Late wilt of maize-\textit{Harpophora maydis}

\textit{Harpophora maydis} is a soilborne and apparently seedborne fungus related to the root-infecting species in the genus \textit{Gaeumannomyces}. It is known from only a few scattered countries, where it can cause significant losses, but may have been unobserved in others in which the primary host, maize, is grown. No dispersal by fungal propagules has been demonstrated, so that, other than in soil, its likely means of spread over borders would be in seed. Importation of the fungus with seed is considered to be the source in Hungary (Pécsi and Németh, 1998). Although research has been done on chemical and biological control methods, development and use of resistant varieties is the most practical means of control. Regulation and testing of imported seed should prevent the pathogen's transport to new regions.

\textit{Harpophora maydis} (Samra, Sabet & Hing.) W. Gams 2000

Culture on PDA white to pale gray, becoming dark gray to black. Mycelium appressed, felty, margin "rhizoidal", the outermost hyphae branching to resemble roots; hyphal "ropes" curving clockwise. Hyphae hyaline, septate, Conidiophores 60-250 µm or longer, mostly branched; conidia formed in phialides at apices, collecting in "heads". Conidia straight, mostly one-celled, hyaline, oblong, 3.5-14 x 3.3-3.6 µm. Sclerotia-like bodies, composed of several thick-walled pigmented cells, formed in old cultures.

For additional details, see Samra et al., 1963; Payak et al., 1970; Gams 2000.

\textbf{Distribution:} Asia (India), Africa (Egypt), Europe (Hungary, Portugal, Spain)

\textit{Zea maydis} (Poaceae)

The fungus was described initially as \textit{Cephalosporium maydis}, based on its production of "heads" of hyaline, non-septate conidia from simple phialides (Samra et al., 1963). Gams (2000) observed that it is similar to anamorphs of species of \textit{Gaeumannomyces} and \textit{Magnaporthe} in conidiogenous cell morphology and in that its colonies are fast-growing, thin and pigmented in culture. He transferred it to the new genus \textit{Harpophora} comprised of those anamorphs. The divergent collarettes which Gams observed on the phialides are not apparent in the earliest photographs of the species (Samra et al., 1963). Molecular studies indicate that \textit{H. maydis} is closely related to species of \textit{Gaeumannomyces}, a genus in the ascomycete family Magnaporthaceae, but support it as a distinct species (Ward and Bateman, 1999, Saleh and Leslie, 2004).

\textbf{DISTRIBUTION}

This pathogen has been known to occur in Egypt (Samra et al., 1963) and India (Payak et al., 1970) for some decades. It is more recently reported from Portugal and Spain (Molinero-Ruiz et al. 2010), Hungary (Pécsi and Németh, 1998) and may be present in Kenya (Ward and Bateman, 1999). These widely scattered locations suggest its probable transmission by seed, but also a failure to distinguish its symptoms from those of other diseases or stresses (Freeman and Ward, 2004). Although the known host, maize, originated in Central America (Maiti and Wesche-Ebeling, 1998), the relatively recent appearance of this disease in the widely grown crop may indicate a different source for the fungus.

\textbf{RISK OF INTRODUCTION}

The risk for introduction of \textit{H. maydis} to new areas lies in its capacity to be seedborne; the risk for establishment in new areas lies in the widespread cultivation of maize as a food crop and in the ability of the fungus to survive in plant debris and possibly in the soil.

\textbf{SIMILARITIES TO OTHER SPECIES/CONDITIONS}

\textit{Harpophora maydis} lacks a known teleomorph but is similar to the Harpophora anamorphic states of \textit{Gaeumannomyces} species in culture (Saleh et al., 2003). It can be distinguished from other pathogenic \textit{Cephalosporium} species due to its fast growth in culture on complex media, minimal growth on Czapeks agar (a defined medium), and eventual dark pigmentation (Samra et al., 1963). The conidiophores can be quite long and the conidia are generally larger than those of other pathogenic \textit{Cephalosporium} species (Samra et al., 1963). The divergent collarettes on the phialides (Gams, 2000) also separate it from \textit{Cephalosporium} and other anamorphic fungi pathogenic to maize.

