Recovery Plan

For

Late Wilt of Corn

Caused by
Harpophora maydis syn. *Cephalosporium maydis*

November 12, 2008

This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension, and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of a plant disease outbreak, and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward pant disease recovery.

Executive Summary

Late wilt, or black bundle disease, poses a moderate to severe threat to corn production in Egypt and India where it occurs endemically, with yield losses approaching 40-70% in non-resistant cultivars. Corn is the most economically important crop in the U.S. with a value of $32.8 billion in 2006. Recent emphasis on biofuels has greatly increased its value in commerce in the U.S. as a primary source for ethanol production. Late wilt is considered a moderate threat to corn production in the U.S., although direct yield losses attributable to late wilt are difficult to predict. Any report of *Harpophora maydis* in the U.S. could result in a long-term quarantine, crop embargo, and restricted movement of agricultural products and equipment resulting in serious economic impact.

In addition to reports of late wilt in Egypt and India, *H. maydis* also has been reported in Israel, Hungary, Portugal and Spain. 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. *H. maydis* is a soilborne vascular wilt pathogen that may move with seed. This fungus over seasons primarily as sclerotia or mycelia on precolonized plant debris. *Lupinus termis* and cotton are the only alternative hosts reported for *H. maydis,* and it is unknown if *Lupinus* spp. or other plants common in the U.S. are collateral hosts. Optimal conditions for corn growth also are optimal for late wilt infection.

Infected seed, biological culture, or a small amount of infected crop residue could provide pathways for introduction of *H. maydis* into the U.S. from which natural increase and subsequent dissemination could occur over a period of years. Contamination of in-coming seed stocks produced outside the U.S. could be inadvertently disseminated by growers over large areas of the Southern Corn Belt. Persistence of *H. maydis* after introduction to southern U.S. corn producing areas is likely, with inoculum increasing for several years - especially where double-cropping and no-till systems are practiced. Secondary dissemination could occur through contaminated seed and the movement of agricultural equipment. Natural increase and secondary spread of *H. maydis* in the Southern Corn Belt could infest up to 75% of the corn production area within 8-10 years if contiguous frequent corn crops continue to be grown under no-till production.

The slow, natural, secondary dissemination of *H. maydis* could permit containment and eradication if the pathogen was introduced into a localized area and was detected rapidly. Effectiveness of containment and quarantine actions is dependent on rapid identification and timely implementation of control measures; however, rapid detection of late wilt in the U.S. at the present time is unlikely since early symptoms are not readily distinguished from abiotic stresses or indigenous pathogens that also produce wilt. Disease symptoms appear late in the season, infected seeds remain symptomless, and the myriad of fungi inhabiting maize tissue make isolation of *H. maydis* and diagnosis difficult. Newly developed molecular techniques may make rapid identification possible; however, prior to deployment, they need to be tested on more than an experimental scale and validated by USDA-APHIS.

The most economically effective management of late wilt is through development of genetically resistant corn lines. Resistant germplasm is known; however, the extent of resistance or

tolerance in corn lines adapted for the U.S to late wilt is not known since this disease is not commonly screened for in U.S. breeding programs. Seed treatment chemicals may limit the spread of late wilt or provide limited control, but are not registered in the U.S. Introduction of late wilt into U.S. corn production systems may require a return to tillage and alternative weed control programs in order to minimize inoculum build-up in affected areas even with the development of resistant cultivars.

Recommended Actions:

- Identify sources of genetic resistance to late wilt and characterize resistance that is present in commercial corn cultivars grown in the U.S.

- Identify potential alternative crop and weed hosts of *H. maydis* present in U.S. corn-producing areas that could serve as a reservoir for this seed, residue and soilborne pathogen.

- Improve cultural and diagnostic techniques for *H. maydis* to facilitate isolation and diagnosis while distinguishing *H. maydis* from closely related *Gaeumannomyces-Harpophora* species. Validate diagnostic techniques (USDA) to insure proper regulatory applicability.

- Evaluate effective cultural modifications to minimize disease severity. Initiate research to determine the impact of predisposing conditions (reduced tillage, herbicide - the almost universal no-till-glyphosate herbicide management practices) in U.S. corn production (74 million acres) on survival and virulence of *H. maydis*, and the subsequent severity of late wilt.

- Establish a core group of people familiar with the disease, its ecology, and control within USDA, academia, and in cooperation with the agricultural industry for diagnosis, survey and timely response.

Late Wilt of Corn or Black Bundle Disease

(caused by *Harpophora maydis*; syn: *Cephalosporium maydis*)

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Reviewer: The American Phytopathological Society Committee (planned in late 2008).

I. Introduction

Late wilt, or black bundle disease, is a vascular wilt disease of corn that is caused by the soilborne fungus, *Harpophora maydis* (Samra et al., 1966) W. Gams with synonyms: *Cephalosporium maydis* Samra, Sabet, & Hingorani and *Acremonium maydis* (CABI, 1999; El-Shafey and Claflin, 1999). This disease is also known as cephalosporiosis del maiz (Spanish), cephalosporiose du mais (French), and Gefaessbuendelkrankheit: mais, Welke: mais (German). Late wilt was first reported as a vascular wilt disease of corn in Egypt in 1960 and is now considered endemic throughout Egypt. Late wilt occurs in Andra Pradesh, Uttar Pradesh, Bihar, and Rajasthan provinces of India (Payak et al., 1970), with unconfirmed reports in Kenya. *Harpophora* (*Cephalosporium*) *maydis* from maize also has been reported from Portugal, Spain, Romania, Israel, Italy, and Hungary (Johal et al., 2004; Pecsi and Nemeth, 1998) (Fig. 1).

The appearance and activity of the pathogen in Hungary has been attributed to global warming and dry early summers (Pecsi and Nemeth, 1998). Serious economic losses from late wilt have been reported in Egypt where 100% infection occurs in some fields, and in India with incidence as high as 70% and economic losses up to 51% (Johal et al., 2004). Based on a limited evaluation of only 11 plant species, *Zea mays* (corn, maize) and *Lupinus* (lupine) are the only known hosts of *H. maydis,* although localized lesions occur on young cotton hypocotyls (Bahteem 185 cultivar). These lesions disappear as the cotton plants mature, and *H. maydis* has not been recovered from them (Sabet et al., 1966). *H. maydis* causes a significant damping-off and stunting of the widely cultivated *Lupinus terminis* in Egypt (Sahab et al., 1985).

Harpophora maydis is considered a distinct species within the *Gaeumannomyces-Harpophora* complex (Saleh and Leslie, 2006). The Egyptian, Indian, and Hungarian isolates of *H. maydis* differ in morphology, pathogenicity, and route of infection (Warren, 1983). The four clonal lineages of Egyptian isolates of *H. maydis* show diversity in amplification fragment length polymorphism (AFLP), and differ in colonization ability and virulence on maize (El-Assiuty et

al, 1999; Saleh et al., 2003; Zeller et al., 2000, 2002). Inoculation with mixed cultures of the different clonal lines may cause less damage to corn than individual pure cultures of the clonal lines in greenhouse inoculations (Zeller et al., 2002).

