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Animal and Plant Health Inspection Service

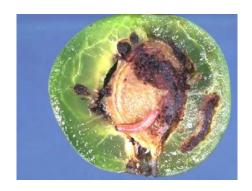
Plant Protection and Quarantine

New Pest Response Guidelines

Plum Fruit Moth (Cydia funebrana)









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CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.



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Cover Images

Top Left—Plum fruit larva damage on plum, R. Coutin, OPIE

Top Right—Adult plum fruit moth, R. Coutin, OPIE

Bottom Left—Plum fruit moth larva damage on plum, R. Coutin, OPIE

Bottom Right—Plum fruit larva damage on plum, R. Coutin, OPIE

Chapter

1

Introduction

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Introduction

Use *New Pest Response Guidelines: Plum Fruit Moth*, Cydia funebrana (*Treitschke*), when designing a program to detect, monitor, control, contain, or eradicate an infestation of this insect 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 contains the necessary information to launch a response to a detection of the plum fruit moth.

If the plum fruit moth 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 plum fruit moth:

- ♦ 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 plum fruit moth 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 *Preparation, Sanitization, and Clean-Up* on page 4-2 before entering and upon leaving each property to prevent contamination.

Scope

The guidelines is divided into the following chapters:

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

8. Pathways on page 8-1

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

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

Authorities

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

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

Program Safety

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

Support for Program Decisionmaking

USDA-APHIS-PPQ-Center for Plant Health, Science and Technology (CPHST) provides technical support to emergency pest response program directors 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.

MARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

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

Lists

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

Disclaimers

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

Table of Contents

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

Control Data

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

Change Bar

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

Decision Tables

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

Table 1-1 How to Use Decision Tables

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

Footnotes

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

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

Heading Levels

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

Hypertext Links

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

Italics

The following items are italicized throughout the guidelines:

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

Numbering Scheme

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

Transmittal Number

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

Acknowledgements

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

How to Cite the Guidelines

Cite the guidelines as follows: U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2011. *New Pest Response Guidelines: Plum Fruit Moth* (Cydia funebrana). 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

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Introduction

Use *Chapter 2: Pest Information* to learn more about the classification, history, host range, and biology of the plum fruit moth, *Cydia funebrana* (Treitschke).

Classification

Cydia funebrana belongs in the phylum Arthropoda, class Insecta, order Lepidoptera, family Tortricidae, subfamily Tortricinae, tribe Grapholitini, and species *Cydia funebrana*. Use *Table 2-1* as a guide to the classification of the plum fruit moth and the names used to describe it in the guidelines.

Table 2-1 Classification of Cydia funebrana

Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera

Table 2-1 Classification of Cydia funebrana (continued)

Family	Tortricidae		
Subfamily	Tortricinae		
Tribe	Grapholitini		
Genus	Cydia		
Full Name	Cydia funebrana (Treitschke)		
Preferred Common Name	Plum Fruit Moth		
Synonyms	Grapholita funebrana Treitschke (Brown et al. 2005) (Zhang 1994); Grapholita cerasana Kozhantshikov (Brown et al. 2005); Carpocapsa funebrana Treitschke (Zhang 1994); Laspeyresia funebrana Treitschke (Zhang 1994); Opadia funebrana Treitschke (Zhang 1994); Tortix funebrana Treitschke (Zhang 1994)		
Common Names	plum fruit moth, red plum maggot, plum fruit maggot		

Historical Information

Cydia funebrana is native to the Palearctic region, predominantly in Europe and Asia (CABI 2010). It feeds on multiple species and is the primary pest of plums in the growing regions of Europe and Asia (Whittle 1984). It has also been infrequently documented as infesting cherry and apple (Venette et al. 2003; CABI 2010).

Ecological Range

Cydia funebrana occurs throughout the Palearctic regions including Europe, Russia, northwestern Africa, and Asia (Whittle 1984); (Zhang 1994) (Table 2.2).

Europe—Albania (EPPO 2007); Armenia (EPPO 2007); Austria (EPPO 2007); Azerbaijan (EPPO 2007); Belgium (EPPO 2007); Belarus (Koltun and Yarchakovskaya 2006); Bosnia and Herzegovina (EPPO 2007); Bulgaria (EPPO 2007); Cyprus (EPPO 2007); Czech Republic (EPPO 2007); Hrdy et al., 1996; Kocourek and Stará, 2005); Denmark (EPPO 2007); Finland (EPPO 2007); France (EPPO 2007); Georgia (EPPO 2007); Germany (EPPO 2007); Hungary (Sáringer, 1967; Sáringer and Deseo, 1972); Italy (Molinari 1995; Butturini et al. 2000; EPPO 2007); Lithuania (EPPO 2007); Netherlands (EPPO 2007); Norway (EPPO 2007); Poland (Pluciennik et al. 1999; EPPO 2007); Romania (EPPO 2007; Oroian et al. 2009); Russia (Saparmamedova 1988; Saparmamedova 1988; Zhang 1994; EPPO 2007); Spain (EPPO 2007);

Sweden (EPPO 2007); Switzerland (Bovey 1966; EPPO 2007); Turkey (EPPO 2007); Ukraine (EPPO 2007); United Kingdom (Bradley et al. 1979; EPPO 2007).

Africa—Algeria (EPPO 2007).

Asia—China (EPPO 2007); Iran (Zhang 1994; EPPO 2007); Japan (Zhang 1994; EPPO 2007); Kazakhstan (EPPO 2007); Kyrgyzstan (EPPO 2007); Syria (EPPO 2007); Tajikistan (EPPO 2007); Turkmenistan (EPPO 2007); Uzbekistan (EPPO 2007).

Potential Distribution

Based on the reported global distribution, it is estimated that *Cydia funebrana* can survive in plant hardiness zones 2 through 11 (*Figure 2-1* on page 2-4). The availability of some economically important hosts including plums and cherries is combined with the climatic suitability to estimate the risk of establishment of *C. funebrana* in the continental United States.

NAPPFAST (North Carolina State University APHIS Plant Pest Forecasting System) maps were used in this section to describe the potential distribution of *Cydia funebrana*.

In a cooperative venture, North Carolina State University (NCSU), USDA—APHIS, and the information technology company ZedX, Inc., developed the Web tool known as NAPPFAST. NAPPFAST uses weather, climate, and soil data, to model pest development. The models supply the predictive pest mapping needs of the Cooperative Agricultural Pest Survey (CAPS) program. In addition, the models produce potential establishment maps for exotic pests, which supports the risk assessment activities of the Plant Epidemiology Risk Assessment Laboratory (PERAL).

Figure 2-1 on page 2-4 was used to describe the relative establishment potential based on the suitability of the climate for the plum fruit moth to grow and survive in the conterminous United States. The map was based on 10 years of daily data from NAPPFAST and developmental data for the plum fruit moth (Charmillot, 1979). In the color scale, the color blue represents a low likelihood of pest growth and survival, while the color red indicates high likelihood of pest growth and survival.

Figure 2-2 on page 2-5 also describes the establishment potential and combines host and climatic suitability information for this species. Climate suitability was based on 10 years of daily data from NAPPFAST and developmental data for the plum fruit moth (Charmillot, 1979).

How to Download Risk Maps from NAPPFAST

The risk maps featured in this section can be downloaded from the NAPPFAST Web site. For further information, refer to *Table 2-2* on page 2-4.

Table 2-2 How to Download Electronic Images from NAPPFAST

If you want to download the following:	Then visit this Web site:	And select this link:
Any host or risk map, including Alaska and Hawaii	http://www.nappfast.org/ caps_pests/ CAPs_Top_50.htm	CAPS AHP 2011 Top 50 and Pest Matrix

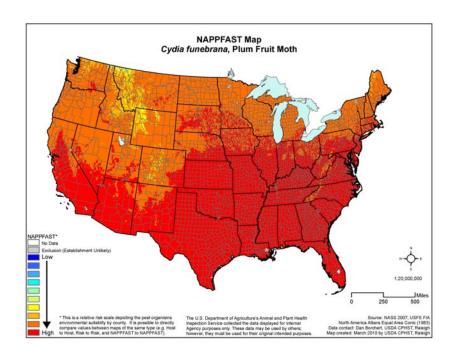


Figure 2-1 NAPPFAST Map of Relative Establishment Potential Based on the Suitability of the Climate for *Cydia funebrana*

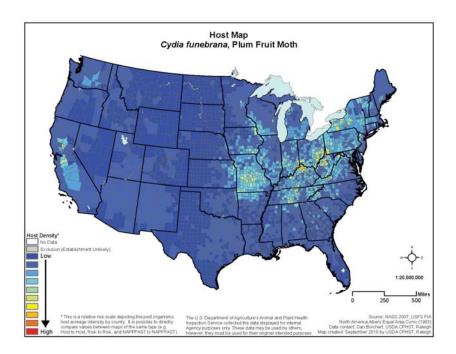


Figure 2-2 NAPPFAST Map of Relative Establishment Potential Combining Host and Climatic Suitability for *Cydia funebrana*

Hosts

Cydia funebrana primarily feeds on plants in the Rosaceae family. Potential host plants, both cultivated and wild, are common in the United States and often occur at high densities.

Primary hosts reported for *Cydia funebrana* were listed in *Table 2-3* on page 2-5. Secondary hosts reported for *C. funebrana* were listed in *Table 2-4* on page 2-6. The hosts were reported from their current distributions, and the host species may not be present in the United States. If pests are introduced into new areas, they may attack native species that have not previously been identified as host plants. Therefore, host species should be surveyed (where applicable) and surveys should be broadened to native species within the host genera.

