United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

New Pest Response Guidelines

Late Wilt of Corn (*Harpophora maydis*)

First Edition Issued 2011

Late Wilt of Corn

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Cover Image

Symptoms of maize plants infected with *Harpophora maydis*. Courtesy of International Maize and Wheat Improvement Center (CIMMYT).

Chapter

1

Introduction

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Introduction

Use *New Pest Response Guidelines: Late Wilt of Corn* (Harpophora maydis), when designing a program to detect, monitor, control, contain, or eradicate, an outbreak of this pathogen in the United States and collaborating territories.

The United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the guidelines through discussion, consultation, or agreement with staff members at the USDA-Agricultural Research Service and advisors at universities.

Any new detection may require the establishment of an Incident Command System to facilitate emergency management. This document is meant to provide the necessary information to launch a response to a detection of *Harpophora maydis*.

If *Harpophora maydis* is detected, PPQ personnel will produce a site-specific action plan based on the guidelines. As the program develops and new information becomes available, the guidelines will be updated.

Users

The guidelines is intended as a field reference for the following users who have been assigned responsibilities for a plant health emergency for late wilt of corn:

- PPQ personnel
- Emergency response coordinators
- State agriculture department personnel
- Others concerned with developing local survey or control programs

Contacts

When an emergency program for late wilt of corn has been implemented, the success of the program depends on the cooperation, assistance, and understanding of other involved groups. The appropriate liaison and information officers should distribute news of the program's progress and developments to interested groups, including the following:

- Academic entities with agricultural interests
- Agricultural interests in other countries
- Commercial interests
- Grower groups such as specific commodity or industry groups
- Land Grant universities and Cooperative Extension Services
- National, State and local news media
- Other Federal, State, county, and municipal agricultural officials
- Public health agencies
- The public
- State and local law enforcement officials
- Tribal governments

Initiating an Emergency Pest Response Program

An emergency pest response program consists of detection and delimitation, and may be followed by programs in regulation, containment, eradication and control. The New Pest Advisory Group (NPAG) will evaluate the pest. After assessing the risk to U.S. plant health, and consulting with experts and regulatory personnel, NPAG will recommend a course of action to PPQ management.

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported
2. The pest is examined and pre-identified by regional or area identifier
3. The pest's identity is confirmed by a national taxonomic authority recognized by USDA–APHIS–PPQ-National Identification System
4. Published New Pest Response Guidelines are consulted or a new NPAG is assembled in order to evaluate the pest
5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators, and trading partners
6. A delimiting survey is conducted at the site of detection
7. An Incident Assessment Team may be sent to evaluate the site
8. A recommendation is made, based on the assessment of surveys, other data, and recommendation of the Incident Assessment Team or the NPAG, as follows:
   - **A.** Take no action
   - **B.** Regulate the pest
   - **C.** Contain the pest
   - **D.** Suppress the pest
   - **E.** Eradicate the pest
9. State Departments of Agriculture are consulted
10. If appropriate, a control strategy is selected
11. A PPQ Deputy Administrator authorizes a response
12. A command post is selected and the Incident Command System is implemented
13. State Departments of Agriculture cooperate with parallel actions using a Unified Command structure

14. Traceback and trace-forward investigations are conducted
15. Field identification procedures are standardized
16. Data reporting is standardized
17. Regulatory actions are taken
18. Environmental Assessments are completed as necessary
19. Treatment is applied for required pest generational time
20. Environmental monitoring is conducted, if appropriate
21. Pest monitoring surveys are conducted to evaluate program success
22. Programs are designed for eradication, containment, or long-term use

Preventing an Infestation

Federal and State regulatory officials must conduct inspections and apply prescribed measures to ensure that pests do not spread within or between properties. Federal and State regulatory officials conducting inspections should follow the sanitation guidelines in the section *Preparation, Sanitization, and Clean-Up* on page 4-2 before entering and upon leaving each property to prevent contamination.

Scope

The guidelines is divided into the following chapters:

1. *Introduction* on page 1-1
2. *Pest Information* on page 2-1
3. *Identification* on page 3-1
4. *Survey Procedures* on page 4-1
5. *Regulatory Procedures* on page 5-1
6. *Control Procedures* on page 6-1
7. *Environmental Compliance* on page 7-1
8. *Pathways* on page 8-1

The guidelines also includes appendixes, a references section, a glossary, and an index.

The Introduction contains basic information about the guidelines. This chapter includes the guideline's purpose, scope, users, and application; a list of related documents that provide the authority for the guidelines content; directions about how to use the guidelines; and the conventions (unfamiliar or unique symbols and highlighting) that appear throughout the guidelines.

Authorities

The regulatory authority for taking the actions listed in the guidelines is contained in the following authorities:

- Plant Protection Act of 2000 (Statute 7 USC 7701-7758)
- Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments
- Fish and Wildlife Coordination Act
- National Historic Preservation Act of 1966
- Endangered Species Act
- Endangered and Threatened Plants (50 CFR 17.12)
- National Environmental Policy Act

Program Safety

Safety of the public and program personnel is a priority in pre-program planning and training and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decisionmaking

USDA–APHIS–PPQ-Center for Plant Health, Science and Technology (CPHST) provides technical support to emergency pest response program directors concerning risk assessments, survey methods, control strategies, regulatory treatments, and other aspects of pest response programs. PPQ managers consult with State departments of agriculture in developing guidelines and policies for pest response programs.

How to Use the Guidelines

The guidelines is a portable electronic document that is updated periodically. Download the current version from its source, and then use Adobe Reader® to view it on your computer screen. You can print the guidelines for convenience. However, links and navigational tools are only functional when the document is viewed in Adobe Reader®. Remember that printed copies of the guidelines are obsolete once a new version has been issued.

Conventions

Conventions are established by custom and are widely recognized and accepted. Conventions used in the guidelines are listed in this section.

Advisories

Advisories are used throughout the guidelines to bring important information to your attention. Please carefully review each advisory. The definitions have been updated so that they coincide with the America National Standards Institute (ANSI) and are in the format shown below.

EXAMPLE Example provides an example of the topic.

Important Important indicates information that is helpful.

CAUTION

CAUTION indicates that people could possibly be endangered and slightly hurt.

DANGER

DANGEROUS indicates that people could easily be hurt or killed.

NOTICE

NOTICE indicates a possibly dangerous situation where goods might be damaged.

⚠ WARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

Boldfaced type is used to highlight negative or important words. These words are: never, not, do not, other than, prohibited.

Lists

Bulleted lists indicate that there is no order to the information being listed. Numbered lists indicate that information will be used in a particular order.

Disclaimers

All disclaimers are located on the unnumbered page that follows the cover.

Table of Contents

Every chapter has a table of contents that lists the heading titles at the beginning to help facilitate finding information.

Control Data

Information placed at the top and bottom of each page helps users keep track of where they are in the guidelines. At the top of the page is the chapter and first-level heading. At the bottom of the page is the month, year, title, and page number. PPQ-Emergency and Domestic Programs-Emergency Programs is the unit responsible for the content of the guidelines.

Change Bar

A vertical black change bar in the left margin is used to indicate a change in the guidelines. Change bars from the previous update are deleted when the chapter or appendix is revised.

Decision Tables

Decision tables are used throughout the guidelines. The first and middle columns in each table represent conditions, and the last column represents the action to take after all conditions listed for that row are considered. Begin with the column headings and move left-to-right, and if the condition does not apply, then continue one row at a time until you find the condition that does apply.

Table 1-1 How to Use Decision Tables

If you:	And if the condition applies:	Then:
Read this column cell and row first	Continue in this cell	TAKE the action listed in this cell
Find the previous condition did not apply, then read this column cell	Continue in this cell	TAKE the action listed in this cell

Footnotes

Footnotes comment on or cite a reference to text and are referenced by number. The footnotes used in the guidelines include general text footnotes, figure footnotes, and table footnotes. General text footnotes are located at the bottom of the page.

When space allows, figure and table footnotes are located directly below the associated figure or table. However, for multi-page tables or tables that cover the length of a page, footnote numbers and footnote text cannot be listed on the same page. If a table or figure continues beyond one page, the associated footnotes will appear on the page following the end of the figure or table.

Heading Levels

Within each chapter and section there can be four heading levels; each heading is green and is located within the middle and right side of the page. The first-level heading is indicated by a horizontal line across the page, and the heading follows directly below. The second-, third-, and fourth-level headings each have a font size smaller than the preceding heading level. The fourth-level heading runs in with the text that follows.

Hypertext Links

Figures, headings, and tables are cross-referenced in the body of the guidelines and are highlighted in boldface type. These appear in blue hypertext in the online guidelines.

Italics

The following items are italicized throughout the guidelines:

- Cross-references to headings and titles
- Names of publications
- Scientific names

Numbering Scheme

A two-level numbering scheme is used in the guidelines for pages, tables, and figures. The first number represents the chapter. The second number represented the page, table, or figure. This numbering scheme allows for identifying and updating. Dashes are used in page numbering to differentiate page numbers from decimal points.

Transmittal Number

The transmittal number contains the month, year, and a consecutively-issued number (beginning with -01 for the first edition and increasing consecutively for each update to the edition). The transmittal number is only changed when the specific chapter sections, appendixes, or glossary, tables, or index is updated. If no changes are made, then the transmittal number remains the unchanged. The transmittal number only changes for the entire guidelines when a new edition is issued or changes are made to the entire guidelines.

Acknowledgements

Writers, editors, reviewers, creators of cover images, and other contributors to the guidelines, are acknowledged in the acknowledgements section. Names, affiliations, and Web site addresses of the creators of photographic images, illustrations, and diagrams, are acknowledged in the caption accompanying the figure.

How to Cite the Guidelines

Cite the guidelines as follows: U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2011. *New Pest Response Guidelines: Late Wilt of Corn* (Harpophora maydis). Washington, D.C.: Government Printing Office. http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

How to Find More Information

Contact USDA–APHIS–PPQ–EDP-Emergency Management for more information about the guidelines. Refer to *Resources* on page A-1 for contact information.

Chapter

2

Pest Information

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Introduction

Use *Chapter 2 Pest Information* to learn more about the classification, history, host range, and biology of *Harpophora maydis*, the etiologic agent of late wilt of corn disease.

Classification

Harpophora maydis is an important soilborne fungal plant pathogen whose taxonomic position relative to other fungi has not been completely resolved. *Table 2-1* on page 2-1 presents the classification for this organism. Based on molecular sequencing data analysis, *H. maydis* represents a distinct taxon within the *Gaeumannomyces-Harpophora* species complex.

Table 2-1 Classification of *Harpophora maydis*

Kingdom	Fungi[1]
Phylum	Ascomycota
Class	Sordariomycetes
Order	*Incertae sedis*
Family	Magnaporthaceae
Genus	*Harpophora*

Table 2-1 Classification of *Harpophora maydis (continued)*

Species	*Harpophora maydis* (Samra, Sabet & Hing.)
Synonyms	*Cephalosporium maydis* Samra, Sabet & Hing. 1963, *Acremonium maydis*
Common Names	late wilt of corn (maize), black bundle disease, 'Shallal' disease of maize

1 CABI 2011, El-Shafey and Claflin 1999.

Historical Information

Late wilt of corn is an important disease in Egypt and parts of India. It was first recorded in Egypt in 1960 and was attributed to a new fungal species, *Cephalosporium maydis* (Samra et al. 1962, Samra et al. 1963). The description was flawed because it did not refer to a type specimen.

Domsch and Gams (1972) suggested that the conidial state of *Cephalosporium maydis* was a *Phialophora* (the anamorph of *Gaeumannomyces*) and that spore production in *Cephalosporium 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 *Gaeumannomyces* 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 *Cephalosporium maydis*.

Damage

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 late-season severe stalkrot of widespread incidence and severity in Egypt, with 100 percent infection reported in some fields (Galal et al. 1979, Samra et al. 1962). Yield losses of up to 40 percent have been reported (El-Shafey and Claflin 1999, Jain et al. 1974). 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 percent (Satyanarayana 1995).

