlorotic fleck as an indicator of infection is more accurate for inbreds than for hybrids in assessing relative resistance.

Hyphae were found consistently in chlorotic spots on leaves of infected seedlings. Sporidia cultured from these spots produced typical fleck symptoms when injected into seedlings, and the same plants produced sori containing spores of *S. reiliana* at maturity. These observations document the etiology of the chlorotic fleck symptom and the systemic nature of head smut infection.

LITERATURE CITED

1. Edmunds, L. K., 1963. Use of sporidial hypodermic injection to test sorghum for head smut resistance. Plant Dis. Rep. 47:909-913.
2. Fienberg, S. E. 1977. The analysis of cross-classified categorical data. Massachusetts Institute of Technology Press, Cambridge. 151 pp.
3. Foster, J. H., and Frederiksen, R. A. 1977. Evaluation of maize for resistance to head smut. (Abstr.) Proc. Am. Phytopathol. Soc. 4:142.
4. Foster, J. H., and Frederiksen, R. A. 1977. Symptoms of head smut in maize seedlings and evaluation of hybrids and inbreds. Tex. Agric. Exp. Stn. Rep. PR3432. 4 pp.
5. Lynch, K. V. 1981. Studies in the control of head smut of corn induced by *Sphacelotheca reiliana*. M.S. thesis. University of Guelph, Guelph, Ontario. 91 pp.
6. Mankin, C. J. 1953. Studies of the biology of *Sphacelotheca reiliana* causing head smut of corn. Ph.D. thesis. Washington State University, Pullman. 65 pp.

7. Stromberg, E. L. 1981. Head smut of maize, a new disease in Minnesota. (Abstr.) Phytopathology 71:906.
8. Stromberg, E. L., Stienstra, W. C., Kommedahl, T., Matyac, C. A., Windels, C. E., and Geadelmann, J. L. 1984. Smut expression and resistance of corn to *Sphacelotheca reiliana*. Plant Dis. 68:880-884.
9. Tyler, L. J., and Shumway, C. P. 1935. Hybridization between *Sphacelotheca sorghi* and *Sorosporium reilianum*. Phytopathology 25:375-376.