



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Plant Protection
and Quarantine

New Pest Response Guidelines

Ash Dieback (Teleomorph: *Hymenoscyphus
pseudoalbidus*; Anamorph: *Chalara fraxinea*)



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Contents

Contents	<i>TOC-1</i>
Figures	<i>LOF-1</i>
Tables	<i>LOT-1</i>
Acknowledgements	<i>AKN-1</i>
Introduction	<i>1-1</i>
Pest Information	<i>2-1</i>
Identification	<i>3-1</i>
Survey Procedures	<i>4-1</i>
Regulatory Procedures	<i>5-1</i>
Control Procedures	<i>6-1</i>
Environmental Compliance	<i>7-1</i>
Pathways	<i>8-1</i>
References	<i>REFERENCES-1</i>
Glossary	<i>GLOSSARY-1</i>
Resources	<i>A-1</i>
Forms	<i>B-1</i>
How to Submit Plant Samples	<i>C-1</i>
Taxonomic Support for Surveys	<i>D-1</i>
Research Needs	<i>E-1</i>

Figures

Ash Dieback

- Figure 2-1 Distribution of *Fraxinus* Species that are Potential Hosts of *Hymenoscyphus pseudoalbidus* Within the United States. Map courtesy of USDA, APHIS, PPQ, CPHST (<http://www.nap-pfast.org/>). 2-8
- Figure 2-2 Lifecycle of *Hymenoscyphus pseudoalbidus* (adapted from Gross et al. (2012a)) 2-11
- Figure 3-1 Necrotic Spots on Leaves and Wilting of Leaves Caused by Ash Dieback (Image Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland) Stems: canker on woody stem, internal discoloration, necrosis leading to dieback 3-3
- Figure 3-2 (A) Stem Canker and (B) Internal Wood Discoloration and Necrosis Caused by Ash Dieback (Images Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland) Growing points and Whole plant: wilting, shoot dieback and tree death 3-4
- Figure 3-3 (A) Wilting Young Tree and (B) Mature Trees Showing Severe Crown Dieback Caused by Ash Dieback (Images Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland) 3-4
- Figure 3-4 (A) Phialophores of *Chalara fraxinea* on Vegetative Hyphae, Mostly Reduced to Phialid and (B) Long and Branched Phialophore in a 4-Week-Old Colony (Kowalski, 2006) 3-5
- Figure 3-5 (A) Conidia of *Chalara fraxinea* in a Chain and (B) Conidia in Slimy Droplets (Kowalski, 2006) 3-6
- Figure 3-6 Apothecia of *Hymenoscyphus pseudoalbidus* on *Fraxinus* spp. Leaf Petioles and Rachises From the previous year in the Forest Litter Sizes of the Disc Flats of the Apothecia Range from 1.5 to about 6 mm (© Thomas Kirisits, IFFF-Boku Vienna, Austria, Keßler et al., 2012) (See Also Insert) Black Pseudosclerotial Plates (Arrowed) on Petioles and Rachises, from which Apothecia Emerge (© Thomas Kirisits, IFFF-Boku Vienna, Austria, Kirisits et al., 2012) 3-7
- Figure 4-1 Rating System to Assess the Severity of Ash Dieback (Kirisits and Freinschlag, 2012) 4-4
- Figure B-1 Example of PPQ Form 391 Specimens For Determination, side 1 B-2
- Figure B-2 Example of PPQ Form 391 Specimens For Determination, side 2 B-3

Figure B-3 Example of PPQ 523 Emergency Action Notification B-7

Tables

Table 1-1	How to Use Decision Tables	1-8
Table 2-1	Classification of <i>Hymenoscyphus pseudoalbidus</i> (Teleomorph)	2-2
Table 2-2	World-wide Distribution of <i>Hymenoscyphus pseudoalbidus</i>	2-6
Table 2-3	Plant Hosts of <i>Hymenoscyphus pseudoalbidus</i>	2-9
Table 3-1	PCR Primers for Detection of <i>Hymenoscyphus pseudoalbidus</i>	3-9
Table A-1	Resources for Ash Dieback	A-1
Table B-1	Instructions for Completing PPQ Form 391, Specimens for Determination	B-5

Acknowledgements

Authors

Wiseborn B. Danquah, PhD., USDA-APHIS-PPQ-CPHST-PERAL

Stefano Costanzo, Ph.D., USDA-APHIS-PPQ-CPHST-PERAL

Reviewers

Bruce D Moltzan, PhD., National Program Leader-Forest Pathology, USDA Forest Service

Kerry O. Britton, PhD., National Pathologist USDA Forest Service

Cover Images

Tree canker caused by the fungus *Hymenoscyphus pseudoalbidus* on European ash (*Fraxinus excelsior* L.) Images courtesy of Andrej Kunca, National Forest Centre-Slovakia, Bugwood.org (<http://www.invasive.org/>)

Acknowledgements

Introduction

Contents

Introduction	1-1
Users	1-2
Contacts	1-2
Initiating an Emergency Pest Response Program	1-3
Preventing an Infestation	1-4
Scope	1-4
Authorities	1-5
Program Safety	1-5
Support for Program Decisionmaking	1-5
How to Use the Guidelines	1-6
Conventions	1-6
Acknowledgements	1-9
How to Cite the Guidelines	1-9
How to Find More Information	1-10

Introduction

Use *New Pest Response Guidelines: Ash Dieback (Teleomorph: Hymenoscyphus pseudoalbidus; Anamorph: Chalara fraxinea)* when designing a program to detect, monitor, control, contain, or eradicate, an outbreak of ash dieback 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, meeting, 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 *Hymenoscyphus pseudoalbidus*.

If *Hymenoscyphus pseudoalbidus* 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 reference for the following users who have been assigned responsibilities for a plant health emergency for small banded pine weevil:

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

Contacts

When an emergency pest response program for *Hymenoscyphus pseudoalbidus* has been implemented, the success of the program depends on the cooperation, assistance, and understanding of other involved groups. The appropriate liaisons 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 [Survey Procedures](#) on page 4-1 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 about risk assessments, survey methods, control strategies, regulatory treatments, and other aspects of pest response programs. PPQ managers meet 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-EDP-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: Ash Dieback (Teleomorph: Hymenoscyphus pseudoalbidus; Anamorph: Chalara fraxinea)*. Washington, D.C. 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.

Pest Information

Contents

Introduction	2-1
Classification	2-2
Historical Information	2-2
Damage	2-3
Economic Impact	2-4
Ecological Range	2-5
Potential Distribution	2-7
Hosts	2-8
Biology and Life Cycle	2-9
Environmental Impact	2-11

Introduction

Use *Chapter 2 Pest Information* to learn more about the classification, history, host range, and biology of the ascomycete fungus *Hymenoscyphus pseudoalbidus* (anamorph *Chalara fraxinea*), cause of ash dieback disease. Over the last two decades this infectious pathogen has become a serious threat to *Fraxinus* spp. across many European countries. Although it is absent from the United States, it poses an eminent threat to *Fraxinus* spp. in forest ecosystems, tree nurseries, and the urban landscape.

Classification

Most commonly referred to by its anamorph state (*Chalara fraxinea*), the ascomycete fungus, *Hymenoscyphus pseudoalbidus* causes severe dieback on several *Fraxinus* spp., especially the European ash (*Fraxinus excelsior* L.) in parts of Europe (Bengtsson *et al.*, 2012). The fungus belongs to the phylum Ascomycota, class Leotiomycetes, order Helotiales, family Helotiaceae and genus *Hymenoscyphus* (Table 2-1 on page 2-2).

Table 2-1 Classification of *Hymenoscyphus pseudoalbidus* (Teleomorph)

Rank	Taxon
Kingdom	Fungi
Phylum	Ascomycota
Class	Leotiomycetes
Order	Helotiales
Family	Helotiaceae
Genus	<i>Hymenoscyphus</i>
Full name and Authority	<i>Hymenoscyphus pseudoalbidus</i> V. Queloz, C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holdenrieder

Note: *Chalara fraxinea* T. Kowalski (Anamorph).

Disease Common Names

Hymenoscyphus pseudoalbidus causes a disease commonly referred to as ash dieback, ash decline or *Chalara* dieback (This disease is different from the ash dieback (ash yellow) disease caused by phytoplasmas and reported in the United States (Sinclair and Griffiths, 1994; Hibben and Silverborg, 1978).

Historical Information

Ash dieback affecting *F. excelsior* was first observed in eastern Poland (Przybyl, 2002; Kowalski, 2006) in the mid-1990s. The disease has spread rapidly since the first report and is now present in a large part of continental Europe (Chandelier *et al.*, 2011; Queloz *et al.*, 2011) and most recently was detected in the British isles (DAFM., 2012; BES, 2012).

A species of *Chalara* was isolated from declining *F. excelsior*, and subsequent artificial inoculation tests proved this anamorph to be the causal agent of ash

dieback (Bakys *et al.*, 2009a; Kowalski and Holdenrieder, 2009a). This fungus could not be assigned to any of the previously known *Chalara* spp. because of its small, short cylindrical phialoconidia which are extruded in chains or in slimy droplets and also on the basis of it being different from other *Chalara* spp. in morphological features of its phialophores, and colony characteristics. It was, therefore, described as *Chalara fraxinea* T. Kowalski, and designated as the causal organism of ash dieback (Kowalski, 2006; Kowalski and Holdenrieder, 2009a).

