Eyespot of Maize, a Disease New to North America

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ABSTRACT

A new foliage disease of maize occurred across the northern part of the United States corn belt in 1968 and 1969. The inciting fungus, *Kabatiella zeae*, originally was described from Hokkaido, Japan, in 1959. Inoculations with conidia from pure cultures induced lesions identical to those observed in the field. The fungus was isolated from overwintered maize debris. Field tests with inbred lines and hybrids indicated a range of reaction to the disease. Phytopathology 61:54-57.

Additional key words: Zea mays.

A new foliage disease of maize appeared over a wide area of north central USA in 1968 (10). It was most common in west central Wisconsin, southeastern Minnesota, northeastern Iowa, northern Illinois, and northern Indiana. Symptoms were unlike any of the known diseases of this host in North America. Maize in Wisconsin fields first developed typical symptoms on the upper leaves in late August; by early September, severely affected plants were dead and substantial yield reductions occurred. No epiphytotic developed in 1969, although symptoms occurred on occasional plants. Severe infections occurred locally and too late to cause economic damage.

Early symptoms consisted of small, 1- to 4-mm, translucent circular-to-oval lesions which occasionally became sufficiently numerous to induce larger areas of necrosis. In the early season, the spots usually developed in zones or patches rather than distributed over the leaf blade. The spots were water-soaked initially, with the centers becoming cream-to-tan colored, surrounded by a brown or purple ring with a narrow yellow halo. This appearance suggested the name "eyespot". Although often numerous on individual leaves, the spots were determinate (Fig. 1). Similar symptoms developed on the leaf sheath and outer husks of the ears. The disease was most intense on the upper leaves of plants approaching maturity. These symptoms were similar to those described by Narita & Hiratsuka (3) and Narita et al. (4) from Hokkaido, Japan.

Early leaf symptoms could readily be confused with some of the many different, noninfectious, so-called "physiological" spots that are observed in maize, particularly among inbred lines. The early symptoms also resemble those incited by species of *Curvularia* in tropical or warm temperate areas (5, 6, A. J. Ullstrup *unpublished data*).

MATERIALS AND METHODS, AND RESULTS.—*Isolation and identification of pathogen.*—Initially, the organism was difficult to isolate because of the numerous saprophytes on the mature maize leaves; although after 24 hr in a moist chamber, curved-to-falcate, single-celled, hyaline conidia were evident on the lesions.

The fungus was first isolated by the Waring Blendor technique described by Stover & Waite (9). Leaves containing lesions were washed in running water, dried, and blended 2-5 min with sterile distilled water. Following three to five successive dilutions (1/10), the suspensions were poured onto petri plates containing acidified Czapek-Dox agar with Tergitol (sodium tetradecyl sulfate, nonionic NPX, Union Carbide Corp., N.Y.) (8), or potato-dextrose agar (PDA) with Novabiocin (Upjohn Co., Kalamazoo, Mich.) at 100 mg/liter (1). After settling, the excess suspension tissue was discarded. After slanting for 1 hr, the excess liquid was removed and the slant left to dry. A slowly developing fungus was consistently isolated from the lesions. The conidia were identical to those present on lesions in the moist chamber.

The fungus also was isolated from mature leaves and overwintered debris by grinding the dry material to a fine powder, dusting this on moist corn seedlings, and maintaining them in a moist chamber for 24-36 hr. Lesions which developed on these seedlings or lesions from relatively young leaves in the field could be surface-disinfested with 5% sodium hypochlorite or 70% ethanol for 0.5 to 1 min and plated on acidified water agar or PDA. Lesions from greenhouse inoculations could be plated without surface disinfestation to obtain pure cultures (Fig. 2).

Inoculations of 5-week-old, greenhouse-grown maize plants by spraying or injection with conidial suspensions from pure cultures induced typical symptoms in 2 weeks (Fig. 3). With one inoculation, the lesions were usually confined to a single intermediate aged leaf. Older plants (6-8 weeks) were more susceptible than young seedlings (3-4 leaf).

The colonies are yellow or pink in early growth in culture, but eventually become dark blue or black; thus, they belong to the group termed the "black yeasts" (2). The growth in culture is tough and leathery.

