Stenocarpella macrospora (=*Diplodia macrospora*) and *S. maydis* (=*D. maydis*) Compared as Pathogens of Corn

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ABSTRACT

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Stenocarpella macrospora, long known as a minor pathogen of corn (*Zea mays*) in the southern United States, traditionally has been considered a weaker pathogen in this country than the closely related and better known *S. maydis*. Recent studies in Latin America and Africa have indicated that *S. macrospora* can cause appreciable losses in yield, stored grain, and poultry fed on infected grain. Although *S. maydis* exhibits some competitive superiority with respect to metabolic and cultural characteristics, our tests showed that *S. macrospora* is actually more aggressive than *S. maydis* in attacking young stalks and ears. Whereas corn plants are susceptible to *S. maydis* only at very early stages (seedlings before the primary node develops) and at very late stages (stalks and ears several weeks after silking), *S. macrospora* can attack all corn tissues vigorously at all stages of growth. Our investigations indicate that the key to successful infection and disease development by *S. macrospora* is presence of inoculum under moderately humid conditions (mean RH = 50% day, 95% night) at typical U.S. Corn Belt temperatures. Implications of our findings are discussed with respect to the increasing production of corn in the United States under minimum tillage practices.

The *Stenocarpella* (*Diplodia*) diseases of corn (*Zea mays* L.) are widespread throughout the world (6,29). The principal pathogens involved are *S. maydis* (Berk.) Sutton, described by Berkeley (2) as *Sphaeria maydis* from an Ohio specimen in 1847, and *Stenocarpella macrospora* (Earle) Sutton, described 50 years later in Alabama by Earle (12). In 1930, Eddins (13) delineated the etiology of the leaf-spot, ear-, and stalk-rot disease caused by *S. macrospora*, comparing it as a corn pathogen with *S. maydis* and the anamorph of *Physalospora zeicola* Ell. & Ev. According to Sutton's (28) recent revision of the Coelomycetes, the two corn pathogen species considered here are properly included in the genus *Stenocarpella* Syd. on the basis of conidiation. Therefore, despite the historical association of these pathogens with an enormous body of phytopathological literature in the genus *Diplodia*, we recognize *Stenocarpella* as the proper genus.

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The importance of *S. maydis* as a stalk- and ear-rotting pathogen of corn is well documented (3,5,14,31). The considerable literature on *S. macrospora* has originated primarily from Africa, India, and Latin America and has been to a great extent concerned with the mycotoxicology of the fungus with respect to poultry and livestock (4,11,20,21) or to physiological comparisons of the fungus with *S. maydis* (22,27,32). In the United States, *S. macrospora* has been considered a minor pathogen of corn, metabolically and pathogenically inferior to *S. maydis* (14,34). The so-called "inferiority" is based on its inability to synthesize biotin (as can *S. maydis*), its lesser sporulating capacity both in vivo and in vitro, and according to Hoppe (15), inability to compete with *S. maydis* when mixed inocula of the two species are injected into corn ears.

In his 1943 report on geographical distribution of the two species in the United States, Stevens (26) showed *S. maydis* to be more widespread, having been found in California and in all but three states east of the Rocky Mountains. *S. macrospora*, though reported from Maryland, Delaware, and Connecticut, occurred primarily in the southern states. Where both species were found, *S. maydis* was greatly preponderant and apparently responsible for the greater amount of ear- and stalk-rot. Until the recent report by Turner and Bell (30), occurrence of *S. macrospora* in the United States has received little attention since Stevens' (26) 1943 study. Young et al (35) identified a number of ecological strains of *S. maydis*, and Hoppe (15) defined 24 strains from 25 isolates on the basis of cultural aversion and pathogenic dominance (or inhibition).