Fig. 1. World Distribution of Late Wilt of Corn caused by *Harpophora maydis*

- Confirmed reports
- Unconfirmed reports (or in the process of confirmation)

Adopted from CABI, 1999 and updated from Johal et al., 2004.

II. Symptoms

Root tips of infected corn plants are stained red during early stages of infection, but above ground parts generally remain symptomless until tasseling when a rapid wilting of lower leaves progresses upward. Leaves appear streaked as tissue between the veins becomes dull green and then chlorotic (Fig. 2) before eventually rolling inward and appearing scorched while retaining somewhat of a green color. Yellow to reddish brown streaks appear on the basal internodes of the stalk (Fig. 3). Wilting can occur suddenly so that non-infected ("escapes") or resistant plants are quite distinct (Fig. 4.). Stalks dry and have a shrunken and hollow appearance with dark yellow to brownish macerated pith and brownish-black vascular bundles (Fig. 5). Lower parts of infected stalks become dry, shrunken and hollow (Fig. 7).

Late wilt is often associated with infection by secondary invaders such as *H. acremonium*, *Sclerotium bataticola*, *Fusarium verticillioides*, and various bacterial rots to present a "stalk rot complex" (El-Shafey and Claflin, 1999). Symptoms may be modified by these secondary

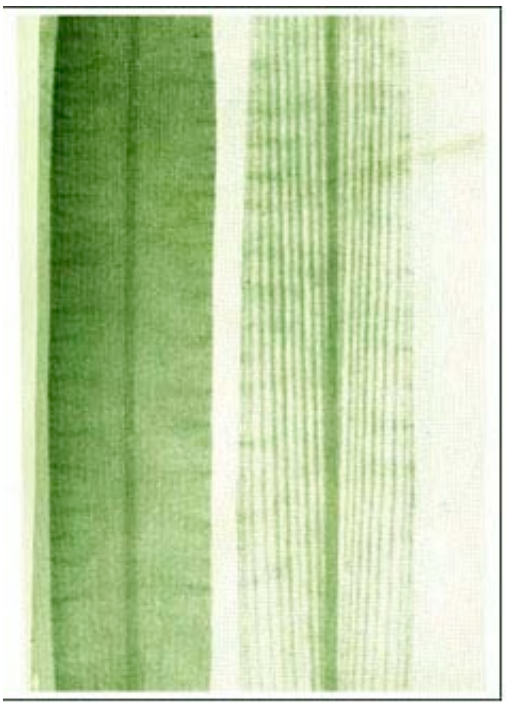


Figure 2. Leaf streaking of *Harpophora* infected plant (R) compared with healthy leaf (L) (Sabet et al., 1966).

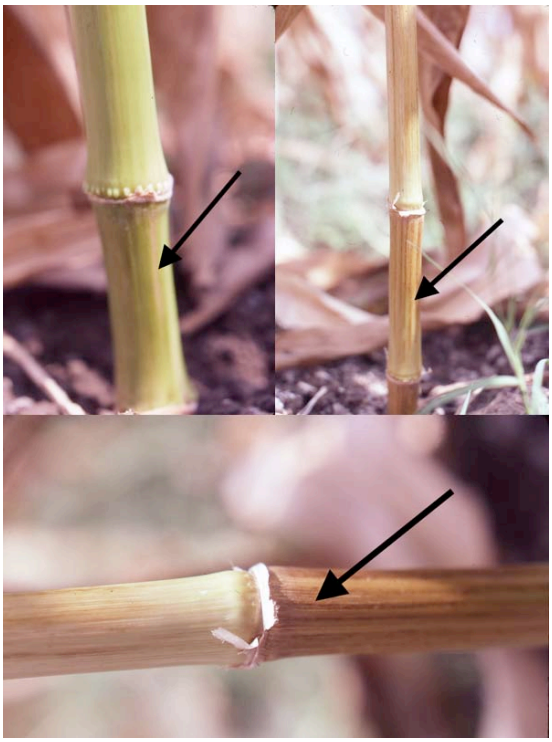


Figure 3. Progressive development of yellow to reddish-brown streaks on *H. maydis* infected lower stalks (photo by H. Warren)

Figure 4. Rapid wilting of infected plant alongside resistant hybrids (photos by H. Warren).

invaders and make identification of late wilt in the field more difficult since some of these pathogens produce wet or soft rot symptoms where basal internodes turn into a soft mass of disintegrating brown tissue. Fewer ears are produced, and kernels that form are poorly developed and may be infected with the pathogen. Seed-borne infection results in seed rot and damping-off of seedlings or seedlings with stunted roots (Fig. 6). Seed rot, preemergence damping-off, delayed seedling emergence, and reduced seedling vigor have been demonstrated when soils are artificially inoculated with high concentrations of inoculum (Payak et al., 1970), but have not been demonstrated with natural seed-borne inoculum (CABI, 1999).

Figure 5. Progressive development of discolored vascular bundles of *H. maydis* infected plants (Photo by A.J. Ulstrup and B.L. Renfro, from Compendium of Corn Diseases, third edition, APS Press).

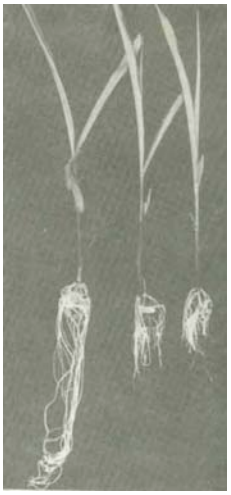


Figure 6. Reduced root development of inoculated maize seedlings (R) compared to uninoculated control (L). (Samra et al., 1972)

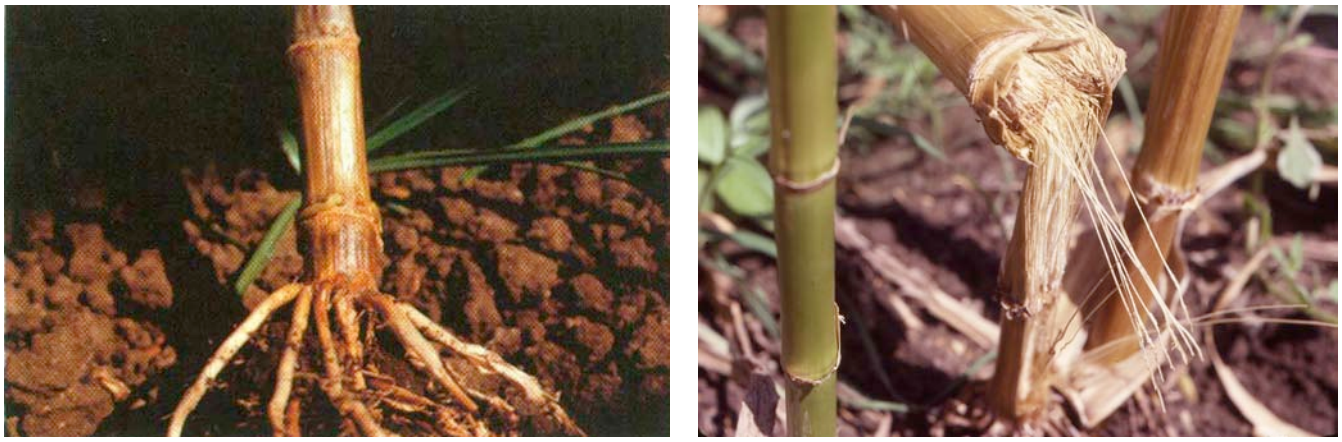


Figure 7. Discolored and necrotic tissue observed with late wilt on lower stem nodes (left, photo by L. Claflin) and pith maceration (right, photo by H. Warren).