Payak et al. (1970) reported that infesting soil with *Harpophora maydis* caused an increased rate of seed rot, and reduced emergence. Seeds obtained from infected plants of the composite variety "Ambar" also had reduced emergence and lower seedling vigor compared to seeds from unwilted plants.

The severity of the late wilt disease has diminished in most corn plantings due to the introduction of new resistant hybrids, through an active breeding program by the Egyptian Ministry of Agriculture (Mostafa et al. 1996) and in India by Ramana et al. (1997) and Satyanarayana (1995). The extent of resistance or tolerance in corn lines adapted for the United States 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 *Harpophora maydis* population in Egypt is clonal, at least four phylogenetic lineages are present (Saleh et al. 2003; Zeller et al. 2000). 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-Assuity 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.

Economic Impact

Corn is the most economically important grain crop in the United States that would be affected by *Harpophora maydis*. Corn is produced across the United States with the greatest concentration in the central U.S. corn belt: Iowa, Indiana, Illinois, Ohio, South Dakota, North Dakota, Nebraska, Kansas, Minnesota, Wisconsin, Michigan, Missouri, and Kentucky. Approximately 50 percent of all corn grown in the United States is from Iowa, Indiana, Illinois, and Ohio.

The National Agricultural Statistics Service (NASS 2010) estimated that 12.4 billion bushels of corn grain were produced in 2010. Grain yield was estimated at 152.8 bushels per acre, and the area harvested for grain was estimated at 81.4 million acres. The estimated total value was $66.7 billion. In that year Iowa, Illinois, Nebraska and Minnesota were the main corn producing States.

Ecological Range

Harpophora maydis is endemic in Egypt and has been reported from India, Hungary, Israel, Portugal, and Spain (*Table 2-2* on page 2-4 and *Table 2-3* on page 2-4) (Bergstrom et al. 2008, Payak et al. 1970, Pesci and Nemeth 1998, Samra et al. 1962, Samra et al. 1963). There also were unconfirmed reports of the disease in Italy, 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).

Table 2-2 Countries in Which *Harpophora maydis* is Present

Country	Reference
Egypt	Samra et al. 1963
India	Payak et al. 1970
Spain	Molinero-Ruiz et al. 2010
Portugal	Molinero-Ruiz et al. 2010
Hungary	Pécsi and Németh 1998

Table 2-3 Countries in Which the Presence of *Harpophora maydis* is Unconfirmed

Country	Reference
Italy	Bergstrom et al. 2008
Romania	Bergstrom et al. 2008
Israel	Bergstrom et al. 2008
Kenya	Ward and Bateman 1999

Potential Distribution

The pathogen *Harpophora maydis* is not known to exist in the United States, but poses a threat to corn production in this country. This fungal 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 from May to June are most conducive. Based on climate models, the southern half of the United States would be favorable for disease development. Most of the continental United States is at a moderate risk of the establishment of *Harpophora maydis*. The Pareto risk map summarizes the overall risk based on climate, host, and pathways data (*Figure 2-1* on page 2-5). Areas of Arkansas, Illinois, Indiana, Mississippi, Missouri, Tennessee, and Texas, have the highest risk for establishment of *H. maydis.*

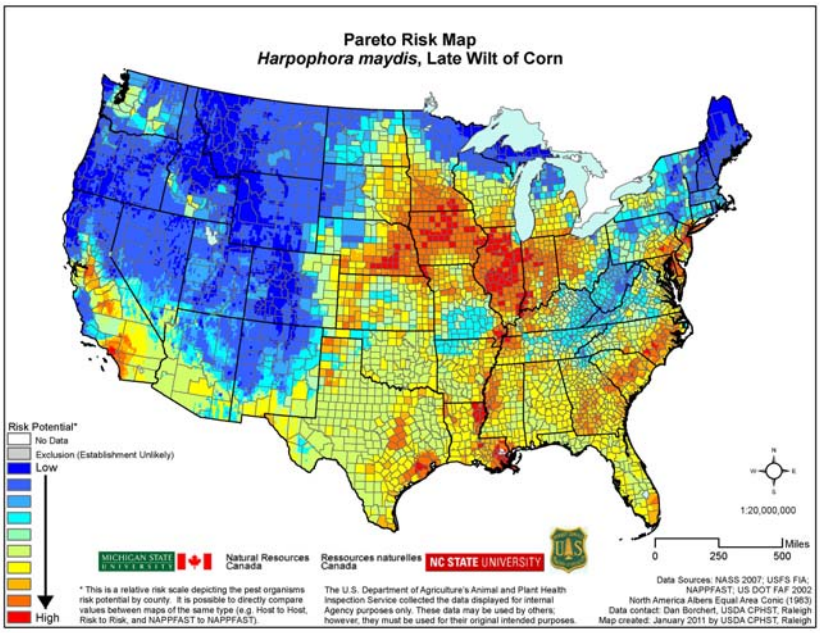

Figure 2-1 Risk Map for *Harpophora maydis* Within the Continental United States (http://www.nappfast.org/)

Hosts

The only reported hosts of *Harpophora maydis* are corn, lupine, and cotton (*Table 2-4* on page 2-6). Disease susceptibility tests have been conducted on a limited number of plant species. Among them, *Gossypium hirsutum* (cotton) develops localized lesions on young hypocotyls that disappear as the plant matures. USDA–APHIS produced a host density map based on corn cultivation data in the United States to help Cooperative Agricultural Pest Survey (CAPS) program cooperators plan surveys and for decision support (*Figure 2-2* on page 2-6, *Figure 2-3* on page 2-7, *Figure 2-4* on page 2-7, and *Figure 2-5* on page 2-8).

Table 2-4 Plant Hosts of *Harpophora maydis*

Scientific Name	Common Name	Geographic Origin	Reference
Zea mays L.	Corn	Egypt	Samra et al. 1962
Lupinus albus L. (*Lupinus termis* Forssk.)	Lupine	Egypt	Sahab et al. 1985
Gossypium hirsutum L.	Cotton	Egypt	Sabet et al. 1966a

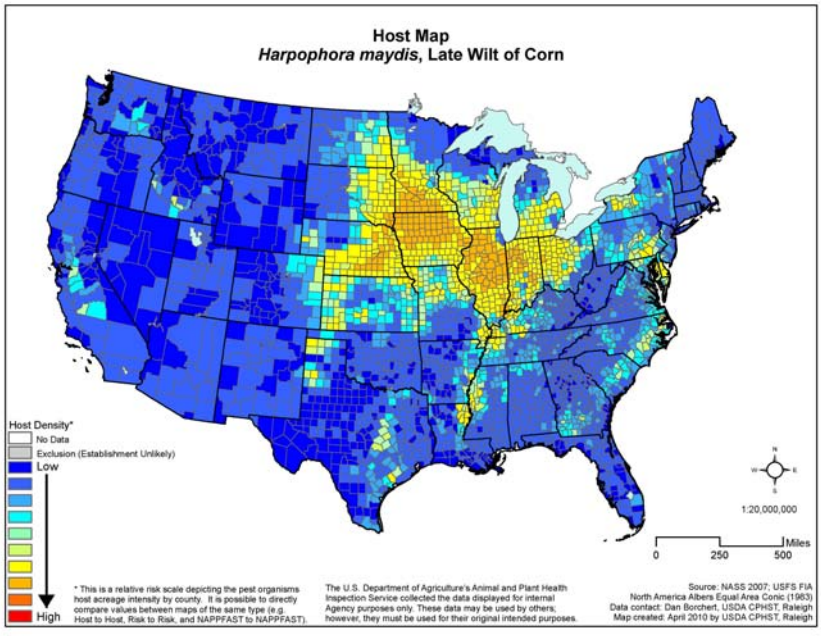

Figure 2-2 Host Map for Establishment Potential of *Harpophora maydis* Within the United States (http://www.nappfast.org/)

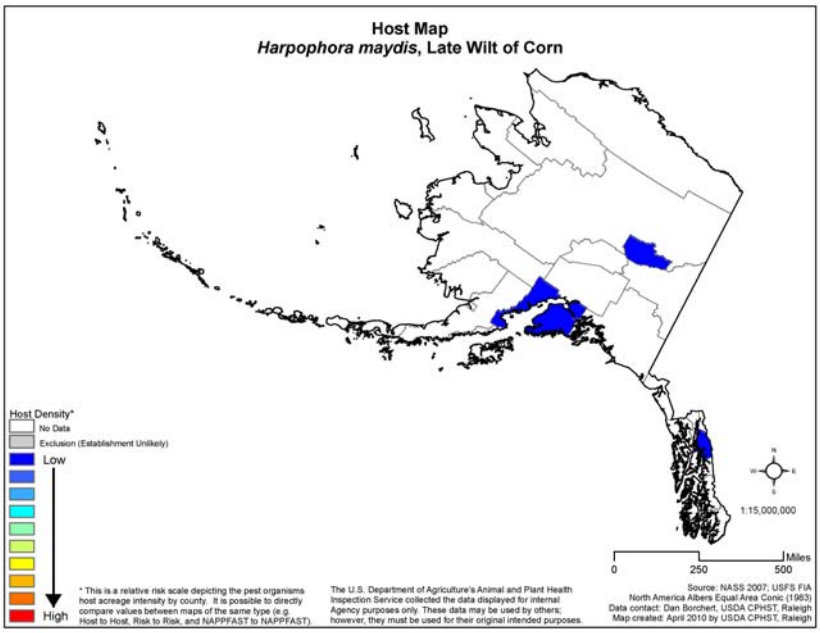

Figure 2-3 Host Map for Establishment Potential of *Harpophora maydis* Within Alaska (http://www.nappfast.org/)

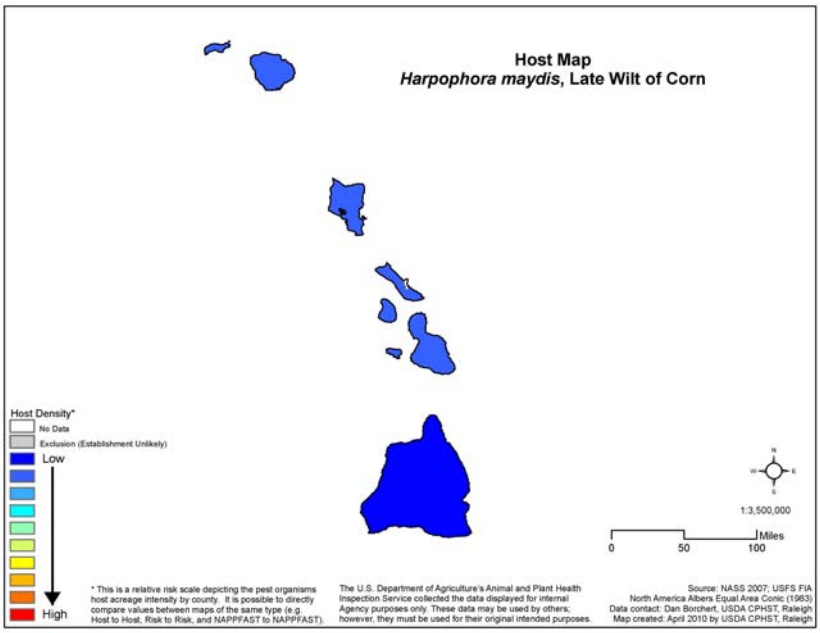

Figure 2-4 Host Map for Establishment Potential of *Harpophora maydis* Within Hawaii (http://www.nappfast.org/)

