Late Wilt Disease of Maize Caused by Cephalosporium maydis

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SUMMARY

The pathogen inciting late wilt of maize is described and the name **Cephalosporium maydis** sp. nov. is proposed. The pathogen was first isolated from the roots and stems of wilting maize in 1960 in Egypt. It is a vascular parasite capable of invading xylem vessels of the entire plant. In a few cases, the pathogen has been isolated from kernels on diseased plants. The optimum temperature for growth is about 30°, the maximum 36°, and the minimum 12°C. *C. maydis* was the only one among 4 pathogenic species of this genus capable of inciting a wilt of maize following artificial inoculation in the greenhouse. Conidia and conidiophores of the fungus are longer than those of other pathogenic species of *Cephalosporium*. Penetration of the host apparently takes place about 6 weeks after planting, whereas symptoms may appear from just prior to tasseling (2 months after planting) until shortly before maturity.

INTRODUCTION.—Pathogenicity of a number of species of *Cephalosporium* has been reported. Reddy and Holbert (6) have reported pathogenicity of *C. acremonium* Corda on corn. Stripe disease of wheat caused by *C. gramineum* was described by Nisikado, *et al.* (5). Allington and Chamberlain (1) found *C. gregatum* as the causal organism of the brown stem rot of soybean. Butler and Khan (3) described *C. sacchari* as the incitant of wilt of sugarcane.

Late wilt of maize was first discovered in Egypt in 1960 (7) and has assumed major importance in that country. The disease was described and the causal fungus tentatively identified as a species of *Cephalosporium* (15). The purpose of this paper is to describe the causal organism in detail and to report recent observations and experiments on the disease.

SYMPTOMS.—The first symptom of this disease is a moderately rapid wilting of plants usually about the time of tasseling; but time of onset may extend from just prior to tasseling until shortly before maturity. Wilting progresses from lower to upper portions of the plant. Leaves become dull green, eventually lose color, and become dry as though suffering from lack of water (Fig. 1-A). Vascular bundles in the stalk become reddish brown; and, within a short period, lower internodes assume this color. In advanced stages, lower portions of the stalk become dry, shrunken, and hollow. Stalk symptoms may be modified depending on extent of invasion by saprophytic organisms. Diseased parts of the lower stalks may become soft and wet when overrun by saprophytes. After the first wilt symptoms appear, progress of the disease is relatively rapid. Because of the delay in appearance of initial symptoms until about flowering, this disease has been designated as "late wilt."

THE PATHOGEN.—*Microscopic characters.*—Hyphae are hyaline, septate, branched, and decumbent. Conidiophores are 30-250 μ long, but occasionally are 400 μ long. They are hyaline, mostly septate, branched, and straight. Conidia are produced successively and exogenously at apices of the conidiophore so that several spores collect in heads (Fig. 1-C, G). Conidia are hyaline, straight, single-celled, oblong, and measure 3.6-14 x 3-3.6 μ (7.2 x 3.5 μ). Conidia germinate rapidly, usually by 2 polar germ tubes, but 1 and sometimes 3 germ tubes may be formed. Anastomosis of germ tubes is unusually frequent in this fungus (Fig. 1-F). After about 3 weeks of growth on potato-dextrose agar (PDA), conidia are difficult to find in the culture. Small botryoid sclerotia-like bodies, consisting of a few thick-walled, dark-colored cells, appear in old cultures (Fig. 1-E).

Cultural studies.—The pathogen grew well and sporulated on oatmeal, prune, PDA, malt extract, and yeast extract-glucose agars. On PDA and on yeast extract-glucose agar, the minimum temperature for growth was about 12°, the optimum 30°, and the maximum 36°C. Growth at 28° was similar to that at 32°C. At 12° and and 36°C, the fungus showed slight growth; colony diameter reached 2.3 and 3.0 cm, respectively, in 2 weeks. No growth was observed at 8° and 38°C, even after 4 weeks. Spores appeared within 2 days after seeding on the media at 22-24°C. Good growth and sporulation were obtained on potato-dextrose broth and on yeast extract-glucose broth in which spores accumulated at the bottom of the flask.