The spores are long, curved to falcate with pointed ends, hyaline, and nonseptate. Spores from the host measured 18-33 × 3-4 μ (27 × 3.6 μ). On the host, spores are produced successively on the tips of short conidiophores. They may also develop along the hyphae near septa (Fig. 4, 5). One to several short conidiophores may emerge from stomates in the necrotic portion of the lesions. The successive production of spores gives rise to mounds of spores around the conidio-

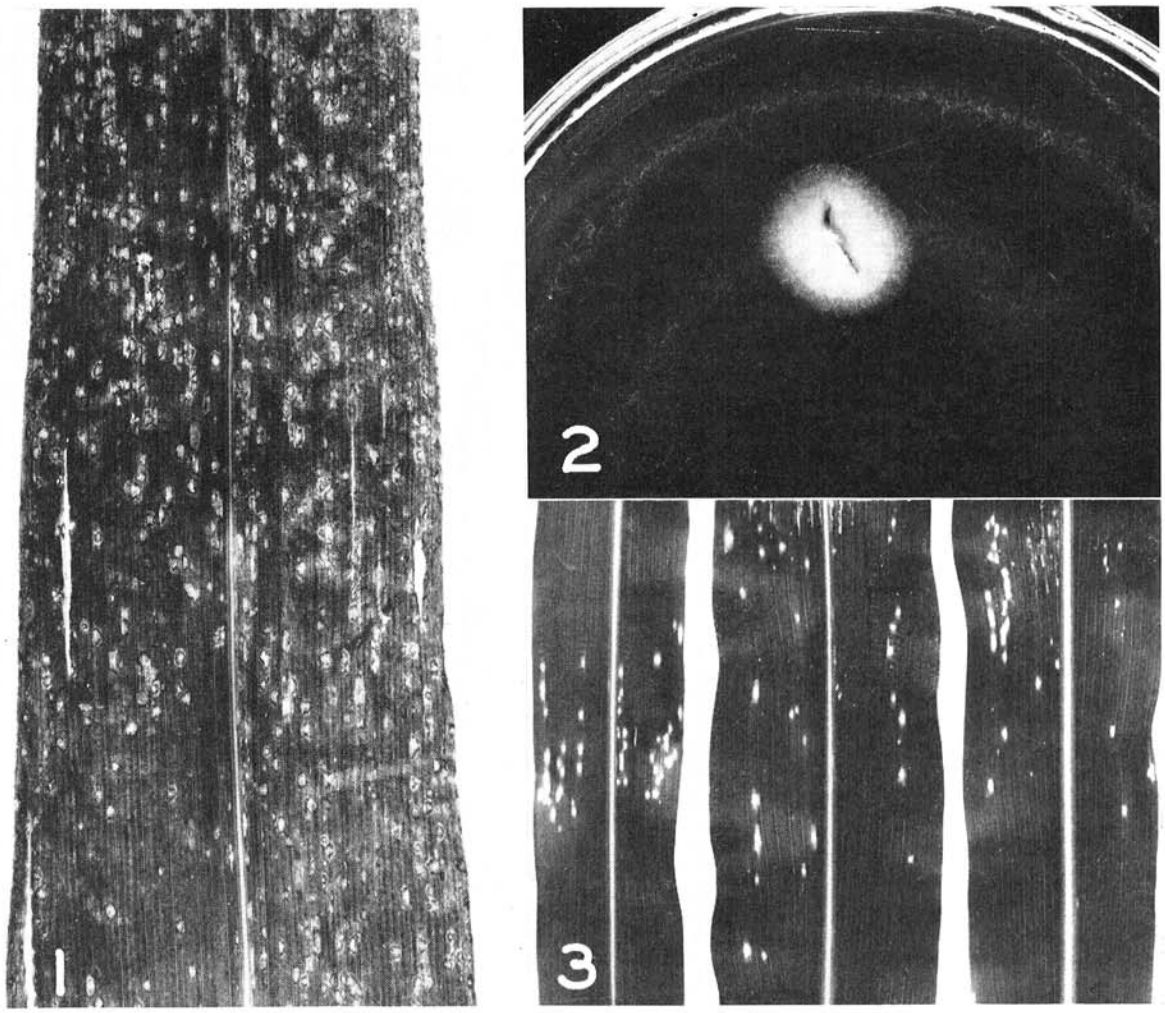

Fig. 1-3. Eyespot disease of maize. **1)** Natural infection by *Kabatiella zeae* on mature leaf. **2)** Growth of *K. zeae* from inoculated leaves. **3)** Symptoms on inoculated young leaves.

phores. In pure cultures, the size of conidia and ratios of length to width vary with the agar substrate.