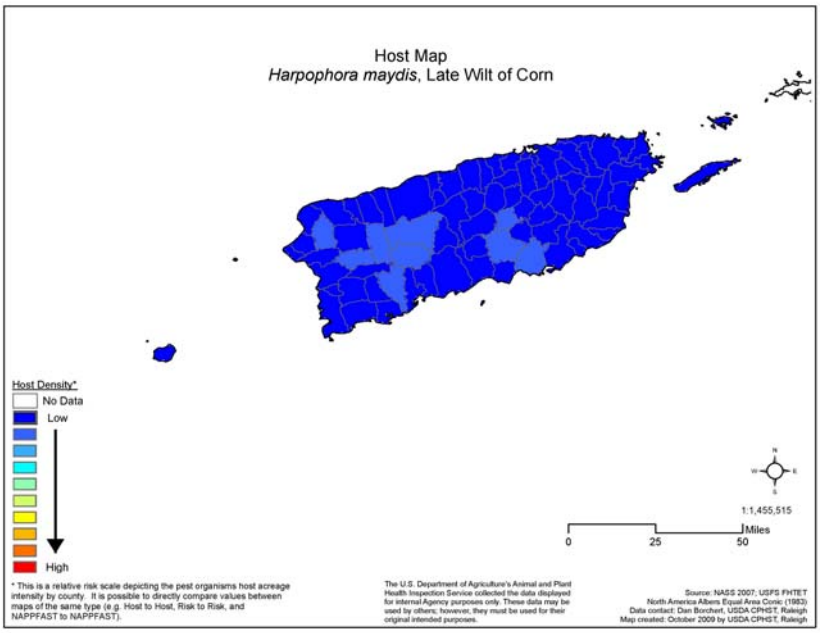

Figure 2-5 Host Map for Establishment Potential of *Harpophora maydis* Within Puerto Rico (http://www.nappfast.org/)

Life Cycle

The fungus is soilborne and causes a vascular wilt disease that most commonly infects seedling plants and has no specific moisture requirements. Initially, the fungus grows superficially on the roots, producing hyphae with short, thick-walled, swollen cells (Sabet et al. 1970b) (*Figure 2-6* on page 2-10). Infection in corn occurs through the roots or mesocotyl (Sabet et al. 1970b). Singh and Siradhana (1987a) found that three irrigations at an interval of 8 hours after inoculation supported maximum rate of disease development. As plants mature, fewer plants are infected, and they become immune about 50 days after planting.

After penetration, *Harpophora 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). *Harpophora maydis* can remain viable in the soil for several years in the absence of a host, and has been shown to persist on corn stubble for 12 to 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 alternate host, can play a role in the survival of the pathogen. The pathogen is most common in hot and humid environments and in heavy textured soils rich in clay or silt. Saturated soils lessen the incidence of *Harpophora maydis*.

Dawood et al. (1979) noted that *Harpophora 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 to 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 70–100 percent. 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 low. Recently formed sclerotia on stalk pieces 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 5 weeks of incubation at 30°C. Viability of these sclerotia was low. *Harpophora maydis* attacks the roots of corn and can allow other pathogens (fungi and viruses) access to the plant. Sabet et al. (1966a) showed that the infection of cotton roots with *H. maydis* decreased the severity of cotton wilt caused by *Fusarium oxysporum*.

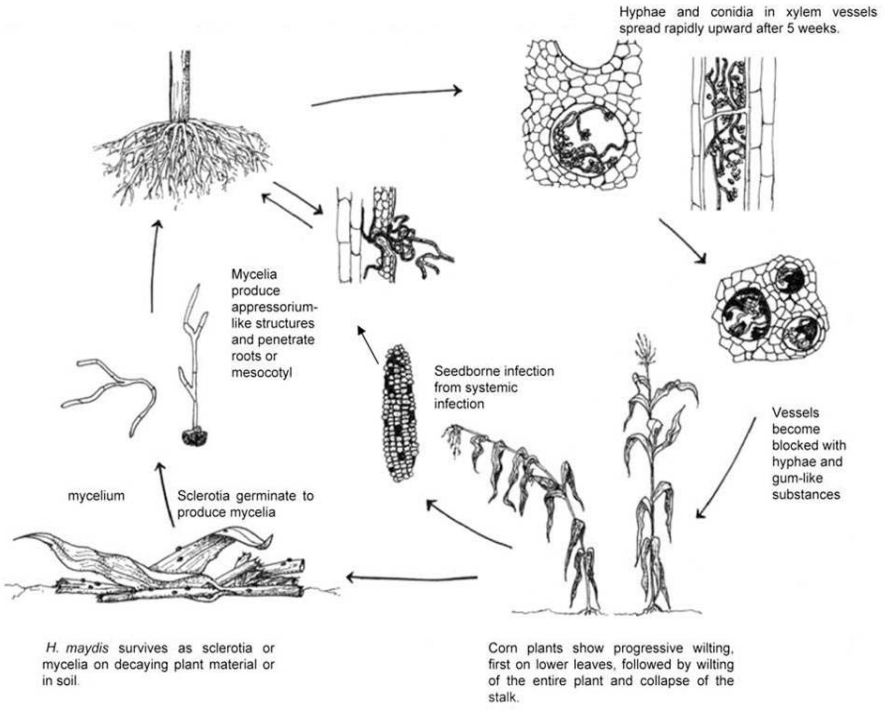

Figure 2-6 Disease Cycle of *Harpophora maydis* on Maize (Johal et al. 2004)

Environmental Impact

The introduction of this pathogen could have some negative effects on the environment. Plant hosts of *Harpophora maydis* may include several *Lupinus* spp. which are listed as federally threatened or endangered (*Table 2-5* on page 2-10) (USFWS 2011). Chemical control programs may be initiated if *H. maydis* is introduced in the United States. The programs can negatively affect non-target pests and the environment.

Table 2-5 Endangered and Threatened[1] Potential Hosts of *Harpophora maydis*[2]

Lupinus species	Common Name	Status	State
aridorum	scrub lupine	Endangered	FL
nipomensis	Nipomo Mesa lupine	Endangered	CA
sulphureus ssp. *kincaidii*	Kincaid's lupine	Threatened	OR, WA
tidestromii	clover lupine	Endangered	CA

1 Endangered and Threatened Plants (50 CFR 17.12).

2 USFWS 2011.

Chapter

3

Identification

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Introduction

Use *Chapter 3 Identification* as a guide to recognizing late wilt of corn, *Harpophora maydis*. Accurate identification of the pathogen is pivotal to assessing its potential risk, developing a survey strategy, and determining the level and manner of control. The recognition of characteristic symptoms on plants is not definitive, and morphological and microscopic characteristics are necessary to identify *H. maydis*.

Authorities

Qualified State, County, or cooperating university, personnel may perform preliminary identification and screening of suspect *Harpophora maydis*. Before survey and control activities are initiated in the United States, an authority recognized by USDA–APHIS–PPQ-National Identification Services must confirm the identity of such pests. Submit specimens to the USDA-National Identification Services (NIS). For further information refer to *How to Submit Plant Samples* on page C-1 and *Taxonomic Support for Surveys* on page D-1.

Reporting

Forward reports of positive identifications by national specialists to PPQ-National Identification Service (NIS) in Riverdale, Maryland, according to Agency protocol. NIS will report the identification status of these tentative and confirmed records to PPQ-Emergency and Domestic Programs (EDP). EDP will report the results to all other appropriate parties. For further information refer to *Taxonomic Support for Surveys* on page D-1.

Characteristic Symptoms

This section describes the plant symptoms that are characteristic of *Harpophora maydis* infection.

Corn

Infected plants do not exhibit symptoms until they reach the tasseling stage and start wilting, generally beginning from the top leaves. Root tips of infected corn plants are stained red during the early stages of infection, but aboveground parts generally remain symptomless until tasseling when a rapid wilting of lower leaves progresses upward (*Figure 3-1* on page 3-3). 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, and become dry as though suffering from lack of water (*Figure 3-2* on page 3-4). Leaves assume a scorched appearance.

Vascular bundles in the stalk become reddish brown and within a short period, lower internodes assume this color (*Figure 3-3* on page 3-5 and *Figure 3-4* on page 3-5) (El-Shafey and Claflin 1999, Samra et al. 1963). In advanced stages, lower portions of the stalk become dry, shrunken, and hollow (*Figure 3-5* on page 3-6).

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 sweet 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 was 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

during harvesting, especially when intensified by delayed harvest or wind damage (Jain et al. 1974).

Figure 3-1 Rapid Wilting of Corn Plant Infected by *Harpophora maydis* and Resistant Hybrids (Bergstrom et al. 2008)

Figure 3-2 Rapid Wilting of Corn Plant Infected by *Harpophora maydis* and Resistant Hybrids (Bergstrom et al. 2008)

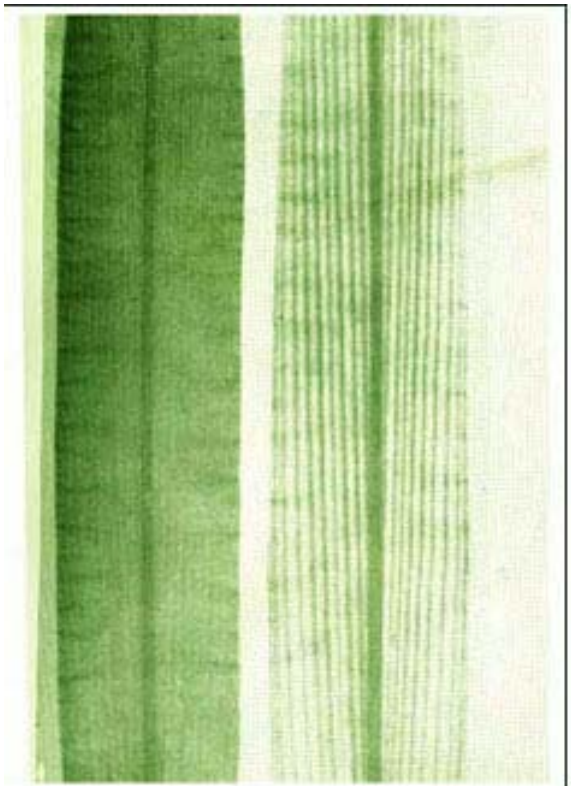

Figure 3-3 Streaking of Leaves of Corn Plant Infected by *Harpophora maydis* (right) Compared with Healthy Leaf (left) (Bergstrom et al. 2008)

Figure 3-4 Progressive Development of Discolored Vascular Bundles of Corn Plants Infected by *Harpophora maydis* (Bergstrom et al. 2008)

Figure 3-5 Progressive Development of Yellow to Reddish-Brown Streaks of Lower Stalks of Corn Infected by *Harpophora maydis* (Bergstrom et al. 2008)

Figure 3-6 Discolored and Necrotic Tissue of Lower Stem Nodes of Corn Infected by *Harpophora maydis* (Bergstrom et al. 2008)

Figure 3-7 Pith Maceration of Corn Infected by *Harpophora maydis* (Bergstrom et al. 2008)

Cotton

Reddish lesions and shallow cracks have been observed on cotton roots of the cultivar "Bahteem 185" grown in inoculated soil. These lesions disappear as the cotton plants mature, and *Harpophora 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 unknown if infections of *H. maydis* on cotton are important to subsequent corn crops.

Lupine

General symptoms of wilt and root rot were reported for the cultivated forage legume in Sabet et al. 1966b. Sahab et al. (1985) indicated that *Harpophora maydis* was also responsible for significant damping-off and stunting of the widely cultivated *Lupinus termis* (*Lupinus albus* L., white lupine) in Egypt.

Diagnostic Tests

Confirmation of *Harpophora maydis* is by morphological identification. This pathogen may be identified morphologically by examination of the shape and size of conidia and conidiophores, color and type of colony, and temperature requirements.

Symptoms are not definitive, and morphological and microscopic characteristics are still used to identify *Harpophora 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) used 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* 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 (*Table 3-1* on page 3-8) (Saleh and Leslie 2004; Zeller et al. 2000).

Table 3-1 PCR Primers for Detection of *Harpophora maydis*[1] [2]

Name	Oligonucleotide Sequence
CMaflp11	5'-TTTCCTGCGGTGCCAA-3'
CMaflp12	5'-TAATGCGGTTAGCCACTC-3'

1 Expected amplicon size 300 bp. Conditions: 67°C annealing temperature and 1.5 mM $MgCl_2$ concentration.

2 Saleh and Leslie 2004.

Successful isolation can usually be obtained by sterilizing the internode of symptomatic plants in 5 percent sodium hypochlorite, splitting them with a sterile knife, and placing a small piece of discolored vascular bundle on PDYA media (Samra and Sabet 1966; Zeller et al. 2002). Use dilution plating to obtain single spore isolates. The recovery of *Harpophora 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. *Harpophora maydis* can be cultured from infected seed by soaking seeds in 1 percent 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).