Table 2-3 Primary Hosts Reported for Cydia funebrana

Family	Latin Name	Common Name	Reference
Rosaceae	Prunus armeniaca L.	Apricot	CABI (2010), EPPO, (2007), Venette et al. (2003), Whittle (1984)
Rosaceae	Prunus domestica L.	European plum	CABI (2010), EPPO (2007), Venette et al. (2003)

Table 2-3 Primary Hosts Reported for Cydia funebrana (continued)

Family	Latin Name	Common Name	Reference
Rosaceae	Prunus avium (L.) L.	Cherry	CABI (2010), EPPO (2007), Venette et al. (2003), Whittle (1984)
Rosaceae	Prunus L.	Plum	EPPO (2007)
Rosaceae	Prunus cerasifera Ehrh.	Cherry plum	Popova (1971), Venette et al. (2003)
Rosaceae	Prunus domestica L. var. insititia (L.) Fiori & Paoletti	European plum	Popova (1971), Venette et al. (2003)
Rosaceae	Prunus japonica Thunb.	Japanese bush cherry	Popova (1971), Venette et al. (2003)
Rosaceae	Prunus cerasus L.	Sour cherry	CABI (2010), Venette et al. (2003)
Rosaceae	Prunus spinosa L.	Blackthorn	Venette et al. (2003), Vernon (1971), CABI (2010)

Table 2-4 Secondary Hosts Reported for Cydia funebrana

Family	Latin Name	Common Name	Reference
Fagaceae	Castanea sativa Mill.	European chestnut	Venette et al. (2003)
Juglanda- ceae	Juglans regia L.	English walnut	Venette et al. (2003)
Juglanda- ceae	Juglans L.	Walnut	EPPO (2007)
Rosaceae	Malus domestica auct. non Borkh.	Apple	CABI (2010), EPPO (2007), Venette et al. (2003)
Rosaceae	Malus sylvestris (L.) Mill.	European crab apple	Venette et al.
Rosaceae	Prunus dulcis (Mill.) D.A. Webb	Bitter almond	Venette et al.
Rosaceae	Pyrus communis L.	Common pear	Venette et al.
Rosaceae	Prunus dulcis (Mill.) D.A. Webb	Sweet almond	CABI (2010), EPPO (2007)
Rosaceae	Prunus persica (L.) Batsch	Peach	CABI (2010), EPPO, (2007), Venette et al. (2003)
Rosaceae	<i>Prunus salicina</i> Lindl.	Japanese plum	CABI (2010)

Life Cycle

Eggs

Egg deposition by *Cydia funebrana* adults occurs at sundown at temperatures around 25°C. The females deposit 3 to 5 eggs per fruit (Popova, 1971). Eggs hatch in about 1 to 2 weeks (Whittle, 1984).

Larvae

After egg hatch, the larvae immediately begin burrowing into the fruit (Whittle, 1984). The exact number of instars is unknown and may vary between three and six (Baker, 1963; Dickler, 1991; Popova, 1971). The larvae remain in the fruit until either diapause or pupation depending on the season. To diapause, the larva moves to outside the fruit as a 2nd or 3rd instar and seeks shelter in bark crevices or soil. Pupation occurs outside of the fruit in the ground or the base of a tree (Popova, 1971).

Pupae

The larvae pupate in bark crevices or protected areas in the soil (Popova, 1971).

Adults

The number of generations per year depends on climatic conditions. The adult moths are difficult to differentiate between others of the same family and may require dissection of the genitalia for proper identification.

Developmental Rates and Day Degrees

Depending on temperature, the larvae complete their growth in 2 to 3 weeks (Popova, 1971). The number of larval instars suggested by researchers varies. Popova (1971) determined that *Cydia funebrana* has only three larva instars. Baker (1963) indicated that the number of instars may vary between four and five; Dickler (1991) reported that the larvae undergo five to six instars.

Based on the developmental threshold of 10°C, the eggs require 75 degree days (DD), 175 DD for larva and 160 DD for pupa to develop. The total life cycle requires 410 DD for complete development (Charmillot et al., 1979). Degree days necessary for additional life stages and events are listed in *Table 2-5* on page 2-8.

Table 2-5 Developmental Threshold and Degree Days for *Cydia funebrana*¹

	Threshold,			
Stage	°C	DD	Notes	References
Egg	10	75	Lab study	Charmillot et al. (1979)
	11	Not speci- fied	Lab study	Butturini et al. (2000)
Larva	10	Not speci- fied	Lab study	Butturini et al. (2000)
	10	175	Lab study	Charmillot et al. (1979)
Pupa	10	160	Lab study	Charmillot et al. (1979)
	10.8	Not spec- ified	Lab study	Butturini et al. (2000)
Adult	Not speci- fied	280	96% emergence overwintering generation	Kocourek et al. (1995)
	Not speci- fied	380-420	5-10% emergence summer generation	Kocourek et al. (1995)
	5.8	Not speci- fied	Lab study, females	Butturini et al. (2000)
	10	30	First male moths caught in phero- mone traps in Swit- zerland	Charmillot et al. (1979)
	10	400-500	Second generation flight begins in Switzerland	Charmillot et al. (1979)
	10 ²	475-540	Flight of 1st summer generation in Hungary with 4 yr. average flight duration of 51 days	Sáringer and Deseo (1972)
	10 ²	810-900	Flight of 2nd summer generation in Hungary with 4 yr. avg. flight duration of 52 days	Sáringer and Deseo (1972)
Adult-Adult	10	390-410	10% ♂ emergence to 10% ♂emer- gence	Hrdy et al. (1996)
	Not speci- fied	387	Between flight peaks of 2 generations	Deseö (1971) in Hrdy et al. (1996)
Male Flight	10	290-320	Onset of flight summer generation; cumulative DD from Jan 1	Hrdy et al. (1996)

Table 2-5 Developmental Threshold and Degree Days for *Cydia funebrana*¹ (continued)

Stage	Threshold, °C	DD	Notes	References
	10	530-760	50% male emer- gence summer generation; differ- ent locations; cumulative DD from Jan 1	Hrdy et al. (1996)
Complete life cycle	10	420	Egg to first egg	Charmillot et al. (1979)

- 1 Venette et al. 2003.
- 2 Biological zero point under laboratory conditions.

How to Calculate Day Degree Values

Day degree values are based on the developmental threshold temperature of an insect and are species-specific. Threshold temperatures can represent either upper or lower limitations, and may be measurements of air or soil temperature, depending on where the insect lives.

To determine degree day values for a pest, use the equations below. For further information, refer to *Potential Distribution* on page 2-3.

Equation 1	Degree Days = [(Average Daily Temperature) – (Developmental Threshold)]	
Equation 2	Degree Days = [(Maximum Temperature + Minimum Temperature)/2] – (Developmental Threshold)	

Behavior

Cydia funebrana can have between 1 to 3 generations a year, as well as possible overlapping generations depending on climatic conditions (Molinari 1995; Molinari et al. 1997). A single generation with a partial second is reported to occur in England (Vernon 1971), two generations in the Czech Republic, Slovak Republic (Hrdy et al. 1996), Poland (Plucienniek et al. 1999) and Switzerland (Charmillot et al. 1979) and three generations in Yugslovia (Batinica 1970), Italy (Butturini et al. 2000), Armenia and Hungry (Popova 1971; Sáringer and Deseo 1972). In areas of multiple generations, it may be difficult to distinguish between the generations due to generational overlap (Sáringer and Deseo 1972; Venette et al. 2003).

Cydia funebrana overwinters as late instar larvae in bark crevices or the soil (Popova 1971; Whittle 1984) and pupate in the early spring (April). After pupation, the moths emerge in early to mid-spring depending on the weather conditions (Sáringer and Deseo 1972). The flight of the overwintered population occurs between April and June with the peak in May (Vernon 1971; Sciarretta 2001). In areas of multiple generations, a second flight may occur later in the summer, and in warmer areas, a third flight may also occur (Sciarretta 2001). The adult moths live 11 to 16 days (Popova 1971).

Cydia funebrana adults are most active between 18 and 22°C (Whittle 1984). The moths rest on the tree leaves during the day, becoming more active after sunset (Whittle 1984). The adult moths are generally sexually active before sunrise and lay most of their eggs in the evening (Charmillot et al. 1979). Most egg laying occurs at 25°C with about 3 to 5 eggs laid per fruit or fruit stalk (Popova 1971). Adult females average 50 eggs during their lifetime (Popova 1971). Eggs hatch in 1 to 2 weeks and the larvae immediately begin feeding on the fruit by burrowing into the flesh (Whittle 1984). Because the larvae bore into the fruit and use frass and webbing to block off the boring hole, they are difficult to control (Popova 1971). The larva remains in the fruit, and only leaves to pupate as a late instar larva (Popova 1971; Whittle 1984).

Diapause is influenced by photoperiod and the ability of an insect to withstand freezing temperatures (Milonas and Savopoulou-Soultani 2004). The diapause for *Cydia funebrana* is primarily influenced by photoperiod during the early larval stages (Sáringer 1967). *Cydia funebrana* diapause is induced by short day length ranging from <17 h; the critical photophase for inducing diapause occurs between 14 and 15 h (Sáringer 1967).

Economic Impact

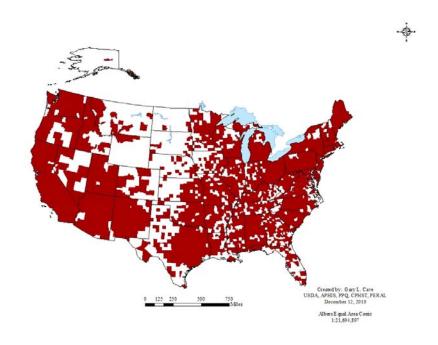
Fruit trees in the United States already suffer from the internal carpophagous feeder *Cydia pomonella* (L.), codling moth, as well as a large complex of tortricids (leafrollers) (Dunley et al. 2006). In the Palearctic region, *Cydia funebrana* is considered to be the key pest of plum (Charmillot et al. 1979) and if introduced into the United States, it could become another major economic pest.

Cydia funebrana causes economic damage as larvae by burrowing into fruiting structures. First generation fruit infection results in a bluish discoloration of the plum. These premature ripened fruits usually drop to the ground in June. Along with immature fruit drop, feeding and frass buildup makes the fruit unmarketable for the fresh market as well as processing (Dickler 1991). Andreev and Kutinokva (2008) reported that damage caused by the first generation of larvae can result in up to 12% damage while the second and third

generation feeding can result in greater than 30% damage. Additionally, Popova (1971) reviewed research articles in which accounts of economic damage caused by *Cydia funebrana* ranged upwards to 90% to plum crops.