During our recent surveys of corn diseases in Central America, conducted with a view toward anticipating potential disease problems in the United States, we observed *S. macrospora* as a severe leaf-spotting and ear-rotting pathogen under semitropical conditions (17). Latin American workers have reported appreciable losses in yield, stored grain, and poultry fed on grain infected by *Stenocarpella* spp. (11,18). At harvest time in humid areas of these countries, we have observed instances where the grains of every ear were covered by mycelium of *S. macrospora*.

Our observations of the sometimes devastating effects of *S. macrospora* in Latin America under semitropical conditions led us to undertake the investigations reported in this paper. We have attempted to determine experimentally how these two species compare as pathogens of corn under conditions favorable to development of the host in the more humid areas of the U.S. Corn Belt, with mean RH of 50% day and 95% night.

MATERIALS AND METHODS

Sources of cultures. Two cultures were selected for use in most of our tests among several isolates of each species tested initially for comparison of growth habit and pathogenicity. Preliminary tests of isolates of *S. maydis* from several 1976–1978 fields in Maryland and Delaware and of *S. macrospora* from Tennessee, Costa Rica, and Mexico indicated no differences within species as to cultural habit, no intraspecific aversion (15), and no observable differences in pathogenic vigor when inoculated onto greenhouse plants at several stages of growth. The isolate of *S. maydis* used in most tests was from a 1977 Maryland field showing a high percentage of severely rotted cornstalks. We selected the Tennessee isolate of *S. macrospora* (received from M. T. Turner, Bloomington, IL 61701) to avoid using an exotic strain.

Preparation of inocula. Inasmuch as this report is not concerned with behavior in culture, we present only the methods and media most satisfactory for preparing comparable inocula of the two species. The nutritional studies by Wilson (34) and others provide in-depth information on all aspects of nutritional requirements for these fungi (22,27). Only solid media were used for production of pycnidia. Oatmeal agar (OA) prepared by the method of Riker and Riker (25) was the most satisfactory agar for culturing both fungi. *S. maydis* conidia could be harvested readily from OA plates, but those of *S. macrospora* were so embedded in mycelium on OA that harvest of conidia was difficult. Other agar media tested were V-8 juice, rice polish, cornmeal, potato-dextrose, yeast extract dextrose, malt extract dextrose, Sachs', and Richard's. For both species, pycnidia produced on autoclaved corn leaf pieces placed either on OA or on sterile moist filter paper proved to yield conidia most readily.

Leaf-piece plates (with OA or filter paper) were seeded with single pycnidia (for quantitative studies) by transfer with a fine needle or with wefts of hyphae from young cultures (1–2 wk). Cultures were incubated at 26 C in alternating light (200 ft-c fluorescent) and dark (12/12 hr) for 7–14 days, keeping filter paper moist by adding sterile water.

Conidial suspensions were prepared as follows: A leaf piece bearing pycnidia was lifted from a plate with forceps and laid onto the inner wall of a 50-ml beaker with pycnidia exposed. Several milliliters of distilled water with one drop of Tween 20 per 200 ml were sprayed from a wash bottle, then the leaf surface was stroked gently with a bent glass rod and rinsed to release conidia into the water. This concentrated suspension was filtered to remove leaf debris and diluted with distilled water to make the desired concentration and quantity of inoculum. The number of plates harvested depended on the number of plants to be inoculated and method of inoculation. The ratio of plates needed for comparable conidial concentrations was about 5:1 for *S. macrospora* to *S. maydis*.

Pathogenicity studies. Most of the pathogenicity tests were conducted on commercial hybrids (DeKalb XL43 and XL66, and Pioneer Brand 3184, 3334A, and 3369A) rather than experimental lines because the purpose of this investigation was to compare the two species of *Stenocarpella* rather than to search for disease resistance to either species in corn. Plants were grown in soil (2:1:1, soil-sand-peat) in 7.6- or 15-L pots and in a greenhouse bed. They were fertilized as needed for maintaining good nutrition until maturity. The greenhouse bed used in most of these studies was planted with 12 rows, three plants per row, allowing six rows for each species of *Stenocarpella*. The number of replicates ranged from two to six for plants in pots or in beds, depending upon the number of hybrids or lines, treatments, and available space. Field testing was limited to 10 5-m rows of plants in order to remove those inoculated with *S. macrospora* before pycnidia formed because this species has not been found recently in Maryland. Experiments were conducted over a 6-yr period (1976–1982) with many repetitions, especially of those that gave results contrary to those of previous workers.