Although drought and other stalk- or root-rotting pathogens can cause wilt, the late appearance of the symptoms under conditions of adequate soil moisture are characteristic of this disease.

\textbf{DETECTION AND INSPECTION METHODS}

The symptoms of wilt, external and internal discoloration of stems, and stalk rot are not particularly
distinctive and may be obscured due to drought, over-irrigation or other pathogens.

**DIAGNOSTIC METHOD**

Saleh and Leslie (2004) found a unique 300 bp sequence of rDNA in all strains of *H. maydis* tested indicating that the PCR primer pair for that sequence could be used in a specific identification test. Molecular methods can also be used to distinguish the species from *Gaeumannomyces* and *Phialophora* pathogens of maize stems (Ward and Bateman, 1999). Sequences of the ITS and other regions of nuclear DNA are available in GenBank for comparison (NCBI, 2010).

**NOTES ON CROPS/OTHER PLANTS AFFECTED**

*Zea mays* L. is the major crop so far known to be damaged, but the fungus may have other hosts, particularly if it originates from Egypt or India rather than with *Zea mays* from the western hemisphere. *Lupinus termis* Forssk. ( = *L. albus* L. var. *albus*), a cultivated forage legume, has been reported as a host in Egypt (Sahab et al., 1985).

**SYMPTOMS**

Leaves wilt moderately rapidly beginning in the tasseling (flowering) period or later. Progressing upward from the lower part of the plant, leaves become dry and dull green, rolling inward and eventually losing color. Vascular bundles in the stalk turn reddish-brown and then internodes also become discolored. Lower portions of the stalk are dry, shrunked, and hollow (Samra et al., 1962). Some plants develop yellowish to purple or dark brown streaks on the lower stem (Payak et al., 1970; El-Shafey and Claflin, 1999). Rotting of roots and lower internodes may involve secondary organisms (Sabet et al., 1961; Samra et al., 1962).

**BIOLOGY AND ECOLOGY**

**Life Cycle**

Soilborne or seedborne *H. maydis* infects the roots of young plants or the seedling mesocotyl, invades the vessels, and grows or is translocated from the roots up the stalk and into the ear stalks and grain (Samra et al., 1963; Sabet et al., 1970b). Initial superficial growth on the roots consists of short, brown, thick-walled swollen cells (Sabet et al., 1970b). In India, maximum disease occurred at a constant 24 °C or when the temperature varied naturally between 20 and 32 °C. Less disease was obtained at a constant temperature of 36 °C (Singh and Siradhana, 1987a). These parameters correspond approximately to earlier observations of an optimum temperature of 30 °C for growth on PDA and yeast-extract-glucose agar, with the maximum for growth at 36 °C; no growth occurred at 8 or 38 °C (Samra et al., 1963).

The fungus was found to remain viable and virulent in/on seeds in the laboratory for up to ten months. Mycelium added to soil survived three months if the soil was sterilized, but only half as long if it were untreated (Singh and Sirhana, 1987b). Other studies found *H. maydis* to have low competitive saprophytic ability in soil and to be inhibited by the growth of soil microorganisms (Sabet et al., 1970a). In infected stems kept inside at 20-35 °C, the fungus survived and retained pathogenicity for up to 24 months. In the field stem pieces on the surface of soil retained the pathogen for twelve months, but it could not be recovered after ten months from pieces buried at 10 cm (Singh and Sirhana, 1987b). The researchers suggested that survival with seed would be longer in cooler climates.

**Physiology and phenology**

This pathogen was distinguished initially by its rapid growth in culture (Samra et al., 1963). A slow-growing variant was reported by Payak et al. (1970) in India, but because conidia and conidiophores were not observed, its identification cannot be assured.

Zeller et al. (2000) and Saleh et al. (2003) found four distinct genetic lineages among Egyptian isolates. No gene flow between them was apparent, suggesting clonality of generation and the absence of sexual reproduction (Saleh et al., 2003).

Virulence and competitive ability are not the same in *H. maydis*, varying independently among the four lineages of the pathogen in Egypt (Zeller et al., 2002). The most virulent lineage was least competitive in mixed inoculum and one of the less virulent was present in 70% of infections.