Although *Cydia funebrana* is polyphagous, the primary hosts are in the Rosaceae family and more specifically, the *Prunus* spp, (Whittle 1984). A large portion of the U.S. contains *Prunus* spp. (*Figure 2-1* on page 2-4). There was approximately 263 million dollars of utilized production for plums and prunes in 2009 in California, Idaho, Michigan, Oregon and Washington combined. About 18 tons fresh plums per acre were produced resulting in 625,000 tons produced in those five states (NASS 2010). *Cydia funebrana* has also been described as also feeding on *Malus* spp, but less frequently. In the United States, apple acreage was 347,800 acres with a fresh market value at more than 2 billion dollars in 2009.

This pest has established populations in geographic areas with climates closely following the USDA Plant Hardiness Zones 2 to 11. This would cover most of the United States.



Source of data: USDA-NRCS 2010

Figure 2-3 Counties in the United States Containing Prunus species

Environmental Impact

Cydia funebrana is a common pest of the Rosaceae family. Secondary hosts include plants in the Fagaceae and Juglandaceae family (Venette, Davis et al. 2003). Additionally, chemical control programs may be initiated in the event of an introduction of *C. funebrana* in the United States, which may negatively impact non-target pests and the environment. Those plants included on the Federal List of Endangered and Threatened Plants (50 CFR 17.12) (USFWS 2011) that may be attacked by *C. funebrana* are listed in *Table 2-6* on page 2-12.

Table 2-6 Potential Host Plants of *Cydia funebrana* Included in List of Endangered and Threatened Plants (50 CFR 17.12)¹

Family	Species	Common Name	U.S. Range	Status ²
Fagaceae	Quercus hinckleyi C.H. Mull.	Hinckley's oak	TX	Т
Juglanda- ceae	Juglans jamaicensis C. DC.	Nogal or West Indian wal- nut	PR, Cuba, Hispaniola	E
Rosaceae	Acaena exigua A. Gray	Liliwai	HI	E
Rosaceae	Cercocarpus traskiae Eastw.	Catalina Island mountain- mahogany	CA	E
Rosaceae	Geum radiatum Michx.	Spreading avens	NC, TN	E
Rosaceae	Ivesia kingii var. ere- mica S. Watson	Ash Meadows ivesia	NV	Т
Rosaceae	Potentilla hickmanii Eastw.	Hickman's potentilla	CA	Е
Rosaceae	<i>Prunus geniculata</i> Harper	Scrub plum	FL	E
Rosaceae	Purshia subintegra (Kearney) Henrick- son	Arizona cliffrose	AZ	Е
Rosaceae	<i>Spiraea virginiana</i> Britton	Virginia spiraea	GA, KY, NC, OH, PA, TN, VA, WV	Т

¹ USFWS 2011.

² T = Threatened; E = Endangered.

Chapter

Identification

3

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Introduction

Use *Chapter 3 Identification* as a guide to recognizing the plum fruit moth, *Cydia funebrana* (Treitschke). Accurate identification of the pest is pivotal to assessing its potential risk, developing a survey strategy, and determining the level and manner of control.

Authorities

Qualified State, County, or cooperating University, personnel may perform the preliminary identification and screening of suspect *Cydia funebrana*. Before survey and control activities are initiated in the United States, an authority recognized by USDA–APHIS–PPQ-National Identification Services must confirm the identity of such pests. Submit specimens to the USDA-National Identification Services (NIS). For further information refer to *How to Submit Insect Specimens* on page C-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.

Diagnostic Aids

A paper by Baker (1963) gives detailed descriptions of identification for both *Cydia funebrana* and oriental fruit moth, *Grapholita molesta* (Busck).

Due to the difficulty in identifying many of these carpophagous species, researchers have developed molecular tools to distinguish some common species (Chen and Dorn 2009). For molecular identification of tortricids, refer to *Tortricidae Molecular Protocols* on page E-1.

Characteristics

Use the morphological characteristics described in this section to identify *Cydia funebrana*.

Adults

According to Dickler (1991):

The adult moth has a wingspan of 11-15 mm. Its dark-brown forewing is equipped with a shiny lead-grey "mirror" at the base, bearing four short, dark striae. The hindwing is grey, suffused with brown. In mixed populations, *C. funebrana* and *C. molesta* can only be distinguished from each other on the basis of their genitalia.

According to Bradley et al. 1979:

Labial palpus, frons grayish fuscous. Forewing mainly overlaid with fuscous brown except obscure pairs of white interspaces between poorly defined blackish-brown costal strigulae; fasciate marking blackish brown, indeterminate except outer edge of subbasal fascia weak dorsally; discocellular spot minute, indistinct,

White; distal area, especially ocellus, irrorate (tips of scales) with white or grayish white, similar irroration mediodorsally forms indistinct blotch; ocellus comprising usually four black dots, edged laterally by thick plumbeous stria on inner margin, thinner stria on outer margin; cilia concolorous with wing basally, otherwise gray, with black sub-basal line indented subapically. Hindwing fuscous, lighter basally and along termen; cilia grayish white, fuscous sub-basal line.

Distinguished from *C. tenebrosana* (Duponchel) (a tortricid moth) by darker grayish fuscous labial palpi and frons, and whitish irroration in distal and mediodorsal areas of forewing in *C. funebrana*.

According to Alford (1978):

Female genitalia (Fig. 2) of *C. funebrana*. Male genitalia of *C. funebrana* (Fig. 3) distinguished from *C. tenebrosana* by symmetrical projection on sacculus, and peglike projection at orifice of aedeagus. *C. tenebrosana*, the projection on the sacculus asymmetrical and directed towards the valva, and the aedeagus geniculate.



Peter Tilley, http://:www.moths-of-homes.info

Figure 3-1 Plum Fruit Moth Adult

Eggs

Eggs are about 0.7 mm across (Alford 1981). Dickler (1991) reported that each egg measures 0.7 x 0.6 mm and is flat, oval and transparent. They are lenticular-ovate and translucent white, becoming yellow later (Bradley et al. 1979).

Larvae

According to Dickler (1991):

The body of the full-grown larva has a reddish colour and reaches 10 to 12 mm in length. The head is dark chocolate brown, while the triangular prothoracic shield is a lighter shade of brown. The anal plate is light brown with darker markings and has a weak anal comb.

According to Baker (1963):

The larval head is dark brown to black in all instars. The body is white up to the last instar, when the body becomes bright pink as it matures.



R. Coutin, OPIE

Figure 3-2 Plum Fruit Moth Adult

Pupae

According to Dickler (1991):

The pupa is light brown and 6-7 mm long.

According to Baker (1963):

Pupa uniformly pale brown in colour, with labial palps and with prominent double transverse rows of spines on the dorsal surface of each abdominal segment. Only a few setae, no covering of short hairs. Spiracles on abdominal segment 2 relatively smaller and more nearly circular (diameter approximately 0.07 X 0.06 mm). Pupa with dorsal arc of spines on last abdominal segment composed of small sharply-pointed spines, but often only weakly developed. Lengths of larger spines usually less than one-fifth distance between dorsal end of anal scar and base of dorsal spines. Number of spines variable.

Similar Species

Anthophila fabriciana (L.) and Nola cucullatella (L.) appear similar to Cydia funebrana. There are also difficulties distinguishing between Grapholita molesta, oriental fruit moth, and C. funebrana unless dissecting the male genitalia. Baker (1963) gives a detailed description of the life stages for both the moths. Electronic versions of the screening aids are also available at the Web site of the Cooperative Agricultural Pest Survey (CAPS).



Survey Procedures

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Introduction

Use *Chapter 4 Survey Procedures* as a guide when conducting a survey for the plum fruit moth, *Cydia funebrana* (Treitschke).

Preparation, Sanitation, 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 inspection unsafe. 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 greenhouses.
- **2.** Conduct the survey at the proper time. The schedule should be on a regular time interval that coincides with weather and temperature conditions most suitable for *Cydia funebrana*. For further information, refer to *Developmental Rates and Day Degrees* on page 2-7.
- **3.** Obtain permission from the landowner before entering a property.
- **4.** Determine if quarantines for other pests of apple or other host crops are in effect for the area being surveyed. Comply with any and all quarantine requirements.
- **5.** When visiting apple orchard, nursery or landscape planting sites to conduct surveys or to take samples, everyone must take strict measures to prevent contamination by *Cydia funebrana* or other pests between properties during inspections. Before entering a new property, make certain that clothing and footwear are clean and free of pests and soil to avoid moving pests from one property to another. Wash hands with an approved antimicrobial soap.
- **6.** Before entering a new property, make certain that clothing and footwear are clean and free of pests and soil to avoid moving soil-borne pests and arthropods from one property to another. Wash hands. Change clothes if clothing is covered with insects.
- **7.** Mark the apple 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 infested host plant location so that the area or plant may be re-sampled if necessary.
- **8.** Survey task forces should consist of an experienced survey specialist familiar with the plum fruit moth and the symptoms of its damage.

Survey Types

Plant regulatory officials will conduct detection, delimiting, monitoring, targeted, traceback, trace-forward, and sentinel site surveys, for *Cydia funebrana*. The survey types are described in detail in this chapter. At the time of writing, pheromone trappings are the recommended survey method for *Cydia funebrana*.

Surveyors will also use the following common tools and techniques when surveying for this pest:

- ◆ Visual Inspection of Plants on page 4-8
- ◆ Sweep Net Sampling on page 4-10
- ◆ **Pheromone Lures** on page 4-11
- ◆ Trapping With Pheromone Lures on page 4-13

Detection Survey

Use a detection survey to determine whether a pest is present in a defined area where it is not known to occur. The detection survey can be broad in scope, as when assessing the presence of the pest over large areas or it may be restricted to determining if a specific pest is present in a focused area (i.e., a greenhouse).

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 the 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

Use the following tools singly or in any combination to detect the presence of *Cydia funebrana*:

- **1.** Check plants for the presence of the pest and its damage. Refer to *Visual Inspection of Plants* on page 4-8.
- **2.** Focus on high risk areas where *Cydia funebrana* is more likely to be found. See *Targeted Survey* on page 4-7 and for detailed information.
- **3.** Establish regular sites to inspect along your normal surveying route. See *Sentinel Site Survey* on page 4-7 for detailed information.