Similar Species

Late wilt of corn does not occur in the United States and may not be readily recognized or distinguished initially from abiotic stresses without some training. The pathogen is closely related and similar to endemic soilborne fungi in the *Gaeumannomyces-Harpophora* complex. Specifically, *Harpophora maydis* lacks a known teleomorph but is similar to the *Harpophora* anamorphic states of *Gaeumannomyces* species in culture (Saleh et al. 2003).

Late wilt of corn can be distinguished from other pathogenic *Cephalosporium* species due to its fast growth in culture on complex media, minimal growth on Czapek's agar, and eventual dark pigmentation (Samra et al. 1963). The conidiophores can be long and the conidia are generally larger than those of other pathogenic *Cephalosporium* spp. (Samra et al. 1963).

The divergent collarettes on the phialides (Gams 2000) also separate it from *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. Species-specific PCR primers may be used for specific identification.

Chapter

4

Survey Procedures

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Introduction

Use *Chapter 4 Survey Procedures* as a guide when conducting a survey for late wilt of corn caused by the pathogen *Harpophora maydis*. The pathogen also has the potential to infect hosts in the genus *Lupinus*.

Survey Types

Plant regulatory officials will conduct detection, delimiting, and monitoring surveys for *Harpophora maydis*. Conduct a detection survey to determine if *Harpophora maydis* is present or absent in a corn production field or other natural areas where host plants may be present. After a new detection in the United States, or when a detection in a new area is confirmed, conduct a delimiting survey to define the extent of potentially infected plants in the United States. Conduct a monitoring survey to determine the success of control or mitigation activities conducted against the pathogen.

Preparation, Sanitization, and Clean-up

This section provides information that will help personnel prepare to conduct a survey, procedures to follow during a survey, and instructions for proper cleaning and sanitizing of supplies and equipment after the survey is finished.

1. Before starting a survey, determine if there have been recent pesticide applications that would make it unsafe to inspect the agricultural field. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields.
2. Conduct the survey at the proper time. Studies have shown that late wilt of corn is easier to detect at the corn tasseling stage VT. Based upon the pathogen reported global distribution, scientists believe *Harpophora maydis* could establish throughout the corn producing area in the United States with greater potential damages occurring in the southern States. General surveys should focus on months when host plants are growing, although host height in the field and symptoms caused by other common problems such as nitrogen deficiency may complicate the detection.
3. Obtain permission from the landowner before entering a property.
4. Determine if quarantines for other pests, or other crops, are in effect for the area being surveyed. Comply with any and all quarantine requirements.
5. When visiting the area to conduct surveys or to take samples, everyone must take strict measures to prevent contamination by *Harpophora maydis* or other pests between properties during inspections.

Before entering a new property, make certain that clothing and footwear are clean and free of pests and soil to avoid moving soilborne pests and arthropods from one property to another.

Wash hands with an approved antimicrobial soap. If not using an antimicrobial soap, wash hands with regular soap and warm water to remove soil and debris. Then use an alcohol-based antimicrobial lotion, with an equivalent of 63 percent ethyl alcohol. If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, antimicrobial lotions are less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.

6. Gather together all supplies. Confirm the equipment and tools are clean. When taking plant samples, disinfest tools with sodium hypochlorite (bleach) to avoid spreading diseases or other pests. A brief spray or immersion of the cutting portion of the tool in a 5 percent solution of sodium hypochlorite is an effective way to inactivate bacterial and other diseases and prevent their spread. For further instructions, refer to the formulas and instructions for mixing sodium hypochlorite in the PPQ *Manual for Agricultural Clearance*.
7. Mark the plant, tree or sampled location with flagging whenever possible, and draw a map of the immediate area and indicate reference points so that the areas can be found in the future if necessary. Do not rely totally on the flagging or other markers to re-locate a site as they may be removed. Record the GPS coordinates for each trap or infested tree location so that the area or plant may be re-sampled if necessary.
8. Survey task forces should consist of an experienced survey specialist or plant pathologist familiar with *Harpophora maydis* and late wilt of corn symptoms.

Detection Survey

The purpose of a detection survey is to determine whether a pest is present in a defined area. This can be broad in scope as when assessing the presence of the pest over large areas or it may be restricted to determining if a specific pest is present in a focused area.

Based strictly on a negative result in a detection survey, it is not valid to claim that a pest does not exist in an area if results are negative. However, negative results are valuable for providing clues as to mode of dispersal, temporal occurrence, or industry practices, particularly when considered with results from similar areas or proximities.

Procedure

Follow this procedure when conducting a detection survey for *Harpophora maydis*:

1. Use visual inspection to examine the cultivated host plants corn, cotton, and *Lupinus* spp. for wilting symptoms. Refer to *Visual Inspection for Detection Survey* on page 4-8 for further information on inspection procedures.

Important Detection surveys for corn infected by *Harpophora maydis* or other cultivated hosts in fields should be conducted by State inspectors in conjunction with Federal PPQ inspectors.

2. To confirm disease, collect plants showing typical symptoms. Place samples in plastic bags. Keep samples cool. Double bag the samples and deliver promptly to a diagnostic laboratory.

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. Symptoms start from lower leaves moving rapidly upwards during the flowering period. Some plants varieties and particular hybrid combinations are tolerant and can be symptomless carriers of the disease.

Environmental conditions and seasonal changes in plants can complicate the recognition of induced symptoms. Mechanical disruption to the xylem of plants can cause symptoms similar to those associated with infection. It is important to inspect symptomatic shoots for damage to the vascular tissue due to breakage, as well as damage to vascular tissue by boring insects.

Delimiting Survey After Initial U.S. Detection

If *Harpophora maydis* is detected in the United States, surveys will be conducted in the area to determine the distribution of the infected plants. In large areas, locating the actual source of an infestation could be difficult depending on season, age of infected plants, and time elapsed from the initial infection.

Procedure

Follow the same procedure used for *Detection Survey* on page 4-3. Once *Harpophora maydis* has been confirmed surveys should be most intensive around the known positive detections and any discovered through traceback and trace-forward investigations.

Traceback and Trace-Forward Survey

Traceback and trace-forward investigations help determine priorities for delimiting survey activities after an initial detection in the United States. Use traceback investigations to determine the source of infection. Trace-forward investigations attempt to define further potential dissemination through means of natural and artificial spread such as the commercial or private distribution of infected plant material. Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

Trace Forward Survey

Target seed associated with positive testing lots. Consider any distribution channels or irrigation water that might be pathways for further disseminating the pathogen, including but not limited to the following:

- Associated seed lots on a farm, in storage areas, or bins, that may have come in contact with positive testing lots
- Harvesting equipment or other vehicle movement history in fields and nearby fields planted with positive tested lots
- Irrigation or associated waterways running to other areas from fields with positive testing lots
- Weed hosts around contaminated waterways associated with positive testing fields

Analyzing Information

Use traceback information gathered from seed lot tags and invoices to determine the origin of the corn seed. With timely submitted records from growers and corn storage facilities, planning staffs on site can construct prioritized lists for further surveys. Information available from local water companies or farm organizations can be obtained to construct maps of water sources, irrigation channels, and connections in areas with suspect corn plants and potentially infected corn seeds. Contact local water companies and farm organizations to obtain the information, and use it to assess the need for further surveys of fields associated with irrigation water.

Due to reports indicating that *Harpophora maydis* is seedborne, systematic seed testing and certifications should be implemented to prevent its accidental introduction into new areas or countries. However, *H. maydis* can also enter through the illegal importation of seed.

For seed companies, a list of facilities associated with infected seeds from those testing positive for *Harpophora maydis* will be compiled by CPHST.

Important A list of facilities associated with infected seeds will be distributed by the State to the field offices, and are not to be shared with individuals outside the regulatory cooperators working with APHIS–PPQ . Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited. Each State is only authorized to see locations within their State and sharing of confidential business information may be restricted between State and Federal entities. Check the privacy laws with the State Plant Health Director for the State. Refer to *Resources* on page A-1 for contact information.

Positive Detection in Corn

Traceback surveys for positive corn detections should determine the source of infection by examination or analysis of the following:

- Certified seed documentation
- Corn storage facilities
- Irrigation practices and water sources
- Corn processing plants and water handling practices
- Any other potential movement of plant material, water, or machinery that could contribute to tracing the source of contamination

Water Sources and Irrigation Methods

If fields test positive or are directly associated, gather information on irrigation water distribution systems documenting the water source for each area. Obtain this information from local commercial water companies or irrigation district organizations. Maps of irrigation distribution systems should be available from the grower to overlay on agriculture field maps.

Water Sources

Water sources can include the following:

- Dry land farming without irrigation systems
- Ground water that is pumped from wells
- Surface water that is distributed through canals and irrigation ditches

Irrigation Methods

Irrigation methods can include the following:

- Flood
- Center pivot
- Solid set with pipe and fixed risers for sprinklers
- Side roll with pipes and sprinklers that can be rolled on large wheels

When notifying growers on the list, be sure to identify yourself as a USDA or State regulatory official conducting an investigation of facilities that may have received material infested with *Harpophora maydis*. Speak to the growers or farm managers and obtain proper permission before entering private property. Check nursery records to obtain names and addresses for all sales or distribution sites if any sales or distribution has occurred from infested nursery during the previous 6 months.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. If *Harpophora maydis* is detected in the United States, CPHST personnel will assemble a technical working group to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments on the pest population. Refer to *Control Procedures* on page 6-1 for further information on control options.

Procedure

Once *Harpophora maydis* has been confirmed from a particular field, and infected and potentially infected plants have been destroyed, additional monitoring will be necessary. Use the following tools:

- Visual inspection in the field
- Collection of samples from soil, potential weed hosts, and water, for several years and multiple times per season

Refer to *Visual Inspection for Detection Survey* on page 4-8 and *Visual Inspection for Delimiting Survey* on page 4-8 for further information concerning the inspection of host plants.

Visual Inspection for Detection Survey

Use visual inspection as a tool when surveying for late wilt of corn in field crops.

Conduct a visual inspection in corn fields by looking for plants with wilting symptoms. Wilting and other symptoms may appear in several plants or all plants in a particular row or section of the field. The absence of wilt symptoms, however, does not mean that *Harpophora maydis* is not present in the inspected area. Some infected plants, including cultivars that are less sensitive to late wilt, may not express symptoms depending on the severity of the infection.

The following symptoms can be found on corn plants infected with *Harpophora maydis*:

- Infected leaves dry and dull green
- Leaves rolling inward and eventually loosing color
- Infected vascular bundles in the stalk reddish-brown
- Stalk internodes discolored
- Lower portions of the stalk dry, shrunken, and hollow

Some plants develop yellowish to purple or dark brown streaks on the lower stem. Continued colonization of the vascular bundles eventually results in death of the plant. Rotting of roots and lower internodes may involve secondary organisms.

Important Other diseases, as well as drought, can cause symptoms of wilt that are similar, so diagnostic tests must be performed on samples from symptomatic plants in order to confirm the presence of *Harpophora maydis*.

For further information, refer to *Identification* on page 3-1.

Visual Inspection for Delimiting Survey

Construct delimiting surveys in an area—based on known positive testing, associated positive testing, or potentially infested areas—from investigations of distribution channels and shared irrigation water. However, it may be necessary to do random samples in a general growing area to detect new infestations not discovered through investigations.

The delimiting survey in a general growing area can include random sampling of stored seeds and fields throughout a geographical area, with more intensive sampling near known infestations. As the distance away from the epicenter of a known infestation increases, decrease the rate of random sampling. Based on the epidemiology and grower practices, an evaluation of risk and resources available will help determine the extent of these random sampling surveys.

