Mycosphaerella zeae-maydis sp. n., the Sexual Stage of Phyllosticta maydis

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The authors thank Prof. R. P. Korf, Department of Plant Pathology, Cornell University, Ithaca, New York, for a critical reading of the manuscript and W. J. Dress, Prof. of Botany in the L. H. Bailey Hortorium, Cornell University, Ithaca, New York, for composing the Latin description.

Accepted for publication 1 November 1972.

ABSTRACT

Mycosphaerella zeae-maydis sp. n. is described as the sexual stage of *Phyllosticta maydis*. Pseudothecia were observed in naturally infected corn leaf debris, and the fungus was readily cultured from overwintered plant material and from leaves infected during the growing season. Pseudothecia with mature ascospores form in culture at 21 C in darkness. The pseudothecia are about 140 μ in diam, the asci 45 to 65 × 9.6 to 12 μ, and the ascospores 15 to 20 × 5 to 6 μ. The ascospores are hyaline and two-celled, with the apical cell broader and longer than the lower cell and with a marked constriction at the septum. Cultures from single conidia and from single ascospores form pycnidia and conidia at 24 C in the light and produce symptoms characteristic of yellow leaf blight when used in the inoculation of corn. Characters differentiating the new species from *M. zeae* (=*M. zeicola*) and *M. maydis* are noted.

Phytopathology 63:529-532

Additional key words: Zea mays, corn, maize, yellow leaf blight.

Yellow leaf blight of corn (*Zea mays* L.) has been reported in mid-western and northeastern USA and in Canada, and the pathogen has been identified as *Phyllosticta maydis* Arny & Nelson (1, 2, 3, 4, 5, 8, 10). In the spring of 1970 and 1971, pseudothecia of a species of *Mycosphaerella* were found in corn leaf debris collected at Aurora, New York, during routine sampling for overwintering propagules of *P. maydis*. Isolation of *P. maydis* from these specimens was relatively easy. There was no definite proof that the fungus isolated was the same as the *Mycosphaerella*, however, and an attempt was made to induce *P. maydis* to produce a sexual stage in culture. Ascocarps of *Mycosphaerella* developed in cultures of *P. maydis*, and this sexual stage resembled that seen in field material. Similar mature pseudothecia formed in cultures from 27 different monoconidial isolates of *P. maydis*. This paper reports the methods used in obtaining the ascocarps and in proving pathogenicity of isolates from single ascospores from these ascocarps. The morphology and taxonomy of the species are also described.

MATERIALS AND METHODS.—*The conidial stage and pathogenicity*.—Twenty-seven monoconidial slant cultures were prepared from isolates derived from fresh diseased corn plants collected in the field. Their distribution included 17 New York counties, one county in Pennsylvania, and one province in Canada. The cultures were maintained on potato-dextrose agar (PDA) by mass transfer. Three of these monoconidial isolates were selected for work with the sexual stage: one from Cayuga County, N.Y. (P-1), one from Delaware County, N.Y. (P-4), and one from Canada (P-6).

Pathogenicity of all monoconidial isolates was tested on seedlings of a corn inbred (W182B) in the fifth leaf stage. Plants were inoculated with conidial suspensions from pycnidia produced in culture and were kept in a humidity chamber at 18 C for 36 hr and in the greenhouse for 7 days before examination for yellow leaf blight.

Production of pseudothecia and pathogenicity of monoascospore isolates.—Mature pseudothecia were produced by two methods. In the first, all leaves from 20 noninoculated corn plants in the fifth leaf stage were chopped into small pieces and placed in the upper compartment of a desiccator, and 100 ml of propylene oxide solution (v/v:propylene oxide/distilled water) were pipetted into the lower compartment. The desiccator was sealed and left standing overnight. Sterile leaves were transferred to a sterile vessel, and after 5 hr of aeration, the leaves were portioned into two units. One portion was ground for 5 min in a Waring Blendor containing a slant culture of *P. maydis* and 50 ml of distilled water. The other was ground in 50 ml of distilled water, but with no culture, as a control. Excess fluid was expressed through cheesecloth and the residue was placed in petri plates for incubation.

In the second method, a small block of mycelium and pycnidia from a slant culture of *P. maydis* was placed at the side of a 2 × 5 cm section of sterile, autoclaved, senescent corn leaf in the center of a petri plate containing water agar.

All conidial isolates of *P. maydis* were maintained in darkness at 21 C in plates prepared by one of the above methods. Isolates of P-1, P-4, and P-6 were incubated in darkness for 15 days at 15, 18, 21, and 24 C.