III. Spread

H. maydis is a soilborne vascular wilt pathogen that also is seed-borne (Michail et al., 1999). The pathogen survives as sclerotia on corn debris and infects seedlings through the roots or mesocotyl. *H. maydis* initially grows epiphytically on roots and produces short, thick-walled hyphae with swollen cells similar to hyphopodia of *Gaeumannomyces*. Penetration can occur anywhere on the root system or mesocotyl (except root tips), but is most common where lateral roots originate or in the zone of root elongation. As epidermal cells start to collapse, the fungus penetrates directly and grows intra- and intercellularly to the xylem. Root injury predisposes plants to the disease and insect or nematode damage provides additional avenues for entry (Payak et al., 1970; Singh and Siradhana, 1988).

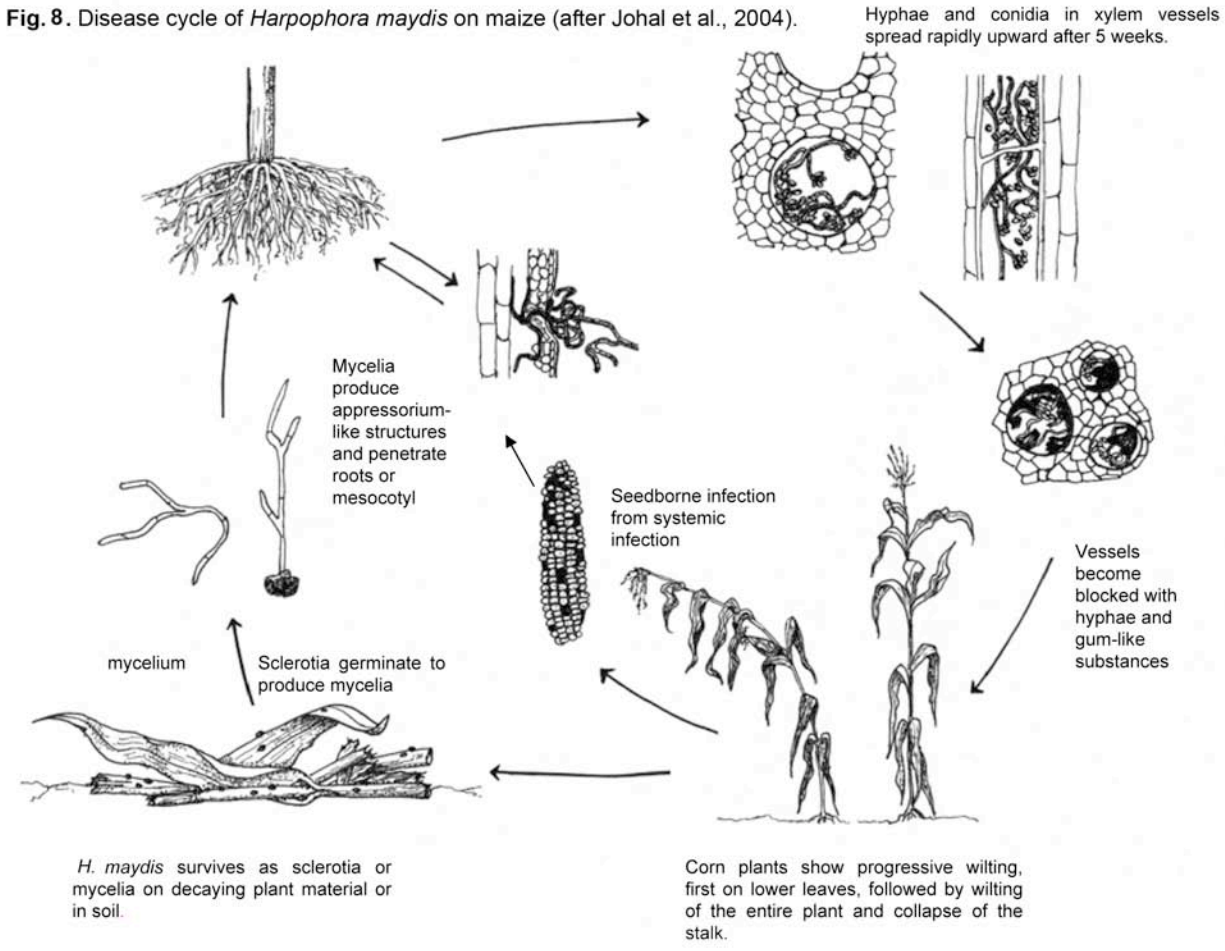


Fig. 8. Disease cycle of *Harpophora maydis* on maize (after Johal et al., 2004).

The fungus spreads slowly the first five weeks after corn germination before growing rapidly upward throughout the plant (Fig. 8). By flowering (anthesis at 9-10 weeks), it is distributed throughout the stalk and many vessels are blocked with hyphae and a dark gum-like substance (Sabet et al., 1970). Vascular occlusion appears to be the principle cause of late wilt symptom development. *H. maydis* can be isolated from cobs 12-13 weeks after planting from which it moves through the pedicels to seed embryos (Michail et al., 1999).

Plants become more resistant to infection as they mature, and 50-day old plants were not susceptible to infection unless roots were injured (Sabet et al., 1970); however, screening for infection routinely occurs by bypassing the most likely resistance to natural infection by injecting *H. maydis* into the 1st or 2nd stem node of 50-60 day old plants (Payak et al., 1970; Pecsi and Nemeth, 1998; Singh and Siradhana, 1988).