Although initial studies led to *Chalara fraxinea* being associated with *Hymenoscyphus albidus* (Roberge ex Desm.) W. Phillips (a widespread saprophytic fungus known in Europe as a decomposer of shed *Fraxinus* leaves) (Kowalski and Holdenrieder 2009b), further molecular studies based on ribosomal DNA (rDNA) internal transcribed spacer (ITS), *calmodulin gene and translation elongation factor 1- α* as well as differences in inter simple sequence repeat (ISSR) markers revealed the true teleomorph of *C. fraxinea* to be *Hymenoscyphus pseudoalbidus* (Queloz *et al.*, 2011; Bengtsson *et al.*, 2012), a cryptic species of the non-pathogenic *H. albidus*. The widespread invasion by *H. pseudoalbidus* has resulted in *H. albidus* becoming a rare species in places such as Denmark. This is because these two organisms share a common ecological niche and the pathogenic *H. pseudoalbidus* tends to exclude *H. albidus* from their shared niche (McKinney *et al.*, 2012).

Damage

Infection of *Fraxinus* spp. by *H. pseudoalbidus* results in necrotic lesions and cankers. These necrotic lesions may extend into the stem of the tree causing wood discoloration and killing plant tissue in the wake of its spread. The lesions girdle the stem preventing nutrients from being effectively transported around the plant subsequently resulting in the dieback of shoots, twigs, branches and smaller stems. The disease has been observed on trees of all ages (Kowalski and Łukomska, 2005; Schumacher, 2011).

Tree mortality due to *H. pseudoalbidus* damage is greatest in saplings and young trees as well as natural regeneration (Kirisits *et al.*, 2009; Kirisits and Freinschlag, 2012). Although older trees are also severely damaged, they are usually able to withstand the effects of the disease for extended periods and may eventually die as a result of secondary infections by pathogens such as *Armillaria* spp. (Bakys *et al.*, 2009a; Bakys *et al.*, 2009a). Ash dieback is a widespread and serious disease in many European countries causing damage not only in forest trees but also in urban areas (parks and gardens) and nurseries (Kowalski and Łukomska, 2005; Kirisits *et al.*, 2009; Schumacher, 2011).

In Lithuania, approximately 60 percent of all *Fraxinus* spp. stands throughout the country showed symptoms of ash dieback in 2002. In parts of the country only about two percent of *F. excelsior* trees were visually healthy (Visaitis and Lygis, 2008). The disease has been so devastating in countries such as Denmark, Switzerland and Poland such that between 2003 and 2009, the disease had spread to all parts of these countries (Skovsgaard *et al.*, 2009; McKinney *et al.*, 2011; Pautasso *et al.*, 2013). Since the first report of the disease in 2002, between 60 and 90 percent of *Fraxinus* trees had been damaged by ash dieback throughout Denmark by 2010 (Skovsgaard *et al.*, 2009; McKinney *et al.*, 2011). Approximately 60 percent of the total *Fraxinus* stands in Lithuania covering an approximate area of 30, 000 ha had been damaged by ash dieback in 2008 (Visaitis and Lygis, 2008). Although the total percentage of *F. excelsior* trees in terms of the total number of trees in Sweden is just about one percent (Fischer and Lorenz, 2011), about a fourth of all *Fraxinus* spp. in southern Sweden were either killed or severely damaged by ash dieback by 2009 (Fischer *et al.*, 2010). This has resulted to the declaration of *F. excelsior* as a threatened species in Sweden (Stenlid *et al.*, 2011).

Economic Impact

Fraxinus spp. are important hardwood resources in the United States. White (*F. americana* L.) and green (*F. pennsylvanica* Marsh.) ash are the most important *Fraxinus* spp. throughout the eastern United States and southern Canada, while Black ash (*F. nigra* Marsh.) is an important timber species in the northeastern United States and southeastern Canada (Solomon *et al.*, 1993). These *Fraxinus* spp. collectively make up over seven percent and five percent of all hardwood and tree species, respectively, throughout the northeastern United States and eastern Canada (Gould and Bauer, 2009). The estimated number of *Fraxinus* trees growing in United States timberlands is in excess of 7.5 million (Gould and Bauer, 2009). On a national scale, the value of *Fraxinus* spp. produced by the nursery industry in the United States is estimated to be \$140 million annually (Pennsylvania Department of Agriculture, 2006). Approximately 275 million board feet of *Fraxinus* spp. sawtimber is produced in the United States annually (Solomon *et al.*, 1993). Exports of *Fraxinus* spp. lumber from the United States were in excess of \$132 million in 2011 (FAS, 2011).

The loss in residential property value and timber to forest land owners due to emerald ash borer (*Agrilus planipennis*) infestation in the United states is estimated to be \$380 million and \$60 million respectively each year (Aukema *et al.*, 2011). The total loss as a result of a complete loss of *Fraxinus* trees in the state of Ohio has been estimated to be approximately \$7.5 billion (Sydnor *et al.*, 2007). Although the extent of ash dieback damage in Europe has not been quantified, the extensive damage observed in the countries where the disease has been reported (Skovsgaard *et al.*, 2009; McKinney *et al.*, 2011;

Visaitis and Lygis, 2008) indicate that the disease can be as devastating as the emerald ash borer.

Ash dieback caused by *H. pseudoalbidus* can potentially alter the landscape ecosystem of the Midwestern United States where according to Gould and Bauer (2009) the tree cover in some areas is composed of between 20 to 40 percent *Fraxinus* trees. The loss of *Fraxinus* spp. in the national urban landscape could lead to a potential loss of between 0.5 to 2% of the total leaf area (30-90 million trees). This translates into a financial loss of between \$20 to 60 billion (Pennsylvania Department of Agriculture, 2006). Additionally, the United States Forest Service estimates the cost to state, local governments, and landowners to remove and replace dead and dying *Fraxinus* trees in urban and suburban areas to be approximately \$7 billion over the next 25 years (Gould and Bauer, 2009).

Ecological Range

Inferring from its pattern of spread across Europe, *H. pseudoalbidus* has been described as an aggressive invasive species which has only recently been introduced into Europe (Timmermann *et al.*, 2011). According to Zhao *et al.* (2013) the fungus previously identified as *Lambertella albida* (Gillet) Korf, a synonym of *H. albidus* in Japan is actually *H. pseudoalbidus*. The Japanese *H. pseudoalbidus* populations have shown higher genetic variation than European populations, and have not been reported as pathogenic to indigenous *Fraxinus* spp. Studies have also failed to confirm the presence of *H. albidus* in Japan leading to the conclusion that *H. pseudoalbidus* is the correct name for what had previously been misidentified as *L. albida*.

Kowalsi and Bartnik (2010) classified *C. fraxinea* as a mesophilic fungus because isolates can grow *in vitro* at temperatures ranging between 5° and 30°C, with optimum temperatures for colony growth between at 20° and 25°C. Conidial sporulation is favoured by lower temperatures between 5° and 15°C (Kowalsi and Bartnik, 2010). According to Ogris (2010) the minimum temperature for apothecia development is 1.1°C with an optimum growth temperature of 22°C. High air humidity and adequate sunlight are also essential for the growth and maturation of apothecia (Ogris, 2010).

The known worldwide distribution of the *H. pseudoalbidus* ([Table 2-2](#) on page [2-6](#)) is restricted to Asia (Japan), parts of continental Europe, and the British

isles. There is no evidence of its presence in Africa, Oceania, North or South America as well as the Caribbean Region.

Table 2-2 World-wide Distribution of *Hymenoscyphus pseudoalbidus*

Geographic Region	Country	References
Asia	Japan ¹	(Zhao <i>et al.</i> , 2013)
Europe	Austria	(Halmschlager and Kirisits, 2008)
Europe	Belarus	(Timmermann <i>et al.</i> , 2011)
Europe	Belgium	(Chandler <i>et al.</i> , 2011)
Europe	Croatia	(Timmermann <i>et al.</i> , 2011)
Europe	Czech Republic	(Jankovsky and Hold-enrieder, 2009)
Europe	Denmark	(Timmermann <i>et al.</i> , 2011)
Europe	Estonia	(Dernkhan and Hanso, 2010)
Europe	Finland	(Rytköen <i>et al.</i> , 2011)
Europe	Guernsey	(EPPO, 2012)
Europe	France	(loos <i>et al.</i> , 2009)
Europe	Germany	(Schumacher <i>et al.</i> , 2007)
Europe	Great Britain	(BES, 2012)
Europe	Hungary	(Szabo, 2009)
Europe	Italy	(Ogris <i>et al.</i> , 2010)
Europe	Ireland	(DAFM., 2012)
Europe	Latvia	(Rytköen <i>et al.</i> , 2011)
Europe	Lithuania	(Lygis <i>et al.</i> , 2005)
Europe	The Netherlands	(EPPO, 2010)
Europe	Norway	(Talgø <i>et al.</i> , 2009)
Europe	Poland	(Przybyl, 2002)
Europe	Romania	(Timmermann <i>et al.</i> , 2011)
Europe	Russia ²	(Timmermann <i>et al.</i> , 2011)
Europe	Slovakia	(Kunca <i>et al.</i> , 2011)
Europe	Slovenia	(Ogris <i>et al.</i> , 2009)
Europe	Sweden	(Bakys <i>et al.</i> , 2009a)
Europe	Switzerland	(Queloz <i>et al.</i> , 2011)

1 Previously identified as *Lambertella albida* (Gillet) Korf

2 present in Kaliningrad Oblast only

Potential Distribution

Hymenoscyphus pseudoalbidus is not known to be established in the United States; however, it poses a serious threat to *Fraxinus* forest habitats within the country ([Figure 2-1](#) on page 2-8). The spread of the disease almost at the same time from central-east Europe where it was first detected to almost every part of continental Europe including Finland in the north, Belgium in the west, and Italy in the south is an indication of the wide climatic adaptation of *H. pseudoalbidus*.

