Harpophora maydis

Scientific Name
Harpophora maydis (Samra, Sabet and Hingorani) Gams

Synonyms:
Cephalosporium maydis and *Acremonium maydis*

Common Name(s)
Late wilt of corn, 'Shallal' disease of maize, and black bundle disease

Type of Pest
Fungal Pathogen

Taxonomic Position
Class: Ascomycetes **Order:** Incertae sedis **Family:** Magnaporthaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2009 & 2010

Pest Description
Taxonomic note: Late wilt is an important disease in Egypt and parts of India. It was first recorded in Egypt in 1960 and described as a new species, *Cephalosporium maydis* (Samra et al., 1962, 1963). The description was flawed in that it did not refer to a type specimen. Domsch and Gams (1972) suggested that the conidial state of *C. maydis* was a *Phialophora* (the anamorph of *Gauemannomyces*) and that spore production in *C. maydis* was typical of that genus (Ward and Bateman, 1999). Most members of the genus *Cephalosporium* were transferred to the genus *Acremonium,* a genus of hyaline hyphomycete with aculeate (spine-like) phialides unrelated to either *Phialophora* or *Harpophora*. Gams (2000) introduced *Harpophora* as a new genus (contains anamorphs of *Gauemannomyces* and *Magnaporthe*) that is distinct from *Phialophora*. *Harpophora* spp. are characterized by fast-growing, thin colonies with sickle-shaped conidia. Older hyphae are heavily pigmented, younger hyphae are nearly hyaline, and phialides are intermediate in pigmentation relative to the older and younger hyphae. When he introduced *Harpophora,* Gams (2000) also introduced the new combination *Harpophora maydis* (Samra, Sabet, and Hingorani) Gams as a replacement for C*ephalosporium maydis*.

Hyphae of *H. maydis* are hyaline, septate, branched, and decumbent. Conidiophores may develop terminally or laterally and contain five to eight conidia clustered in heads. Conidiophores are 30-250 µm long, but occasionally are 400 µm 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. Conidia are hyaline, straight, single-celled, oblong, and measure 3.6-14 x 3-3.6 µm

(avg. 7.2 x 3.5 µm). Conidia germinate rapidly by two polar germ tubes, but one and sometimes three germ tubes may be formed. Anastomosis of germ tubes in unusually frequent in this fungus. After about 3 weeks of growth on potato dextrose agar (PDA), conidia are difficult to find in culture. Small botryoid sclerotia-like bodies, consisting of a few thick-walled, dark colored cells, appear in old cultures (Samra et al., 1963).

The pathogen grew well and sporulated on oatmeal, prune, PDA, malt extract, and yeast extract-glucose agar. The minimum temperature for growth was 12°C (54°F), the optimum 25-30°C (77-86°F), and the maximum 30°C. No growth was observed at 8 and 38°C, (46 and 100°F) even after 4 weeks, Spores appeared within 2 days after seeding on the media at 22-24°C (72-75°F) (Samra et al., 1963). The pathogen may be successfully isolated onto potato dextrose agar amended with 0.2% yeast extract and incubated at 29-30ºC (84-86ºF) (Michail et al., 1999). Young colonies are white, low growing and dense like felt, but may turn gray or black over time. Colony margins have a characteristic “rhizoid” appearance. Small sclerotia may develop in culture (Payak et al., 1970).

Biology and Ecology

The fungus is soilborne and causes a vascular wilt disease that most commonly infects seedling plants and has no specific moisture requirements. Singh and Siradhana (1987a) found that three irrigations at an interval of eight hours after inoculation supported maximum disease. Infection in corn occurs through the roots or mesocotyl (Sabet et al., 1970b). Infections have been shown to occur through stalks in India.

Figure 1. Rapid wilting of infected plant alongside resistant hybrids. Photo courtesy of H. Warren.

As plants mature, fewer are infected, and they become immune about 50 days after planting. Initially, the fungus grows superficially on the roots, producing hyphae with short, thick-walled, swollen cells (Sabet et al., 1970b). After penetration, *H. maydis* colonizes xylem tissue and is rapidly translocated to the upper parts of the plant. When infections are severe, the fungus colonizes the kernels, resulting in seedborne dissemination and also causes seed rot and damping off (El-Shafey and Claflin, 1999; Michail et al., 1999). No perfect (sexual) state has been identified (Saleh and Leslie, 2004).