Plants were inoculated at all stages of development from four-leaf stage to 3 wk after silk emergence or soft dough stage. Several inoculation techniques were used, depending on age of the plants and object of the experiment. For testing pathogenicity to leaves, plants were sprayed at four- to 12-leaf stages with conidial suspensions (about 1×10^4/ml). Young plants in pots were incubated in dew chambers (23) for 16 hr at 26 C. Plants in the bed were exposed at night to 12–14 hr simulated dew effected by two jets of steam from nozzles installed 3 m above the floor in an air-conditioned greenhouse, resulting in RH of 100% at 26–28 C. Daytime RH averaged 50%, and temperatures ranged from 30 to 34 C.

For tests of sheath and stalk susceptibility to the pathogens, plants were inoculated at all stages from four-leaf to soft dough stage by dropping 0.25 ml of conidial suspension (1×10^4/ml) behind sheaths at one or more blade-sheath junctures per plant, varying with the experiment. Care was taken not to cause mechanical injury to the tissues. In a few tests, stalks were injected with a hypodermic needle just above the first node. Ears were injected at or before silk emergence at both tip and butt ends with conidial suspensions of each species, alternating ends for each, and in other ear tests with mixtures of conidia of the two species in equal concentrations, with water controls in all tests.

RESULTS

Pathogenicity to leaf blades. The two *Stenocarpella* species produced similar lesions (gray-green, elliptical, 3–5 mm long, with water-soaked appearance) on leaf blades of all hybrids and lines after conidial suspensions were sprayed onto plants at the three- to six-leaf stage. *S. maydis* did not produce lesions on later-developing leaves, and those on the lower leaves did not enlarge further. In contrast, *S. macrospora* produced lesions on leaves of all ages and most lesions elongated to streaks as long as 10 cm (Fig. 1), with pycnidia forming in later stages. The striking symptom of leaf-striping (Fig. 2) resulted 10 days after plants were sprayed with conidial suspensions at the five-leaf stage.

Pathogenicity to other tissues. At stages from first stalk elongation until anthesis, *S. macrospora* was by far the more aggressive pathogen, rapidly inducing sheath necrosis that resulted in progressive death of leaf blades as fungal growth proceeded up the stalk (Fig. 3). At elevated RH (50% day, 100% night), these effects were more severe and heavy mycelial growth appeared on stalks and ear husks; superficial growth of *S. maydis* occurred only on interior ear husks (Fig. 4). *S. macrospora* produced more pycnidia on the stalks (Fig. 5), whereas pycnidium production on the ear husks was much greater by *S. maydis*.

In tests of pathogenicity to ears either by injection at both tip and butt ends with conidial suspensions of each species or with mixed inocula of both species in equal concentrations, *S. macrospora* showed far greater aggressiveness. In the former group of 50 inoculated ears, *S. macrospora* invariably grew to cover two-thirds to three-fourths of the husk surface, with mycelial growth extending into the grains and cob. In not a single instance did *S. maydis* exceed the mycelial development of *S. macrospora*. Similarly, in tests with mixed inocula, only *S. macrospora* was found sporulating and could be reisolated at the end of the test.

In field tests, severe ear rot resulted from inserting inoculum of either species in the sheath cavity behind young ear shoots at silk emergence. Ear husk tissues were killed by both pathogens and turned brown prematurely while the remainder of the plant was still green. In those inoculated with *S. macrospora*, the ear-shoot leaf was always blighted with a characteristic blanching of the entire blade (Fig. 6), a symptom not observed in plants inoculated with *S. maydis*.