Optimum temperature and moisture conditions for corn growth also are optimal for disease development (Warren, 1983). Approximately 90% of the 79 million acres of corn in the United States matches the ecological range of the *H. maydis* isolate from Hungary (Figs 9, 10, after Johal et al., 2004). Thus, late wilt develops rapidly at 20-32°C, with optimum disease development at 21-27°C (Singh and Siradhana, 1987a). Growth of *H. maydis* in soil is sharply inhibited above 35 C, but this fungal pathogen can grow over a wide range of soil pH from 4.5-10, with an optimum at pH 6.5 (Singh and Siradhana, 1987b). The natural incidence of late wilt in India is highest when rainfall is above average or with frequent irrigation (Singh and Siradhana, 1987a). In contrast, frequent irrigation decreased infection in Egypt where irrigation intervals of 9-10 days are recommended and excessive soil moisture reduced wilt (Satyanarayana, 1996). Low soil moisture (25% saturation) favors sclerotial survival.

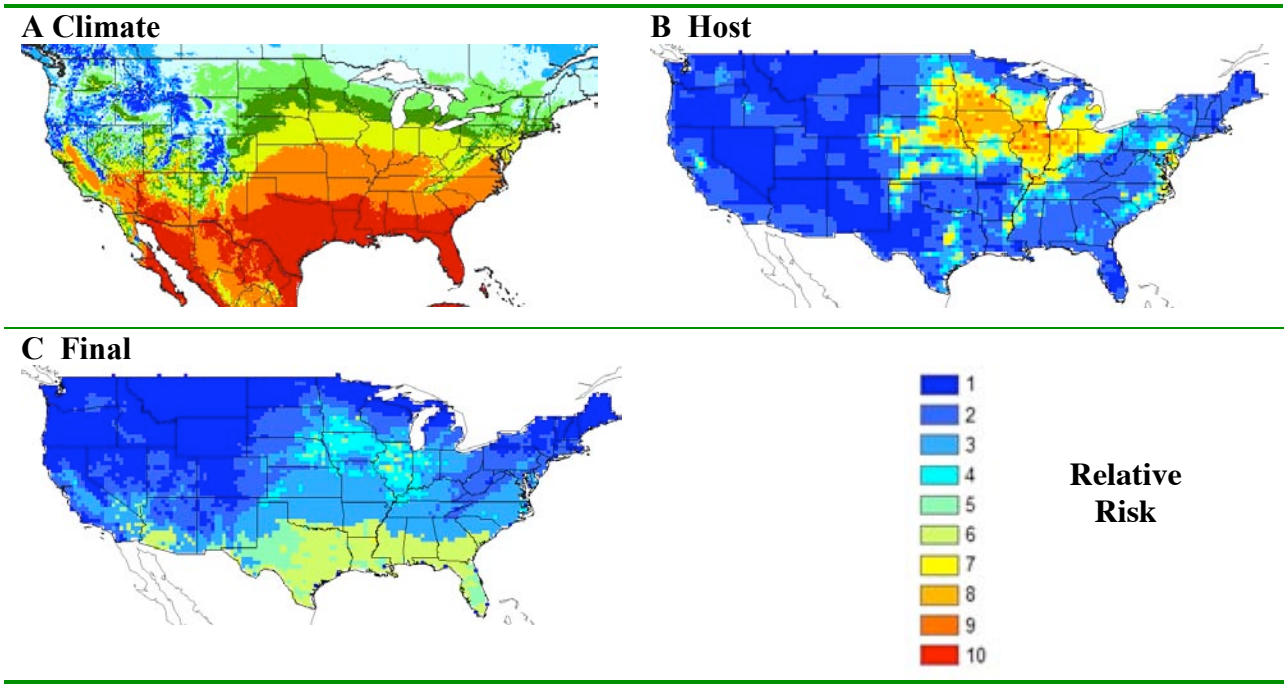


Figure 9. Risk maps for maize wilt based upon a) climate, b) host, and c) climate and host for growth of *H. maydis* causal agent of maize wilt during May and June based on 10 years of climate data (after Magarey, 2008).

Spread is primarily through movement of infested soil, crop residue, or seed-borne inoculum. Spread within a field often is associated with mechanical operations such as cultivation that move soil. *H. maydis* can persist on corn stubble for 12-15 months (Sabet et al., 1970; Singh and Siradhana, 1987b). Inoculum survival in soil is generally poor and restricted to the top 20 cm of soil. A low competitive saprophytic ability in soil and poor saprophytic colonization of fresh substrate indicate that survival is primarily in parasitically infected plant residues. Sclerotia (Fig. 9) are produced under low humidity and insure long-term survival of *H. maydis* (up to 15 months) in no-till residues on the soil surface; but low viability is reported when infected stalk pieces are buried in the soil, especially at temperatures above 10°C, soil moisture above 50%, and low C/N ratio. The role that no-till management (common for U.S. corn production) would have on persistence and spread of late wilt needs further research since disease management in Egypt involves deep plowing to bury sclerotia below 20 cm. Lupine facilitates parasitic survival of the pathogen under at least some field conditions (Botros et al., 1990; Johal et al., 2004).

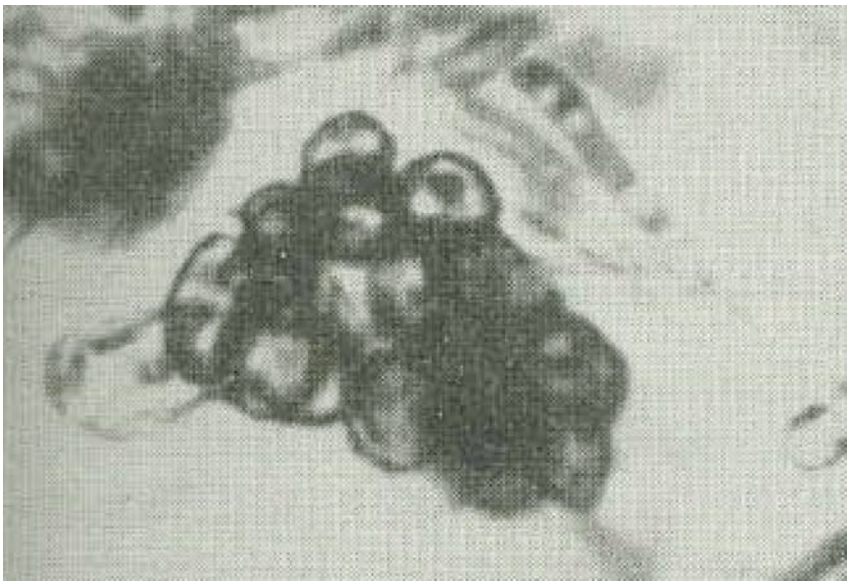


Figure 10. Botryoform sclerotia-like bodies formed in old cultures of *H. maydis* on PDA. (Samra et al., 1963)

H. maydis can be isolated from the seed coat, endosperm and embryo of freshly harvested and stored seed corn (Michail et al., 1999; Sabet et al., 1966). Severely infected seeds rot or seedlings damp-off, and emergence may be delayed and seedling vigor reduced in *H. maydis*-infested soils (Payak et al., 1970). Infected seed can produce plants with late wilt symptoms, infest soil, and result in subsequent development of late wilt in healthy seeds grown in that soil. *H. maydis* can survive in seed for 10 months at high temperature and low humidity in India, but longer survival is predicted at low temperatures (Singh and Siradhana, 1987a).