The fungus was first identified as *Kabatiella zeae* Narita & Hiratsuka (3) as the cause of a leaf spot of maize. In 1959, Narita & Hiratsuka (3) tested the organism on a number of grasses and two *Trifolium* species, but found only maize to be susceptible. In Japan, the disease was called "Kappanbyo" (brown spot). No subsequent reports of the disease in Japan or elsewhere have been found. The name "eyespot" has been used in the USA rather than "brown spot", since in the USA the latter refers to the disease incited by *Physoderma maydis*.

Spore germination and growth.—Spores were collected from 2-week-old oatmeal agar cultures, and washed in sterile water, and a suspension was streaked over water agar, Czapek's agar, cornmeal agar, and PDA. The agar plates were incubated in the dark. Optimum temp for germination was 24 C; the min was between 4 and 8 C; the max about 36 C. At the extremes, germ tubes were less than one-half the length of the spores after 72 hr.

Radial growth was measured on oatmeal agar, cornmeal agar, Czapek's agar, and PDA. The best growth was at 24 C; the min temp for growth was between 4 and 8 C, and the max between 32 and 36 C. Maximum radial growth and spore production was attained on oatmeal agar in 2 to 3 weeks. Sporulation was also good within 48-72 hr on V-8 juice agar plates inoculated by flooding with a spore suspension. Concentrated spore suspensions were obtained in the same length of time with shake culture in a carboxymethyl-cellulose (CMC) liquid medium (CMC, 15 g; NH_4NO_3, 1 g; yeast extract, 1 g; KH_2PO_4, 1 g; $MgSO_4$, 0.5 g; H_2O, 1 liter). The fungus grew on sterilized whole oats or sorghum kernels, but did not sporulate; however, when these colonized kernels were partially dried and then placed in a moist atmosphere good sporulation occurred. The fungus lost virulence with continued culture on artificial substrates.

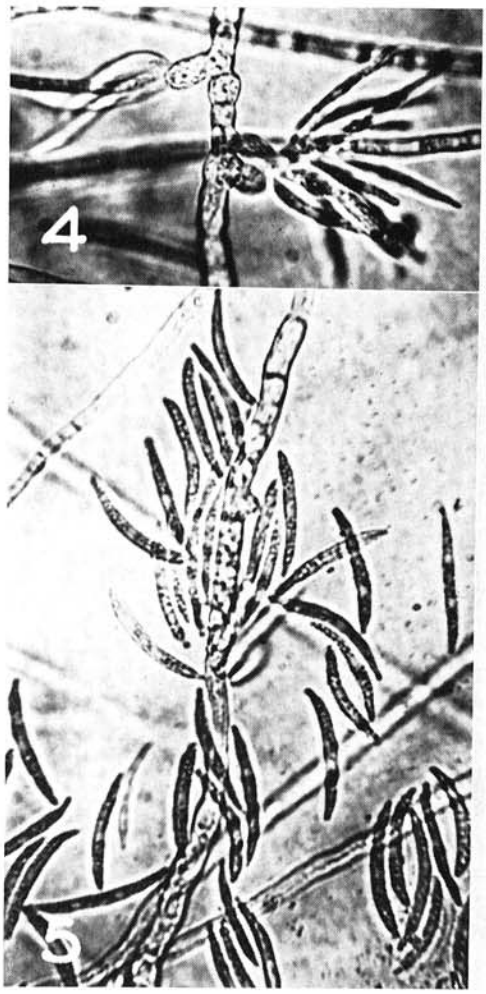

Fig. 4-6. *Kabatiella zeae.* **4)** Conidia and conidiophores (× ca. 550). **5)** Conidia and mycelium (× ca. 550). **6)** Natural infection by *K. zeae* of maize leaves from young plants growing among debris of previous maize crop as observed on 26 June 1969. *Phyllosticta* sp., incitant of yellow leaf blight, was also isolated from yellow areas of same leaves.

Inoculation.—Injection of a concd spore suspension into the leaf whorl of greenhouse-grown maize seedlings resulted in moderate infection (Fig. 3). Spraying of seedlings induced only sparse infection, although age of plants and the level of spore concn were important. In a greenhouse test with 24-inch plants, inoculating with 2.8×10^5 propagules/ml resulted in lesions on 15 of 15 plants; 2.8×10^4 and 2.8×10^3, lesions on four plants; and 2.8×10^2, lesions on none. In the field, good infection was obtained by spraying with a spore suspension just after tasseling. Infection also occurred after placing colonized oat kernels in the whorl of plants in the field. The fungus was most aggressive on maturing leaves.