Petri plates containing water agar were prepared for single ascospore isolations. The inner side of a plate top was smeared with Vaseline and a piece of corn leaf containing pseudothecia developed by the second method was fastened onto it. Ascospores were shed onto the medium below, and by rotating the lid every hour, ascospores were scattered on the water agar. Single ascospores were picked up with a needle and placed on PDA in test tubes. The pycnidial stage

resulting from these monoascospore isolates was used to inoculate corn seedlings in the manner described above.

Morphology and taxonomy of the sexual stage.—A comparative study was made of herbarium specimens of three possibly related species previously described on corn (7, 9, 11, 13): *Mycosphaerella maydis* (Pass.) Lobik, Type, New York Botanical Garden; *M. zeae* (Sacc.) Woronow, Type, Saccardo Herbarium; *M. zeicola* Stout, Type and 12 other Illinois collections, State Nat. Hist. Herb. Slides were prepared by soaking small pieces of corn leaf specimen containing pseudothecia in 0.2 M aqueous KOH and then mounting individual pseudothecia in 1% acid fuchsin in lactophenol (w/w). Sliding microtome sections were similarly prepared by soaking leaf fragments in the KOH solution and then mounting sections in acid fuchsin.

RESULTS.—*The conidial stage and pathogenicity.*—Symptoms characteristic of leaf blight appeared on corn seedlings inoculated with each of the 27 isolates of *P. maydis* obtained from New York, Pennsylvania, and Canada. There were no marked differences in appearance of the symptoms.

Production of pseudothecia and pathogenicity of monoascospore isolates.—Mature pseudothecia formed in quantity in tissue sterilized by propylene oxide and in pieces of corn tissue placed on water agar (Fig. 1-A). In an experiment on the effect of temperature on development of the sexual stage of three monoconidial isolates P-1, P-4, and P-6, ascocarps were produced most abundantly at 18 C and 21 C. Considerably fewer ascocarps developed at 15 C, and no pseudothecia were observed at 24 C. At 21 C, most of the ascocarps were mature after 15 days of incubation in the dark. Other monoconidial isolates produced pseudothecia readily in darkness at 21 C.

Single ascospore isolates of P-1, P-4, and P-6 were obtained. The cultures grew rapidly and closely resembled the monoconidial cultures from which they originated, producing pycnidia and conidia in abundance. Mature pseudothecia developed in each monoconidial isolate from the 30 clones investigated. For the three clones in which monoascospore isolates were made, all 72 single ascospore cultures developed mature pseudothecia, indicating that this fungus is homothallic. Inoculation of corn seedlings with conidia from these monoascospore isolates resulted in the development of characteristic yellow leaf blight symptoms. Isolations from diseased leaves gave cultures similar to *P. maydis*.

Morphology and taxonomy of the sexual stage.—Pseudothecia develop within corn leaf tissue, and at maturity the tips of these fruiting bodies are exposed. Mature pseudothecia are dark brown and globose, measuring 86.4 to 192.0 μ (mean 141.2 μ) in diameter (Fig. 1-A, C). The pseudothecial wall consists of several layers of irregular and isodiametric pseudoparenchymatous cells 8 to 25 μ (mean 15.8 μ) in diameter. Young ascocarps have no ostioles. Mature ascocarps have round papillate ostioles 14.4 to 24.0 μ (mean 18.5 μ) in diameter. These ostioles seem to open wide at time of spore discharge. Asci are borne at the bottom of the pseudothecium and are cylindrical or clavate, straight or curved, and have thick hyaline walls. The asci are rounded at the apex and slightly narrowed at the base, with short stipes. Mature asci are 40.8 to 64.8 × 9.6 to 12 μ (mean 49.5 × 11.3 μ). Each ascus contains eight biseriately arranged ascospores (Fig. 1-D). Ascospores are hyaline, straight or curved, and 13.5 to 20.0 × 5.0 to 6.0 μ (mean 16.0 × 5.1 μ). They are two-celled and the cells have rounded ends. The upper cell is markedly larger than the lower, being broader and longer. There is a marked constriction at the septum, and each cell contains two or three guttules. Both cells of the spore may germinate, and one or more germ tubes may develop from each cell (Fig. 1-B).

Diagnostic morphological characters include the size and color of the pseudothecium; size of the pseudothecial cells; shape of the asci; shape, size, and position of septa and presence of guttules in ascospores. Both *M. zeae* and *M. zeicola* have brown pseudothecia 80 to 110 μ in diameter, and small pseudothecial wall cells 7 to 15 μ in diameter. The asci of both fungi are cylindrical to clavate and 38 to 55 μ long. The ascospores are mucronate, each with a median septum and very slight constriction at the septum. Ascospores are 14 to 19 × 4 to 6 μ. *M. zeicola* should be considered a synomym of *M. zeae*.