High levels of genetic variation are characteristic for sexually reproducing organisms with a wide geographical distribution (James *et al.*, 1999). Studies based on Random amplified microsatellites (RAMS) markers have shown isolates of *H. pseudoalbidus* from Finland and Estonia (Rytönen *et al.*, 2011) as well as Poland (Kraj *et al.*, 2012) to have considerable genetic variability. This high genetic variation is not expected for a pathogen which has been introduced only recently into Europe (Queloz *et al.* 2010). According to Kraj *et al.* (2012) the genetic variability of *C. fraxinea* isolates is not connected to the geographic distance or regions of their occurrence but rather related to the need to adapt to climatic conditions. There is, therefore, the possibility that this high genetic variability within populations which possibly evolved in response to climatic conditions can contribute to differences in virulence (Kraj *et al.*, 2012). Isolates of *C. fraxinea* have shown significant temperature dependent variation in colony characteristics and growth rate *in vitro* (Kowalski and Bartnik 2010). These differences were not only present in isolates from distant origins, but also among isolates deriving from the same forest.

The full host range of *H. pseudoalbidus* is currently unknown. It is thus impossible to know if the fungus can cause disease on all the native *Fraxinus* spp. within the United States. It may infest other species of *Fraxinus* considering the high genetic variability it has shown in Europe. Refer to [Figure 2-1](#) on page 2-8 for the distribution of all *Fraxinus* spp. in the continental United States. The map indicates that the eastern half of the United States has

areas of moderate to high risk for establishment of *H. pseudoalbidus* based on the presence of all *Fraxinus* spp.

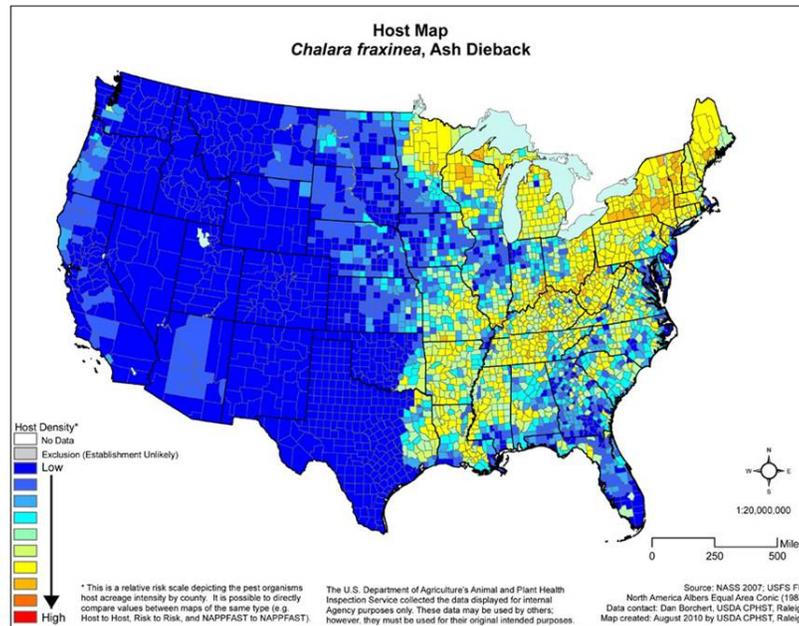


Figure 2-1 Distribution of *Fraxinus* Species that are Potential Hosts of *Hymenoscyphus pseudoalbidus* Within the United States. Map courtesy of USDA, APHIS, PPQ, CPHST (<http://www.nappfast.org/>).

Hosts

All the identified hosts of *H. pseudoalbidus* belong to the genus *Fraxinus*. *Fraxinus excelsior* is regarded as the most susceptible host of *H. pseudoalbidus* while *F. ornus* and *F. pennsylvanica* are considered as moderately susceptible (Kirisits *et al.*, 2009; Drenkhan and Hanso, 2010; Queloz *et al.*, 2011). *Fraxinus americana* and *F. mandshurica* display leaf wilting but only minor bark necrosis with limited dieback of shoots (Drenkhan and Hanso, 2010). Refer to [Table 2-3](#) on page 2-9 for the current known hosts of *H. pseudoalbidus*.

Table 2-3 Plant Hosts of *Hymenoscyphus pseudoalbidus*

Scientific name	Common name	References
<i>Fraxinus excelsior</i> L.	European ash	(Kowalski, 2006; Queloz <i>et al.</i> , 2011)
<i>Fraxinus excelsior</i> subsp. <i>excelsior</i>	<i>F. excelsior</i> 'Pendula'	(Kirisits <i>et al.</i> , 2009)
<i>Fraxinus angustifolia</i> subsp. <i>danubialis</i>		Kirisits <i>et al.</i> , 2009)
<i>Fraxinus ornus</i> L.	Flowering ash	(Kirisits <i>et al.</i> , 2009)
<i>Fraxinus angustifolia</i> Vahl	Narrow-leafed ash	(Kirisits <i>et al.</i> , 2010)
<i>Fraxinus nigra</i> Marsh.	Black ash	(Drenkhan and Hanso, 2010)
<i>Fraxinus pennsylvanica</i> Marsh.	Green ash	(Drenkhan and Hanso, 2010)
<i>Fraxinus americana</i> L.	White ash	(Drenkhan and Hanso, 2010)
<i>Fraxinus mandshurica</i> Rupr.	Manchurian ash	(Drenkhan and Hanso, 2010)

Biology and Life Cycle

Recent studies have indicated that apothecia of *H. pseudoalbidus* first appear at the end of May, June or early July in the year following infection (Kowalski and Holdenrieder, 2009b; Kirisits and Cech, 2009; Kirisits *et al.*, 2009; Queloz *et al.*, 2011; Timmermann *et al.*, 2011). The apothecia formed on the previous year's fallen leaf petioles and rachises in the leaf litter produce ascospores (Queloz *et al.*, 2011) which are windborne and the main source of long distance dispersal (Bengtsson *et al.*, 2012; Gross *et al.*, 2012a; Timmermann *et al.*, 2011). According to Timmermann *et al.* (2011) the period of highest ascospore deposition is between 6 and 8 a.m. which coincides with high air humidity to facilitate germination.

The pathogen can survive even exceptionally dry summers and postpone sporulation by its ability to remain dormant for at least two years in pseudosclerotial plates which provide a durable barriers preventing desiccation as well as the permeation of antimicrobial compounds (Gross and Holdenrieder, 2013). Petioles which bear apothecia can continue to produce spores for at least two successive years under favorable conditions (Gross and Holdenrieder, 2013).

When the ascospores land on green leaves, they colonize the leaves and start new infections (Kowalski and Holdenrieder, 2009b; Timmermann *et al.*, 2011).

The fungus can grow from infected leaves to the leaf petioles, rachises, and subsequently into the phloem and xylem tissues of *Fraxinus* spp. to cause necrotic phloem lesions and wood discoloration (Kirisits and Cech, 2009; Kirisits *et al.*, 2009). Shoot infections do not result in multiplication of the fungus. According to McKinney *et al.* (2011) only a small proportion of leaf infections actually result in shoot infections as usually the leaves are shed before the pathogen makes its way to the main stem and also fruiting bodies are seldom formed on shoots.

Attempts to germinate the conidia or to induce disease by introducing them into young *Fraxinus* plants have been unsuccessful (Kirisits and Cech 2009) and no clonal populations have been observed (Bengtsson *et al.*, 2012; Kraj *et al.*, 2012). It has been suggested that conidia are not infectious and may act exclusively as spermatia in the process of teleomorph formation (Kirisits & Cech, 2009; Kirisits *et al.*, 2009; Gross *et al.*, 2012a&b). The fungus produces a characteristic black pseudosclerotial plate on the surface of the petiole and overwinters inside (Kowalski and Holdenrieder, 2009b). Following fertilization, in the summer of the next growing season new apothecia develop and start a new infection cycle (Gross *et al.*, 2012a).

Due to its slow growth in culture on malt extract agar supplemented with 100 mg/L of streptomycin sulphate, colonies of the anamorph, *C. fraxinea* are sometimes overgrown by colonies of other fast-growing saprotrophic fungi within the host tissue (Kowalski, 2006; Ioos *et al.*, 2009; Chandelier *et al.*, 2010). To overcome this, *C. fraxinea* form an inhibition zone with width ranging between 3 and 12 mm around its colonies (Kowalski and Bartnik, 2010). The fungus produces secondary metabolites as white crystalline substances (Kowalski and Bartnik, 2010) which contain viridian and viridiol (Grad *et al.*, 2009). Viridin is mycotoxic and appears to be responsible for the formation of the inhibition zone around the fungus (Kowalski and Bartnik, 2010) while viridiol is phytotoxic (Howell and Stiponovic, 1984). Refer to [Figure 2-2](#) on page 2-11 for a schematic representation of the lifecycle of *H. pseudoalbidus*.

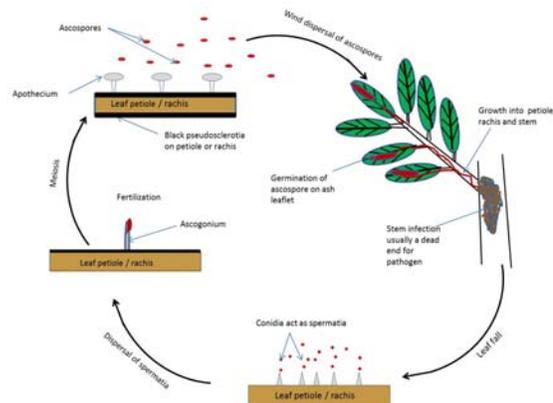


Figure 2-2 Lifecycle of *Hymenoscyphus pseudoalbidus* (adapted from Gross et al. (2012a))

Environmental Impact

Introduction of *H. pseudoalbidus* could have some negative impacts on the environment including forests, parks, urban areas, and gardens. None of the *Fraxinus* spp. present in the United States is listed as a species of concern or endangered (USFWS, 2011). According to Pautasso *et al.* (2013) the loss of a high proportion of *Fraxinus* spp. will result in adverse ecological effects including reduced bio-diversity and changes in community structure. For organisms that are dependent on *Fraxinus* spp., the loss or a drastic reduction in the number of these trees due to ash dieback will mean a loss of their habitat (Pautasso *et al.*, 2013). Furthermore, the establishment of *H. pseudoalbidus* in the United States may trigger the initiation of chemical control programs which may negatively impact non-target organisms within the environment.