DISCUSSION

Among several contrasting physiological characteristics exhibited by *S. maydis* and *S. macrospora*, *S. maydis* has been shown by workers in this country to be the more vigorous species (22,27,32). In pathogenicity, *S. macrospora* has been conceded superiority only in its ability to attack leaf blades. Whereas *S. maydis* has long been recognized by U.S. workers as a major stalk- and ear-rotting pathogen of corn (3,5,14,31), *S. macrospora* has been considered a minor and weaker pathogen (14,15,34). In the more humid corn-producing areas of Latin America and Africa, however, *S. macrospora* and *S. maydis* are both of great concern as ear-rotting pathogens because of potent mycotoxins they produce in infected grain, which may be used in feed for livestock and poultry (4,8,9,11,20,21,24).

Ears and stalks are reported to be resistant to *S. maydis* until several weeks after silking, or until the soft dough stage (3,7,31). We have found no reports on these aspects with regard to *S. macrospora*. Our tests corroborated the reports with respect to *S. maydis*, but we found

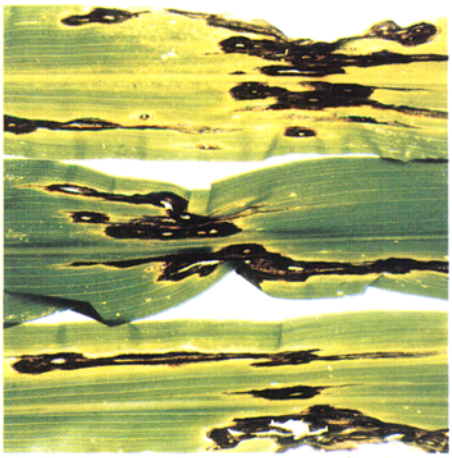

Fig. 1. *Stenocarpella macrospora* leaf lesions 12 days after inoculation.

Fig. 2. Leaf striping by *S. macrospora* (artificial inoculation).

Fig. 3. Plants inoculated at five-leaf stage with (center) *S. maydis* and (right) *S. macrospora*. (Left) Control.

Fig. 4. Contrast in stalk response to (left) *S. macrospora* and (right) *S. maydis* 2 wk after inoculation at early silk. Outer husk removed from *S. maydis* ear.

Fig. 5. Conidia of *S. macrospora* extruding in cirri from pycnidia on stalk.

Fig. 7. Plants inoculated at five-leaf stage with (left) *S. maydis* and (right) *S. macrospora*; no stalk rot developed in *S. maydis* plant. Note that mycelium obscures pycnidia.

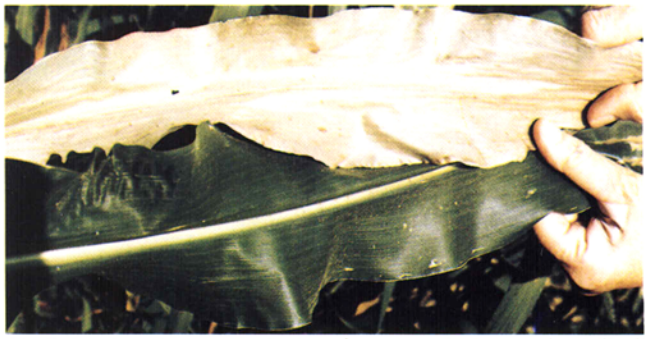

Fig. 6. Ear-shoot leaves of field plants inoculated behind ear shoot: (top) blanching by *S. macrospora* and (bottom) none by *S. maydis*.

no such period of resistance to *S. macrospora*. When inoculum of both species was provided in equal concentrations, *S. macrospora* was the more aggressive pathogen toward all tissues tested, including leaves from the four- to 12-leaf stage, stalks from first elongation to 3 wk after silking, and ears at emergence of silks.