Overwintering and spread.—Maize debris, primarily leaves, was collected in April of 1969 from fields which had been planted to maize the previous year. When this material was finely ground and dusted on seedlings placed under moist conditions, lesions developed in a number of cases. *Kabatiella zeae* was isolated from the lesions produced by several of the collections. Additional evidence for survival in debris was obtained from plots at one location planted to maize in 1969 in undisturbed 1968 stubble and debris. In late June, when the corn plants were about 1 ft high, abundant eyespot lesions appeared in zones on the older leaves (Fig. 6). Plants only 10 ft away, where no debris was present, were free from the disease at this stage. Yellow leaf blight (*Phyllosticta* sp.) (7) was also present in these debris plots.

The eyespot disease has not been limited to fields with debris, and observations indicate that the spores of the fungus are readily carried by air currents. Scattered eyespot lesions were found in late June to early August 1969 in Wisconsin and Indiana. In only a few fields, however, was there a substantial buildup in late August or September. This was in contrast to the heavy epiphytotic which developed late in the season of 1968 in Wisconsin, but not in Indiana. The late season of 1969, however, was relatively dry in comparison with that of 1968.

Distribution.—Distribution of the disease in North

America includes Illinois, Indiana, Iowa, Minnesota, Ohio, and Wisconsin. Symptoms characteristic of the disease have been reported from Nebraska, South Dakota, and Ontario, but its actual presence has not been confirmed. Similar symptoms were also observed in the province of Styria, Austria, first in 1960 by one of the authors and again in 1962 by Director F. Beran (*personal communication*) of the Bundesanstalt für Pflanzenschutz. In 1962 there were appreciable reductions in yield.

Varietal reactions.—A number of hybrids and inbred lines were grown in the field in 1969 in an attempt to determine their reaction to the eyespot disease. One trial, planted in the debris of a plot in which maize plants had been severely diseased in 1968, developed considerable natural infection; a second trial, sprayed with a spore suspension after tasseling, became severely infected. A range of reaction to the disease was noted, although the bulk of the material was moderately to highly susceptible. The inbred lines and crosses which appeared to have some tolerance were relatively late maturing. Resistance appeared to be at least partially dominant.

DISCUSSION.—We consider the eyespot disease herein described to be the same as the "Kappanbyo" reported in Hokkaido, Japan (3, 4). In both regions, the disease appears to be favored by cool, humid weather, as evidenced by its appearance only in the northern half of the US corn belt and on the island of Hokkaido, Japan, which has a cool and humid climate. Narita et al. (4) indicated that in 1956 the disease was generally more severe than in 1957 or 1958, and that 1956 was more humid than 1957 or 1958. The symptoms, the development of the disease in the field, and the descriptions of the causal organism are similar. The spore size reported (3), (average $32.4 \times 2.6\ \mu$) compares favorably with that ($27 \times 3.6\ \mu$) found in the present work. Our findings as to growth of the organism agree reasonably well with their report (4) that PDA gave better growth than did corn extract on Czapek's agar, that 24 C gave opt radial growth on PDA, and that best germination was at 28-30 C.

Maturing leaves appear to be more susceptible to *K. zeae* than do young leaves. Few lesions appeared on inoculated young leaves in the greenhouse. In the field during the early part of the season a few lesions are found, usually concd in small areas, but as the plants approach maturity infection becomes more general, particularly on the upper leaves.

Certain inbred lines and hybrids appeared to have a measure of tolerance to the disease. Limited evidence suggests that for some of the inbreds at least, the tolerance was transmitted to the F_1 and was partially dominant. The apparent relationship between lateness of inbreds and tolerance to eyespot needs study. Cool, moist weather conditions late in the season appeared to favor the disease, yet tolerance was found most often in the late-maturing material.

As was the case in northern Japan, the origin in N. America of this newly recognized disease is unknown. It may have been present on maize or other grasses earlier in amounts which escaped detection, and conditions in 1968 were particularly favorable for its development. With primary inoculum now present over a wide area, the chances for future epiphytotics appear to depend on weather conditions and the susceptibility level of hybrids being grown.

Our knowledge of control measures is inadequate. Clean plowing or crop rotation have reduced early infection, but the disease has built up in clean-plowed fields and where corn was not grown the previous year. Some hybrids have shown a degree of tolerance; however, because the make-up of most commercial hybrids is unknown to experiment stations and to the buyer, the problem of identifying such hybrids rests with the seed corn companies at the present time.

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