Identification

Contents

Introduction	3-1
Authorities	3-1
Reporting	3-2
Characteristic Symptoms	3-2
Summary of Symptoms	3-3
Description	3-4
Diagnostic Test	3-8
Similar Species	3-10

Introduction

Use *Chapter 3 Identification* as a guide to recognizing ash dieback (*Hymenoscyphus pseudoalbidus*). Accurate identification of the pathogen is important in assessing its presence, potential risk, developing a survey strategy, and determining the level and manner of control. Recognition of symptoms is not definitive and morphological, microscopic features and molecular diagnosis are necessary to identify *H. pseudoalbidus*.

Authorities

Qualified State, County, or cooperating university, personnel may perform preliminary identification and screening of suspect *H. pseudoalbidus*. 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 pathogens. 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 ash dieback caused by *H. pseudoalbidus* in *Fraxinus* spp. Both *Chalara fraxinea* and *H. pseudoalbidus* are listed as reportable in the PEST ID database (queried January 23, 2013), and *C. fraxinea* is listed as a pest of concern on the 2012 PPQ Prioritized Offshore Pest List.

The ash dieback disease caused by *H. pseudoalbidus* is characterized by a number of symptoms (Kirisits *et al.* 2009; Kowalski 2006; Kräutler and Kirisits, 2012) which include necrosis of leaves, buds and leaf stalks resulting in wilting and premature shedding of leaves followed by necrotic lesions and cankers in the bark, shoots, branches and stems coupled with wood discoloration and eventual dieback of shoots (Bakys *et al.*, 2009a,b; Kirisits *et al.*, 2009; Kowalski, 2006; Kräutler and Kirisits, 2012).

The initial symptoms include the appearance of small necrotic lesions on leaf petioles and leaflet veins and the formation of lesions on the rachises (Kowalski and Łukomska, 2005; Schumacher *et al.*, 2007; Halmschlager and Kirisits, 2008; Kirisits *et al.*, 2009). These leaf infections are very important infection courts from which the fungus grows into the shoots and also the infected leaves and rachises after they are shed support the growth of the fruiting bodies (Kirisits and Cech, 2009; Kirisits *et al.*, 2009; Schumacher, 2011; Kräutler and Kirisits, 2012). Infection on the shoots and stems begin with small necrotic spots. These lesions enlarge and frequently girdle the stem leading to dieback of branches, shoots, and twigs, and particularly in the death of the top of the crown (Bakys *et al.*, 2009a; Kirisits *et al.*, 2009; Kowalski, 2006). Affected trees may show a proliferation of epicormic shoots on twigs, branches, and the stem possibly as a means of compensating for the loss in leaf area due to shoot dieback (Kirisits and Freinschlag, 2012).

Summary of Symptoms

Leaves: necrotic areas, abnormal colors, wilting and premature leaf fall
([Figure 3-1](#) on page 3-3).



Figure 3-1 Necrotic Spots on Leaves and Wilting of Leaves Caused by Ash Dieback (Image Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland)
Stems: canker on woody stem, internal discoloration, necrosis leading to dieback



Figure 3-2 (A) Stem Canker and (B) Internal Wood Discoloration and Necrosis Caused by Ash Dieback (Images Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland) Growing points and Whole plant: wilting, shoot dieback and tree death



Figure 3-3 (A) Wilting Young Tree and (B) Mature Trees Showing Severe Crown Dieback Caused by Ash Dieback (Images Courtesy of Tadeusz Kowalski, Department of Forest Pathology, Agricultural University of Cracow, Poland)

Description

Use the signs of the disease described in this section to verify *Hymenoscyphus pseudoalbidus* and its anamorph *Chalara fraxinea*.

Colony Morphology

According to Kowalski and Bartnik (2010) colonies formed by *H. pseudoalbida* isolated from diseased necrotic tissue differ greatly in color, growth rate and interactions from other fungi. Colonies of the anamorph formed on malt extract agar (MEA) appear effuse, cottony, dull white to fulvous brown (dull reddish-yellow/orange or brownish-yellow) with dark grey local spots. The colonies grow to between 9 and 28 mm in diameter after 21 days at 20°C (68°F) in the dark. The vegetative hyphae are subhyaline to olivaceous brown about 1.2 to 3.0 µm wide. They have rare swellings up to 4.2 µm and thin-walled, septate with septae 5 to 21 µm apart (Kowalski, 2006).

Phialophores

These arise directly on the superficial or slightly immersed hyphae on pseudoparenchymatous stromata. They are often reduced to phialides or are cylindrical to obclavate with up to three septate in the basal part. They are olivaceous brown, erect, straight or slightly bent, smooth-walled, unconstructed at the septa. They are mainly between 24 to 37 µm long and terminate in a phialide. In few-week-old colonies, phialophores are up to 96 µm long and between 3.0 to 4.2 µm wide at base. They are simple or have between one and five branches. Phialides occur also terminally on undifferentiated hyphae (*Figure 3-4* on page 3-5) (Kowalski, 2006).

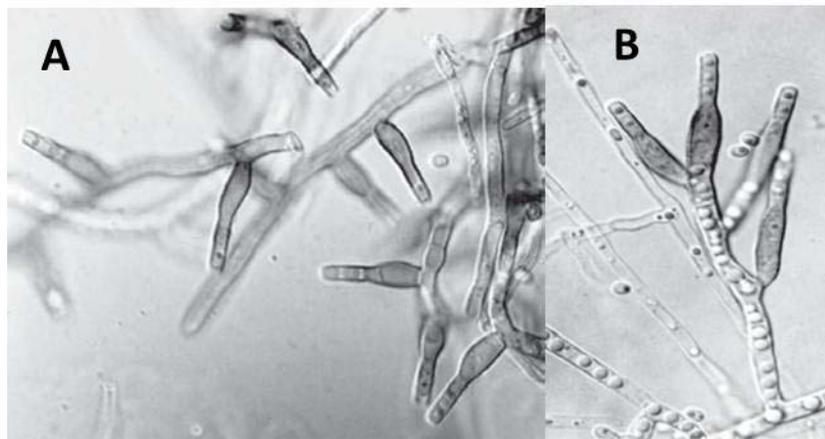


Figure 3-4 (A) Phialophores of *Chalara fraxinea* on Vegetative Hyphae, Mostly Reduced to Phialid and (B) Long and Branched Phialophore in a 4-Week-Old Colony (Kowalski, 2006)

Phialoconidia

Conidia are produced in culture and on artificially inoculated young trees but sporulation has seldom been seen to occur in natural lesions (Kowalski and Holdenrieder, 2009b). The conidia are extruded in short chains or more frequently in slimy droplets (*Figure 3-5* on page 3-6). They have short-cylindrical ends, rounded or blunt, and sometimes with a truncate base bearing small marginal frills. They are unicellular, hyaline to subhyaline and filled with one or two oil droplets. They are smooth-walled, 3.2 to 4.0 μm by 2.0-2.5 μm (mean conidium length/width ratio 1.4:1) (Kowalski, 2006).

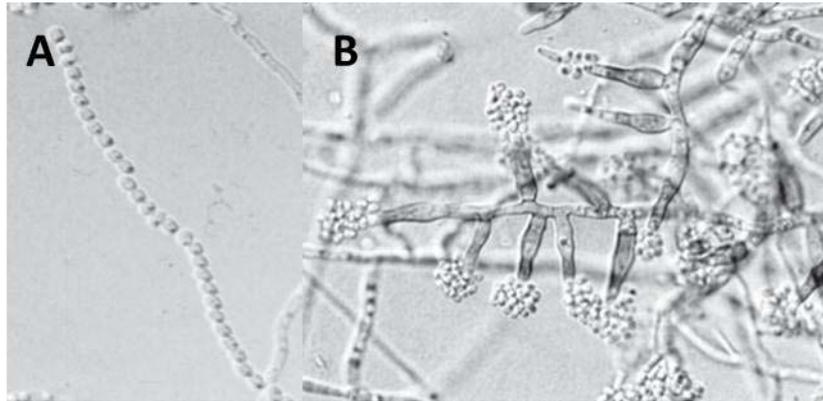


Figure 3-5 (A) Conidia of *Chalara fraxinea* in a Chain and (B) Conidia in Slimy Droplets (Kowalski, 2006)

Apothecium

Apothecia arise from black pseudosclerotial plates on fallen petioles or dead shoots (Kirisits *et al.*, 2009). They are nail shaped, white to cream, becoming cinnamon brown with age and drying (Hosoya *et al.*, 1993). The receptacle of the apothecium is flat, 1.5-3.0 mm diameter, stipe 0.4-2.0 x 0.2-0.5 mm, enlarged or narrow at base (Kowalski and Holdenrieder, 2009b). The basal region is frequently black. Paraphyses cylindrical, 2.0-2.5 μm thick, enlarged to 3 μm at apex, septate, hyaline, slightly yellowish. Asci are cylindrical-clavate, stipitate, 96 to 126 by 8 to 10 μm (Hosoya *et al.*, 1993) and eight-spored (Kowalski and Holdenrieder, 2009b). Ascospores are irregularly biserial, fusiform-elliptical, broadly rounded above, narrow below, straight or slightly curved (Kowalski and Holdenrieder, 2009b). They occasionally become brown before discharge (Hosoya *et al.*, 1993) ([Figure 3-6](#) on page 3-7).