Delimiting Survey Following Initial U.S. Detection

If *Cydia funebrana* is detected in the United States, surveys will be conducted in the area to determine the distribution of the pest. Use a delimiting survey to determine the type and extent of control measures to apply. In large areas, locating the source of an infestation could be difficult.

Procedure

Use the procedure in *Detection Survey* on page 4-3 as a guide. Additional surveys should continue in nearby areas in order to determine the full extent of the infestation. Inspections should encompass continually larger areas particularly where hosts are known to occur. Surveys should be most intensive around the known positive detections and any discovered through traceback and trace-forward investigations, if possible.

Cydia funebrana primarily moves locally. Adult moths are capable of dispersing by flight, but the larvae do not disperse; upon hatching, the larva burrows immediately into the fruit (Popova 1971).

Table 4-1 Delimiting Survey Decision Table for Cydia funebrana

If you find:	In an area that is:	Take this action:	And supplement with:
One or more adults	ne or more adults Within the original infestation site		Visual survey
	Within a 1 mile square area	Set 36 traps per square mile in 9 square miles around the core area	Visual survey and trapping of 100 hosts per square mile in the 9 square mile area.
One or more (any stage)	Within a 6 square mile area	Set 36 traps per square mile in 25 square miles around the core area	Visual survey and trapping of 100 hosts per square mile in the 25 square mile area.

Use the site of the detection as the focal point. Begin by setting 36 traps per square mile in the core area where *Cydia funebrana* has been detected. Each block represents one square mile (*Figure 4-1* on page 4-5). Set out traps at the focal point and in each square mile in the first and second buffer areas in a standard grid array. In tree crops, traps should be suspended from tree limbs within the canopy for the highest number of moth catches (Gut et al. 2009). If traps are placed in a wild host, follow these guidelines to determine placement, but try to follow grid spacing as closely as possible.

Once a delimiting survey area has been established, the area beyond the last buffer zone will be trapped at a minimum rate of nine traps per square mile for two life cycles where hosts are available, up to 10 miles from the epicenter.

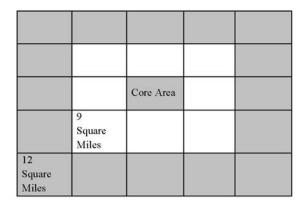


Figure 4-1 Trapping Scheme for Plum Fruit Moth

Traceback and Trace-Forward Surveys

Traceback and trace-forward investigations help surveyors to set priorities for delimiting survey activities after an initial detection. Use traceback investigations to determine the source of an infestation. Use trace-forward investigations to determine the potential dissemination of the pest, through means of natural and artificial spread (commercial or private distribution of infested plant material). Once a positive detection is confirmed, conduct investigations in order to determine the extent of the infestation or suspect areas in which to conduct further investigations.

Procedure

If this pest is found attacking nursery stock, surveyors should compile a list of facilities associated with nursery stock infested with *Cydia funebrana*. The lists will be distributed by the State to the field offices.

Important

The lists will be distributed by the State to the field offices, and are not to be shared with individuals outside USDA-APHIS-PPQ and State regulatory cooperators. Grower names and field locations on the 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 material infested with *Cydia funebrana*. Speak to the growers or farm managers and obtain proper permission before entering private property. If any sales or distribution has occurred from an infested nursery during the previous six months, surveyors should check nursery records to obtain names and addresses for all sales or distribution sites.

Infestations of *Cydia funebrana* may go undetected if populations are small and breeding insects are in the tree canopy, or resting on nearby plants. Typically, if a single moth is found in an area far removed from a port of entry or host plant, it is likely that it was transported to the site. The same is true for isolated detections during cool seasons. *Cydia funebrana* is inactive at air temperatures lower than 13°C (55°F) (Popova 1971).

Use wind field maps to plot the possible path of the *Cydia funebrana* adult moth. Calculate the estimated day and time of arrival (based on the circumstances at the site and likely air mass movements) and work backward in time and space to construct a logical path. Site circumstances that provide clues to the estimated time of arrival include the following detections:

- ◆ Associated with the arrival of a weather system
- ◆ Adults with no evidence of larval feeding
- ◆ Located inland at locations away from obvious ports of entry
- Populations that end abruptly outside a given area
- New generation or stage in the life cycle
- Sudden outbreaks or increases in numbers not associated with local breeding populations

Once the path of the moth is plotted, carry out surveys along the path until the likely introduction site is located. Likely origins include port environs, areas where over-wintering is possible, or agricultural areas where hosts are abundant. Allowing for the imprecision of this method, surveys add weight to conjecture about the origin of an introduction.

Computer generated atmospheric trajectory analyses are available to help identify potential sources of infestation and to trace the probable movement of plant pests with air masses. One such program is the Branching Atmospheric Trajectory (BAT). For further information, refer to *Resources* on page A-1.

Monitoring Survey

Perform a monitoring survey to determine the success of control or mitigation activities conducted against a pest.

Procedure

If *Cydia funebrana* 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 pest population.

Targeted Survey

Conduct targeted surveys at facilities associated with high risk pathways, and in areas where introduction of *Cydia funebrana* may be considered more likely. This may include orchards near ports of entry for fruit and nursery stock. Areas with regular traffic from countries with known infestations that may carry insect hitchhikers should also be targeted for regular surveys.

Procedure

At the time of publication, a defined method was unavailable.

Sentinel Site Survey

In case of *Cydia funebrana* introduction, sentinel sites may need to be established to monitor population spread. Cooperators and researchers can survey these areas during times of possible establishment to determine presence or absence of *Cydia funebrana* in an area.

Procedure

At the time of publication, a defined method was unavailable.

Visual Inspection of Plants

This section contains instructions for inspecting plants for infestations of *Cydia funebrana* as well as the damage caused by this pest. Refer to *Table 4-2* on page 4-8 for advantages and disadvantages of visual inspections.

Table 4-2 Advantages and Disadvantages of Visual Inspections

Advantages	Disadvantages
◆Locates pupae, eggs or larvae that would not be detected by other survey methods	◆Labor intensive
◆Inexpensive and simple	◆Time intensive
	◆Search efficiency varies greatly by habitat

Procedure

- **1.** Inspect plum trees or other potential host plants and nearby resting places for aggregations of *Cydia funebrana*. Review images of *Cydia funebrana* in *Characteristics* on page 3-2.
- **2.** Disturb plants if necessary to incite the flight of adults.
- **3.** Collect samples of tortridids while inspecting potential host plants. Review the images in *Characteristics* on page 3-2.

Important Do not move live insects from survey sites.

- **4.** Follow the instructions described in *Processing Samples* on page 4-13 when preparing specimens.
- **5.** Submit specimens and plant material to the proper authority. Refer to *How to Submit Insect Specimens* on page C-1 for further information.
- **6.** If *Cydia funebrana* is detected in an area, a technical working group for this pest will be assembled; the group will provide further guidance concerning additional surveys.

What to Look For

Check orchards, fencerows, nearby trees and other habitats for suitable hosts. Be sure to check field edges, since hosts favored by *Cydia funebrana* may be there, especially brambles. Areas with damaged or poorly growing plants should receive priority in the survey. Look for host fruits, berries, with a larva inside the fruit with a large amount of frass (excrement) near the entrance (Whittle 1984). Hosts from the core area are normally examined at the site.

Follow a similar sampling pattern for each field or orchard surveyed. Collect samples at least 75 feet from the edge of 5 different locations (*Table 4-2* on page 4-9).

At each sample location, inspect at least 10 plants from 3 adjoining rows (or at equally spaced intervals). Note that *Cydia funebrana* larva feeds internally on the fruit of the host, resulting in many external symptoms. It may be helpful to search for some of the following: plants showing signs of poor growth; rotting or abnormally fallen fruit or leaves; holes in fruit; adults hidden in foliage.

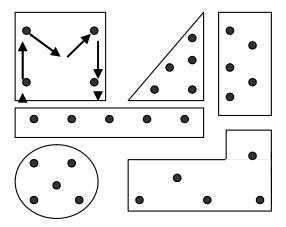


Figure 4-2 Standard Survey Sampling Pattern

Visual Symptoms

Look for eggs on the surface of the fruit, leaves or on the base of the stalks (Whittle 1984). The infested fruit will have holes in the fruit near the stalks (Whittle 1984). Infested fruit may ripen faster (Vernon 1971). The feeding tunnels will be full of frass (excrement) (Whittle 1984). Refer to *Figure 4-3* on page 4-10.







Source: R. Coutin, OPIE

Figure 4-3 Cydia funebrana Larval Damage on Plum

Feeding damage by *Cydia funebrana* on fruit usually appears as a boring hole in the fruit with gummy ooze or frass. In the process of host examination, the surface of the fruits, berries, twigs, stems, and leaves of the host plants should be examined for eggs and fruit should be cut open and examined for larvae.

Important

Any specimens collected should be held in colony for at least one life cycle of *Cydia funebrana*. The facility where the samples are held must be secure to prevent any inadvertent release of moths. Security measures must be equal to those established for a quarantine insect rearing facility.

Sweep Net Sampling

Sweep-net sampling will be effective for sampling of *Cydia funebrana* adults if the host being sampled is one of the hosts listed in *Table 2-3* on page 2-5 and *Table 2-4* on page 2-6, or any other host discovered during program operations. Look for fruit that indicate larval feeding and since larvae burrow into the fruit, sweep-nets will not be an effective tool for collecting larvae. Sweeping may be a useful method for collecting *Cydia funebrana* adults only. Sweeping at dusk or dawn, in synchrony with adult activity, will produce the best yield.

Sweep net sampling can be performed in combination with visual inspection. While walking forward, swing the net rapidly from side to side over the tops of the foliage. A typical sample unit is 25 to 100 sweeps (*Figure 4-4* on page 4-12). When performing aerial sweeps for adults, move the net in a horizontal figure-8 path, passing the handle from hand to hand at the body mid-point during the down stroke.

Similar Pest Species

If damaged or stuck on a sticky trap, then many species may appear similar to *Cydia funebrana* (Alford 1978).