Seed produced outside the U.S. may allow for the accidental introduction of *H. maydis* as a moderate risk since infected seeds cannot be visually distinguished from healthy ones and U.S. seed handling procedures do not include tests for late wilt. The inadvertent introduction of *H. maydis* into the United States on lupine used for erosion control or forage also should be considered as a potential source of risk.

IV. Monitoring and Detection

Isolation and Identification:

Late wilt does not occur in the United States and may not be readily recognized or distinguished initially from abiotic stresses without some training. Symptom recognition is based on the dull green, desiccated (scorched) leaves, streaked and "collapsed" stalk, and discolored pith tissues. 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. 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, 2006; Zeller et el., 2000).

Isolation of *H. maydis* from plants is difficult because of its slow growth in culture and the relative abundance of other more rapidly growing fungi such as *Fusarium* spp. in the stalk rot complex (Saleh et al., 2003). Successful isolation can usually be obtained by sterilizing the internode of symptomatic plants in 5% sodium hypochlorite, splitting them with a sterile knife, and placing a small piece of discolored vascular bundle on PDYA media (PDA + 0.2% yeast extract) (Zeller et al., 2002). Single spore isolates can be obtained by dilution plating.

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% hypochlorite for 3 minutes, plating on PDYA, incubating at 20 ^{o}C 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, 2006).

Monitoring:

Diagnosing an exotic disease in the field is critical for containment and eradication. Untrained university extension personnel, growers, scouts, crop specialists and plant pathologists may not identify late wilt early in the field because there are no distinct symptoms of this disease that separate it from the myriad of other abiotic or fungal diseases present to varying degrees in corn production areas. The pathogen is closely related and similar to endemic soilborne fungi in the *Gaeumannomyces-Harpophora* complex. Species-specific PCR primers may be used for specific identification if they become validated and commercially available (Saleh and Leslie, 2006).

The National Plant Diagnostic Network (NPDN) is designed to provide a coordinated and distributed diagnostic laboratory and information system to quickly detect pests and pathogens that have been introduced and report to appropriate responders and decision makers. NPDN is made up of experts at land-grant universities and USDA facilities as part of the Homeland Security effort. Each of the five NPDN regions have a regional hub where a Web-based diagnostic and reporting system is in place to provide an effective communication network between diagnostic labs and regulatory agencies. This system should be prepared to facilitate

identification of an *H. maydis* introduction, especially in the event of simultaneous outbreaks at many locations from infested seed. To help facilitate this, late wilt and its causal fungal pathogen are listed as #43 on the Cooperative Agricultural Pest Survey (CAPS) List. Select data collected from the NPDN regions are archived in the NPDN National Data Repository located at Purdue University. A response pathway is outlined in Figure 11.

Infested seed, distributed early throughout the Southern Corn Belt, could initiate a localized epidemic and provide sequential inoculum through movement of agricultural commodities and equipment for gradual contamination of much of the Corn Belt with *H. maydis*. "No-till" and "eco-fallow" management practices generally used throughout the Corn Belt could facilitate establishment and persistence of this disease. Reports of *H. maydis* in Hungary indicate it could successfully overwinter in the Northern Corn Belt similar to a closely related established pathogen on wheat, *Cephalosporium gramineum*. The widespread distribution of lupine species in the U.S. may make them important alternative perennial sources of inoculum since at least one species of lupine can serve as a host for *H. maydis*. Serious market damage would result from any occurrence of this exotic disease within a region and result in long-term quarantine, embargo of crop produced, movement of equipment, etc.

V. Response

<u>Containment and eradication:</u>
While this plan is focused primarily on recovery, there is a continuum of activities involved in the detection of a new pathogen such as *H. maydis* from response to recovery. The response to all plant health emergencies is under USDA-APHIS-Plant Protection and Quarantine's authority (USDA-APHIS-PPQ) delegated from the Secretary of Agriculture under the Plant Protection Act of 2000.

After a detection of *H. maydis* has been confirmed by the USDA-APHIS-PPQ recognized authority, APHIS, in cooperation with the appropriate State Department(s) of Agriculture is in charge of the response. The response will begin with an initial assessment. This may be immediate in the form of a rapid assessment team consisting of state and federal *H. maydis* experts and regulatory personnel. The team will be sent to the site of initial detection to delimit (if possible) the infestation, to prevent the movement of regulated articles, conduct investigations, and initiate delimiting surveys to establish the scope of the affected area. Actions that may be taken include regulatory measures to quarantine infested or potentially infested areas, stop the movement of infested or potentially infested articles in commerce, and implement control measures, which may include plant removal and destruction, and/or insuring adherence to required sanitary practices as well as the application of appropriately labeled chemicals and disinfectants. APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant diseases or regulated articles, and works in conjunction with states to impose these actions parallel to state regulatory actions that restrict intrastate movement.

Response will depend on where and when *H. maydis* is found (state, region, etc.) and how widespread it occurs based on an initial assessment by the rapid assessment team. When results of a delimiting survey are known, a decision can be made to continue or expand regulatory

actions if containment and eradication are deemed possible, or to modify or remove such regulatory actions in the event of widespread establishment,

There is a low likelihood of early detection of *H. maydis,* in the absence of a focused first detector education and outreach effort, because most agricultural workers, county agents, and field agronomists are not familiar with symptoms of this disease or its causal exotic pathogen. Several endemic pathogens of corn produce wilt symptoms, so an exotic pathogen is not likely to be suspected initially. The absence of external symptoms until flowering or later further complicates diagnosis, and variability of local soil and environmental conditions in an infested field can mask disease expression. Identification of diseased samples submitted to professional plant disease diagnosticians at the onset of symptoms may take weeks rather than days since many different fungi and bacteria can be isolated from dying corn plants. Although molecular techniques may make rapid identification possible (Saleh and Leslie, unpublished), late wilt is currently unlikely to be diagnosed rapidly enough to prevent infected seed or inoculum from making its way into additional commercial production areas. Cooperation of public and private entities is important, especially where international private personnel have experience with late wilt through their commercial enterprise in areas where late wilt occurs naturally. Significant emphasis should be placed on identifying resistant germplasm and effective management systems because establishment of this disease is a high probability should it be introduced.