Figure 3-6 Apothecia of *Hymenoscyphus pseudoalbidus* on *Fraxinus* spp. Leaf Petioles and Rachises From the previous year in the Forest Litter Sizes of the Disc Flats of the Apothecia Range from 1.5 to about 6 mm (© Thomas Kirisits, IFFF-Boku Vienna, Austria, Keßler *et al.*, 2012) (See Also Insert) Black Pseudosclerotial Plates (Arrowed) on Petioles and Rachises, from which Apothecia Emerge (© Thomas Kirisits, IFFF-Boku Vienna, Austria, Kirisits *et al.*, 2012)

Diagnostic Test

The CAPS (2010) approved method for identification of *C. fraxinea* the anamorph of *H. pseudoalbidus* involves a morphological identification of the pathogen. It is isolated from the leading edge of local necrotic lesions from stems and leaf petioles on malt extract agar (Kowalski, 2006).

Literature-Based Methods

Sampling Procedure for Ash Dieback (*Chalara fraxinea*) (morphological)

The pathogen is isolated from pieces of stem or twig. The plant material is first surface sterilizing by rinsing in 95 percent ethanol for one minute, four percent NaOCl for five minutes and 96 percent ethanol for 30 seconds and drying in sterilised blotting paper (Kowalski and Bartnik, 2010). The surface bark is removed and pieces of shoots 5 x 2 x 2 mm are plated on Petri plates containing two percent malt extract agar supplemented with 100 mg/L of streptomycin sulphate. According to Kowalski and Bartnik (2010) the optimum temperature for colony growth is 20°C in the dark, while conidial sporulation is favoured by lower temperatures (5°-15°C). Other authors have also used different media to isolate the pathogen. Bakys *et al.* (2009a) used Hagem agar medium (Kalm and Kalyoncu, 2008) to isolate fungi from diseased *Fraxinus* spp. while Talgo *et al.* (2009) used potato dextrose agar and water agar for pathogen isolation.

Due its slow growth in culture, colonies are sometimes overgrown by colonies of other fast-growing saprotrophic fungi within the host tissue. The production of phialophores and conidia also takes several weeks making classical isolation and identification techniques time consuming and inefficient (Kowalski, 2006; Ioos *et al.*, 2009; Bakys *et al.*, 2009b; Chandelier *et al.*, 2010) Thus the need for molecular method adopted to high throughput analysis to complement morphological identifications.

Molecular Analysis

The presence of *H. pseudoalbidus* in *F. excelsior* seeds (Clearly *et al.*, 2012) and in culture (Jankovsky and Holdenrieder, 2009; Kowalski. and Holdenrieder, 2009b) were detected using PCR involving the amplification of the ITS region of the rDNA. The PCR products were purified, sequenced and compared with sequences from databases to determine the species of fungus present.

Bakys *et al.* (2009b) used a combination of terminal restriction fragment length polymorphism (T-RFLP) and ITS rDNA sequencing previously described by Lindahl *et al.* (2007) to detect *H. pseudoalbidus* directly from plant tissue.

They cloned and sequenced the polymerase chain reaction (PCR) products from the ITS region of the rDNA and compared the sequences from cloned fragments with database sequences in order to identify the taxa in the T-RFLP profiles. Johansson *et al.* (2010) also developed a PCR method to detect *H. pseudoalbidus* from tissue without the need to first culture the fungus. A set of species-specific primers based on the ITS sequence of *H. pseudoalbidus* isolates obtained by Bakys *et al.* (2009b) were developed and used in this study.

Chandelier *et al.* (2010) developed a real-time PCR for the detection of *H. pseudoalbidus* from woody tissues of *Fraxinus* spp. using PCR primers and Taqman probes, based on the ITS region of the multi-copy gene rDNA. To facilitate the routine application of this method, sawdust obtained from drilling into infected wood material was used for DNA extraction without further grinding. Ioos *et al.* (2009) also developed a real-time PCR for the detection of *C. fraxinea* in plant tissue using species-specific polymorphisms present within the ITS region to design a primer pair and a dual-labeled probe.

Table 3-1 PCR Primers for Detection of *Hymenoscyphus pseudoalbidus*

Name	Oligonucleotide sequence (5'-3')	Product size (bp)	Reference
ITScf-F	AGCTGGG- GAAACCTGACTG		
ITScf-R	ACACCGCAAG- GACCCTATC	456	(Johansson <i>et al.</i> , 2010)
Cfrax-F	ATTATATTGTTGCT TTAGCAGGTC		
Cfrax-R	TCCTCTAGCAGG- CACAGTC	67	
C-frax-P	6-FAM- CTCTGGG- CGTCGGCCTCG- BHQ-1		(Ioos <i>et al.</i> , 2009)
Cf-F	CCCTTGTTATAT- TATATTGTTGCTTT AGC		
Cf-R	GGGCCTCTAG- CAGGCACAGT	81	
Cf-S	6-FAM –TCTGGGC- GTCGGCCTCGG– BHQ-1		(Chandelier <i>et al.</i> , 2010)

Other Methods

Pham *et al.* (2013) used Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) to rapidly detect *Chalara fraxinea* *in vitro* and *in vivo* in tissues of diseased *Fraxinus*. They used a range of novel secondary metabolites produced by the fungus as chemical markers to determine the presence of the pathogen. These metabolites have molecular weights between 900 to 1250 Da and are collectively named chalarafraxinines.

Similar Species

The symptoms of ash dieback are usually visible on infected trees, but these symptoms can be confused with those of diseases caused by other fungi and insects such as the emerald ash borer. The anamorph of *H. pseudoalbidus* (*Chalara fraxinea*) can be present in symptomless leaves and shoots (Bakys *et al.*, 2009a b; Schumacher *et al.*, 2010) indicating latent infections. It can be differentiated from other fungal species that cause canker and dieback in *Fraxinus* spp. because it does not sporulate in pycnidia or perithecia and also it does not produce obvious stroma on infected stems or branches (Sinclair and Lyon, 2005). It is differentiated from other species of *Chalara* by its small, short cylindrical phialoconidia extruded in chains or in slimy droplets and the morphological features of the phialophores as well as colony characteristics (Kowalski, 2006).

The apothecia of the teleomorph, *H. pseudoalbidus* produced on detached petioles in the leaf litter of *Fraxinus* spp. (Kowalski and Holdenrieder, 2009b) cannot easily be distinguished morphologically from that of *H. albidus*. These cryptic species can be differentiated based on molecular characteristics including differences in the internal transcribed spacers of the rDNA genes sequence (Queloz *et al.*, 2011).

Survey Procedures

Contents

Introduction	4-1
Survey Types	4-1
Preparation, Sanitization, and Clean-up	4-2
Detection Survey	4-3
Delimiting Survey after Initial United States Detection	4-5
Trace-back and Trace-Forward Investigations	4-5
Monitoring Survey	4-6
Visual Inspection for Detection Survey	4-7
Sentinel Sites	4-8
Targeted Surveys	4-8
Survey Records	4-8
Data Collection	4-9
Cooperation with Other Surveys	4-9

Introduction

Use *Chapter 4 Survey Procedures* as a guide when conducting a survey for *Hymenoscyphus pseudoalbidus* in potentially infected *Fraxinus* tree hosts.

Survey Types

Plant regulatory officials will conduct detection, delimiting, and monitoring surveys for *H. pseudoalbidus*. Detection surveys will be conducted to ascertain the presence or absence of *Hymenoscyphus pseudoalbidus* in an area where it is not known to occur. After a new detection in the United States, or when detection in a new area is confirmed, a delimiting survey should be conducted to define the extent and geographic location where the disease is present. In addition, when a control procedure is applied and there is a need to measure its effectiveness, consider conducting a monitoring survey.

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 plants and leaf litter. 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 or nurseries.
2. Conduct the survey at the proper time. Studies have shown that *H. pseudoalbidus* apothecia, from which air borne ascospores that infect plants are produced, appear on *Fraxinus* leaf petioles and rachises in the forest during warmer months. General survey should focus on months when host plants are easily accessible and during active growing phases.
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 *H. pseudoalbidus* or other pests between properties during inspections.
6. Before entering a new property, make certain that clothing and footwear are clean and free of pests, soil and litter to avoid moving soil borne pests and arthropods from one property to another.
7. 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.
8. Gather together all supplies. Confirm the equipment and tools are clean. When taking plant samples, disinfest tools with 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 (bleach) is an effective way to inactivate bacterial and other diseases and prevent their spread.

9. 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.
 10. Survey task forces should consist of an experienced survey specialist or plant pathologist familiar with *H. pseudoalbidus* and the symptoms of their damage.
-

Detection Survey

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

Statistically, a detection survey is not a valid tool to claim that a pest does not exist in an area, even if results are negative. Negative results can be used to provide clues about mode of dispersal, temporal occurrence, or industry practices. Negative results are also important when compared with results from sites that are topographically, spatially, or geographically similar.

Procedure

Follow this procedure when conducting a detection survey for *Hymenoscyphus pseudoalbidus*.

1. Use visual inspection to examine the host plants for symptoms. Refer to [Visual Inspection for Detection Survey](#) on page 4-7 for further information on inspection procedures.

Important

Detection surveys for *Fraxinus* tree infected by *Hymenoscyphus pseudoalbidus* should be conducted by State inspectors in conjunction with Federal PPQ inspectors.

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

The CAPS (2010)-approved survey method for *Hymenoscyphus pseudoalbidus* is based on visual survey. For visual survey, collect shoots, twigs, leaves and stem samples from symptomatic plants with characteristic symptoms of the pathogen including shoot, twig, and branch dieback, wilting, leaf and bark lesions, and gray to brown discoloration of the wood.

Literature-Based Methods

Generally trees showing symptoms of crown decline are sampled (Rytokonen *et al.*, 2011; Bakys *et al.*, 2009a). To determine the severity of ash dieback on the tree crown, Kirisits and Freinschlag (2012) devised a rating system. The crown of each individual tree was divided into thirds. Each section of the crown was given a rating based on the severity of dieback using the class means (0, 2.5, 12.5, 35, 65, 90 and 100). The values of all three sections were averaged to get the ash dieback severity rating in percent for each tree (Figure 4-1 on page 4-4).