Targeted Surveys

Conduct targeted surveys at nurseries associated with high risk pathways. Areas with regular traffic from countries with known infestations, that may carry hitchhiker insect vectors, should also be targeted for regular surveys.

Procedure

A defined method is unavailable.

Survey Records

Records should be kept for each survey site. Negative survey data must be recorded even in the absence of *Harpophora maydis.* Record also the absence of samples at surveyed sites. Survey records and data recording formats should be consistent, to allow for standardized collection of information.

If automated field collection devices are used, such as the Integrated Survey Information System (ISIS), ensure that all surveyors are trained in the technology before beginning the survey. Use the appropriate ISIS templates for this pathogen. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders before working in the field. At the end of the survey, all survey data should be entered into a designated State or national pest database.

Data Collection

Surveyors visiting sites to place holds or take samples should collect the following information:

- Date of collection or observations
- Collector’s name
- Grower’s field identification numbers
- GPS coordinates

- Variety of host plants grown
- Methods of irrigation
- History of farm machinery usage
- Observations of wilt
- Other relevant information

In the absence of inspection officials, take the following actions immediately if wilting symptoms are noticed:

1. Mark the location
2. Remove the plants and flag the location in the field
3. Notify the State or PPQ inspector
4. Place the whole plant inside two resealable plastic bags
5. Label the sealed bags with the following information:
   A. Date
   B. Name of person responsible
   C. Location of sample collection
6. Keep bagged plants cool or refrigerated until the inspector arrives
7. Do not freeze the sample

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize infestations of *Harpophora maydis*.

Chapter

5

Regulatory Procedures

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Introduction

Use *Chapter 5 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against late wilt of corn, caused by the pathogen *Harpophora maydis*. The pathogen also has the potential to infect hosts in the genus *Lupinus*.

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in accordance with labeling restrictions. During all field visits, please ensure that proper sanitation procedures are followed as outlined in *Preparation, Sanitization, and Clean-up* on page 4-2.

Regulatory Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification may be issued to hold articles or facilities, pending positive identification by a USDA–APHIS–PPQ-recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register.*

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides the authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under State authority.

State departments of agriculture normally work in conjunction with Federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the States measures are inadequate, USDA can take intrastate regulatory action provided that the governor of the State has been consulted and a notice has been published in the Federal Register. If intrastate action cannot or will not be taken by a State, PPQ may find it necessary to quarantine an entire State.

PPQ works in conjunction with State departments of agriculture to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property in the absence of owner permission. PPQ prefers to work with the State to facilitate access when permission is denied, however each State government has varying authorities regarding entering private property.

A General Memorandum of Understanding (MOU) exists between PPQ and each State that specifies various areas where PPQ and the State department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected State. Refer to *Resources* on page A-1 for information on identifying SPHD's and SPRO's.

Tribal Governments

USDA–APHIS–PPQ also works with federally-recognized Indian Tribes to conduct surveys, enforce regulations and take control actions. Each Tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to State governments. Permission is required to enter and access Tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Indian Tribal governments about actions that may have substantial direct effects on Tribes. Whether an action is substantial and direct is determined by the Tribes. Effects are not limited to Tribal land boundaries (reservations) and may include effects on off-reservation land or resources which Tribes customarily use or even effects on historic or sacred sites in States where Tribes no longer exist.

Consultation is a specialized form of communication and coordination between the Federal and Tribal governments. Consultation must be conducted early in the development of a regulatory action to ensure that Tribes have opportunity to identify resources which may be affected by the action and to recommend the best ways to take actions on Tribal lands or affecting Tribal resources. Communication with Tribal leadership follows special communication protocols. For additional information, contact PPQ's Tribal Liaison. Refer to *Table A-1* on page A-1 for information on identifying PPQ's Tribal Liaison.

To determine if there are Federally-recognized Tribes in a State, contact the State Plant Health Director (SPHD). To determine if there are sacred or historic sites in an area, contact the State Historic Preservation Officer (SHPO). For clarification, check with your SPHD or State Plant Regulatory Official (SPRO) in the affected State. Refer to *Resources* on page A-1 for contact information.

Overview of Regulatory Program After Detection

Once an initial U.S. detection is confirmed, holds will be placed on the property by the issuance of an Emergency Action Notification. Immediately place a hold on the property to prevent the removal of any host plants of the pest.

Traceback and trace-forward investigations from the property will determine the need for subsequent holds for testing and/or further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures prescribed.

Record-Keeping

Record-keeping and documentation are important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by the owners, researchers or manager for information on destination of shipped plant material, movement of plant material within the facility, and any management (cultural or sanitation) practices employed.

Keep a detailed account of the numbers and types of plants held, destroyed, and/or requiring treatments in control actions. Consult a master list of properties, distributed with the lists of suspect nurseries based on traceback and trace-forward investigations, or nurseries within a quarantine area. Draw maps of the facility layout to located suspect plants, and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout, and document plant propagation methods, labeling, and any other information that may be useful for further investigations and analysis.

Keep all written records filed with the Emergency Action Notification copies, including copies of sample submission forms, documentation of control activities, and related State issued documents if available.

Issuing an Emergency Action Notification

Issue an Emergency Action Notification to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested, or testing determines there is no risk, the material may be released and the release documented on the EAN.

Regulated Area Requirements Under Regulatory Control

Depending upon decisions made by Federal and State regulatory officials in consultation with a Technical Working Group, quarantine areas may have certain other requirements for commercial or research fields in that area, such as plant removal and destruction, cultural control measures, or plant waste material disposal.

Any regulatory treatments used to control this pest or herbicides used to treat plants will be labeled for that use or exemptions will be in place to allow the use of other materials.

Establishing a Federal Regulatory Area or Action

Regulatory actions undertaken using Emergency Action Notifications continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfestation orders or longer term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers, and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing, and risk assessment will determine the area to be designated for a Federal and parallel State regulatory action. Risk factors will take into account positive testing, positive associated, and potentially infested exposed plants. Boundaries drawn may include a buffer area determined based on risk factors and epidemiology.

Regulatory Records

Maintain standardized regulatory records and databases in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

Use of Chemicals

The PPQ *Treatment Manual* and the guidelines identify the authorized chemicals, and describe the methods and rates of application, and any special application instructions. For further information refer to *Control Procedures* on page 6-1. Concurrence by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for this pest.

Chapter

6

Control Procedures

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Introduction

Use *Chapter 6 Control Procedures* to learn more about controlling an outbreak of late wilt of corn caused by the pathogen *Harpophora maydis*. Consider the treatment options described within this chapter when taking action to eradicate, contain, or suppress late wilt of corn.

This pathogen is generally controlled by implementing a combination of strategies including use of genetic resistance, cultural practices and chemical treatments.

Overview of Emergency Programs

APHIS–PPQ develops and makes control measures available to involved States. United States Environmental Protection Agency-approved treatments will be recommended when available. If the selected treatments are not labeled for use against the pest or in a particular environment, PPQ's FIFRA Coordinator is available to explore the appropriateness in developing an Emergency Exemption under Section 18, or a State Special Local Need under section 24(c) of FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act), as amended.

The PPQ FIFRA Coordinator is also available upon request to work with EPA to rush the approval of a product that may not be registered in the United States, or to obtain labeling for a new use-site. The PPQ FIFRA Coordinator is available for guidance pertaining to pesticide use and registration. Refer to *Resources* on page A-1 for information on contacting the Coordinator.

Treatment Options

Consider the treatment options described within this chapter when taking action to eradicate or control late wilt of corn. Treatments may include the following:

- *Fungicides* on page 6-4
- *Cultural Control* on page 6-5
- *Biological Control* on page 6-6
- *Genetic Resistance* on page 6-7

Environmental Documentation and Monitoring

Obtain all required environmental documentation before beginning. For further information, refer to *Environmental Compliance* on page 7-1. Contact Environmental Services staff for the most recent documentation. Refer to *Resources* on page A-1 for contact information.

Efficacy of Treatment

Eradication measures should be continued for several years to ensure that populations of exotic *Harpophora maydis* have been eliminated. Once the pest has been eradicated, monitoring of the site should be continued for 1 to 2 years. For further information, refer to *Monitoring Survey* on page 4-7.

Site Assessment

When visiting a site keep a log of observations, flag the infested areas, and record the coordinates. Record also the name of the property owner. Some of this information may have been recorded during the survey. Communicate frequently with the person responsible for the site.

Classification

Information on the type of property needs to be recorded to help develop a control plan. Site access, security, containment, and ownership type may dictate a particular direction in control options. Prepare a concise overview of the infested area. Record information about the infested property, including the following:

- Location
- Type of property ownership (government, private, Tribal, commercial, residential, or agricultural)
- Current and past users of the property
- Distribution of infected plants
- Status of security and containment
- Modes of artificial movement

Safeguarding Against Artificial Movement

Harpophora maydis is most likely to be moved in infested seeds, decaying plant material, and soil. Bales and ears of corn moved on agricultural equipment may be a secondary means of dissemination for this pathogen. There is the potential for risk from the movement of biological culture and infected crop residues. If plants infected with *H. maydis* are found in an area, then host material moving out of the immediate area should be checked carefully. If necessary, quarantines can be put in place to prevent the spread of the target pest to uninfested areas. For further information, refer to *Issuing an Emergency Action Notification* on page 5-4.

Fungicides

Important All treatments listed in the guidelines should only be used as a reference to assist in the regulatory decision making process. It is the National Program Manager's responsibility to verify that treatments are appropriate and legal for use. Upon detection and when a chemical treatment is selected, the National Program Manager should consult with PPQ's FIFRA Coordinator to ensure that the chemical is approved by EPA for use in the United States prior to application. Refer to *Resources* on page A-1 for contact information.

Important The cost and labor required for frequent fungicide applications to corn make chemical control prohibitively expensive in the United States, and contemporary fungicides have not been evaluated against late wilt or registered for this use in this country.

Use fungicides approved by the PPQ FIFRA Coordinator to treat seeds or storage facilities. In India, seed treatments of the following fungicides significantly reduced the severity of late wilt of corn and increased yields 11 to 91 percent (Begum et al. 1989, Satyanarayana and Begum 1996):

- Captan
- Carbendazim
- Carboxin
- Thiram

Captan provided the most effective and economic return to growers. In contrast, benomyl was not effective as a dust or dip and consistently failed to control late wilt in Egyptian trials (Sabet et al. 1972).

Failures in Egypt may be due to differences in the chemical sensitivity or virulence of *Harpophora maydis* isolates, the chemical formulations evaluated, environmental conditions, or the complexity of the stalk-rot disease complex in Egyptian soils. Additionally, Pécsi and Németh (1998) tested a number of fungicides in vitro that effectively inhibited growth of the fungus, specifically benomyl (as Chinoin Fundazol 50 WP), cyproconazole (as Alto 100SL), carbendazim + cyproconazole (as Alto combi 420), difenoconazole + propiconazole (as Rias 300EC) and flusilazole + carbendazim (as Alert), tested at six different dilutions (10, 50, 100 200, 400, 800 ppm).

Systemic fungicides and their fungitoxic products are translocated to corn leaves within 2 days and can persist in corn roots for 90 days; however, results in the field have been disappointing unless the fungicide was applied several times during the growing season (Singh and Siradhana 1989).

Labeling

While the proposed formulation is approved for an effective eradication program, it may not be labeled at the time of pest detection, for the specific site where treatment is required. If a formulation is not labeled for the needed use, it may be possible to request a Emergency Exemption, or a State Special Local Need under section 24(c) of Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) from the U.S. Environmental Protection Agency (EPA).

For further information refer to *Regulatory Procedures* on page 5-1.

Cultural Control

The use of disease-free seed that is inspected and certified could limit further distribution of *Harpophora maydis* and reduce the levels of potential inoculum. 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. The widespread use of no-till corn systems in the United States could eventually result in the build-up of inoculum in soil or the increased virulence of the pathogen.