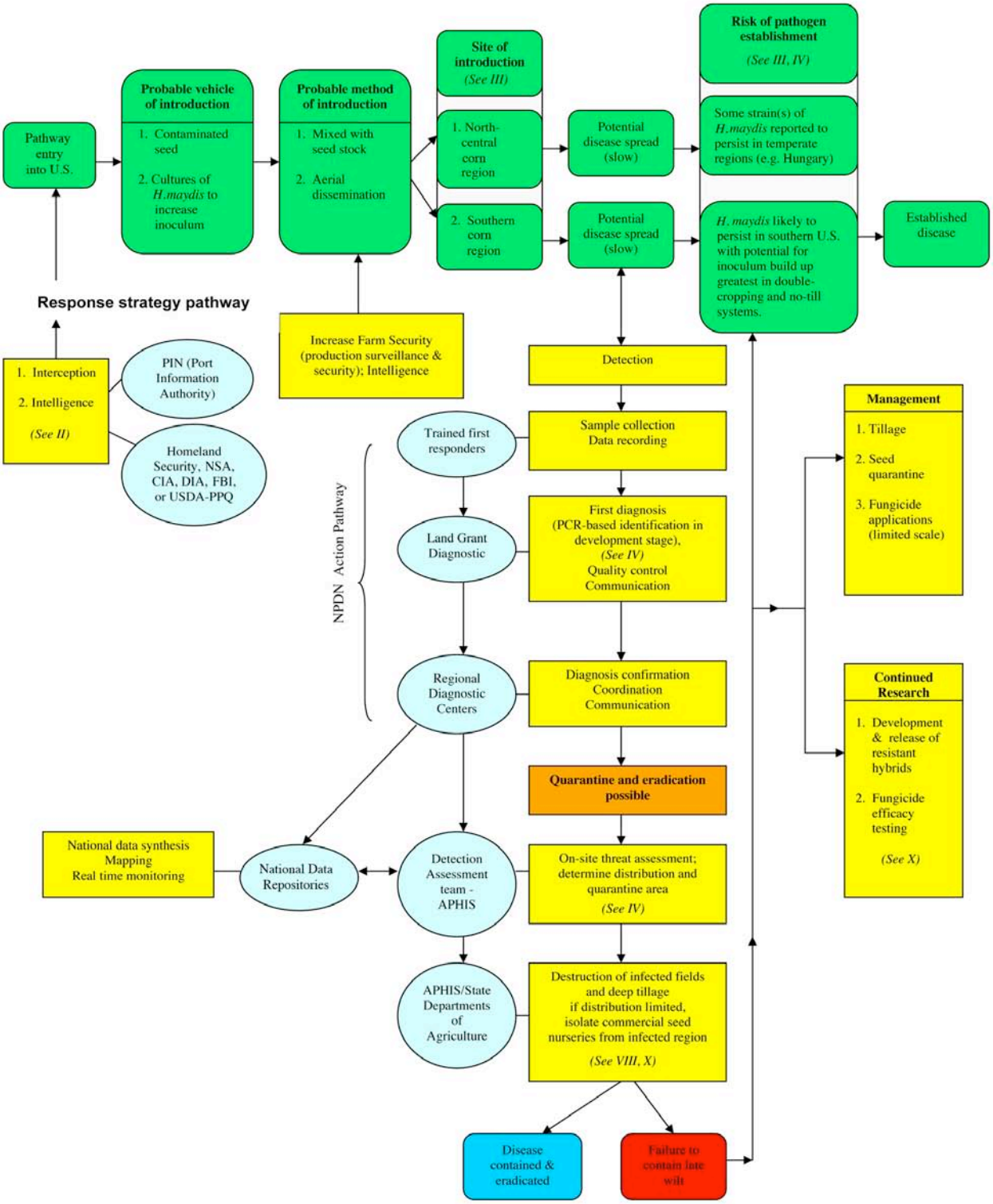
VI. USDA Pathogen Permits

USDA-APHIS-PPQ permit and registration requirements for plant diseases and laboratories fall under the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331).. The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status, that are shipped interstate and also requires that the receiving laboratory have a permit: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ permit services at (301) 734-8758. This procedure may limit early detection of late wilt since it complicates sending and receiving samples for identification (confirmation), although there are diagnostic laboratories in every state and all NPDN laboratories have APHIS permits to handle 'unknown' diagnostic samples. Concerted efforts to educate first detectors will insure the proper handling and identification of potential late wilt materials in the event that a suspect high consequence or quarantined sample is found.

VII. Economic Impact and Compensation

Late wilt has been considered the most economically important fungal disease of corn in Egypt where 100% infection occurs in some fields, and yield losses approached 40% before the introduction of resistant cultivars. Late wilt also has been destructive in India, with incidence as high as 70% and economic losses up to 51%. Although the disease does not occur in the U.S., it is considered a potentially significant pathogen (Warren, 1983) especially in the Southern and Western portions of the Corn Belt where environmental conditions are nearly optimal for the establishment and expression of this disease. In the absence of identified tolerance or genetic resistance in commercial corn lines used in the U.S., all areas could be seriously impacted by this persistent, soilborne pathogen because of favorable no-till management and favorable environmental conditions.

Figure 11. Pathway and response to the introduction of *H. maydis*, cause of late wilt of corn (after Johal et al., 2004).



Compensation by the USDA Risk Management Agency (RMA) for a loss caused by late wilt is available, but would not be covered if due to insufficient or improper application of disease control measures.

VIII. Mitigation and Disease Management

Because *H. maydis* dissemination from a localized (point) introduction would be moderately slow, containment and eradication may be possible, but would depend on rapid identification of the pathogen and eradication by timely implementation of control measures. In order to improve the likelihood of early detection, first responders and diagnosticians will need to be familiarized with late wilt symptoms. Diagnosis is complicated by difficulties in isolating *H. maydis* in dying corn tissues. Any seed production would need to be moved out of the potential quarantine area because of the seed-borne nature of this pathogen. Mitigation measures include genetic resistance and cultural or chemical disease controls.

Genetic Resistance: The most effective control of late wilt is with resistant germplasm (El-Shafey et al., 1988; Zeller et al., 2000), although some cultural and chemical controls can reduce its impact on commercial production. There has been little evaluation of late wilt resistance in commercial breeding programs in the U.S. because *H. maydis* is an exotic pathogen. The absence of definitive symptoms of late wilt make selection for resistance in breeding programs more difficult than with other diseases, and resistant plants cannot always be separated from escapes (Dr. A. Ellingboe, personal communication). The National Maize Program at the Agricultural Research Center in Giza, Egypt has identified many sources of resistance through their screening of thousands of local and exotic germ lines since 1963. Their release of resistant varieties since 1980 has significantly reduced late wilt losses in Egypt (El-Shafey et al., 1988; Soliman and Sadek, 1998). Egyptian lines could serve as an important source of late wilt resistance to introduce resistance into U.S. hybrids (Fig. 3). Late wilt resistance also is known in various proprietary germ lines of international seed companies doing business in late wilt infested areas of the world (Dr. W. Dolezal, Pioneer-DuPont Company, personal communication)

New virulent strains of *H. maydis* have developed so that breeding for resistance will remain a continuous process. Lineage IV of *H. maydis* appears to be evolving faster than other lineages and may be responding to the extensive use of resistant varieties in the Nile River Delta since there is greater variability of *H. maydis* isolates in this intensively cropped area. (El Assiuty et al., 1999; Dr. J. Leslie, personal communication). Inbred Egyptian lines Gm. 4, Gm. 5, Gm. 6, Gm. 13, and Gm. 26 exhibit late wilt resistance and high yield characteristics. The cross of Gm. 26 x Gm. 30 was the most superior cross with a resistance rating of 99% (Soliman and Sadek, 1998). Resistant lines developed in India include X102, CM111, CM202, and (CM104xWL) (Satyanarayana, 1995).