Pheromone Lures

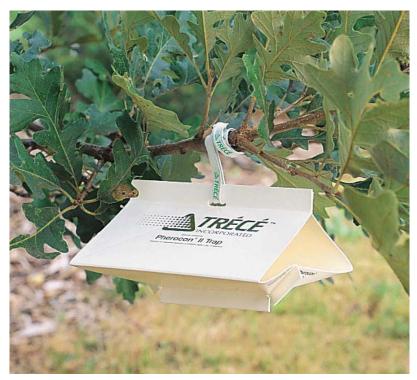
Many pheromone lures are available commercially to monitor pest populations. Pheromone traps are commonly used to monitor activity of *Cydia funebrana* (*Table 4-3* on page 4-11) (Cravedi and Molinari 1993; Hrudova 2003). Paper delta traps (*Figure 4-4* on page 4-12) have been used to monitor *C. funebrana* populations using Z-8-dodecene-1-yl acetate, E-8-dodecene-1-yl acetate. These traps were placed in the middle of the fruit tree crowns at about 20 m apart (Hrudova 2003). Pheromone trapping is also used to determine if the pest has reached the threshold level of ten males per trap (Molinari 1995). Hrdy et al. (1996) used pheromone trap catch data to create a predictive model for flight activity of *C. funebrana*. Additionally, mass trapping using pheromone based glue traps has been successful at reducing *C. funebrana* populations (Koltun and Yarchakovskaya 2006). The use of pheromone traps for monitoring *C. funebrana* populations may be useful upon introduction into the United States.

Table 4-3 Pheromones for Cydia funebrana Trapping

Compound	Abbreviation	Reference
Dodecyl acetate, <i>E</i> -8-Dodecenyl acetate, <i>Z</i> -8-Dodecenyl acetate, <i>Z</i> -8-Dodecen-1-ol, Tetradecyl acetate, <i>Z</i> -8-Tetradecenyl acetate, <i>Z</i> -10-Tetradecenyl acetate, Hexadecyl acetate, Octadecyl acetate, Eicosyl acetate	12:Ac, E8-12:Ac, Z8- 12:Ac, Z8-12:OH, 14:Ac, Z8-14:Ac, Z10-14:Ac, 16:Ac, 18:Ac, 20:Ac	Guerin et al. (1986)
Z-10-Dodecenyl acetate	Z10-12:Ac	Arn et al. (1974)
Z-8-Dodecenyl acetate	Z8-12:Ac	Granges and Baggiolini (1971)

Table 4-3 Pheromones for Cydia funebrana Trapping

Compound	Abbreviation	Reference
Z-8-Dodecenyl acetate, E-10-Dodecenyl acetate	Z8-12:Ac, E10-12:Ac	Witzgall et al. (1996)
Z-8-Dodecenyl acetate, E-8-Dodecenyl acetate	Z8-12:Ac, E8-12:Ac	Arn et al. (1976), Biwer and Descoins (1978)



Source: Gemplers, 2010

Figure 4-4 Paper Delta Trap

Trapping With Pheromone Lures

Pheromone trapping can be used to determine the presence of an insect (*Table 4-4* on page 4-13) (Venette et al. 2003). Lures for *Cydia funebrana* are available commercially. The gray rubber septum dispenser has a 2 to 12 week length of effectiveness depending on the brand and external temperature (Gut et al. 2009).

Table 4-4 Advantages and Disadvantages of Trapping with Pheromone Lures

Advantages	Disadvantages
◆Specific attractant	◆Effectiveness may depend on climatic or wind conditions
◆Low maintenance	◆May be phase dependant
◆Active but does not require energy input	

Processing Samples

This section contains instructions for preparing and shipping insect and plant specimens.

Preparing Samples

Preserve *Cydia funebrana* larva in 70 per cent isopropyl alcohol and sent for identification and preservation. Adults should be pinned or sent in cotton to not damage identifiable characteristics on the wings.

Shipping Samples

Call the laboratory prior to shipping the samples via overnight delivery service. Instructions and contact information are located in *How to Submit Insect Specimens* on page C-1 and *Taxonomic Support for Surveys* on page D-1.

Data Collection

Recording negative results in surveys is just as important as positive detections since it helps define an area of infestation. A system of data collection should include an efficient tracking system for suspect samples such that their status is known at various stages and laboratories in the confirmation process. If available, use pre-programmed hand-held units with GPS capability. Data collected during surveys should include the following:

♦ Date of survey

- ◆ Collector's name and affiliation
- ◆ Full name of business, institution, or agency
- ◆ Full mailing address including country
- ◆ Type of property (commercial nursery, hotel, natural field, residence)
- GPS coordinates of the host plant and property
- ♦ Host species and cultivar
- General conditions or any other relevant information
- Positive or negative results from specimen collection

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize infestations of *Cydia funebrana*. Large larval populations feeding on host plants may occur on host plants during the spring and summer.

Chapter 5

Regulatory Procedures

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Introduction

Use *Chapter 5 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against the plum fruit moth, *Cydia funebrana* (Treitschke).

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, Sanitation, 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 *Table A-1* on page A-1 for information on identifying PPQ's Tribal Liaison.

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

Overview of Regulatory Program After Detection

Once an initial U.S. detection is confirmed, holds will be placed on the property by the issuance of an Emergency Action Notification. Immediately 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

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Introduction

Use *Chapter 6 Control Procedures* as a guide to controlling the plum fruit moth, *Cydia funebrana* (Treitschke). Consider the treatment options described within this chapter when taking action to eradicate, contain, or suppress the plum fruit moth.

Carpophagous insects are low threshold pests (Dorn and Pinero 2009), requiring an extensive management program. A successful integrated pest management (IPM) system will consider chemical, biological and cultural techniques to reduce pest populations.

Researchers recommend a variety of insecticide classes to control *Cydia funebrana*. These include insect growth regulators, organophosphates and pyrethroids. Biological insecticides including *Bacillus thuringiensis* Berliner, baculoviruses and spinosad can also be integrated into a management program. Biological control organisms are present in the environment and may also help reduce *C. funebrana* population.

The biology of *C. funebrana* is similar to that of *Cydia pomonella* (L), codling moth; therefore, control measures for codling moth have also been researched for this section. A successful IPM program will consider chemical, biological and cultural techniques to reduce pest populations.

Overview of Emergency Programs

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

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

Treatment Options

Consider the treatment options described within this chapter when taking action to eradicate, contain, or suppress *Cydia funebrana*. There are various chemical control measures available for use against *C. funebrana*, although it has been found that codling moth and many species of leafrollers and are developing resistance to insecticides used in various regions of the world (Dunley et al. 2006; Kehrli et al. 2009; Sial et al. 2010).

All treatments listed in the guidelines should only be used as a reference to assist in the regulatory decisionmaking process. It is the National Program Manager's responsibility to verify that treatments are appropriate and legal for use. Upon detection and when a chemical treatment is selected, the National Program Manager should consult with PPQ's FIFRA Coordinator to ensure that the chemical is approved by EPA for use in the United States prior to application.

Eradication

Eradication is the first action to consider with the introduction of a new pest. Eradication may be feasible under some conditions, but if it fails then other strategies will be considered. Eradication may be feasible when the following conditions exist: pest population is confined to a small area, detection occurs soon after the introduction, or pest population density is low.

If an infestation of *Cydia funebrana* is discovered that meets the above named conditions, eradication will be attempted. Measures will include but may not be limited to removal and destruction of all infested plant material, removal of host material within 2 miles (3.2 km) of the find, and treatment of the soil and surrounding vegetation with an approved pesticide after removal of the infested plants.

Treatment Area

Table 6-1 Decision Table for Eradication Treatment Area of Plum Fruit Moth

If this number PFM:	Are detected in an area of this size:	Then treatment will commence and extend:
1 to 5 larvae, pupae or gravid females OR 2 to 5 males or virgin females	Less than 6 square miles	200 yards beyond the detection
6 or more of any stage	Greater than 6 square miles	2½ miles beyond the detection

Cultural Control

Sanitation

When visiting fields to conduct surveys or take samples, everyone (including regulatory officials) must take strict measures to prevent contamination by *Cydia funebrana* between properties during inspections. Before entering a new property make certain that footwear and clothing are clean and free of soil and insects to avoid moving *C. funebrana* from one property to another.

Carry out sanitation in nurseries, gardens, landscapes, fields, and other establishments where hosts are present within the core and buffer areas. Clear out all infected fruit and debris from orchards (Scholz 2009). Depending on the circumstances and equipment available, use the following techniques:

- Clean cultivation
- Burning of host plants
- Field sanitation

Mulching

Mulching may be used to enhance alternative hosts for parasitoids of *Cydia funebrana* and to improve, the natural control of this species. Mulching methods which encourage undergrowth of nectar plants and habitat conditions (vegetation height) in the orchard on longevity, fertility and abundance (habitat preference) of parasitoids should be investigated. Based on such data, vegetation management might be used as a tool to enhance parasitoid efficiency in biological control (Kienzle et al. 1997).

Insecticides

Scholz (2009) recommends using forecasting models and pheromone monitoring of *Cydia funebrana* to determine appropriate insecticide spray applications. For all applications, it is important to coincide with the appropriate life stage in the life cycle.

The insecticides used to control *C. funebrana* are summarized in *Table 6-2* on page 6-5.