Hot water seed treatment (60°C for 10 to 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 of corn (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 and frequent watering or saturated soils reduced late wilt (Samra and Sabet 1966). Corn did not develop late wilt following paddy-cultivated rice, which increases the availability of manganese for subsequent crops, although *Harpophora maydis* also is sensitive to low oxygen conditions (Samra and Sabet 1966). The management of moisture, and flood fallowing, may be useful cultural controls for late wilt where they are economically practical (Samra and Sabet 1966; Singh and Siradhana 1988).

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 potassium soils of Egypt (Samra et al. 1972, Samra and Sabet 1966). Phosphorus, organic amendments (straw, cotton cakes, and brodret) and micronutrients (Cu, Fe, Mn, and Zn) also reduce disease severity (Singh and Siradhana 1990).

Biological Control

If the pathogen population was discovered too late for eradication measures to be effective, new measures should rely on containment or management options. Containment means keeping the target population of infected plants confined to a specific area, and perhaps later developing tools to eradicate it. In contrast, management is used when the population of the pathogen is so large or widely spread that resources are better directed at limiting the impacts caused by the infestation.

The following control options are best suited for both containment programs and long term management. They could be used in an eradication program if the intent is to bring population numbers down to better achieve this goal. Biological control agents are useful for suppressing pathogen populations, but do not eradicate them. Biological control can be useful if rigorous screening on non-target organisms is tested. Obtain the proper permits from PPQ-Plant, Organism, and Soil Permits, prior to testing.

Since *Harpophora maydis* is a poor saprophytic competitor (Sabet 1970, Sabet et al. 1970a), 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 1991, El-Assiuty et al. 1991, El-Mehalowy 2004, El-Mehalowy et al. 2004, Singh 1988, Singh and Siradhana 1988).

The application of biological control was tested on stored maize grains against various pathogenic fungi. The inoculation of culture filtrate or total suspension of *Streptomyces* spp. with or without seed surface-disinfection was shown to significantly reduce the incidence of pathogenic fungi generally associated with corn seeds in storage (Bressan 2003, Bressan 2003).

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 United States because *Harpophora 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.

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 important sources of late wilt resistance to introduce resistance into U.S. hybrids. 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.

During 2001 to 2004, a breeding program in India in collaboration with Asian Regional Maize Program of CYMMYT evaluated two-hundred inbred lines for sources of resistance against post-flowering stalk rots of maize, caused among others fungi by *Harpophora maydis*, *Fusarium moniliforme* and *Macrophomina phaseolina* (Shekhar et al. 2010). Three resistant maize lines, namely PFSR-13-5, JCY2-2-4-1-1-1-1 and JCY3-7-1-2-1-b-1, were identified. Additionally, resistance level of five pools/populations was improved to an

overall acceptable level (PFSR (Y)-C1, PFSR (white), extra-early (white), P-100, P-300 and P-345).

New virulent strains of *Harpophora maydis* have been observed 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-Assuity et al. 1999). 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 × Gm. 30 was the superior cross with a resistance rating of 99 percent (Soliman and Sadek 1998). Resistant lines developed in India include X102, CM111, CM202, and (CM104×WL) (Satyanarayana 1995).

Harpophora maydis lineages differ in their ability to colonize maize plants and in their relative aggressiveness in single culture inoculations (El-Assuity 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.

Chapter

7

Environmental Compliance

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Introduction

Use *Chapter 7 Environmental Compliance* as a guide to the environmental regulations concerning late wilt of corn caused by the pathogen *Harpophora maydis*. The pathogen also has the potential to infect hosts in the genus *Lupinus*.

Overview

Program managers of Federal emergency response or domestic pest control programs must ensure that their programs comply with all Federal Acts and Executive Orders pertaining to the environment, as applicable. Two primary Federal Acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may commence.

Program managers should also seek guidance and advice as needed from Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development (PPD) staff. ERAS is available to provide guidance

and advice to program managers and prepare drafts of applicable environmental documentation.

In preparing draft NEPA documentation ERAS may also perform and incorporate assessments that pertain to other acts and executive orders described below, as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), will assist ERAS in the development of documents, and will implement any environmental monitoring.

Leaders of programs are strongly advised to consult with ERAS and/or ECT early in the development of a program in order to conduct a preliminary review of applicable environmental statutes and to ensure timely compliance. Environmental monitoring of APHIS pest control activities may be required as part of compliance with environmental statutes, as requested by program managers, or as suggested to address concerns with controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition, or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from ECT will work with the program manager to develop an environmental monitoring plan, conduct training to implement the plan, provide day-to-day guidance on monitoring, and provide an interpretive report of monitoring activities.

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all Federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decisionmaker prior to taking action, and to inform the public of the decision. Actions that are excluded from this examination, that normally require an Environmental Assessment, and that normally require Environmental Impact Statements, are codified in APHIS' NEPA Implementing Procedures located in 7 CFR 372.5.

The three types of NEPA documentation are Categorical Exclusions, Environmental Assessments, and Environmental Impact Statements.

Categorical Exclusion

Categorical Exclusions (CE) are classes of actions that do not have a significant effect on the quality of the human environment and for which neither an Environmental Assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have been built into the actions themselves (7 CFR 372.5(c)).

Environmental Assessment

An Environmental Assessment (EA) is a public document that succinctly presents information and analysis for the decisionmaker of the proposed action. An EA can lead to the preparation of an environmental impact statement (EIS), a finding of no significant impact (FONSI), or the abandonment of a proposed action.

Environmental Impact Statement

If a major Federal action may significantly affect the quality of the human environment (adverse or beneficial) or the proposed action may result in public controversy, then prepare an Environmental Impact Statement (EIS).

Endangered Species Act

The Endangered Species Act (ESA) is a statute requiring that programs consider their potential effects on federally-protected species. The ESA requires programs to identify protected species and their habitat in or near program areas, and document how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act

The statute requires that programs avoid harm to over 800 endemic bird species, eggs, and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

Clean Water Act

The statute requires various permits for work in wetlands and for potential discharges of program chemicals into water. This may require coordination with the Environmental Protection Agency, individual States, and the Army Corps of Engineers. Such permits would be required even if the pesticide label allows for direct application to water.

Tribal Consultation

The Executive Order requires formal government-to-government communication and interaction if a program might have substantial direct effects on any federally-recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed prior to general public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed Federal actions intersect with Tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act

The statute requires programs to consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local State Historic Preservation Offices. Documentation under this act involves preparing an inventory of the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may require public involvement and comment prior to the start of program activities.

Coastal Zone Management Act

The statute requires coordination with States where programs may impact Coastal Zone Management Plans. Federal activities that may affect coastal resources are evaluated through a process called Federal consistency. This process allows the public, local governments, Tribes, and State agencies an opportunity to review the Federal action. The Federal consistency process is administered individually by states with Coastal Zone Management Plans.

Environmental Justice

The Executive Order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to consider if the actions might disproportionally impact minority or economically disadvantaged populations and if so, how such impact will be avoided.

Protection of Children

The Executive Order requires Federal agencies to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. If such a risk is identified, then measures must be described and implemented to minimize such risks.

Chapter

8

Pathways

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Introduction

Use *Chapter 8 Pathways* as a source of information on the pathways of introduction of late wilt of corn, *Harpophora maydis*, at U.S. ports. The fungus could potentially enter the continental United States through commerce, or the movement of seed, soil, or plant material. *Harpophora maydis* is a moderate threat to the production of corn in the United States. The pathogen also has the potential to infect hosts in the genus *Lupinus*.

Natural Movement

The natural spread of *Harpophora maydis* into the continental United States is a rare possibility. Conidia are the only form of spore 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 as well as decaying plant material and could be unintentionally dispersed by soil movement (Dawood et al. 1979).

Commerce

Plant parts liable to carry the pest in trade or transport are seeds; fresh corn on the cob; fresh or dried corn stalks (leaves, cobs and husk); micropropagated plants; roots; and all plant tissues containing xylem conducting elements. The presence of the fungus in or on seed has been established. The importation of infected seed is considered the cause of the pathogen's appearance in Hungary (Pécsi and Németh 1998). Movement of soil on machinery or tools could carry the fungus locally.

Harpophora maydis is an imminent threat that could be introduced into the United States with imported leaves, stems, and roots of infected plants (*Figure 8-1* on page 8-2) (CABI 2005). Due to the relatively slow rate of disease spread through natural secondary dissemination, containment and eradication should be actively pursued. The success of such strategy would rely on accurate and timely identification of the pathogen matched by quarantine action and targeted eradication implementing all necessary control measures.

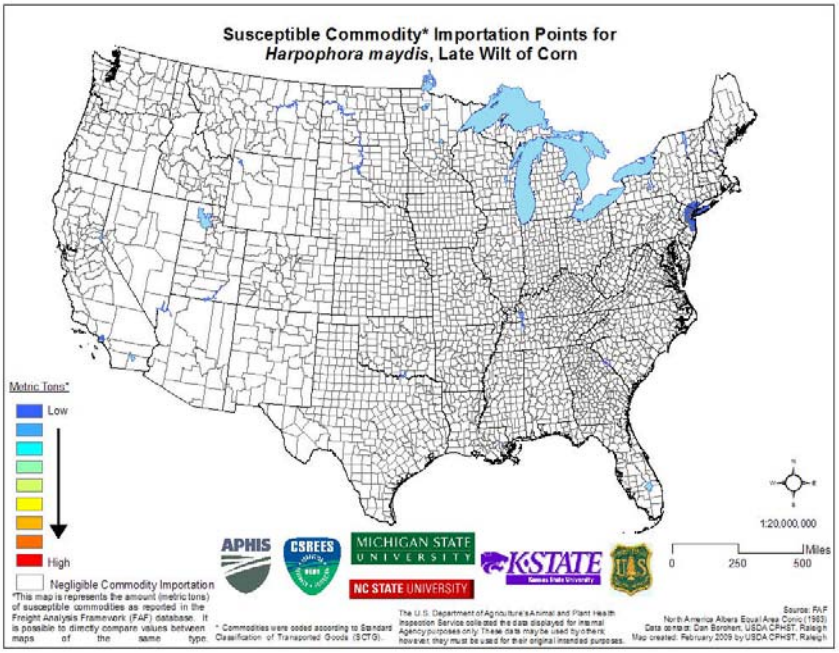

Figure 8-1 Points of Importation for *Harpophora maydis* Within the Conterminous United States (https://www.nappfast.org)

Once a positive identification has been made confirming the infection of plants by *Harpophora maydis*, initiate investigations to determine the probable origin of the initial infections as well as the extent of the distribution of potentially infected plants in the U.S. Territory.

After investigations are performed and the risk of the establishment of the pathogen is evaluated, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific actions under the Plant Protection Act. The Plant Protection Act of 2000 provides for authority for emergency quarantine action.

Program personnel must maintain records and maps noting the location of all detections, the number and type of plants subjected to control actions, and the materials and chemical formulations used in each treated area.

Late Wilt of Corn

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Use the References section to learn more about the publications, Web sites, and other resources, that were consulted during the production of the guidelines.

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Late Wilt of Corn

Glossary

Use this glossary to find the meaning of specialized words, abbreviations, acronyms, and terms used by PPQ–EDP. To locate where in the manual a given definition, term, or abbreviation is mentioned, refer to the Index.