One to four symptomatic twigs or branches are collected from symptomatic trees (Rytokonen *et al.*, 2011). In each stand, branches with various severity of dieback are cut from three to five trees, individually packaged into plastic bags for transport (Bakys *et al.*, 2009a). Visually examine *Fraxinus* trees for characteristic ash dieback symptoms. The fallen leaf litter may be examined for the presence of *H. pseudoalbidus* apothecia.

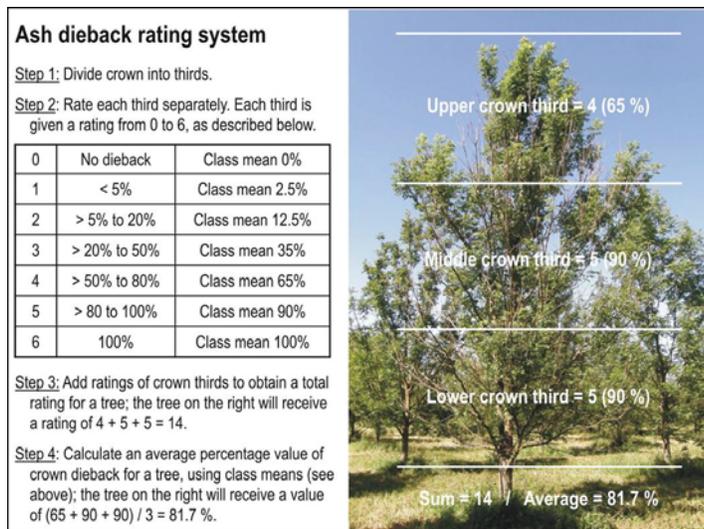


Figure 4-1 Rating System to Assess the Severity of Ash Dieback (Kirisits and Freinschlag, 2012)

Delimiting Survey after Initial United States Detection

If *H. pseudoalbidus* is detected in the United States, surveys will be conducted in the disease center 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 *H. pseudoalbidus* have been confirmed, surveys should be most intensive around the known positive detections and any discovered through trace-back and trace-forward investigations.

Trace-back and Trace-Forward Investigations

Trace-back and trace-forward investigations help determine priorities for delimiting survey activities after an initial detection. Trace-back investigations attempt to determine the source of infection. Trace-forward investigations attempt to define further potential dissemination through means of natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, efforts should be made to determine the extent of the infestation or potentially infected areas in which to conduct further investigations. The transportation of seeds (Cleary *et al.*, 2012), seedlings for planting and wood of *Fraxinus* trees may disseminate *H. pseudoalbidus* over long distances (Husson *et al.*, 2012). However, due to the risk of further emerald ash borer introductions, USDA has prohibited the importation of plants for planting of the listed host genera, with the exception of seed.

Homeowner Properties

For positive detections on homeowner properties, ask the owner of the infected material to determine where it originated (nursery, neighbors, *etc.*) and where it might have been further distributed.

Nursery Properties

For nursery hosts, a list of facilities associated with potentially infected nursery stock from those testing positive for *H. pseudoalbidus* will be compiled. These lists will be distributed by the State to the field offices, and are not to be shared with individuals outside USDA, APHIS, PPQ regulatory cooperators. 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.

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 *H. pseudoalbidus* -infected material. Speak to the growers or farm managers and obtain proper permission before entering private property.

Several actions need to occur immediately upon confirmation that a nursery sample is positive for *H. pseudoalbidus*:

- ◆ 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).
- ◆ Evaluate the disease situation, including identification and inspection of the budwood source(s) of the diseased tree(s), the location within the nursery, and the disease severity.
- ◆ Check nursery records to identify potential sources of the infection including sources of seed and budwood outside the nursery.

See [Regulatory Procedures](#) on page 5-1 and [Control Procedures](#) on page 6-1 for more information.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. If *H. pseudoalbidus* is detected in the United States, a technical working group will be assembled to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments on the pathogen. Refer to [Control Procedures](#) on page 6-1 for further information on control options.

Procedure

Once *H. pseudoalbidus* has been confirmed from a particular field sample and control measures have been implemented, additional monitoring will be necessary. Use the following tools:

- ◆ Visual Inspection of Trees
 - ◆ Collection of samples from forest litter and potential hosts for several years and multiple times per season. Refer to [Visual Inspection for Detection Survey](#) on page 4-7 and [Visual Inspection for Delimiting Survey](#) on page 4-7 for further information concerning the inspection of host plants.
-

Visual Inspection for Detection Survey

Use visual inspection as a tool when surveying for ash dieback (*Hymenoscyphus pseudoalbidus*) in forest, nurseries and urban landscapes.

Conduct a visual inspection in a field by looking for plants with typical ash dieback symptoms. The absence of symptoms, however, does not necessarily mean *H. pseudoalbidus* is not present in the area inspected. Some infected plants may not express symptoms, depending on the time and severity of the infection and in particular, less sensitive *Fraxinus* spp.

Visual Inspection for Delimiting Survey

Conduct delimiting surveys in an area—based on known positive testing, associated positive testing, or potentially infested areas to define the geographic location of the pathogen population. The delimiting survey in a general growing area can include random sampling of wild and cultivated host species 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.

Sentinel Sites

Sentinel sites are locations that are regularly inspected along the surveyor's normal route. The sites can be established using a known host plant. The plant used as a sentinel site should be inspected for visual signs of damage; if available, test the host plant. Use GPS to record the location of the host plant, and draw a map of the immediate area that includes reference points so that the area can be found by others if necessary. Once the sentinel site is established the surveyor should re-inspect the site on a regular basis (bimonthly or monthly) as permitted by the person's regular survey schedule. GIS can be used to map the sentinel site locations to help visualize an even coverage, particularly in high risk areas.

Other Diseases

Other diseases can cause symptoms that are similar, so diagnostic tests must be performed on samples from symptomatic plants in order to confirm the presence of *H. pseudoalbidus*. See [Identification](#) on page 3-1 for more information.

Targeted Surveys

Conduct regular targeted surveys at nurseries as well as areas with regular traffic from countries with known infestations, urban street trees, parks, and forests.

Survey Records

Records should be kept for each survey site. 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
- ◆ Host plant species
- ◆ History of machinery usage
- ◆ Observations of symptoms
- ◆ Other relevant information

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

1. Mark the location
2. Take samples of diseased plant parts and flag the location within the field
3. Notify the State or PPQ inspector
4. Place the samples from the infected 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 samples 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 outbreaks that could be associated with *H. pseudoalbidus*.

Regulatory Procedures

Contents

Introduction	5-1
Instructions to Officials	5-1
Regulatory Actions and Authorities	5-2
Overview of Regulatory Program After Detection	5-3
Record-Keeping	5-4
Issuing an Emergency Action Notification	5-4
Regulated Area Requirements Under Regulatory Control	5-4
Establishing a Federal Regulatory Area or Action	5-5
Regulatory Records	5-5
Use of Chemicals	5-5

Introduction

Use *Chapter 5 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest control programs against the small banded pine weevil, *Pissodes castaneus* (DeGeer, 1775).

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 people interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in line with labeling restrictions. During all field visits, 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 without 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 more information, contact PPQ's Tribal Liaison. Refer to [Resources](#) 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 put 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.

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 instructions. For further information refer to [Control Procedures](#) on page 6-1. Agreement 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.

Control Procedures

Contents

Introduction	6-1
Overview of Emergency Programs	6-1
Treatment Options	6-2
Eradication	6-2
Cultural Control and Sanitary Measures	6-3
Chemical Control	6-4
Application	6-4
Labeling	6-4
Biological Control	6-5
Host Resistance	6-5

Introduction

Use *Chapter 6 Control Procedures* as a guide to control an outbreak of ash dieback (*H. pseudoalbidus*) in the United States. Consider the treatment options described within this chapter when taking action to manage or contain an infestation by *H. pseudoalbidus*. The control of this pathogen must involve an integrated approach that entails the use of cultural and management control, chemical treatments, biocontrol, as well as the use of genetic resistance.

Overview of Emergency Programs

Plant Protection and Quarantine (PPQ) develops and makes control measures available to involved States. Environmental Protection Agency (EPA) approved treatments will be recommended when available. If selected treatments are not labeled for use against the organism 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 and Pesticide Use Coordinators are also available upon request to work with EPA to expedite approval of a product that may not be registered in the United States, or to obtain labeling for a new use. Refer to *Resources* on page [A-1](#) for information on contacting the Coordinator.

Treatment Options

Although there is currently no information on an effective control method for *H. pseudoalbidus* (Kunca *et al.*, 2011), consider the treatment options described within this chapter when taking action to eradicate or control *H. pseudoalbidus*. Treatments may include the following:

- ◆ *Eradication* on page 6-2
- ◆ *Cultural Control and Sanitary Measures* on page 6-3
- ◆ *Chemical Control* on page 6-4
- ◆ *Biological Control* on page 6-5
- ◆ *Host Resistance* on page 6-5

Eradication

When a pathogen has been newly introduced, eradication is usually the first action to consider. However, eradication is only feasible where the infestation is confined to a small area and detection of the pathogen occurs soon after the introduction.

If an infestation of *H. pseudoalbidus* is discovered that meets the above conditions, eradication should be attempted. Measures will include but may not be limited to the removal and destruction of all infested plant material including leaf litter. Studies on the spread of ash dieback in Norway have indicated that the pathogen has a potential dispersal rate of 20 to 30 km per year (Solheim *et al.* 2011). Therefore, depending on the time of detection, removal of host material within an appropriate distance of the find is critical. There is no information available regarding the persistence of the pathogen within the soil. Measures may also include a treatment of the soil and surrounding vegetation with an approved pesticide after the removal of the infested plants. Eradication measures should be continued for several years to ensure that populations of *H. pseudoalbidus* have been eliminated. Once the pathogen has been eradicated, monitoring of the site should be continued for 2-5 years. For further information, refer to *Monitoring Survey* on page 4-6.