**Associations**

The rot of the base of the affected stem and the associated roots is partly due to secondary organisms. Sabet et al (1961) identified the fungi *Rhizoctonia bataticola*, *Fusarium moniliforme* and other *Fusarium* species, as well as some bacteria, from stalks primarily affected by late wilt. *Fusarium graminearum* and *F. moniliforme* are stalk rot-causing fungi that have been frequently found together with *H. maydis* in seed samples of maize in Egypt (Mohamed et al., 1967).
As a "temporary root-surface inhabitant" that does not penetrate beyond the epidermis, *H. maydis* can reduce root infection and disease in cotton caused by *F. oxysporum f. vasinfectum* in Egypt (Sabet et al., 1969). The protective effect was greater when *H. maydis* was added to the soil of potted plants before the cotton pathogen.

**MOVEMENT AND DISPERSAL**

**Natural dispersal:** Conidia are the only spore form produced, thus they could be the means of dispersal but this has not been demonstrated to occur in nature. The spores have been observed in xylem vessels (Samra et al., 1962). Sclerotia are a means of survival in soil and are dispersed with soil (Dawood et al., 1979).

**Vector transmission:** Not reported.

**Accidental introduction:** The presence of the fungus in and/or on seed has been established (see Seedborne Aspects). Importation of infected seed is considered the cause of the pathogen's appearance in Hungary (Pécsi and Német, 1998). Movement of soil on machinery or tools could carry the fungus locally.

**SEEDBORNE ASPECTS OF DISEASE**

**Incidence**

*Harpophora maydis* was detected in 39 out of 42 seed samples in Egypt (Mohamed et al., 1967). Seed infection was induced in plants inoculated at planting time (Samra et al., 1963). In Hungary Michail et al. (1999) detected *H. maydis* in a higher percentage of white maize seeds (1-9%) than in yellow cultivars (1-3%). The fungus was detected in different ear parts, i.e., ear branch, cob, seeds, ear husks, and silk, of naturally infected maize cultivars. It was manifested most in the branch and less in the cob, seeds, husks and silk, although no part was infected at a level greater than 10%. The fungus was detected in the embryo, the endosperm and seed coat of seed in 12 of the 13 tested samples, with the exception of that of cv. Amon (Michail et al., 1999)

**Effect on Seed Quality**

Seed rot and reduced emergence resulted from infesting soil with *H. maydis* (Payak et al., 1970). Seeds obtained from infected plants of the composite variety Ambar also had reduced emergence and lower seedling vigor compared to seeds from unwilted plants.

**Pathogen Transmission**

There is no direct evidence for transmission through seeds to mature plants, but it is likely (Warren, 1983). *Harpophora maydis* is also commonly soilborne, with sclerotia surviving on maize debris (Dawood et al., 1979).

**Seed Treatment**

Seed treatment with carbendazim or captan gave effective control of late wilt of maize in India (Satyanarayana and Begum, 1996), but benomyl was not effective as a dust or dip in Egypt (Sabet et al., 1972). When applied to seeds, certain actinomycete and yeast isolates were antagonistic to *H. maydis* and significantly reduced the incidence of late wilt disease under controlled greenhouse conditions (El-Mehalawy et al., 2004).

**Seed Health Tests**

**Culture plate (Mohamed et al., 1967)**

1. Wash seeds in tap water.
2. Surface disinfect seeds in 0.1% mercuric chloride, 2 minutes.
3. Rinse in sterile water.
4. Incubate on PDA at 27°C, 10 days.

**Culture plate (Michail et al., 1999)**

1. Soak seeds in 1% sodium hypochlorite solution for 3 minutes.
2. Place ten seeds in each plates of potato dextrose-yeast extract agar medium; total of 200 seeds per variety.
3. Incubate for 2 days at 20°C under 12 h alternating cycles of near ultraviolet light (NUV) and darkness.
4. Observe plates under low magnification beginning at 24 hours after start of incubation.

**IMPACTS**

**Economic impact:** This is a late-season disease of widespread incidence and severity in Egypt, with 100% infection reported in some fields (Samra et al., 1962; Galal et al., 1979). Yield losses up to 40% in susceptible cultivars are reported (El-Shafey and Claflin, 1999).