Mycosphaerella maydis differs from the above two species in having black pseudothecia, 72 to 180 μ in diameter. The pseudothecial wall cells often exceed 15 μ in diameter. Asci are saccate and 48 to 80 × 13 to 18 μ. Ascospores tend to be larger, usually exceeding 20 μ in length. They are oblong, each with a median septum, or sometimes the upper cell shorter than the lower cell. They have two to three guttules per cell but no constriction at the septum.

The sexual stage of the fungus under consideration differs from *M. zeae* (inclusive of *M. zeicola*) in having larger pseudothecia and larger pseudothecial wall cells. The ascospores are clearly unequal, with the upper cell much broader and longer than the lower cell and marked constrictions at each septum in contrast to the spores of *M. zeae* which are mucronate with a median septum and have little constriction at the septum. The tips of ascospores are rounded and not mucronate. It differs from *M. maydis* in having nonsaccate asci, smaller ascospores, and a marked constriction at the septum of the ascospores.

Since the perfect stage of *Phyllosticta maydis* is morphologically distinct from *M. maydis* and *M. zeae*, it is described as a new species as follows:

Mycosphaerella zeae-maydis Mukunya & Boothroyd, sp. nov.

Pseudothecia in textu folii evoluta, maturitate atrobrunnea, globosa, 86.4-192.0 μ ($\bar{\mu}$ 141.2 μ) diam. Pseudothecii paries ex stratis pluribus cellularum pseudoparenchymaticarum irregularium et isodiametricarum 8-25 μ ($\bar{\mu}$ 15.8 μ) diam constans. Ascocarpi juveniles ostiolis carentes, maturi ostiola rotunda papillata 14.4-24.0 μ ($\bar{\mu}$ 18.5 μ) diam praebentes. Ostiola dimissione sporarum hiantia ut videtur. Asci ad basin prolati, cylindrici vel clavati, recti vel curvati, parietibus crassis hyalinis praediti, apice rotundati, ad basin in stipitem breven aliquantum angustati, maturitate