Definitions, Terms, and Abbreviations

amplicon. Piece of DNA synthesized using amplification techniques such as PCR
approved landfill. State licensed municipal or private landfill managed under state regulation to prevent leaching of potential pollutants into groundwater
APHIS. USDA-Animal and Plant Health Inspection Service
APA. American Phytopathological Society
bp. base pair
CAPS. Cooperative Agricultural Pest Survey Program, a partnership between all 50 States and USDA to detect and monitor exotic pests of economic impact
chlorosis. Yellowing of normally green tissue due to chlorophyll destruction in infected plants
CIMMYT. International Maize and Wheat Improvement Center
CPB. U.S. Department of Homeland Security-Customs and Border Protection
CPHST. PPQ-Center for Plant Health Science and Technology
decontamination. Application of approved chemical or other treatment to contaminated implements, material, or buildings for killing or deactivating a pathogen
detection survey. Survey conducted in an environmentally favorable area where *Harpophora maydis* is not known to occur
DHS. U.S. Department of Homeland Security
dieback. Death of branches on woody plants, shrubs, trees; typically young shoots, twigs, and distal portions of branches die progressively toward older plant parts
disposal. Method used to eliminate diseased plant material or material associated with diseased plant material, usually at an approved landfill
EDP. PPQ-Emergency and Domestic Programs
host. Plant which is invaded by a parasite or pathogen and from which it obtains its nutrients
EM. PPQ-Emergency Management
FIFRA. Federal Insecticide, Fungicide, and GLOSSARYRodenticide Act
ICS. Incident Command System
infection. Establishment of a parasite on or within a host plant
ISIS. Integrated Survey Information System

monitoring survey. Survey conducted at a site where a disease was found and where an eradication program is being performed; *also known as evaluation survey*
necrosis. Dead or discolored plant tissue
NEPA. National Environmental Policy Act
NIS. PPQ-National Identification Service
NPAG. PPQ New Pest Advisory Group
NPRG. New Pest Response Guidelines
pathogen. Any organism that can incite a disease
PCR. Polymerase chain reaction, a laboratory technique that amplifies DNA sequences in order to determine if a host is infected with a known pathogen.
PCR-primers. Short fragments of single stranded DNA (15 to 30 nucleotides in length), complementary to DNA sequences that flank the target region of interest; necessary components for the polymerase chain reaction
PDYA media. PDA + 0.2 percent yeast extract
PERAL. Plant Epidemiology and Risk Analysis Laboratory
pest. includes insects, weeds, plant disease agents, and microorganisms
PPQ. APHIS-Plant Protection and Quarantine
SEL. USDA–ARS-Systematic Entomology Laboratory
SPHD. State Plant Health Director
SPRO. State Plant Regulatory Official
symptom. External and internal reactions or alterations of a plant as the result of a disease
traceback. To investigate the origin of infested plants through intermediate steps in commercial distribution channels to the origin
trace-forward. To investigate where infected plants may have been distributed from a source through steps in commercial distribution channels
TWG. Technical Working Group
USDA. United States Department of Agriculture
VT. tasseling stage of corn growth

Appendix

A

Resources

Use *Appendix A Resources* to find the Web site addresses, street addresses, and telephone numbers of resources mentioned in the guidelines. To locate where in the guidelines a topic is mentioned, refer to the index.

Table A-1 Resources for *Harpophora maydis*

Resource	Contact Information
Center for Plant Health, Science, and Technology (USDA–APHIS–PPQ–CPHST)	http://www.aphis.usda.gov/plant_health/cphst/index.shtml
Emergency and Domestic Programs, Emergency Management (USDA–APHIS–PPQ–EDP–EM)	http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml
PPQ *Manual for Agricultural Clearance*	http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml
PPQ *Treatment Manual*	http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml
Host or Risk Maps	http://www.nappfast.org/caps_pests/CAPs_Top_50.htm
Plant, Organism, and Soil Permits (APHIS–PPQ	http://www.aphis.usda.gov/plant_health/permits/index.shtml
National Program Manager for Native American Program Delivery and Tribal Liaison (USDA–APHIS–PPQ)	14082 S. Poston Place Tucson, AZ 85736 Telephone: (520) 822-544
Biological Control Coordinator (USDA–APHIS–CPHST)	http://www.aphis.usda.gov/plant_health/cphst/projects/arthropod-pests.shtml
FIFRA Coordinator (USDA–APHIS–PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: (301) 734-5861
Environmental Compliance Coordinator (USDA–APHIS–PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: (301) 734-7175
PPQ Form 391(Fillable)	http://www.aphis.usda.gov/library/forms/
List of State Plant Health Directors (SPHD)	http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml
List of State Plant Regulatory Officials (SPRO)	http://nationalplantboard.org/member/index.html

Appendix

B

Forms

Use *Appendix B Forms* to learn how to complete the forms mentioned in the guidelines. To locate where in the guidelines a form is mentioned, refer to the index.

Contents

PPQ Form 391 Specimens For Determination **B-2**
PPQ 523 Emergency Action Notification **B-7**

PPQ Form 391 Specimens For Determination

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions. *See reverse for additional OMB information.*

FORM APPROVED
OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete Items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete Items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.

PRIORITY

1. COLLECTION NUMBER

2. DATE — MO | DA | YR

3. SUBMITTING AGENCY
☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER

6. ADDRESS OF SENDER

ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*

7. NAME AND ADDRESS OF PROPERTY OR OWNER

COUNTRY/ COUNTY

PURPOSE

8. REASON FOR IDENTIFICATION *("x" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS*)*
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
F. ☐ Possible Immigrant *(Explain in* REMARKS*)*
G. ☐ Survey *(Explain in* REMARKS*)*
H. ☐ Other *(Explain in* REMARKS*)*

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

HOST DATA

10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure and indicate* ☐ Number ☐ Percent*):*

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

13. PLANT PARTS AFFECTED
☐ Leaves, Upper Surface ☐ Leaves, Lower Surface ☐ Petiole ☐ Stem
☐ Trunk/Bark ☐ Branches ☐ Growing Tips ☐ Roots
☐ Bulbs, Tubers, Corms ☐ Buds ☐ Flowers ☐ Fruits or Nuts
☐ Seeds

PEST DATA

14. PEST DISTRIBUTION
☐ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD

17. TYPE OF TRAP AND LURE

18. TRAP NUMBER

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

SIGNATURE

DATE

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

PPQ FORM 391 *Previous editions are obsolete.*
(AUG 02)

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

Figure B-1 Example of PPQ Form 391 Specimens For Determination, side 1

OMB Information
According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0010. The time required to complete this information collection is estimated to average .25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

Instructions
Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS
1	1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number **EXAMPLE** In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001 2. Enter the collection number
2	Enter date
3	Check block to indicate Agency submitting specimens for identification
4	Enter name of sender
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)
6	Enter address
7	Enter name and address of property owner
8A-8L	Check all appropriate blocks
9	Leave Blank
10	Enter scientific name of host, if possible
11	Enter quantity of host and plants affected
12	Check block to indicate distribution of plant
13	Check appropriate blocks to indicate plant parts affected
14	Check block to indicate pest distribution
15	• Check appropriate block to indicate type of specimen • Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Enter X in block to indicate isolated or general plant symptoms
20	Enter X in appropriate block for weed density
21	Enter X in appropriate block for weed growth stage
22	Provide a brief explanation if Prompt or URGENT identification is requested
23	Enter a tentative determination if you made one
24	Leave blank

Distribution of PPQ Form 391
Distribute PPQ Form 391 as follows:
1. Send Original along with the sample to your Area Identifier.
2. Retain and file a copy for your records.

Figure B-2 Example of PPQ Form 391 Specimens For Determination, side 2

Purpose

Submit PPQ Form 391, Specimens for Determination, along with specimens sent for positive or negative identification.

Instructions

Follow the instructions in *Table B-1* on page B-5. Inspectors must provide all relevant collection information with samples. This information should be communicated within a State and with the regional office program contact. If a sample tracking database is available at the time of the detection, please enter collection information in the system as soon as possible.

Distribution

Distribute PPQ Form 391 as follows:

Send the original along with the sample to your area identifier

Retain and file a copy for your records

Table B-1 Instructions for Completing PPQ Form 391, Specimens for Determination

Block		Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: 2-letter State code–5-digit sample number (Survey Identification Number in Parentheses) Example: PA-1234 (04202010001) 2. CONTINUE consecutive numbering for each subsequent collection 3. ENTER the collection number
2	DATE	ENTER the date of the collection
3	SUBMITTING AGENCY	PLACE an X in the PPQ block
4	NAME OF SENDER	ENTER the sender's or collector's name
5	TYPE OF PROPERTY	ENTER the type of property where the specimen was collected (farm, feed mill, nursery, etc.)
6	ADDRESS OF SENDER	ENTER the sender's or collector's address
7	NAME AND ADDRESS OF PROPERTY OR OWNER	ENTER the name and address of the property where the specimen was collected
8A-8H	REASONS FOR IDENTIFICATION	PLACE an X in the correct block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE blank; ENTER remarks in *Block 22*
10	HOST INFORMATION NAME OF HOST	If known, ENTER the scientific name of the host
11	QUANTITY OF HOST	If applicable, ENTER the number of acres planted with the host
12	PLANT DISTRIBUTION	PLACE an X in the applicable box
13	PLANT PARTS AFFECTED	PLACE an X in the applicable box
14	PEST DISTRIBUTION FEW/COMMON/ ABUNDANT/EXTREME	PLACE an X in the appropriate block
15	INSECTS/NEMATODES/ MOLLUSKS	PLACE an X in the applicable box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage
16	SAMPLING METHOD	ENTER the type of sample
17	TYPE OF TRAP AND LURE	ENTER the type of sample
18	TRAP NUMBER	ENTER the sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check the appropriate box; otherwise LEAVE blank
20	WEED DENSITY	If applicable, check the appropriate box; otherwise LEAVE blank

Table B-1 Instructions for Completing PPQ Form 391, Specimens for Determination (continued)

Block		Instructions
21	WEED GROWTH STAGE	If applicable, check the appropriate box; otherwise LEAVE blank
22	REMARKS	ENTER the name of the office or diagnostic laboratory forwarding the sample; include a contact name, email address, phone number of the contact; also include the date forwarded to the State diagnostic laboratory or USDA–APHIS–NIS
23	TENTATIVE DETERMINATION	ENTER the preliminary diagnosis
24	DETERMINATION AND NOTES (**Not** for Field Use)	LEAVE blank; will be completed by the official identifier

PPQ 523 Emergency Action Notification

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED - OMB NO. 0579-0102

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION | 2. DATE ISSUED

3. NAME AND QUANTITY OF ARTICLE(S)

4. LOCATION OF ARTICLES

5. DESTINATION OF ARTICLES

6. SHIPPER

7. NAME OF CARRIER

8. SHIPMENT ID NO.(S)

9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. | FAX NO.

SS NO. | TAX ID NO.

10. PORT OF LADING | 11. DATE OF ARRIVAL

12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)

12a. PEST ID NO. | 12b. DATE INTERCEPTED

13. COUNTRY OF ORIGIN | 14. GROWER NO.

15. FOREIGN CERTIFICATE NO.

15a. PLACE ISSUED | 15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT:

☐ RE-EXPORTATION:

☐ DESTRUCTION:

☐ OTHER:

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):

18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE: | DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER: | DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

Figure B-3 Example of PPQ 523 Emergency Action Notification

Purpose

Issue a PPQ 523, Emergency Action Notification (EAN), to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested, or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision to destroy plants is made, or in the case of submitted samples, once positive confirmation is received, the same EAN which placed plants on hold also is used to document any actions taken, such as destruction and disinfection. Additional action may be warranted in the case of other fields testing positive for this pest.

Instructions

If plant lots or shipments are held as separate units, issue separate EANs for each unit of suspected plant material and associated material held. EANs are issued under the authority of the Plant Protection Act of 2000 (statute 7 USC 7701-7758). States are advised to issue their own hold orders parallel to the EAN to ensure that plant material cannot move intrastate.

When using EANs to hold articles, it is most important that the EAN language clearly specify actions to be taken. An EAN issued for positive testing and positive-associated plant material must clearly state that the material must be disposed of, or destroyed, and areas disinfected. Include language that these actions will take place at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, then document on the same EAN, any disposal, destruction, and disinfection orders resulting from investigations or testing.

Find additional instructions for completing, using, and distributing this form in the PPQ *Manual for Agricultural Clearance*.

Appendix

C

How to Submit Plant Samples

Plant Samples for Plant Pathology Analysis

1. Sampling

   Please submit adequate amounts of suspect leaf material when possible. This helps ensure that there is sufficient material if downstream diagnostic techniques are required. Twelve or more leaves per sample are desired.

2. Storing

   Refrigerate samples while awaiting shipment to the diagnostic laboratory. Place leaves without paper towel in a sealed and labeled ziplock bag.