The macroscopic characters of the pathogen are, unless otherwise stated, those observed in 1-week-old cultures on PDA (Difco) at 24°C. Mycelial growth is moderately rapid, covering the agar surface of a 9-cm petri dish in 9 days. Colonies are low-growing and felty; at first white, but turning gray with age. Some cultures become slate gray to black after 2-4 weeks of growth. The margin of the colony has a characteristic

"rhizoid" appearance (Fig. 1-D) and "hyphal ropes" are often oriented in a clockwise direction.

Comparison of growth of Cephalosporium acremonium, C. gramineum, C. gregatum, and the late wilt organism on different media.—Three species of *Cephalosporium* and the late wilt fungus were grown on 6 media at 22-24°C. As shown in Table 1 and Fig. 2-A, the 4 organisms are distinct. The late wilt fungus grew most rapidly and the diameter of the colony reached 9 cm in 9 days on media 1, 2, and 3. On Czapek's agar and on glucose-casein hydrolysate agar, growth was very poor. The growth of *C. acremonium* and *C. gramineum* was about the same on all media. *C. gregatum* grew slowly, but was favored in this respect by yeast extract-glucose and by soybean stem extract agar.

C. acremonium produced a white, floccose orbicular mycelial mat that later developed a typical pinkish tinge. *C. graminium* produced dense, decumbent, flesh-colored colony with a smooth margin. *C. gregatum* produced a flat, dense, radially folded mat of putty-colored mycelium.