H. maydis can remain viable in the soil for several years in the absence of a host. *H. maydis* has been shown to persist on corn stubble for 12-15 months (Sabet et al., 1970b; Singh and Siradhana, 1987b). Inoculum survival in soil is generally poor and restricted to the top 20 cm of soil. Although it is a weakly competitive saprophyte (Sabet

et al., 1970a), the production of sclerotia in infested host debris ensures its long-term survival. Lupine, an alternative host, can play a role in the parasitic survival of the pathogen. The pathogen is most common in hot and humid environments and in heavy-textured soils. Saturated soils lessen the incidence of *H. maydis.*

Dawood et al. (1979) noted that *H. maydis* produced no sclerotia on infected plants during its parasitic phase. It also did not form sclerotia on infected dead plants kept at room temperature for about six months. Sclerotia, however, were produced abundantly in pure cultures grown on enriched maize stalk pieces for 30-45 days at 30°C (86°F), followed by drying under electric air fan. Maximum number of sclerotia was produced at 30°C, followed by 35°C (95°F), and then 25°C (77°F). Considerably less sclerotia were produced at 20°C (68°F). The number of sclerotia decreased as the atmospheric humidity increased from 70 to 100%. Sclerotia also formed on stalk pieces of naturally infected, field grown plants when such pieces were buried in the soil. However, the viability of these sclerotia was rather low.

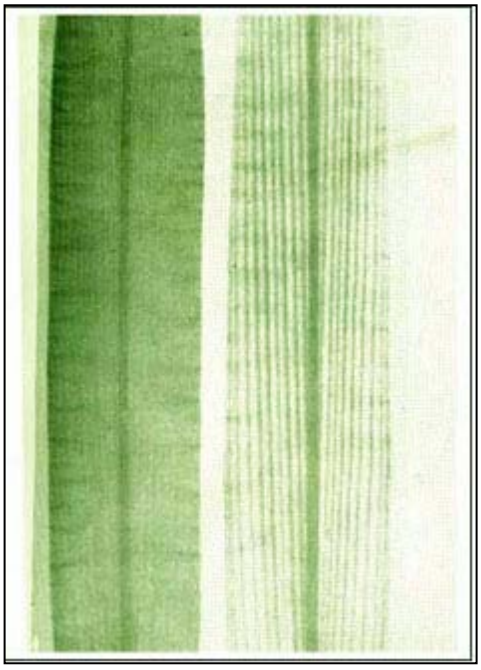

Figure 2. Leaf streaking of *Harpophora* infected plant (R) compared with healthy leaf (L). Photo from Sabet et al., 1966c.

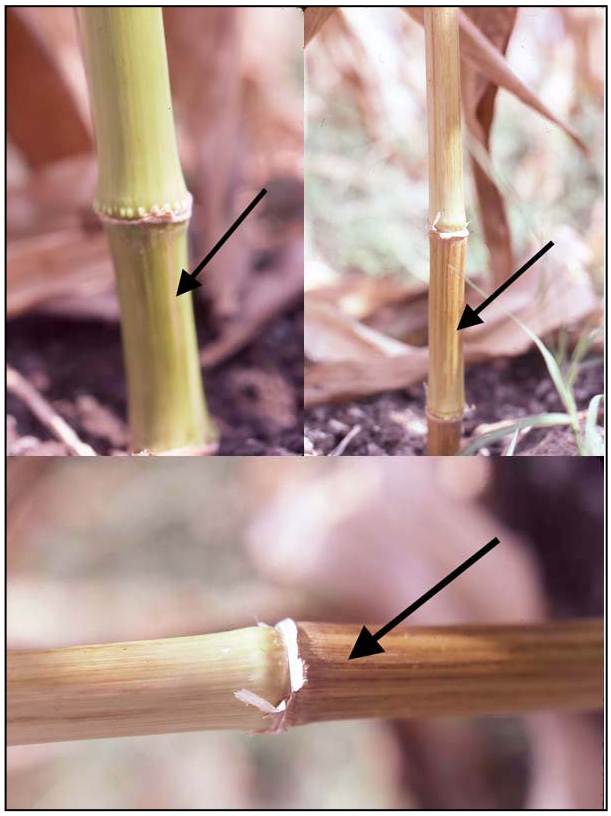

Figure 3. Progressive development of yellow to reddish-brown streaks on *H. maydis* infected lower stalks. Photo courtesy of H. Warren.