When the disease is wide spread, a sanitary felling program to control or eradicate *H. pseudoalbidus* is likely to be both impractical and ineffective (EPPO, 2008; Kirisits *et al.*, 2012). This is because the ascospores are wind-borne and are transported over long distances (Solheim *et al.*, 2011). An example of such a situation was observed in Norway where the extent of disease spread was so extensive that no eradication measures were taken

(EPPO, 2008). Also the felling of trees will have a negative ecological impact on species that are associated with *Fraxinus* trees (Pautasso *et al.*, 2013).

When the disease is wide spread, new measures should rely on containment or management options. Containment means keeping the target population of infected plants confined to a specific area, to allow time to develop tools to manage the disease in the long term. Using this approach requires strong regulatory procedures. A variation of containment is known as Slow-the-Spread (STS) (USDA, APHIS, PPQ, 2003). In STS, the spread of the pest population is slowed as much as possible, resources permitting. 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 may be used for both containment programs and long term management.

Cultural Control and Sanitary Measures

Sanitation of equipment used on or near infected trees may reduce spread of the fungus (Norwegian Food Safety Authority, 2008). Refer to [Preparation, Sanitization, and Clean-up](#) on page 4-2 for more details. Schumacher (2011) indicated that ash dieback intensity is lower in drier sites possibly due to the fact that sporulation and infection by *H. pseudoalbidus* is favoured by high soil moisture and air humidity. The establishment of seed beds in areas with drier microclimate will reduce the severity of the disease.

Ascospores produced during summer (between June and October in Europe) in apothecia on fallen *Fraxinus* spp. rachises in the litter from the previous year are the main source of *H. pseudoalbidus* infection (Kirisits and Cech, 2009; Timmermann *et al.*, 2011; Gross *et al.*, 2012a). Routine removal and destruction of shed *Fraxinus* leaves may help to reduce the source of inoculum and thus to decrease infections in nurseries and urban environments.

Chemical Control

There are no fungicides that have been labeled for use or proven to be effective in the control of *H. pseudoalbidus*. Although chemical control methods have been successful against other *Chalara* spp. including the use of the triazole fungicides, Etaconazole and Propiconazole in the control of *C. elegans* (Labuschagne and Kotzé, 1996), and may be effective against *C. fraxinea*, Schumacher, (2011) indicated that because the fungus invades woody tissues very effectively, the development and application of fungicidal compounds to control the pathogen will be difficult. Furthermore, the use of fungicides in ash dieback control may only be practical on plantation, nursery and garden trees (Moricca and Ragazzi, 2008). Contrary to a crop environment, forests are made up of thousands of species that might be a hindrance to fungicide application in terms of accessibility and application and detrimental effects on non-target organisms.

Application

At the initiation of an eradication or control program, evaluate the available fungicides for their use in program operations. Select a fungicide after considering local conditions along with survey results.

Labeling

Although a proposed formulation may be approved for an effective eradication or control program, it may not be labelled, at the time of pest detection, for the specific use where treatment is required. If a formulation is not labelled for the needed use, it may be possible to request a Federal Crisis or Quarantine Exemption from the EPA under Section 18 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). For further information refer to [Regulatory Procedures](#) on page 5-1. The prescribed formulation must be labelled for use on the site where it is to be applied and must be registered for use in the State where the eradication program is occurring. All applicable label directions must be followed, including requirements for personal protection equipment, maximum treatment rates, storage and disposal.

Biological Control

There are currently no biological control methods that are effective in the control of *H. pseudoalbidus*. Preliminary studies by Orgis (2010) have identified the fungus, *Pacilomyces marquandii* and fungus gnats as natural enemies of *H. pseudoalbidus*. There is the need for further studies into the feasibility of using these organisms in a biological control program in the management of ash dieback.

Host Resistance

All known natural hosts of *H. pseudoalbidus* belong to the genus *Fraxinus*. Complete resistance to the pathogen has not been observed in any of the identified hosts. There is evidence to show that some individual trees of *F. excelsior* are less susceptible to *H. pseudoalbidus* (Pliura *et al.*, 2011; McKinney *et al.*, 2011). Preliminary assessments of some Lithuanian *F. excelsior* populations showed high heritability (0.40) in their tolerance to *H. pseudoalbidus* infection although only 10 percent of individual trees survived after 8 years (Pliura *et al.*, 2011). A similar evaluation in Denmark indicated a high susceptibility in the Danish populations providing evidence for the existence of genetic variation in resistance against *H. pseudoalbidus* (Kjær *et al.*, 2012).

Studies by McKinney *et al.* (2011) suggested that the reduced susceptibility in some individual trees might not be due to the presence of resistance genes but rather phenological characteristics such as early leaf senescence in autumn which reduces susceptibility. This is possible due to the fact that the pathogen enters the trees through their leaves indicating that leaf senescence prior to the establishment of infections in the shoot will reduce the severity of the disease. McKinney *et al.* (2012), however, observed the existence of an active defense mechanism in some *F. excelsior* trees in Danish field trials where partially resistant trees showed significantly less infection and in the presence of infections, a reduction in the development of necrotic lesions. This mechanism is more likely to provide a durable resistance to the pathogen. There is the need for long term research to evaluate these resistant clones for their potential in breeding programs for resistance against *H. pseudoalbidus*.

Environmental Compliance

Contents

Introduction	7-1
Overview	7-1
National Environmental Policy Act	7-2
Endangered Species Act	7-3
Migratory Bird Treaty Act	7-3
Clean Water Act	7-3
Tribal Consultation	7-4
National Historic Preservation Act	7-4
Coastal Zone Management Act	7-4
Environmental Justice	7-4
Protection of Children	7-5

Introduction

Use *Chapter 7 Environmental Compliance* as a guide to environmental regulations pertinent to the small banded pine weevil, *Pissodes castaneus* (DeGeer, 1775).

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 begin.

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 give 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 meet 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 carry out the plan, give 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 before taking action, and to tell 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 needed 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 before 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 need public involvement and comment before 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 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 affect children. If such a risk is identified, then measures must be described and carried out to minimize such risks.

Pathways

Contents

Introduction	8-1
Overview	8-1
Natural Movement	8-2
Human Assisted Spread	8-2

Introduction

Use *Chapter 8 Pathways* as a source of information on the pathways of introduction of the ash dieback fungus, *Hymenoscyphus pseudoalbidus* (anamorph; *Chalara fraxinea*) into the United States.

Overview

The entry and establishment of *H. pseudoalbidus* poses a serious threat to the United States *Fraxinus* forests, urban plantings of *Fraxinus* spp, and to those industries that rely on *Fraxinus* spp. Several *Fraxinus* spp. within the United States that have significant value for timber and firewood as well as in the making of tool handles and quality wooden baseball bats are hosts or potential hosts to this pathogen (Gould and Bauer, 2009; Pautasso *et al.*, 2013). With the increased volume of international trade and passengers travelling to the United States, there is an increased risk of accidental introductions of *Hymenoscyphus pseudoalbidus* through transport of infected seedlings, saplings, or on wood while symptoms are still latent (Bakys *et al.*, 2009a; Husson *et al.*, 2012).

Natural Movement

The known distribution of *Hymenoscyphus pseudoalbidus* in Europe and Asia makes unaided spread of the pathogen into the United States very unlikely. Conidia produced in mucilaginous droplets or chains by the anamorph have a very low potential for aerial dispersion and may require a vector to be transmitted (Kowalski and Holdenrieder, 2009b); however, no known vectors have been associated with *H. pseudoalbidus* (Kowalski and Holdenrieder, 2009b), although various *Chalara* species have been shown to be vectored primarily by insects (Kile and Walker, 1987). This makes the possibility of introducing the pathogen into the United States through conidia transport very unlikely. Attempts to germinate the conidia or to induce disease by introducing them into young *Fraxinus* spp. have also been unsuccessful (Hosoya *et al.*, 1993; Kirisits and Cech 2009) and no clonal populations have been observed (Kirisits *et al.*, 2009). It has thus been suggested that conidia are not infectious and might act exclusively as spermatia in the process of teleomorph formation (Kirisits & Cech, 2009; Kirisits *et al.*, 2009; Gross *et al.*, 2012a).

There is, however, evidence to support an aerial mode of dispersal of *H. pseudoalbidus* through ascospores. Ascospores are produced during summer (between June and October in Europe) in apothecia on fallen *Fraxinus* spp. rachises in the litter from the previous year and are known to be germinable and dispersed by wind (Kirisits and Cech, 2009; Timmermann *et al.* 2011; Gross *et al.*, 2012a). Fungal spores have been shown to travel over long distances and have the ability to easily jump landscape patches without the presence of hosts, as demonstrated by modeling studies (Shaw *et al.*, 2006; Mundt *et al.*, 2009). The specific distance over which *H. pseudoalbidus* ascospores can be dispersed by wind is unknown, however, studies of the spread of the disease in Norway have indicated that the pathogen has a potential dispersal rate of 20 to 30 km per year (Solheim *et al.*, 2011).

Human Assisted Spread

Plants for planting (Kirisits *et al.*, 2009; Kirisits *et al.*, 2012) and wood (Bakys *et al.*, 2009a; Husson *et al.*, 2012) are considered likely pathways for long-range spread of *Hymenoscyphus pseudoalbidus*. Both *C. fraxinea* and *H. pseudoalbidus* are listed as reportable in the PEST ID database (USDA-AQAS, 2013), and *C. fraxinea* is listed as a pest of concern on the 2012 PPQ Prioritized Offshore Pest List.