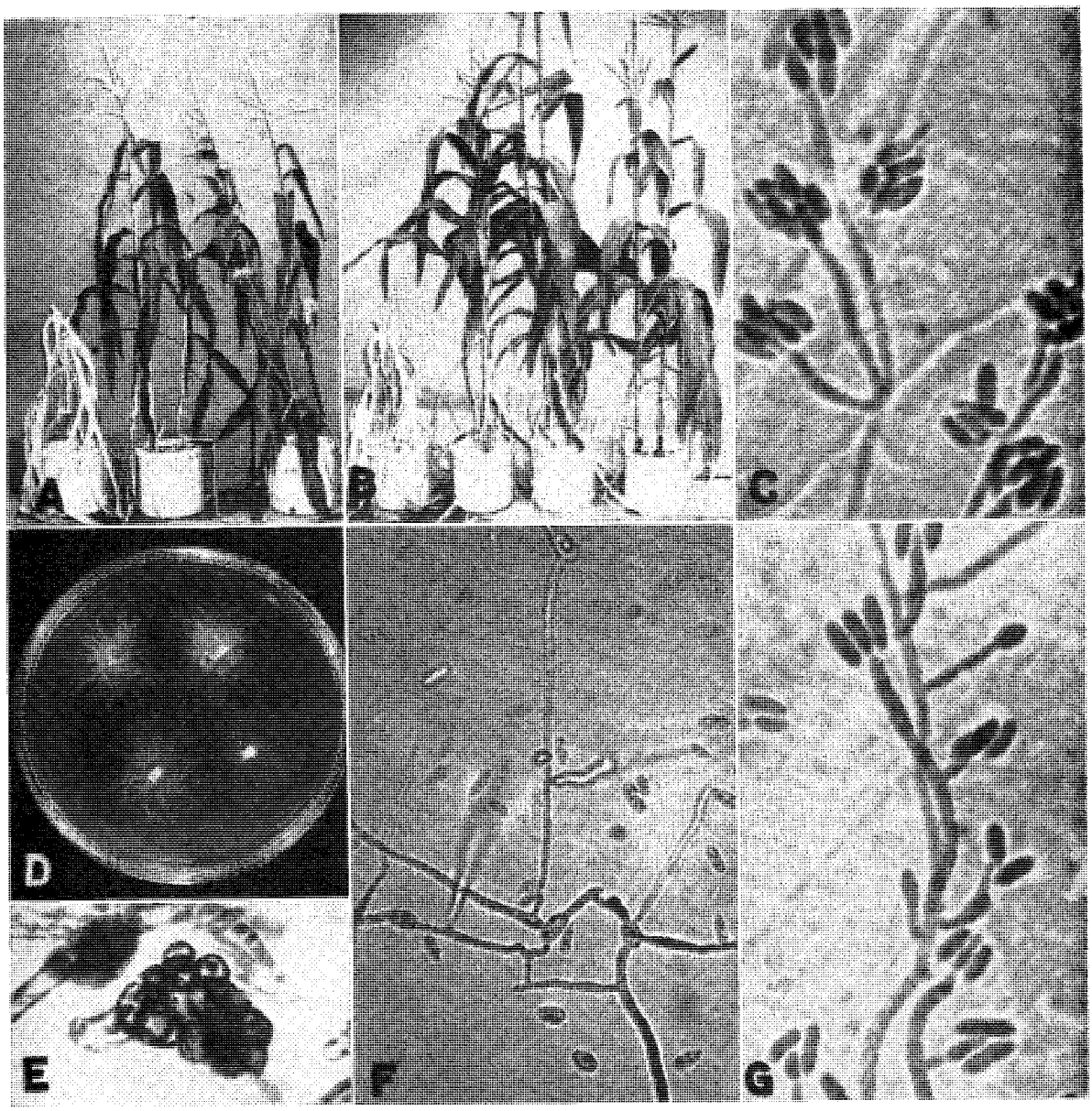

Fig. 1. A) Typical symptoms in the plant at the left following inoculation with *Cephalosporium maydis*. Non-inoculated controls at right. **B)** From left to right: plants inoculated with *C. maydis, C. acremonium, C. gramineum,* and *C. gregatum,* respectively. **C, G)** Formation in culture of clusters of spores at apices of conidiophores of *C. maydis*. **D)** Rhizoid appearance of young colonies of *C. maydis* on potato-dextrose agar (PDA). **E)** Botryoform sclerotia-like bodies consisting of thick-walled, dark-color cells in old cultures of the maize wilt pathogen growing on PDA. **F)** Germination of spores and characteristic anastomosis of germ tubes.

TABLE 1. Growth of 4 species of *Cephalosporium* on 6 different media after 9 days of incubation at 22-24°C

Culture media	Diam of 9-day-old colonies: Maize wilt fungus (cm)	*C. acremonium* (cm)	*C. graminium* (cm)	*C. gregatum* (cm)
Glucose-yeast extract agar	9.0	3.8	2.4	2.0
Malt extract agar	9.0	3.6	1.6	1.0
Potato-dextrose agar	9.0	3.6	1.5	1.1
Czapek's agar		3.7	2.2	.9
Soybean stem extract agar	9.0	3.8	3.0	3.0
Glucose-casein hydrolysate agar		3.7	2.3	.6

Effect of temperature on mycelial growth.—The relationship of temperature to mycelial growth of the maize wilt pathogen was compared with that of *C. acremonium, C. gramineum* and *C. gregatum*. Cultures were seeded on glucose-yeast extract agar and incubated in the dark at specific temperatures.

Table 2 shows that *C. acremonium* grows from 8-38°C, whereas the wilt fungus range was 12-34°C. *C. gramineum* and *C. gregatum* did not grow at 30°C which is about optimum for *C. acremonium* and the maize wilt fungus.

Pathogenicity on maize.—To test pathogenicity of the 4 organisms, a double cross hybrid (Indiana 620) was grown in a nutrient solution (Hoagland and Arnon's No. 1) (4), using Perlite as a substrate. Plants were inoculated by placing a suspension of spores and mycelium 1 in. below the seeds before planting. Only the late wilt pathogen incited disease. Symptoms appeared 2 months after planting and no plants escaped the disease. No symptoms were evident on plants inoculated with the other pathogens (Fig. 1-B). Roots are apparently penetrated about 6 weeks after planting. Microscopic examinations and isolations made at weekly intervals throughout the growing period have consistently revealed the organism in root vessels prior to stem invasion. Amount of mycelium in the vessels varies from a few stands to a compact mass of hyphae, sometimes occupying the entire lumina (Fig. 2-B, C). The organism is predominantly a vascular parasite, progressing upward through stem vessels. In a few cases, the fungus has been isolated from seed of killed plants, suggesting that the organism may be seed-borne.

Taxonomy.—The late wilt pathogen apparently belongs in the genus *Cephalosporium* Corda. No other organism of the genus has previously been described causing wilt on maize. Morphological characteristics of the maize wilt pathogen do not conform precisely to the description of *Cephalosporium* species listed by Buchanan (2) and Saccardo (8, 9, 10, 11, 12, 13, 14). The 3 pathogenic *Cephalosporium* spp. cited above resemble the late wilt pathogen in general shape of spores. Conidia of the late wilt parasite, however, are appreciably longer (Table 3). The most striking difference is in length of branched conidiophore (30-250 μ and occasionally 400 μ) of the late wilt pathogen. The maximum length of the others is not more than 30 μ. According to Butler and Khan (3), conidia of *C. sacchari* are curved on one side and 2-3 septate; whereas conidia of the corn wilt are almost always straight and nonseptate.