H. maydis lineages differ in their ability to colonize maize plants and in their relative aggressiveness in single culture inoculations (El-Assiuty et al., 1999; Zeller et al., 2002). Maize germplasm in Egyptian resistance breeding programs has been challenged primarily with isolates from two of the four (II and III) genetic lineages (Zeller et al., 2000). While lineage IV is highly

virulent when inoculated alone on some cultivars resistant to lineages I-III, it appears to be a relatively poor competitor when applied in a mixed inoculum containing all lineages; thus, all four lineages of *H. maydis* should be used independently and in combination to challenge new lines during the development of resistant germplasm (Saleh et al., 2003; Zeller et al., 2002).

Limited information is available on the inheritance of resistance. Most studies have used traditional quantitative genetic approaches and find that resistance is under polygenic control; however, one study claimed resistance was controlled by a single dominant gene (Shehata, 1976). Resistance has been reported as being partially dominant with five loci controlling resistance, additive with at least three loci controlling resistance, or involving three major genes (El-Itriby et al., 1984). Dominance and epistasis have been cited as major contributors to resistance, with additive effects of lesser importance (Shehata, 1976). The development of specific genetic markers for resistance to late wilt would greatly facilitate incorporation of resistance into adapted hybrids.

Cultural and management controls: Disease-free seed (inspected, certified seed) could limit further distribution of the pathogen and reduce potential inoculum levels. Various cultural measures such as soil solarization, balanced soil fertility, and flood fallowing can reduce disease severity and losses. Inoculum survival is restricted to the top 20 cm of soil, and survival depends primarily on the persistence in infected crop residues. Flood-fallowing increases anaerobic conditions, stimulates lytic organisms to degrade sclerotia, and reduces survival potential. Sanitation measures such as deep tillage may have a significant impact on disease, and double and triple cropped corn fields in Egypt are plowed at least annually (Dr. J. Leslie, personal communication). The widespread use of no-till corn systems in the U.S., and the unknown impact of extensive glyphosate application under U.S. cropping systems that increases severity of several related *Gaeumannomyces-Harpophora* type diseases, could eventually result in inoculum build-up in soil or increased virulence of the pathogen.

Hot water seed treatment (60^{o}C for 10-15 minutes) can reduce seed transmission, but would not generally be practical except for breeding stock. Soil solarization to increase temperatures above 35 C with transparent polyethylene film has also reduced late wilt in Egypt, but would be limited practically to seed production in only a few areas of the U.S. Corn Belt.

Early sowing of corn in Egypt reduced late wilt (El-Shafey et al., 1988), while late summer planting reduced disease severity in India (Singh and Siradhana, 1988). Unfavorable soil conditions with low rainfall may be the determining factor with reported date of seeding effects (Singh and Siradhana, 1988). Moisture stress is a major predisposing factor for late wilt (Abdel-Rahim et al., 1984) and frequent watering or saturated soils reduced late wilt (Samra et al., 1966). Corn did not develop late wilt following paddy-cultivated rice, which increases the availability of Mn for subsequent crops, although *H. maydis* also is sensitive to low oxygen conditions (Samra et al., 1966). Moisture management and flood-fallowing may be useful cultural controls for late wilt where they are economically practical (Singh and Siradhana, 1988; Samra et al., 1966).

Balanced fertility can reduce disease severity, although it does not provide complete control. Low levels of nitrogen fertilization (60 kg/ha) increased wilt (Singh and Siradhana, 1990) even

though yields were increased overall; however, higher nitrogen levels (120 kg N/ha) needed for optimal yield reduced late wilt (Singh and Siradhana, 1990). A physiological sufficiency of potassium also is reported to reduce late wilt in low K fields of India (Singh and Siradhana, 1990), but not in the higher K soils of Egypt (Samra et al., 1966, 1972). Phosphorus, organic amendments (straw, cotton cakes, and brodret) and micronutrients (Cu, Fe, Mn, and Zn) also reduce disease severity (Singh and Siradhana, 1990). The extensive use of glyphosate in U.S. no-till corn production, which can immobilize Mn in soil and restrict plant uptake and transport of Cu, Fe, Mn, Zn and other essential micronutrients, may have a serious predisposing effect on this disease through reduced plant resistance or increased pathogen virulence (Eker et al, 2007).

Since *H. maydis* is a poor saprophytic competitor (Sabet et al., 1970), various attempts at biological control by inoculating corn seed with competitive or antagonistic organisms (*Macrophomina phaseolina*, *Trichurus spiralis*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Verticillium tricorpus*) have been evaluated; however, success on a field scale has not been demonstrated consistently (El-Assiuty et al., 1991; El-Mehalawy et al., 2004; Singh and Siradhana, 1988).

Chemical controls: In India, captan, carbendazim, carboxin and thiram seed treatments significantly reduced late wilt and increased yields 11-91%, with captan providing the most effective and economic return to growers (Begum et al., 1989; Satyanarayana and Begum, 1996). In contrast, seed treatments consistently failed to control late wilt in Egyptian trials. Failures in Egypt may be due to differences in chemical sensitivity or virulence of *H. maydis* isolates, chemical formulations evaluated, environmental conditions, or complexity of the stalk-rot disease complex in Egyptian soils. Systemic fungicides and their fungitoxic products are translocated to corn leaves within 2 days and can persist in corn roots for 90 days; however, field results generally have been disappointing unless the fungicide is applied several times during the growing season (Singh and Siradhana, 1989). The cost and labor required for frequent fungicide applications to corn make chemical control prohibitively expensive in the U.S., and contemporary fungicides have not been evaluated against late wilt or registered for this use in the U.S.

IPM strategy to mitigate late wilt: The most economically effective management of late wilt is through development of genetically resistant corn lines. Resistant germplasm is known; however, it is not known which corn lines adapted for the U.S. have tolerance or resistance to late wilt since this disease is not commonly screened for in U.S. breeding programs. Seed treatment chemicals may limit the spread of late wilt or provide limited control, but are not registered in the U.S. Introduction of late wilt into U.S. corn production systems may require a return to tillage and alternative weed control programs in order to minimize inoculum build-up in affected areas even with the development of resistant cultivars.

IX. Infrastructure and Experts

Corn genetics programs are active in all of the land-grant university systems in the corn grain and seed producing areas of the U.S., and several corn geneticists-breeders have consulted and traveled to areas where late wilt is endemic. They also have visiting scientists and graduate

students from these areas that are familiar with late wilt and, in some cases, conducted extensive research on the disease in their native countries. USDA-ARS personnel at the Foreign Disease and Weed Science Laboratory are equipped to study exotic pathogens in containment facilities and have traveled or conducted cooperative research with countries where late wilt is endemic.