Important

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

Table 6-2 Insecticides Used to Control *Cydia funebrana* and Available for Use in the United States

MOA	Chemical	Pome ¹	Stone ²	U.S. ³	Comments	Reference
4A	acetamiprid	Yes	Yes	Yes	Tested on codling moth in the U.S.	Dunley and Welter (2000)
11	Bacillus thuringiensis	Yes	Yes	Yes	Tested on codling moth in U.S. and PFM	Tălmaciu et al. (2006), Brunner et al. (2010)
11	Bacillus thuringiensis aizawai	Yes	Yes	Yes	Tested on leafrollers.	Pollini (2009)
11	Bacillus thuringiensis kurstaki	Yes	Yes	Yes	Tested on PFM	Tălmaciu et al. (2006), Pollini (2009)
3A	Lambda-cyha- lothrin	Yes	Yes	Yes	Tested on PFM	Tălmaciu et al. (2006)
28	chlorantranilip- role	Yes	Yes	Yes	Tested on leafrollers in U.S. pre-bloom only	Sial and Brunner (2010)
1B	chlorpyrifos	Yes	Yes	Yes	Tested on leafrollers in the U.S. pre- bloom only	Dunley et al. (2006)

Table 6-2 Insecticides Used to Control *Cydia funebrana* and Available for Use in the United States (continued)

	the officed 5					
MOA	Chemical	Pome ¹	Stone ²	U.S. ³	Comments	Reference
6	emamectin benzoate	Yes	No	Yes	Tested on codling moth in the U.S.	Brunner et al. (2010)
7B	fenoxycarb	No	No	Yes	Tested on PFM and codling moth. Approved on ornamen- tals and some flow- ers.	Charmillot (1991), Charmillot (1992), Cross (1997)
22A	indoxacarb	Yes	Yes	Yes	Tested on codling moth in U.S. and PFM	Olszak and Plucien- nik (2001), Charmil- lot et al. (2006), Dunley et al. (2006), Brunner et al. (2010)
N/A	kaolin clay	Yes	Yes	Yes	Tested on coding moth in the U.S.	Brunner et al. (2010)
18	methoxyfeno- zide	Yes	Yes	Yes	Tested on codling moth in U.S., PFM and lea- frollers.	Olszak and Plucien- nik (2001), Bylemans et al. (2003), Cantoni et al. (2004); Dunley et al. (2006); Brunner et al. (2010)
1B	methyl-para- thion	No	No	Yes	Tested on leafrollers in the U.S.	Dunley et al. (2006)
7C	pyriproxyfen	Yes	Yes	Yes	Tested on leafrollers and codling moth in the U.S.	Brunner et al. (2010)
5	spinetoram	Yes	Yes	Yes	Tested on leafrollers in U.S.	Sial et al. (2010)
5	spinosad	Yes	Yes	Yes	Tested on codling moth and plum fruit moth.	Olszak and Plucien- nik (2001)

¹ Approved on pome fruit.

² Approved on stone fruit.

³ Approved in the United States.

Oxadiazine

Indoxacarb

Indoxacarb is effective through both oral and dermal contact; it blocks the sodium channels in the nervous system. As a newly registered insecticide in the United States, it is registered for use on pome fruit to control leafrollers and codling moth. Research has determined that it is not an effective control of codling moth (Brunner et al. 2010). Due to *Cydia funebrana*'s similar biology, it would likely also be as ineffective on the plum fruit moth as on the codling moth.

Insect Growth Regulators

An insect growth regulator (IGR) is a compound that mimics a natural chemical that is produced by the insect. IGR's mimic insect hormones and therefore interrupt normal biological processes. Applications of these compounds can lead to premature molts and growth deformities. IGR's have been tested for efficacy against tortricids and have been found to be effective (Charmillot et al. 2001; Bylemans et al. 2003; Brunner et al. 2010).

Difluenzuron

Diflubenzuron is a chitin synthesis inhibitor. It has been reported as being as efficacious as both methoxyfenozide and indoxacarb at the rates of 0.5 l/ha and 0.2 kg/ha (Olszak and Pluciennik 2001). It is also an effective ovicide against codling moth eggs, but there have also been documented reports of resistance development in Europe. As an insecticide, diflubenzuron is a better ovicide than larvicide (Charmillot et al. 2001).

Methoxyfenozide

Methoxyfenozide is a molt accelerating compound (MAC) and must be ingested to be toxic. It is lethal to lepidopteran larva and may have ovicidal properties against the eggs (Bylemans et al. 2003; Brunner et al. 2010). Hoelscher and Barrett (2003) also reported that application of this compound disrupts adult moth communication and female reproduction. These sublethal effects can play an important role in the control of *Cydia funebrana*.

Timing applications of methoxyfenozide simultaneously with egg hatch and egg lay may offer a new approach to codling moth control (Charmillot et al. 2001; Brunner et al. 2010) and decrease adult female fecundity (Bylemans et al. 2003). Methoxyfenozide is an effective ovicide and larvicide at LC₅₀ values of about 0.6 and 0.8 ppm, respectively on codling moth (Charmillot et al. 2001). In commercial apple orchards in Poland, methoxyfenozide reduced *C. funebrana* damage by 49 percent at 0.4 kg/ha, but up to 85 percent for 0.5 kg/ha (Olszak and Pluciennik 2001). Additionally, applications of methoxyfenozide resulted in control of eggs and larvae of codling moth (Bylemans et al. 2003). Methoxyfenozide has low toxicity to bees and natural

enemies on pome fruit including lacewings, parasites and predatory bugs (Bylemans et al. 2003).

Resistance to Methoxyfenozide and Diflubenzuron

Insecticides containing methoxyfenozide are approved for use in the United States. However, in Washington state, methoxyfenozide is regularly applied to apple trees to control codling moth. These frequent applications resulted in reports of codling moth resistance to methoxyfenozide (Dunley and Welter 2000; Brunner et al. 2010). Cross-resistance between methoxyfenozide and diflubenzuron has also been described for codling moth (Bylemans et al. 2003). Similar results may be exhibited in *Cydia funebrana*; therefore, research to control resistance development should be a priority.

Tebufenozide

Tebufenozide is an ecdysone agonist. It is selective primarily to lepidopteran pests and is thus harmless to beneficials (Dhadialla et al. 1998). This product is has not been found to be effective at controlling codling moth in Washington State (Brunner et al. 2010). Brunner et al. (2010) reported that is not as effective on leafrollers as methoxyfenozide. It also may require two applications to control higher densities of leafrollers. Additionally, Charmillot et al. (2001) reported that it is much more effective as a larvicide than an ovicide and determined that the LC₅₀ is 0.4 ppm on codling moth larva. There have also been reports of tebufenozide resistance developing in some leafrollers in Europe (Cross 1997). At the time of writing this document, no literature was unearthed of the effects of tebufenozide on *Cydia funebrana*.

Fenoxycarb

Fenoxycarb, an IGR, works as a juvenile hormone mimic causing premature and deformed molting to occur. It has been used in Europe to control codling moth on fruit trees since the 1980s (Cross 1997). Charmillot et al. (2001) reported that fenoxycarb was an excellent ovicide of codling moth eggs with an LC₅₀ value of 0.05 ppm. Charmillot (1991) also reported that fenoxycarb is good for ovicidal control of *Cydia funebrana*, however a complete understanding of the biology is necessary for proper ovicidal control.

Due to its broad-spectrum activity, this insecticide can also have deleterious effects to natural enemies (Dhadialla et al. 1998). In the United States, fenoxycarb has not been approved for use on tortricids in apples and pears. It is approved for use on ornamentals, flowers, and non-bearing citrus, fruit and nut trees. If *C. funebrana* is introduced to the United States, fenoxycarb may be approved for a Section 18.

Pyrethroids

Pyrethroids are nerve poisons that activate the sodium channels. The effects of pyrethroids have been tested on codling moth (Dunley and Welter 2000), oriental fruit moth (Trimble et al. 2001) and plum fruit moth (Tălmaciu et al. 2006). Tălmaciu et al. (2006) reported that pyrethroids performed better than *Bt* at controlling *Cydia funebrana* with up to 100 percent control of their populations.

Organophosphate Resistance Management

The use of broad-spectrum organophosphate applications in tree fruit is being phased out (Sial and Brunner 2010). The primary reason for the phase-out is because resistance and cross resistance has been documented for codling moth to organophosphates (Dunley and Welter 2000). In Washington state, resistance to the organophosphate, azinphosmethyl, was found in some populations of codling moth and the leafrollers *Choristoneura rosaceana* (Harris) and *Pandemis pyrusana* Kearfott. Dunley et al. (2006) also reported that these tortricids exhibited cross-resistance to the organophosphate, azinphosmethyl, to the IGRs, tebufenozide and methoxyfenozide.

Azinphosmethyl has been applied and successfully used to control *Cydia funebrana* (Vernon 1971). Researchers recommend that an appropriate cross resistance program would consist of not applying insecticides of the same class against two consecutive generations.

Biological Insecticides

Spinosad

Spinosad was derived from a soil bacterium. It acts as a nerve poison and is effective via ingestion or contact. It is most efficacious against Lepidoptera, Diptera and Thysanoptera. Upon application to crops, spinosad is highly toxic to bees. After drying, however, it no longer causes mortality to bees (Thompson et al. 2007). It is a relatively selective insecticide and has low activity against many predatory mites and bugs (Anthocoridae) (Bylemans and Schoonejans 2000).

Spinosad has been reported as being successful in controlling both the codling moth and plum fruit moth (Pluciennik and Olszak 2005). Two applications of spinosad during a growing season offered 94 percent control of plum fruit moth when compared to an untreated orchard in Poland; it was also considered to be as effective as the organophosphate, fenitrothion (Olszak and Pluciennik 2001). However, researchers at Washington State University indicate that spinosad was effective on leafrollers, but not codling moth (Brunner et al. 2010). Sail et al. (2010) and Sail and Brunner (2010) documented that there is

cross resistance between spinosad and spinetoram for the obliquebanded leafroller *Choristoneura rosaceana* (Harris).

Bacillus thuringiensis

Bacillus thuringiensis Berliner (Bt) is a naturally occurring bacterium that is considered to be an effective insecticide against Lepidoptera (van der Geest 1971; van der Geest 1981). There have been mixed results reported of Bt efficacy on Tortricids (Olszak and Pluciennik 2001). Research performed at Washington State University determined that Bt is currently effective against leafrollers in the United States, but was ineffective against codling moth (Brunner et al. 2010). Conversely, Bt reduced damage by codling moth up to 98 percent in commercial apple orchards in Poland (Olszak and Pluciennik 2001).

There are two forms of Bt available: *Bacillus thuringiensis aizawai* and *Bacillus thuringiensis kurstaki*, both are available for use in the United States, and both have been tested on leafrollers (Pollini 2009).

Fumigation

Follow the guidelines in the USDA-APHIS-PPQ Treatment Manual (PPQ, 2009) for using methyl bromide fumigation.