Only the late wilt pathogen was capable of inciting disease in maize under artificial inoculation in the greenhouse. This differs markedly from anything described for any known member of this genus. The late wilt pathogen, however, appears more closely related to that genus than to any other.

Accordingly, the name **Cephalosporium maydis** sp. nov. is proposed.

Cephalosporium maydis sp. nov.—Mycelium decumbentibus, primo albam denique griseam colore; margine colonium rhizoidibus; condiophoris hyalinis, 60-250 μ longis, simplicibus vel ramosis; conidiis hyalinis, oblongis vel ellipticis, continuis 3.6-14 x 3.3-3.6 μ, capitatus non catenulatus, capitibus irregularibus aeriis vel decumbeutibus.

TABLE 2. Comparison of colony color and cardinal temperatures for mycelial growth of 4 species of *Cephalosporium*

Name of fungus	Color of colony	Temperature and mycelial growth: Minimum (°C)	Optimum (°C)	Maximum (°C)
C. acremonium	White to flesh-colored	8	30	Above 38
C. gramineum	Flesh-colored	Below 8	20	28
C. gregatum	White to flesh-colored or gray	Below 8	24	Below 30
C. maydis	White to pale gray becoming slate gray with age	Below 12	30	Above 34

TABLE 3. Comparative size of conidia and conidiophores of 5 species of *Cephalosporium*

Name of author	Species of *Cephalosporium*	Range in size of conidia (μ)	Avg (μ)	Length of conidiophores (μ)
Reddy and Holbert	*C. acremonium*	3-6 x 1-1.8	4.3 x 1.3	
Nisikado, Y., *et al.*	*C. gramineum*	5-11 x 1.5-3	7.4 x 2.7	5-20
Butler and Khan	*C. sacchari*	4-12 x 2-3 (septate)		6-30
Allington and Chamberlain	*C. gregatum*	3.4-7.6 x 1.7-3.4		4-25
Authors	*C. maydis*	3.6-14 x 3.0-3.6	7.2 x 3.5	30-420