We cannot reconcile our results with those of Hoppe (15), who after inoculation of 33 ears at milk stage with mixed inocula of an isolate of *S. macrospora* and three strains of *S. maydis*, was able 6 wk later to recover the three strains of *S. maydis* in varying proportions but failed to reisolate *S. macrospora* from any of the ears. Our results were just the opposite, using one strain of each species. We recovered only *S. macrospora* after 6 wk. The answer may lie in Hoppe's (15) own data, namely that distinct strain differences occur in *S. maydis*. Although in his discussion he reports that he found intraspecific aversion and hence strain differences in both *S. maydis* and *S. macrospora*, he mentions work with only one strain of the latter species. This discrepancy notwithstanding, it may be that *S. macrospora* also consists of various strains, perhaps with concomitant variations in pathogenic vigor. Reconciliation of our divergent results with those of Hoppe (15) awaits further testing of additional isolates, especially from various geographic areas.

The apparent competitive superiority of *S. maydis* as a corn pathogen may be related not so much to its innate pathogenic vigor or to its greater capacity to sporulate than that of *S. macrospora* but to the greater availability of its conidia resulting from a lesser tendency to form mycelium on stalks and ears at RH >50%. *S. macrospora* can produce abundant pycnidia that extrude multitudes of conidia in cirri (Fig. 5), but both in vivo and in vitro pycnidia are often buried in such dense mycelium (Fig. 7) that conidia are not readily released either for initiating subsequent infections or for use as inoculum.

Davis et al (10) were among the first to look for a physiological basis for stalk-rot resistance of corn. They reported inhibition of *S. maydis* in culture by hot aqueous extracts of dried stalk tissue. Subsequent investigators have extracted and isolated various substances from immature corn stalks that likewise retard growth of *S. maydis* (1,16,19,32,33). Our observations of the superior ability of *S. macrospora* to attack young stalks suggest the desirability of further work to determine whether this pathogen might be less retarded than *S. maydis* by such stalk extracts. The possible presence of such substances in ear husks and leaves also might explain the differential response of these tissues to *S. maydis* and *S. macrospora*.

Leaf-striping by *S. macrospora* (Fig. 2) occurred only occasionally in our tests, apparently when conidia arrived at a certain area of the sheath-blade juncture. Extremely long leaf streaks are common in fields in Mexico and Central America, each streak bearing abundant pycnidia.

The successive blighting of entire leaves as stalk infections progressed upward was a characteristic symptom resulting from artificial inoculations with *S. macrospora* (Fig. 3). The leaves were blighted without evidence of mycelial growth in the tissues, indicating possible action of a phytotoxin. Our investigations (*unpublished*) indicate that more than one phytotoxin may be involved because culture filtrates applied to corn plants have shown activity and thus differed from the plant growth inhibitor chaetoglobosin K recently isolated from *S. macrospora* by Cutler et al (9). The blighting of whole leaves was not observed in plants inoculated with *S. maydis*.

In view of the vigor with which *S. macrospora* infects and invades corn tissues under experimental conditions as reported, its obscurity as a corn pathogen in the United States is problematic. In our area, *S. maydis* has been observed increasingly often, especially under such stress conditions as unbalanced nutrition or summer drought followed by fall rains. Other fungal pathogens of corn, eg, *Cercospora zeae-maydis* Tehon & Daniels and *Kabatiella zeae* Narita & Y. Hiratsuka, have also increased in importance recently in direct relationship to the increased use of minimum tillage practices. The buildup earlier in the growing season of inoculum from debris remaining from previous crops provides pathogen propagules in time for serious damage to crops that, under conventional tillage, would have escaped early infection. Conceivably, *S. macrospora* likewise may be a potentially threatening pathogen, at least in the more humid corn-growing regions of the United States, being limited currently only by timely availability of inoculum.

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