Much of the U.S. commercial corn seed development is performed by international components of Monsanto, DuPont-Pioneer, and DowAgroSciences who have personnel that are familiar with late wilt because they are screening and developing corn lines for commercial production in areas where late wilt is endemic. This provides a critical core of experts who can prepare and respond to an introduction of late wilt into the U.S., or conduct preemptory research necessary for recovery after introduction of *H. maydis*.

Some international and domestic scientific experts on late wilt of corn include:

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X. Research, Extension, and Education Priorities

Research Priorities (ranked)

The following lines of research are needed to effectively evaluate the threat, enhance detection, and manage late wilt on introduction. They highlight important gaps in our present knowledge of this potentially significant disease. Most of these areas are common to many exotic pathogens and indicate specific needs to understand the biological, cultural, and economic aspects of the disease as it could impact U.S. agriculture.

1. Define the area of likely adaptation of *H. maydis* in the U.S. based on climatic, edaphic, and crop cultural factors; and identify predisposing management practices favorable for establishment, survival, and spread of *H. maydis* in the U.S.

It is not clear how extensively *H. maydis* will become established in the corn growing areas of the U.S. since much of the U.S. corn is grown in more temperate climates with colder winters than the climates of Egypt (arid, hot) and India (arid, hot or savannah) which are the regions reporting serious epidemics of late wilt. Although late wilt is reported in Hungary, Spain, and several other more temperate countries, its persistence and economic impact are increasing, but not generally quantitatively evaluated. This

information is necessary since closely related pathogens, *Cephalosporium gramineum*, that causes a wilt of wheat, and take-all, caused be *Gaeumannomyces graminis*, are well established throughout the U.S. temperate areas; and no-till management of corn provides an optimal environment for establishment, survival, and inoculum buildup. Understanding survival and virulence under various cultural-management systems is needed since U.S. corn production is predominantly under a minimum or no-till glyphosate weed control system with the rapid adoption of glyphosate-resistant hybrids. Whether the extensive use of glyphosate will predispose corn to late wilt as seen with several other similar diseases on other crops needs evaluation. What economical tillage or other modifications can be made that will reduce inoculum buildup, susceptibility, and disease loss within the concept of environmental stewardship?

2. Evaluate resistance of U.S. corn germplasm to late wilt.

The evaluation of U.S. germplasm for late wilt resistance, and incorporation into public and private breeding programs should be a significant part of any response plan because resistance has proven to be the most effective means of reducing the economic impact of late wilt in India, Israel, and Egypt. Identification of sources of resistance and incorporation of those sources into adaptable corn lines could shorten the late wilt recovery time by years. Developing this information will require public/private cooperation and could be implemented through memoranda of agreements as well as through specific off-shore cooperative projects in areas where late-wilt is endemic.

3. Develop and commercialize a reliable diagnostic test to identify *H. maydis* and differentiate it from related species of *Cephalosporium*, *Gaeumannomyces*, and *Harpophora*.

Commercialization (availability) of USDA-APHIS validated rapid diagnostic tests for *H. maydis* will be critical to early containment. This could be especially important for seed introduction and domestic production to maintain disease-free seed sources available for commercial production. Once established, eradication is not probable with this soilborne pathogen, so exclusion (disease-free seed) and rapid response are very important against any localized introduction. Diagnostic tests that distinguish closely related endemic *Gaeumannomyces* and *Cephalosporium* spp. from *H. maydis* (*Gaeumannomyces-Harpophora* complex) should be available in diagnostic clinics in order to provide a rapid response to *H. maydis* after introduction and to avoid unnecessary confusion.

4. Identify and seek labeling of seed applied fungicides that reduce seed-borne transmission of *H. maydis*.

Identification of seed treatment or other cost-effective chemical controls will be important especially for seed production, and to minimize spread and impact from seed-borne sources. Identifying the effectiveness of chemical seed treatments or other controls currently used for other crops and registration for use against *H. maydis* (late wilt) similar to actions taken on the introduction of soybean rust need consideration. General soil fumigation of most field crops is cost prohibitive; however, development of biological controls for late wilt has shown some promise in limited trials in Egypt and India although consistent control in the field has not been achieved.

5. Characterize susceptibility to *H. maydis* of *Lupinus* spp. and other crops and weed hosts in the U.S.

Characterization of *H. maydis* susceptibility of *Lupinus* spp and other plants that are present in the U.S. to define their potential role in corn production as an alternative host for *H. maydis* will be important information. Since late wilt is not present in the U.S., this information can only be gained in containment facilities or through off-shore cooperative projects to augment this need.

6. Identify cultural practices that minimize crop losses due to late wilt.

Since late wilt likely will become established before it can be contained and eradicated, the development of effective cultural and management practices to minimize disease losses will be very important. Much of this research will need to be done through cooperative programs with research centers where the disease is endemic.

Extension and Education Needs

1. Develop training materials for identification and rapid diagnosis of *H. maydis*.

a. Standard Operating Procedures need to be developed for corn wilts by NPDN and specifically for *H. maydis*. The necessary materials and training in the use of rapid diagnostic techniques for *H. maydis* need to be available since late wilt is an exotic disease that resembles symptoms of various abiotic conditions and general plant disease responses. *H. maydis* also may be confused with the closely related, endemic *C. gramineum* on culturing in the laboratory.

b. Educational resources must be available for seed producers to maintain phytosanitary status and insure disease-free conditions and plant materials.

c. First responder education, outreach materials, and training modules need to be developed and implemented. Disease identification guides and other educational materials on sample collection and delivery need to be available for growers, crop advisors, and other support industry personnel on the nature of the disease and potential impact of introduction of *H. maydis* into the U.S.

2. Develop improved sampling and survey methods for late wilt and *H. maydis*.

H. maydis is slow growing and difficult to isolate and culture from infected tissue so that improved sampling and survey procedures for late wilt must be developed to include improved methods for the isolation and culture of *H. maydis*.

3. Train a core group of U.S. plant pathologists from government, academia, and industry to become knowledgeable of late wilt and *H. maydis*.

A timely response will require an established core group of people familiar with the disease, its ecology, and symptoms for survey and early diagnosis. NPDN personnel especially should be knowledgeable of late wilt. This can be facilitated by incorporating a renewed emphasis on the epidemiology and management of diseases during training of new plant pathologists. Training could be accomplished through workshops for USDA-APHIS, academic extension organizations, and industry training sessions.

4. Identify export markets where phytosanitary permits for *H. maydis* will be required before U.S. corn can be exported.

Although only about 6% of corn produced in the U.S. is exported, it will be important to know which potential export markets would likely require inspection and a phytosanitary permit, or embargo *H. maydis* contaminated corn.

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Web Resources:
Currently, there are no general web references available on late wilt.