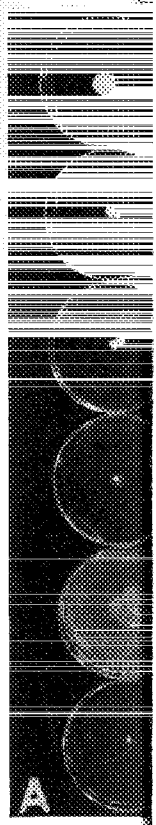

Fig. 2. A) 22-24°C. Colu yeast extract a casein hydroly root of a pla

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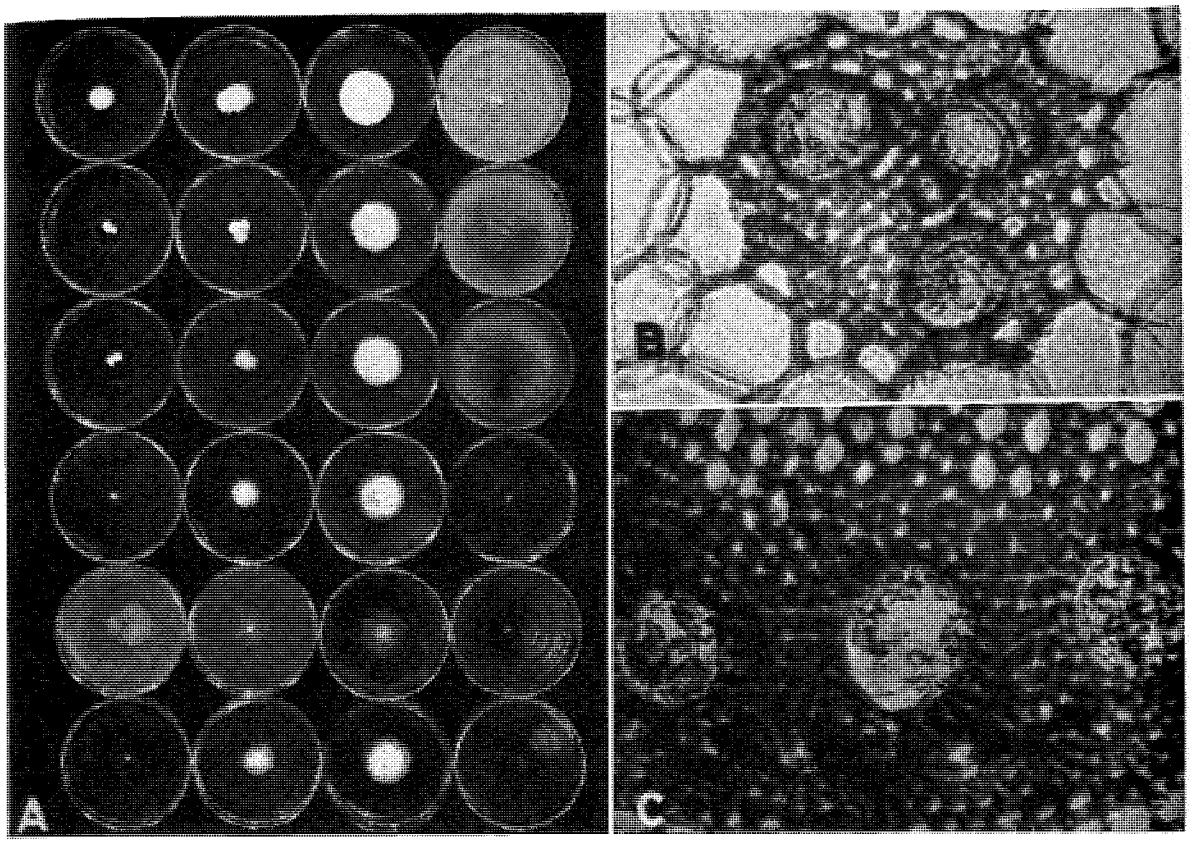

Fig. 2. A) Growth of 4 species of *Cephalosporium* on 6 agar substrates after 9-day incubation in darkness at 22-24°C. Columns, left to right: *C. gregatum, C. gramineum, C. acremonium,* and *C. maydis*. Rows, top to bottom: glucose-yeast extract agar, malt extract agar, potato-glucose agar (Difco), Czapek's agar, soybean stem extract agar, and glucose-casein hydrolysate agar. **B)** Mycelium in xylem of stalk of a plant infected with late wilt. **C)** Mycelium in xylem of root of a plant infected with late wilt.

Habitat in radicibus et caulibus plantarum vivarum (*Zea mays*) et in terris in Egypt.

Mycelium decumbent, felty, low-growing, white to pale gray becoming slate gray or black with age; hyphae hyaline, septate simple or branched. The margin of the colony has a characteristic "rhizoid" appearance and "hyphal ropes" are often oriented in a clockwise direction. Conidiophores usually 60-250 μ, but occasionally longer, mostly branched with conidia forming successively and exogenously at the apices so that several spores may collect in heads. Conidia hyaline, single-celled, straight, oblong, and 3.6-14 x 3.3-3.6 μ (7.2 x 3.5 μ) in size. Small botryoform, sclerotia-like bodies, consisting of a few thick walled, dark-colored cells appear in old cultures. Habitat: In roots and stems of living maize plants and in soil in all provinces of Egypt.

DISCUSSION.—An increasing number of species of *Cephalosporium* formerly considered saprophytic are being associated with vascular disorders in plants. Butler and Khan (3) found *C. sacchari* on sugarcane in 1913. In 1926, Reddy and Holbert (6) gave a detailed description of the black bundle disease of corn incited by *C. acremonium*. Nisikado (5) described *C. gramineum* as the incitant of the stripe disease of wheat in 1934. In 1948, Allington and Chamberlain (1) gave a detailed description of the brown stem rot of soybean caused by *C. gregatum*. The late wilt pathogen is similar to the description of the genus *Cephalosporium* given by Buchanan (2). We distinguished the new pathogen from the known pathogenic species of *Cephalosporium* on the basis of shape and size of the conidia and conidiophores, the color and type of colony, temperature requirements, and the fact that it is the only one that causes wilt of maize. The late wilt disease of maize is becoming of great importance in Egypt. The organism is definitely soil-borne; however, it may be seed-borne. Its parasitic ability on other common crops should be studied and the effect of the crop rotation on the incidence of the disease. The only means of control, as this is a soil-borne disease and can grow saprophytically, is to search for a source of resistance among varieties and inbreds of maize.

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