The fungus is one of the most important pathogens of maize in some parts of India (Payak et al., 1970; Singh and Siradhana, 1987b), causing yield losses of up to 100% (Satyanarayana, 1995). It does not occur in the USA, but is considered to be a potentially important pathogen (Warren, 1983).

**MANAGEMENT**

**SPS measures**

Because the fungus is apparently seedborne, seed testing and certification could help to prevent its introduction into new areas or countries. Maize seed from countries where the pathogen is present should be tested most carefully if it is used.

**Cultural control and sanitary measures**

Crop rotation with rice provides some control, but the fungus may survive several years in soil in Egypt (El-Shafey et al., 1988; El-Shafey and Claflin, 1999). In India, it remained viable in stem pieces on the surface of soil for twelve months, but could not be recovered after ten months from pieces buried at 10 cm (Singh and Siradhana, 1987b). High soil moisture favors disease but saturated soil reduces it (El-Shafey and Claflin, 1999).

**Biological control**

A number of organisms have shown promise as control agents. Six isolates of actinomycetes (Streptomyces graminofaciens, S. gibsonii, S. lydicus, S. nogalater, S. rochei, S. annulatus) and five isolates of yeasts (Candida maltosa, C. glabrata, C. slooffii, Rodotorula rubra, Trichosporon cutaneum) from the rhizosphere of maize in Egypt were antagonistic to H. maydis in vitro, and, when applied to the seed, significantly reduced the incidence of late wilt of maize planted in H. maydis-infested sterilized soil in the greenhouse (El-Mehalawy et al., 2004). The fungus Trichurus spiralis Hasselbr. also was found to inhibit growth of H. maydis in liquid culture, on solid medium, and in soil in pots (Abdel-Hamid et al., 1981). Suspensions of the antagonistic bacterium Bacillus subtilis or its culture filtrate reduced infection when added to infested soil in pots either at the time of sowing or after sowing (Sellam et al., 1978).

**Chemical control**

Benomyl controlled the pathogen in pots and in culture, but was not effective when applied to soil (Sabet et al., 1972). In India, significant reduction of late wilt incidence was obtained with 0.1% Benlate, 0.1% Bavistan, or 0.2% Bayleton applied to the soil as a drench after stem-inoculation of 60-day old plants in pots (Singh and Siradhana, 1989).

**Host resistance**

Use of resistant varieties is considered the best, most practical control (Samra et al., 1963; El-Shafey et al., 1988). Resistance to stalk rot caused by H. maydis and other fungi was observed in different maize varieties, inbred lines and hybrids by Mohamed et al. (1966). The Egyptian resistant hybrid DC-19 was introduced by Labib et al. (1975). Hybrid varieties have been reported to be more susceptible than open-pollinated ones (Sabet et al., 1961). In India, high levels of resistance were detected in lines X102, CM111, and CM202/CM104 x W)-1-1-1 (Satyanarayana, 1995). Resistance is polygenic, quantitatively inherited, and due to additive gene effects (Shehata, 1976; Galal et al., 1979; El-Shafey et al., 1988).

Observing four lineages with different regional distributions among Egyptian isolates of the pathogen, Saleh et al. (2003) suggested that resistant lines could be deployed according to the lineages present in a region. Resistance would need to be tested with all four lineages, individually as well as in the usual combination, because virulence and competitiveness were not linked among the isolates (Zeller et al., 2002).

**GAPS IN KNOWLEDGE/RESEARCH NEEDS**

Possible alternative hosts that could enhance the pathogens survival and spread should be tested. A fast and reliable identification protocol for use on infected tissue should be established. Cultural practices that could minimize disease or contain the spread of the fungus need to be identified. Sources of genetic resistance in maize must be sought.

References
Conidiophore with phialide, 1000x. CBS 662.82A: on potato-dextrose agar

Conidiophores with conidia, 1000x. CBS 662.82A: on potato-dextrose agar.

Conidia, hyphae and conidiophores, 1000x. CBS 662.82A: from potato-dextrose agar.

Broad, pigmented 'runner' hyphae and a hyphal coil, 1000x. CBS 662.82A: from potato-dextrose agar.