3. Documentation

   Each sample should be documented on, and accompanied by its own completed PPQ Form 391 ‘Specimens for Determination’. It is good practice to keep a partially filled electronic copy of this form on your computer with your address and other information filled out in the interest of saving time. Please make sure all fields that apply are filled out and the bottom field (block 24: Determination and Notes) is left blank to be completed by the Identifier. Include the phone number and/or e-mail address of the submitter. Other documentation in the form of notes, images, etc. can be sent along with this if it useful to the determination. It is important that there be a way to cross-reference the sample with the accompanying form. For example, write the “Collection Number” both on the Form 391 and on the sample bag.

4. Packing

   To provide extra insurance against accidental release during shipping, specimens should be double-bagged – i.e. first place the specimen in a self-locking plastic bag and then place that bag within a second self-locking plastic bag. **The Form 391 should not be placed in the bag holding the sample! Rather, it should be placed inside the outer bag**

Place double-bagged samples in a sturdy cardboard box or heavy styrofoam container so that the samples are not damaged during shipping and handling. Ideally, samples should be packed with freezer blocks or wet ice to maintain their integrity during the shipping process. Thoroughly seal all seams on the container with shipping tape.

5. Shipping

   The Identifier Laboratory should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded, what types of samples they are (e.g. SOD-suspect Camellia leaves), when the samples will be shipped, and the package tracking number. Label the shipping box as 'URGENT' and send via overnight express courier (FedEx, UPS, Airborne, DHL, etc) to the appropriate Identifier.

Appendix

D

Taxonomic Support for Surveys

Background

The National Identification Services (NIS) coordinates the identification of plant pests in support of USDA's regulatory programs. Accurate and timely identifications provide the foundation for quarantine action decisions and are essential in the effort to safeguard the nation's agricultural and natural resources.

NIS employs and collaborates with scientists who specialize in various plant pest groups, including weeds, insects, mites, mollusks and plant diseases. These scientists are stationed at a variety of institutions around the country, including federal research laboratories, plant inspection stations, land-grant universities, and natural history museums. Additionally, the NIS Molecular Diagnostics Laboratory is responsible for providing biochemical testing services in support of the agency's pest monitoring programs.

On June 13, 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02 which established the role of PPQ NIS as the point of contact for all domestically- detected, introduced plant pest confirmations and communications. A Domestic Diagnostics Coordinator (DDS) position was established to administer the policy and coordinate domestic diagnostic needs for NIS. This position was filled in October of 2007 by Joel Floyd (USDA, APHIS, PPQ-PSPI,NIS 4700 River Rd., Unit 52, Riverdale, MD 20737, phone (301) 734-4396, fax (301) 734-5276, e-mail: joel.p.floyd@aphis.usda.gov).

Taxonomic Support and Survey Activity

Taxonomic support for pest surveillance is basic to conducting quality surveys. A misidentification or incorrectly screened target pest can mean a missed opportunity for early detection when control strategies would be more viable and cost effective. The importance of good sorting, screening, and identifications in our domestic survey activity cannot be overemphasized.

Fortunately most states have, or have access to, good taxonomic support within their states. Taxonomic support should be accounted for in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the state often may require supplies, develop training materials, or need to hire technicians to meet the needs of screening and

identification. Moreover, when considering whether to survey for a particular pest a given year, it is advisable to consider the challenges of taxonomic support as a factor in choosing that as a survey target in the first place.

Sorting and Screening

For survey activity, samples that are properly sorted and screened prior to being examined by an identifier will result in quicker turn around times for identification.

Sorting

is the first level of activity that assures samples submitted are of the correct target group of pests being surveyed, i.e., after removal of debris, ensure that the correct order, or in some cases family, of insects is submitted; or for plant disease survey samples, select those that are symptomatic if appropriate. There should be a minimum level of sorting expected of surveyors depending on the target group, training, experience, or demonstrated ability.

Screening

is a higher level of discrimination of samples such that the suspect target pests are separated from the known non-target, or native species of similar taxa. For example, only the suspect target species or those that appear similar to the target species are forwarded to an identifier for confirmation. There can be first level screening and second level depending on the difficulty and complexity of the group. Again, the degree of screening appropriate is dependent on the target group, training, experience, and demonstrated ability of the screener.

Check individual survey protocols to determine if samples should be sorted, screened or sent entire (raw) before submitting for identification. If not specified in the protocol, assume that samples should be sorted at some level.

Resources for Sorting, Screening, and Identification

Sorting, screening, and identification resources and aids useful to CAPS and PPQ surveys are best developed by taxonomists who are knowledgeable of the taxa that includes the target pests and the established or native organisms in the same group that are likely to be in samples and can be confused with the target. Many times these aids can be regionally based. They can be in the form of dichotomous keys, picture guides, or reference collections. NIS encourages the development of these resources, and when aids are complete, post them in the CAPS Web site so others can benefit. If local screening aids are developed, please notify Joel Floyd, the Domestic Diagnostics Coordinator, as to their availability. Please see the following for some screening aids available: http://pest.ceris.purdue.edu/caps/screening.php

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a state is not adequate for a particular survey, in some cases other entities may assist including PPQ identifiers, universities and state departments of agriculture in other states, and independent institutions. Check with the PPQ regional CAPS coordinators about the availability of taxonomic assistance.

Universities and State Departments of Agriculture:

Depending on the taxonomic group, there are a few cases where these two entities are interested in receiving samples from other states. Arrangements for payment, if required for these taxonomic services, can be made through cooperative agreements. The National Plant Diagnostic Network (NPDN) also has five hubs that can provide service identifications of plant diseases in their respective regions.

Independent Institutions

The Eastern Region PPQ office has set up multi-state arrangements for Carnegie Museum of Natural History to identify insects from trap samples. They prefer to receive unscreened material and work on a fee basis per sample.

PPQ Port Identifiers

There are over 70 identifiers in PPQ that are stationed at ports of entry who primarily identify pests encountered in international commerce including conveyances, imported cargo, passenger baggage, and propagative material. In some cases, these identifiers process survey samples generated in PPQ conducted surveys, and occasionally from CAPS surveys. They can also enter into our Pest ID database the PPQ form 391 for suspect CAPS target or other suspect new pests, prior to being forwarded for confirmation by an NIS recognized authority.

PPQ Domestic Identifiers

PPQ also has a limited number of domestic identifiers (three entomologists and two plant pathologists) normally stationed at universities who are primarily responsible for survey samples. Domestic identifiers can be used to handle unscreened, or partially screened samples, with prior arrangement through the PPQ regional survey coordinator. They can also as an intermediary alternative to sending an unknown suspect to, for example, the ARS Systematic Entomology Lab (SEL), depending on their specialty and area of coverage. They can also enter into our Pest ID database the PPQ form 391 for suspect CAPS target or other suspect new pests, prior to being forwarded for confirmation by an NIS recognized authority.

PPQ Domestic Identifiers
Bobby Brown
Domestic Entomology Identifier
Specialty: forest pests (coleopteran, hymenoptera)
Area of coverage: primarily Eastern Region

USDA, APHIS, PPQ
901 W. State Street
Smith Hall, Purdue University
Lafayette, IN 47907-2089
Phone: 765-496-9673
Fax: 765-494-0420
e-mail: robert.c.brown@aphis.usda.gov

Julieta Brambila
Domestic Entomology Identifier
Specialty: adult Lepidoptera, Hemiptera
Area of Coverage: primarily Eastern Region
USDA APHIS PPQ
P.O. Box 147100
Gainesville, FL 32614-7100
Office phone: 352- 372-3505 ext. 438, 182
Fax: 352-334-1729
e-mail: julieta.bramila@aphis.usda.gov

Kira Zhaurova
Domestic Entomology Identifier
Specialty: to be determine
Area of Coverage: primarily Western Region
USDA, APHIS, PPQ
Minnie Belle Heep 216D
2475 TAMU
College Station, TX 77843
Phone: 979-450-5492
e-mail: kira.zhaurova@aphis.usda.gov

Grace O'Keefe
Domestic Plant Pathology Identifier
Specialty: Molecular diagnostics (citrus greening, P. ramorum, bacteriology, cyst nematode screening)
Area of Coverage: primarily Eastern Region
USDA, APHIS, PPQ
105 Buckhout Lab
Penn State University
University Park, PA 16802

Lab: 814 - 865 - 9896
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Fax: 814 - 863 – 8265
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Craig A. Webb, Ph.D.
Domestic Plant Pathology Identifier
Specialty: Molecular diagnostics (citrus greening, P. ramorum, cyst nematode screening)
Area of Coverage: primarily Western Region
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Manhattan, KS 66506-5502
Cell (785) 633-9117
Office (785) 532-1349
Fax: 785-532-5692
e-mail: craig.a.webb@aphis.usda.gov

Final Confirmations

If identifiers or laboratories at the state, university, or institution level suspect they have detected a CAPS target, a plant pest new to the United States, or a quarantine pest of limited distribution in a new state, the specimens should be forwarded to an NIS recognized taxonomic authority for final confirmation. State cooperator and university taxonomists can go through a PPQ area identifier or the appropriate domestic identifier that covers their area to get the specimen in the PPQ system (for those identifiers, see table G-1-1 in the Agriculture Clearance Manual, Appendix G link below). They will then send it to the NIS recognized authority for that taxonomic group.

State level taxonomists, who are reasonably sure they have a new United States. record, CAPS target, or new federal quarantine pest, can send the specimen directly to the NIS recognized authority, but must notify their State Survey Coordinator (SSC), PPQ Pest Survey Specialist (PSS), State Plant Health Director (SPHD), and State Plant Regulatory Official (SPRO).

Before forwarding these suspect specimens to identifiers or for confirmation by the NIS recognized authority, please complete a PPQ form 391 with the tentative determination. Also fax a copy of the completed PPQ Form 391 to “Attention: Domestic Diagnostics Coordinator” at 301-734-5276, or send a PDF file in an e-mail to mailto:nis.urgents@aphis.usda.govwith the overnight carrier tracking number.

The addresses of NIS recognized authorities of where suspect specimens are to be sent can be found in The Agriculture Clearance Manual, Appendix G, tables G-1-4 and G-1-5: http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/mac_pdf/g_app_identifiers.pdf

Only use Table G-1-4, the "Urgent" listings, for suspected new United States records, or state record of a significant pest, and Table G-1-5, the "Prompt" listings, for all others.

When the specimen is being forwarded to a specialist for NIS confirmation, use an overnight carrier, insure it is properly and securely packaged, and include the hard copy of the PPQ form 391 marked "Urgent" if it is a suspect new pest, or "Prompt" as above.

Please contact Joel Floyd, the Domestic Diagnostics Coordinator if you have questions about a particular sample routing, at phone number: 301-734-5276, or e-mail: joel.p.floyd@aphis.usda.gov

Digital Images for Confirmation of Domestic Detections

For the above confirmations, do not send digital images for confirmation. Send specimens in these instances. For entry into NAPIS, digital imaging confirmations can be used for new county records for widespread pests by state taxonomists or identifiers if they approve it first. They always have the prerogative to request the specimens be sent.

Communications of Results

If no suspect CAPS target, program pests, or new detections are found, communication of these identification results can be made by domestic identifiers or taxonomists at other institutions directly back to the submitter. They can be in spread sheet form, on hard copy PPQ form 391's, or other informal means with the species found, or "no CAPS target or new suspect pest species found". Good record keeping by the intermediate taxonomists performing these identifications is essential.

All confirmations received from NIS recognized authorities, positive or negative, are communicated by NIS to the PPQ Emergency and Domestic Programs (EDP) staff in PPQ headquarters. EDP then notifies the appropriate PPQ program managers and the SPHD and SPRO simultaneously. One of these contacts should forward the results to the originating laboratory, diagnostician, or identifier.

Data Entry

Cooperative Agricultural Pest Survey (CAPS)

For survey data entered into NAPIS, new country and state records should be confirmed by an NIS recognized authority, while for others that are more widespread, use the identifications from PPQ identifiers or state taxonomists.

Late Wilt of Corn

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