Biological Control

Biological control organisms help suppress and control pest populations, but they do not eradicate them. These organisms can be effective when used in combination with other IPM techniques. They are characterized as predators, parasites, parasitoids, or pathogens. There are many parasitoids documented for *Cydia funebrana* (*Table 6-3*).

Egg Parasitoids

Trichogrammatidae is the only family of Hymenoptera that parasitizes and emerges from tortricid eggs (Cross, Solomon et al. 1999). The rearing and release of egg parasitoids have been successfully used to control Lepidopteran pests (Wiackowski and Wiackowska 1966; Hassan 1992; Li 1994). Wiackowski and Wiackowska (1966) reported that *Trichogramma* release performed better than synthetic insecticides tested including an organochlorine and organophosphate. They also determined that extensive releases of the parasitoid, *Trichogramma cacoeciae* March., resulted in almost 90 percent more egg parasitism of *Cydia funebrana* than the untreated control. The parasitoid release also reduced economic damage more than 90 percent if the parasitoids were released for both generations. The release of the parasitoids must coincide with *C. funebrana* egg lay to attain high parasitism rates.

In the 1960s, the mass production of the parasitoids was more efficient than using synthetic insecticides. More recently, a mass rearing program of Trichogramma egg parasites was completed by Hassan (1993). However, the current cost of mass egg parasitoid rearing is too expensive when compared to the application of insecticides. Rearing programs for *Trichogramma* have been successful worldwide to reduce populations of the European corn borer *Ostrinia nubilalis* (Hb.) (Hassan 1993; Smith 1996).

Larval Parasitoids

Rhabditida

In Russia, the nematode, *Neoaplectana carpocapsae* Weiser, of the Steinernematidae family, was applied to plum trees at a dosage of 1 × 106 per tree and achieved 100 percent control of *Cydia funebrana* larvae (Sledzevskaya, 1980).

Hymenoptera

Several parasitoids have been collected from the larvae of *Cydia funebrana*. The most common recorded parasitoid is Ascogaster quadridentata Wesm.. It has been documented in Turkmenistan as 80 percent of reported parasitoids (Saparmamedova, 1988b), Yugoslavia as 30 percent (Batinica and Muratovic, 1972), and Switzerland as about 10 to 20 percent (Bovey 1937). Other parasitoids include *Ephialtes* spp. (Bovey, 1966) and *Macrocentrus instabilis* Muesebeck (Muesebeck, 1932).

Pupal Parasitoids

There are very few documented pupal parasitoids for *Cydia funebrana*. However, lepidopteran pupal parasitoids are very common and there are probably multiple that exist for *C. funebrana*.

Fungi

Wiackowsk and Wiackowska (1966) tested the efficacy of the entomopathic fungi, *Beauveria* spp., against *C. funebrana*. It was less effective than conventional insecticides with only 21 to 68 percent control.

Viruses

There have been viruses developed for control of codling moth (Laceya, Thomsonb et al. 2008), but not plum fruit moth. The codling moth granulosis viruses have been reported as infecting *Cydia funebrana* (Zimmermann and Weiser 1991), but efficacy was not discussed.

Table 6-3 Biological Control Agents Active Against the Plum Fruit Moth

Action	Family	Species	Reference
Egg para- sitoid	Hymenoptera: Trichogrammati- dae	Trichogramma buluti Bulut & Kilincer, Trichogramma cacoeciae Marchal, Trichogramma dendrolimi (Matsumura), Trichogramma embryophagum Hartig; Trichogramma evanescens Westwood, Trichogramma kilinceri Kostadinov, Trichogramma spp., Trichogramma telengai Sorokina, Trichogramma turkeiensis Kostadinov	Bulut and Kilinçer (1989), CABI (2010), Huber and Hassan (1991), Noyes (2011), Wiackowski and Wiackowska (1966)
Egg-lar- val para- sitoid	Hymenoptera: Braconidae	Ascogaster quadridentata Wesm.	Bovey (1966), Batinica and Muratovic (1972), CABI (2010), Fitton et al. (1988), Saparmamedova (1988), Saparmamedova (1988)
Larval parasitoid	Hymenoptera: Braconidae	Apanteles spp., Bracon hebetor Say, Macrocentrus instabilis Muesebeck	CABI (2010), Muese- beck (1932), Sapar- mamedova (1988), Vernon (1971)
	Hymenoptera: Ichneumonidae	Angitia exareolata Ratzeburg, Ephialtes sp., Liotryphon punctu- latus (Ratz.), Mastrus spp., Mesostenus transfuga Graven- horst, Scambus elegans (Woldst- edt), Venturia canescens (Gravenhorst)	Bovey (1966). CABI (2010), Fitton et al. (1988), Saparmame- dova (1988)
	Diptera: Tachini- dae	Pseudoperichaeta nigrolineata Walker	CABI (2010)
Larval- pupal par- asitoid	Hymenoptera: Chalcididae	Brachymeria rugulosa (Forster)	CABI (2010), Sapar- mamedova (1988)

Table 6-3 Biological Control Agents Active Against the Plum Fruit Moth

Action	Family	Species	Reference
Pupal par- asitoid	Hymenoptera: Ichneumonidae	Pimpla spuria (Gravenhorst)	Saparmamedova (1988)
Parasitoid	Hymenoptera: Chalcididae	Hockeria micula (Nikol'skaya)	CABI (2010), Sapar- mamedova (1988)
Larval nematode	Rhabditida: Steinernemati- dae	Neoaplectana carpocapsae Weiser	Sledzevskaya (1980)
Larval- adult pathogen	Hypocreales: Cordycipitaceae	<i>Beauveria bassiana</i> (BalsCriv.) Vuill	CABI (2010), Wiack- owski and Wiack- owska (1966)
Larval parasitoid	Hymenoptera: Encyrtidae	Copidosoma varicorne	Noyes (2011)
Parasitoid	Hymenoptera: Eulophidae	Hyssopus nigritulus, Tetrastichus sp.	Noyes (2011)
	Hymenoptera: Eurytomidae	Eurytoma verticillata (F.)	CABI (2010)
	Hymenoptera: Pteromalidae	Dibrachys cavus (Walker)	Saparmamedova (1988), CABI (2010)

Sterile Insect Technique

Sterile insect technique (SIT) is an effective tool in certain eradication and suppression programs. SIT employs radiation to sterilize large numbers of male insects. When released, the sterilized insects compete with the viable males. This is most effective when the targeted female mates only once in her lifetime. Upon mating with a sterile male, the female will lay sterile eggs, thereby reducing the reproductive success of the pest. Many factors determine if a particular insect is a good candidate for SIT, including its competitiveness after irradiation, ability to be reared in large numbers, F1 sterility and the development of a pheromone for monitoring (Dyck, Hendrichs et al. 2005).

Sterile insect technique has been researched for codling moth (Carpenter, Bloem et al. 2010). Currently, there is ongoing research for improving SIT for lepidopterans (Simmons, Suckling et al. 2010; Vreysen, Carpenter et al. 2010), but to date there are no stocks of sterile *Cydia funebrana* males available. However, there is a successful SIT program for codling moth in British Columbia, and for pink bollworm in San Joaquin Valley, CA (Bloem, Bloem et al. 2005).

Mating Disruption

Mating disruption has been successful at reducing insecticide use for codling moth and plum fruit moth (Pollini 2009). At low populations it may be possible to reduce damage with pheromones, but still difficult to get damage below economic threshold (Van der Geest and Evenhuis 1991). Charmillot and Blaser (1982) reported successful control with mating disruption resulting in 97 percent control in Switzerland. There have been other additional successful attempts in the Netherlands using mating disruption as a method to control *Cydia funebrana* (Brouwer and van Doornspeek 2008). Alternatively, pheromone traps (600/ha) were placed in trees in plum orchards in Switzerland during a high populations of *C. funebrana*. These traps were not found to be effective in controlling *C. funebrana* and resulted in over 60 percent of the fruit being attacked by harvest (Arn, Delley et al. 1976).

Andreeve and Kutinova (2008) reported that 2 to 3 applications of insecticides per generation are required to control *C. funebrana* resulting in 7 to 8 treatments per year. Chitin synthesis inhibitors were found to not reduce the number of applications, but a combination of mating disruption and systemic insecticides reduced damage by *C. funebrana* below economic injury level (EIL) (< 1.5 percent). The insecticides were only applied when *C. funebrana* damage went above EIL.

In California pear orchards, there has been a successful transition from broad-spectrum insecticides to mating disruption for the control of codling moth (Varela and Elkins 2008). Researchers discovered that by the third year of a mating disruption program, growers were saving up to \$500 per year as compared to conventional insecticides. This control method also reduced the use of organophosphates in the orchards. Judd and Gardiner (2008) determined that the use of Isomate-CM/LR was successful for disrupting mating and control of codling moth and leafrollers *Choristoneura rosaceana* (Harris) and *Pandemis limitata* (Robinson) in British Columbia, Canada. It reduced mating and damage up to 98 percent. It is feasible that a future mating disruption program may help in monitoring and control of *C. funebrana*.

Attract and Kill

The attract and kill method is a valuable IPM technique that combines the use of pheromones and insecticides. This technique is used to reduce populations, but does not substitute for survey trapping (Kirsch, Booysen et al. 2001). To implement this method, both pheromones and insecticides are applied in droplet form to the upper canopy of an orchard. The pheromones attract the male moths to the orchard where they are confused by the amount of pheromones and killed by the insecticide anon.

This technology has been used on codling moth in Washington. It was reported as an effective form of IPM that is less disruptive to predatory insects and mites (Knight 2010). With any IPM method, insecticides need to be applied appropriately with their biological cycle (Knight 2010).

A different method of mass trapping involved sticky glue traps infused with pheromone resulted in decreasing plum fruit moth damage by 84 percent after 5 years (Koltun and Yarchakovskaya 2006).

Pheromones

Mate-find and reproduction by *Cydia funebrana* is a chemically-mediated process. *Cydia funebrana* has more than one compound as its sex pheromone (El-Sayed 2010). There are six documented pheromones of *C. funebrana* (Table 4.5) which are available commercially (http://www.pherobase.com/database/species/species-Grapholita-funebrana.php).