The sclerotia freshly from stalk piece cultures or kept in a refrigerator germinated readily on antibiotic-containing Richards' solution agar. Germination was visible with 48 hours of incubation at 30°C (86°F). Sabet (1984) developed a technique to produce uniform, abundant sclerotia on Farlene-glucose agar, where sclerotia were harvested after five weeks of incubation at 30°C.

Symptoms/Signs

Corn: Root tips of infected corn plants are stained red during early stages of infection, but aboveground parts generally remain symptomless until tasseling when a rapid wilting (Fig. 1) of lower leaves progresses upward. The time of onset may extend from just prior to tasseling until shortly before maturity. The wilting progresses from the lower to the upper portions of the plant. Leaves become dull green, eventually lose color (Fig. 2), and become dry as through suffering form lack of water. Leaves assume a scorched appearance. Vascular bundles in the stalk become reddish brown and within a short period, lower internodes (area between nodes on the stalk) assume this color (Fig. 3) (Samra et al., 1963; El-Shafey and Claflin, 1999).

In advanced stages, lower portions of the stalk become dry, shrunken, and hollow (Fig. 4). Stalk symptoms may be modified depending on the extent of invasion of saprophytic organisms. Secondary infection by other organisms frequently progresses into stalk rot (soft and wet). According to Jain et al. (1974) a 'sweetish smell' often accompanies the wet rot. 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' (Samra et al., 1963). Kernels that form may be poorly developed. Growers often only recognize stalk rot diseases in India during the final stages when the stalks begin to lodge (fall over), especially when intensified by delayed harvest or wind damage (Jain et al., 1974).

Figure 4. Dry hollow *H. maydis* infected stalk. Photo courtesy of CIMMYT.

Cotton: Reddish lesions and shallow cracks have been observed on cotton roots (Bahteem 185 cultivar) grown in inoculated soil. These lesions disappear as the cotton plants mature, and *H. maydis* has not been recovered from them (Sabet et al., 1966a). No aboveground effects are observed throughout the growth of the plants up to maturity. It is not known how important infections of *H. maydis* on cotton are to subsequent infections on corn.

Lupine: Wilt and root rot (Sabet et al., 1966b). *H. maydis* causes a significant damping-off and stunting of the widely cultivated *Lupinus terminis* in Egypt (Sahab et al., 1985).

Pest Importance

Stalk and root rots are, in general, the most serious and widespread group of diseases affecting corn growing regions of the world (Sabet et al., 1966b). *Harpophora maydis* causes a severe stalkrot. In Egypt, 90% of the plants of susceptible cultivars may be infected. Yield losses of up to 40% have been reported (Jain et al., 1974; El-Shafey and

Claflin, 1999). The severity of the late wilt disease has diminished in most corn plantings due to the introduction of new resistant hybrids, which have replaced the local susceptible cultivars, through an active breeding program against late wilt disease by the Egyptian Ministry of Agriculture (Mostafa et al., 1996) and Ramana et al. (1997) and Satyanarayana (1995) in India. The extent of resistance or tolerance in corn lines adapted for the United States to late wilt, however, is not known since this disease is not commonly screened for in U.S. breeding programs (Bergstrom et al., 2008).

Although evidence exists that the *H. maydis* population in Egypt is clonal, at least four phylogenetic lineages are present (Zeller et al., 2000; Saleh et al., 2003). These lineages differ in their ability to colonize maize plants and their relative aggressiveness in single culture inoculations or both (Zeller et al., 2002). They also differed in mixed culture inoculations (El-Assiuty et al., 1999). Adequate understanding of where each lineage is located within a country and using all lineages to challenge host material during the development of resistant germplasm is needed to best deploy host resistance. For example, corn germplasm that is susceptible to lineage IV might be well suited for part of the country where this lineage is not present but not in parts of the country where it is present.

Known Hosts

Zea mays (corn), *Gossypium hirsutum* (cotton), *Lupinus terminus* (lupine)

Known Vectors (or associated insects)

H. maydis is not a known vector, but, it attacks the roots of corn and can allow other pathogens (fungi and viruses) access to the plant. Sabet et al. (1966a) showed that infection of cotton roots with *H. maydis* decreased the severity of cotton wilt, caused by *Fusarium oxysporum*.