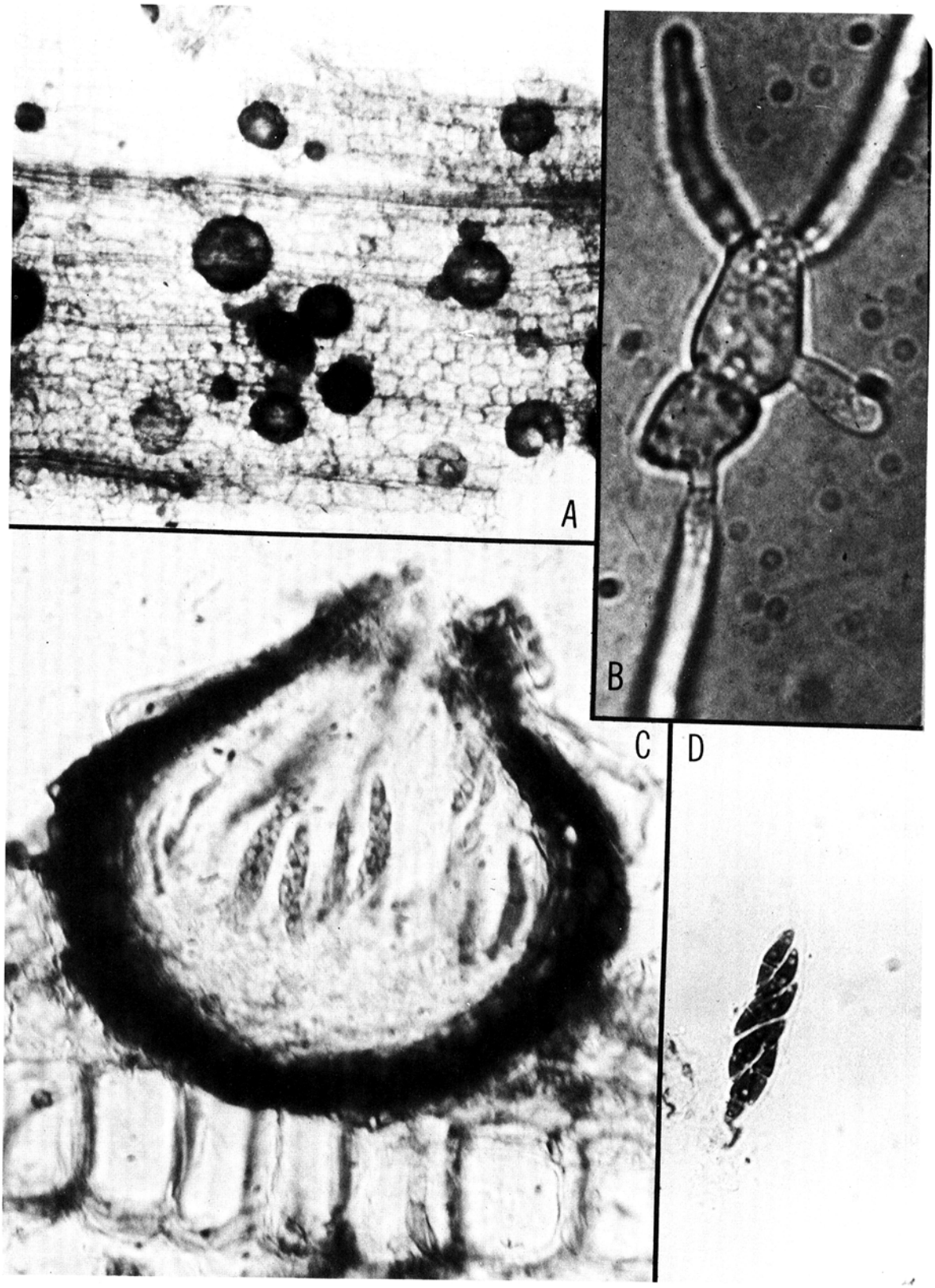

Fig. 1. *Mycosphaerella zeae-maydis*. **A)** Pseudothecia in corn leaf tissue (× 96). **B)** Germinating ascospore (× 2,600). **C)** Section through single pseudothecium (× 625). **D)** Ascus with biseriately arranged two-celled ascospores (× 840).

40.8-64.8 × 9.6-12 μ (μ̄ 49.5 × 11.3 μ). Ascus omnis ascosporas 8 biseriatim dispositas, hyalinas, rectas vel flexas, 13.5-20.0 × 5.0-6.0 μ (μ̄ 16.0 × 5.5 μ), bicellulares continens, cellula supera valde majore, infera angustiore et breviore, utraque ad apices rotundatos angustata, ad septum insigniter constricta, 2 vel 3 guttulos continente. Ascosporarum germinatio ex cellulis ambabus, vel ad polum vel ad latus, frequenter ad locos ambos reperta.

Hab in foliis emortuis *Zea mays* L.; Agronomy Farm, Cornell University, Aurora, New York.

Conidial stage: *Phyllosticta maydis* Arny & Nelson.

A specimen (D. M. Mukunya and C. W. Boothroyd No. 1 Type, on leaves of *Zea mays* L. April 1972) has been deposited in the Plant Pathology herbarium, Cornell University, Ithaca, New York, as CUP 52727. Additional specimens have been entered as CUP 52728, 52729, and 52730.

DISCUSSION.—Asexual reproduction in *M. zeae-maydis* is favored by light (1) and high temperatures (optimum 24 C). In contrast, low temperatures and darkness favor the development of the sexual stage. In winter numerous pseudothecial initials develop in corn debris, but they do not mature until temperatures are ca. 15 to 21 C in spring. A number of genera of ascomycetes respond to low temperatures (6). Wilson (12) found that the most effective temperature for *Venturia inaequalis* pseudothecium initiation in culture and in naturally infected leaves is near 13 C. The optimum temperature for maturation of *V. inaequalis* was reported to be near 20 C and relatively high temperatures (24 C) were unfavorable for maturation of pseudothecia of both *V. inaequalis* and *Pleospora herbarum* (6, 12), a case similar to that of the sexual stage of *M. zeae-maydis*. Leach (6) found that with *P. herbarum* cold induction of pseudothecial initials in the dark occurred when actively growing vegetative colonies were exposed to low temperatures (5 to 15 C) and that maturation required a low temperature (12.5 C).

The discovery of the sexual stage of *M. zeae-maydis* may help to explain the initiation and development of the yellow leaf blight disease of corn in the field. In early spring, the disease builds up very fast. If the development of the sexual stage of the fungus at low temperatures and in darkness in the laboratory are indicative of its behavior under natural conditions, it is probable that the reproductive processes of this fungus are well adapted to these factors in nature. Corn debris is a likely source of inoculum and ascospores are probably responsible for primary infection in the spring. Pycnidia and conidia are formed subsequently during the growing season and conidia are responsible for secondary infection. When the temperature drops in the fall, the formation of pseudothecia in corn leaves is initiated. The fungus overwinters as immature pseudothecia and in the following spring, as the temperature rises, the asci mature and produce ascospores. An effective disease control would be the destruction of corn debris in infested fields. This could be done by deep plowing in late fall after harvesting or in early spring before seeding.

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