The most likely means of introducing *H. pseudoalbidus* into the United States would thus be through assisted introductions via the importation of infected plants for planting, wood and other plant material such as bark and leaves,

(Husson *et al.*, 2012) and possibly seed (Cleary *et al.*, 2012). This risk is, however, greatly reduced as a result of the USDA's prohibition of the importation of *Fraxinus* spp. for planting except seed from any foreign country (except portions of Canada) due to the presence of the emerald ash borer (Importation of Ash Plants, 2008). Thus the most likely method of entry into the United States is intentional (smuggling) or unintentional introduction of host material infected with the pathogen through the international trade of prohibited planting material.

There is a need for further studies into the potential for imported *Fraxinus* seeds to act as a source of inoculum for *H. pseudoalbidus* as a recent study by Cleary *et al.* (2012) on the occurrence of *H. pseudoalbidus* in seeds from declining *F. excelsior* trees collected from Latvia revealed the presence of the pathogen in 8.3 percent of seeds tested. In Norway, all propagative materials of are prohibited from entry in many areas of the country (Norwegian Food Safety Authority, 2008).

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

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References

Glossary

Ash Dieback

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

anamorph. asexual form of a fungus

APA. American Phytopathological Society

APHIS. USDA–Animal and Plant Health Inspection Service

approved landfill. State licensed municipal or private landfill managed under state regulation to prevent leaching of potential pollutants into groundwater

autoecious. parasitic fungus that completes the entire life cycle on a single host

CAPS. Cooperative Agricultural Pest Survey Program, 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

CPB. United States 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 the pathogen is not known to occur

DHS. United States 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

EM. PPQ–Emergency Management

FIFRA. Federal Insecticide, Fungicide, and Rodenticide Act

ICS. Incident Command System

heteroecious. parasitic fungus that develops different stages of the life cycle on different host species.

host. plant which is invaded by a parasite or pathogen and from which it obtains its nutrients.

infection. establishment of a parasite on or within a host plant

ISIS. Integrated Survey Information System

macrocyclic. rust fungi that display a long life cycle with five stages, each with a characteristic type of spore.

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*

NASS. National Agricultural Statistics Service

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. An 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

PERAL. Plant Epidemiology and Risk Analysis Laboratory

pest. 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

teleomorph. sexual form of a fungus.

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

USFWS. United States Fish and Wildlife Service

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 Ash Dieback

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 <i>Manual for Agricultural Clearance</i>	http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml
PPQ <i>Treatment Manual</i>	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	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
National Climatic Center, Data Base Administration, Box 34, Federal Building, Asheville, North Carolina 28801	http://www.ncdc.noaa.gov/oa/ncdc.html
CAPS Survey Manuals	http://caps.ceris.purdue.edu/
Leafhopper and treehopper genera in New Zealand	http://www1.dpi.nsw.gov.au/keys/leafhop/deltocephalinae/opsiini.htm
GenBank®	http://www.ncbi.nlm.nih.gov/
iPhyClassifier	http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi

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 IIB/III USE LOT NO.	
1. COLLECTION NUMBER		2. DATE MO DA YR		3. SUBMITTING AGENCY <input type="checkbox"/> State <input type="checkbox"/> PPQ <input type="checkbox"/> Other _____ Cooperator	
SENDER AND ORIGIN	4. NAME OF SENDER		INTERCEPTION SITE	5. TYPE OF PROPERTY (<i>Farm, Feedmill, Nursery, etc.</i>)	
	6. ADDRESS OF SENDER			7. NAME AND ADDRESS OF PROPERTY OR OWNER	
	ZIP			COUNTRY/ COUNTY	
8. REASON FOR IDENTIFICATION ("x" ALL Applicable Items)					
PURPOSE	A. <input type="checkbox"/> Biological Control (Target Pest Name _____)		E. <input type="checkbox"/> Livestock, Domestic Animal Pest		
	B. <input type="checkbox"/> Damaging Crops/Plants		F. <input type="checkbox"/> Possible Immigrant (<i>Explain in REMARKS</i>)		
	C. <input type="checkbox"/> Suspected Pest of Regulatory Concern (<i>Explain in REMARKS</i>)		G. <input type="checkbox"/> Survey (<i>Explain in REMARKS</i>)		
	D. <input type="checkbox"/> Stored Product Pest		H. <input type="checkbox"/> Other (<i>Explain in REMARKS</i>)		
9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".					
HOST DATA	10. HOST INFORMATION NAME OF HOST (<i>Scientific name when possible</i>)			11. QUANTITY OF HOST NUMBER OF ACRES/PLANTS	
	11. QUANTITY OF HOST PLANTS AFFECTED (<i>Insert figure and indicate</i> <input type="checkbox"/> Number <input type="checkbox"/> Percent):				
	12. PLANT DISTRIBUTION <input type="checkbox"/> LIMITED <input type="checkbox"/> SCATTERED <input type="checkbox"/> WIDESPREAD		13. PLANT PARTS AFFECTED <input type="checkbox"/> Leaves, Upper Surface <input type="checkbox"/> Trunk/Bark <input type="checkbox"/> Bulbs, Tubers, Corms <input type="checkbox"/> Seeds <input type="checkbox"/> Leaves, Lower Surface <input type="checkbox"/> Branches <input type="checkbox"/> Buds <input type="checkbox"/> Petiole <input type="checkbox"/> Growing Tips <input type="checkbox"/> Flowers <input type="checkbox"/> Stem <input type="checkbox"/> Roots <input type="checkbox"/> Fruits or Nuts		
PEST DATA	14. PEST DISTRIBUTION <input type="checkbox"/> FEW <input type="checkbox"/> COMMON <input type="checkbox"/> ABUNDANT <input type="checkbox"/> EXTREME		15. <input type="checkbox"/> INSECTS <input type="checkbox"/> NEMATODES <input type="checkbox"/> MOLLUSKS		
			NUMBER SUBMITTED	LARVAE	PUPAE
			ALIVE	ADULTS	CAST SKINS
			DEAD	EGGS	NYMPHS
16. SAMPLING METHOD		17. TYPE OF TRAP AND LURE		18. TRAP NUMBER	
19. PLANT PATHOLOGY - PLANT SYMPTOMS ("X" one and describe symptoms) <input type="checkbox"/> ISOLATED <input type="checkbox"/> GENERAL					
20. WEED DENSITY <input type="checkbox"/> FEW <input type="checkbox"/> SPOTTY <input type="checkbox"/> GENERAL		21. WEED GROWTH STAGE <input type="checkbox"/> SEEDLING <input type="checkbox"/> VEGETATIVE <input type="checkbox"/> FLOWERING/FRUITING <input type="checkbox"/> MATURE			
22. REMARKS					
23. TENTATIVE DETERMINATION					
24. DETERMINATION AND NOTES (<i>Not for Field Use</i>)				FOR IIB/III USE DATE RECEIVED	
				NO. LABEL SORTED PREPARED DATE ACCEPTED	
SIGNATURE _____ DATE _____				RR	

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

This is a 6-Part form. Copies must be disseminated as follows:

- | | | |
|---|--|---|
| <input type="checkbox"/> PART 1 - PPQ | <input type="checkbox"/> PART 2 - RETURN TO SUBMITTER AFTER IDENTIFICATION | <input type="checkbox"/> PART 3 - IIB/III OR FINAL IDENTIFIER |
| <input type="checkbox"/> PART 4 - INTERMEDIATE IDENTIFIER | <input type="checkbox"/> PART 5 - INTERMEDIATE IDENTIFIER | <input type="checkbox"/> 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	<p>1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number</p> <p>EXAMPLE In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001</p> <p>2. Enter the collection number</p>
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	<ul style="list-style-type: none"> • 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 shared 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:

1. Send the original along with the sample to your area identifier
2. Keep and file a copy for your records

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

Block	Description	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 GIVE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE blank; ENTER remarks in <i>Block 22</i>
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
21	WEED GROWTH STAGE	If applicable, check the appropriate box; otherwise LEAVE blank

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

Block	Description	Instructions
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. <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">1. PPQ LOCATION</td> <td style="width: 50%;">2. DATE ISSUED</td> </tr> </table>	1. PPQ LOCATION	2. DATE ISSUED
1. PPQ LOCATION	2. DATE ISSUED		
3. NAME AND QUANTITY OF ARTICLE(S)	4. LOCATION OF ARTICLES		
6. SHIPPER	5. DESTINATION OF ARTICLES		
9. OWNER/CONSIGNEE OF ARTICLES	7. NAME OF CARRIER		
Name: _____ Address: _____ _____ _____ PHONE NO. _____ FAX NO. _____ SS NO. _____ TAX ID NO. _____	8. SHIPMENT ID NO.(S)		
	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. More 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 EAN's for each unit of suspected plant material and associated material held. EAN's 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 EAN's 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 more instructions for completing, using, and distributing this form in the *PPQ Manual for Agricultural Clearance*.

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.

Taxonomic Support for Surveys

Contents

[Background](#) **D-1**

Background

The National Identification Services (NIS) coordinates the identification of plant pests in support of USDA's regulatory programs. Accurate and timely identifications are the foundation of 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. As well, when considering whether to survey for a particular pest a given year, consider the challenges of taxonomic support.

Sorting and Screening

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

Sorting

Sorting is the first level of activity that assures samples submitted are of the correct target group of pests being surveyed, that is, 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

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
Cell: 814 - 450- 7186
Fax: 814 - 863 - 8265
e-mail: grace.okeefe@aphis.usda.gov

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
USDA, APHIS, PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
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.gov> with 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.

Research Needs

1. Determine the host range and environmental requirements for *Hymenoscyphus pseudoalbidus*
2. Evaluate the risk of imported seeds of *Fraxinus* spp. to serve as a pathway for international spread of *H. pseudoalbidus*
3. Assess potential sources of resistance in the known host plants within the United States.
4. Begin assessments of potential biological control agents such as *Pacilomyces marquandii* and fungus gnats.
5. There is the need to develop rapid diagnosis and identification methods.