Known Distribution

H. maydis has been reported from Egypt, India, Hungary, Israel, Italy, Portugal, Spain (Samra et al., 1962, 1963; Payak et al., 1970; Pecsi and Nemeth, 1998; Bergstrom et al., 2008). There also are unconfirmed reports of the disease in Romania and Kenya which imply that some strain(s) of the pathogen are capable of surviving climates similar to U.S. corn production regions (Bergstrom et al., 2008).

Potential Distribution within the United States

The pathogen is not currently known to exist in the United States, but poses a serious threat to corn production in this country. The organism can be easily moved in shipments that contain either infested soil or seed. Its ability to withstand high temperatures would allow it to survive in the southern United States. Growing conditions during May-June are most conducive. Based on climate models alone, the southern half of the country would be favorable for disease development. When the climatic model is combined with the geographic distribution of corn production in a recent risk analysis by USDA-APHIS-PPQ-CPHST, most of the continental United States is at moderate risk of *H. maydis* establishment. Areas of Arkansas, Illinois,

Indiana, Mississippi, Missouri, Tennessee, and Texas, have the highest risk for establishment of *H. maydis*.

Survey

CAPS-Approved Method: Visual survey is the method to survey for *H. maydis* by collecting symptomatic plant material.

Literature-Based Methods:
Visual survey: surveying for disease is difficult because symptoms cannot be identified until tassel emergence when host height makes viewing large numbers of plants difficult. At tasseling, fields should be monitored visually for characteristic symptoms. Symptom recognition is based on the dull green, desiccated (scorched) leaves, streaked and "collapsed" stalk, and discolored pith tissues. Identification may be complicated by the similarity of symptoms caused by other common problems, such as nitrogen deficiency, but which may occur over larger areas and following excessively wet weather when nitrogen sources may be leached beyond the reach of growing roots.

Key Diagnostics

CAPS-Approved Method: Confirmation of *H. maydis* is by morphological identification. Pathogen may be identified morphologically by examination of the shape and size of conidia and conidiophores, color and type of colony, and temperature requirements.

Literature-Based Methods: Symptoms are not definitive and morphological and microscopic characteristics are still used to identify *H. maydis* (El-Shafey and Claflin, 1999). Isolates can differ in virulence and competitiveness (Zeller et al., 2002); thus, isolation, culture, direct microscopic evaluation, pathogenicity tests, or PCR are required for positive identification. Ward and Bateman (1999) use a pair of PCR primers that amplify a segment of the ribosomal gene locus from many members of the *Gaeumannomyces*- and *Phialophora* fungal pathogens from maize and other host plants. The PCR product from *Harpophora maydis* (=*Cephalosporium maydis*) can be distinguished from that of other members of the group on the basis of its unique size (490 bp) relative to that of other species. Species-specific PCR primers capable of distinguishing *H. maydis* from other species in the *Gaeumannomyces-Harpophora* complex have been developed and can be used for identification, but need to be validated for regulatory purposes (Saleh and Leslie, 2004; Zeller et el., 2000).

Successful isolation can usually be obtained by sterilizing the internode of symptomatic plants in 5% sodium hypochlorite (bleach), splitting them with a sterile knife, and placing a small piece of discolored vascular bundle on PDYA media (PDA + 0.2% yeast extract) (Samra and Sabet, 1966; Zeller et al., 2002). Single spore isolates can be obtained by dilution plating. **Recovery of *H. maydis*, even from heavily infested material, is difficult due to its slow growth and to the relative abundance of other more rapidly growing fungi, most commonly *Fusarium* spp.** (Saleh et al., 2003).

Infected seeds do not show discernible external symptoms and cannot be identified visually. *H. maydis* can be cultured from infected seed by soaking seeds in 1% sodium

hypochlorite for 3 minutes, plating on PDYA, incubating at 20°C (68°F) under 12 hour cycles of alternating near-ultraviolet light and darkness, and examining after 24 hours. Identification of cultures is accomplished by spore morphology and pathogenicity tests. The pathogen can be identified in tissue using PCR techniques that are not influenced by secondary fungal invaders (Saleh and Leslie, 2004).

Easily Confused Pests

Late wilt does not occur in the United States and may not be readily recognized or distinguished initially from abiotic stresses without some training.

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