Pheromone lures are commonly used for monitoring, detection (Cravedi and Molinari 1993; Cross 1996) and control (attract and kill) (Kirsch, Booysen et al. 2001). They may also be used in mating disruption by interfering with the ability of the male to find the female, thereby, disrupting the reproductive success of *Cydia funebrana*. Large concentrations of the pheromones are required to confuse the adults.

Summary

The most effective control program for suppression of *Cydia funebrana* likely incorporates the use of cultural control measures (e.g. removing and destroying infested plants) and chemical control of the residual population.

If an established population is found in an apple production area, a science advisory panel will be asked to determine the best course of action. If

eradication is not possible, as determined by the science advisory panel, it will be the responsibility of university extension services to determine the best management practices.

Environmental Documentation and Monitoring

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

Chapter

Environmental Compliance

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Introduction

Use *Chapter 7 Environmental Compliance* as a guide to the plum fruit moth, *Cydia funebrana* (Treitschke).

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 general public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed Federal actions intersect with Tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act

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

Chapter

Pathways

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Commodity Imports 8-1
Risk of Establishment 8-2

Introduction

Use *Chapter 8 Pathways* as a source of information on the pathways of introduction of the plum fruit moth, *Cydia funebrana* (Treitschke).

Natural Movement

The known range of *Cydia funebrana* in Europe and Asia means that this pest cannot get to the United States on its own through migratory patterns or other natural means of spread. Additionally, if introduced into the United States, *C. funebrana* would primarily move locally and not disperse great distances.

Commodity Imports

The USDA lists *Cydia funebrana* as a quarantined actionable pest. Officers with USDA-APHIS and the Department of Homeland Security reported seven interceptions of PFM at U.S.ports of entry from 1984 to 2011 (USDA 2011). The interceptions of *Cydia funebrana* were all on fruit that was being brought into the country for consumption. Six of the intercepted specimens were on *Prunus* spp. and a single specimen was intercepted on *Malus sylvestris* Mill. All specimens originated in Europe and were intercepted in baggage (USDA 2011).

During processing and shipping, *Cydia funebrana* would likely remain with the commodity because it is an internal feeder (Cave and Lightfield 1997). *Cydia funebrana* feeds primarily on Rosaceae which is common throughout the United States (*Figure 2-2* on page 2-5). Available food sources and

favorable climatic conditions may result in an establishment of *C. funebrana* if introduced to the United States (Venette et al. 2003).

Risk of Establishment

- **1.** Cargo shipments usually consist of fruit only. Since Cydia funebrana is an internal feeder, processing may not remove the specimens. Introductions of larvae in fruit, as seen in the interception data, is the probable introduction pathway.
- **2.** It is essential that inspections for fruit internal feeders focus on examining for feeding tunnels or frass-filled boring holes in the fruit. The infested fruit may also ripen faster than the uninfested fruit.
- **3.** The majority of the United States has a climate that would support Cydia funebrana. The primary hosts, especially cultivated Rosaceae (e.g., *Prunus* spp.) are common in these climatically suitable areas. Thus, upon arrival into the United States, the chance for establishment is relatively high.
- **4.** Although interception data indicates the arrival rate of *Cydia funebrana* is low, discovery of the species is difficult since it is an internal feeder. It would likely follow a pathway of introduction (i.e., within the fruit).
- **5.** The biology of *Cydia funebrana* would make eradication difficult because it is an internal feeder. Insecticide application corresponding directly with egg hatch would be the most successful method of eradicating the species.



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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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Appendix

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 Cydia funebrana

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

Appendix

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

your cooperation is needed to make an accurate record U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE SPECIMENS FOR DETERMINATION			when han year, follo John J. D	of plant pest conditions. See reverse for additional Oll Instructions: Type or print information requested. Press hard and prin when handwritten. Item 1 - assign number for each collection beginni year, followed by collector's initials and collector's number. Example (in John J. Dingle): 83-JJD-001. Pest Data Section - Complete Items 14, 15 and 16 or 19 or 20 and 21					ginning with ple (collector,	LOT NO.	IBIII USE	
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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				
	Assign a number for each collection beginning the year, followed by the collector's initials and collector's number				
1	EXAMPLE In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001				
	2. Enter the collection number				
2	Enter date				
3	Check block to indicate Agency submitting specimens for identification				
4	Enter name of sender				
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)				
6	Enter address				
7	Enter name and address of property owner				
8A-8L	Check all appropriate blocks				
9	Leave Blank				
10	Enter scientific name of host, if possible				
11	Enter quantity of host and plants affected				
12	Check block to indicate distribution of plant				
13	Check appropriate blocks to indicate plant parts affected				
14	Check block to indicate pest distribution				
15	 Check appropriate block to indicate type of specimen Enter number specimens submitted under appropriate column 				
16	Enter sampling method				
17	Enter type of trap and lure				
18	Enter trap number				
19	Enter X in block to indicate isolated or general plant symptoms				
20	Enter X in appropriate block for weed density				
21	Enter X in appropriate block for weed growth stage				
22	Provide a brief explanation if Prompt or URGENT identification is requested				
23	Enter a tentative determination if you made one				
24	Leave blank				

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

- Send Original along with the sample to your Area Identifier.
 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	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) CONTINUE consecutive numbering for each subsequent collection 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

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

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

PPQ 523 Emergency Action Notification

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

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

EMERGENCY ACTION NOTIFICATION

2. DATE ISSUED

4. LOCATION OF ARTICLES

3. NAME AND QUANTITY OF ARTICLE(S)

		5. DESTINATION OF ARTICLES				
6. SHIPPER		7. NAME OF CARRIER				
		8. SHIPMENT ID NO.(S)				
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OTHER:						
Should the owner or owner's agent fa		n the time specified below, USDA is auth disposal, or other action incurred in co				
 AFTER RECEIPT OF THIS NOTIFICATION WITHIN (Specify No. Hours or No. Days): 	ON COMPLETE SPECIFIED ACTION 18	3. SIGNATURE OF OFFICER:				
	ACKNOWLEDGMENT OF RECEIPT	OF EMERGENCY ACTION NOTIFICATION				
		ceipt of the foregoing notification.				
SIGNATURE AND TITLE:		DATE AND	TIME:			
	19. REVOCATION	ON OF NOTIFICATION				
ACTION TAKEN:						
SIGNATURE OF OFFICER:		l l	DATE:			
SIGNATURE OF OFFICER:			DATE:			

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 Insect Specimens

Contents

Insects and Mites C-1
Liquids C-2
Sticky Trap Samples C-2
Dry Specimens C-3
Documentation C-3

Insects and Mites

Taxonomic support for insect surveys requires that samples be competently and consistently sorted, stored, screened in most cases, and submitted to the identifier. The following are submission requirements for insects.

1. Sorting Trap Samples

Trapping initiative is most commonly associated with a pest survey program, such as Wood Boring and Bark Beetles (WBBB), see Bark Beetle Submission Protocol from the PPQ Eastern Region CAPS program for detailed procedures. As such, it is important to sort out the debris and non-target insect orders from the trap material. The taxonomic level of sorting will depend on the expertise available on hand and can be confirmed with the identifier.

2. Screening Trap Samples

Consult the screening aids on the CAPS website for screening aids for particular groups. The use of these aids should be coupled with training from identifiers and/or experienced screeners before their use. These can be found at: http://pest.ceris.purdue.edu/caps/screening.php

3. Storing Samples

Where appropriate, samples can be stored indefinitely in alcohol, however samples of dried insects such as those in sticky traps may decompose over time if not kept in a cool location such as a refrigerator or freezer. If insect samples have decomposed, do not submit them for identification.

4. Packaging and Shipping

Ensure specimens are dead before shipping. This can be accomplished by placing them in a vial of alcohol or putting the dry specimens in the freezer for at least 1day. The following are a few tips on sorting, packaging and shipping liquids, sticky traps and dry samples.

Liquids

Factors such as arthropod group, their life-stage and the means they were collected determine the way the specimens are handled, preserved and shipped to the identifier. In general mites, insect larvae, soft-bodied and hard-bodied adult insects can be transferred to vials of 75-90 percent Ethanol (ETOH), or an equivalent such as isopropyl alcohol. At times, Lingren funnel trap samples may have rainwater in them. To prevent later decay, drain off all the liquid and replace with alcohol. Vials used to ship samples should contain samples from a single trap and a printed or hand-written label with the associated collection number that is also found in the top right corner of form 391. Please make sure to use a writing utensil that isn't alcohol soluble, such as a micron pen or a pencil. It is important not to mix samples from multiple traps in a single vial so as to preserve the locality association data. Vials can be returned to field personnel upon request.

If sending specimens in alcohol is an issue with the mail or freight forwarder, most of the liquid can be decanted off from the vial and then sealed tightly in the container just before shipping. Tell the identifier that the vials will need to have alcohol added back to them as soon as they are received. During the brief time of shipping, the specimens should not dry out if the vial is properly sealed.

Sticky Trap Samples

Adult Lepidoptera, because of their fragile appendages, scales on wings, etc. require special handling and shipping techniques. Lepidoptera specimens in traps should not be manipulated or removed for preliminary screening unless expertise is available. Traps can be folded, with stickum-glue on the inside, but only without the sticky surfaces touching, and secured loosely with a rubber band for shipping. Inserting a few styrofoam peanuts on trap surfaces without insects will cushion and prevent the two sticky surfaces from sticking during shipment to taxonomists. Also DO NOT simply fold traps flat or cover traps with transparent wrap (or other material), as this will guarantee specimens will be seriously damaged or pulled apart – making identification difficult or impossible.

An alternative to this method is to cut out the area of the trap with the suspect pest and pin it securely to the foam bottom of a tray with a lid. Make sure there is some room around the specimen for pinning and future manipulation. For larger numbers of traps, placing several foam peanuts between sticky surfaces (arranged around suspect specimens) can prevent sticky surfaces from making contact when packing multiple folded-traps for shipment. DO NOT simply fold traps flat or cover traps with transparent wrap (or other material), as this will guarantee specimens will be seriously damaged or pulled apart – making identification difficult or impossible.

Dry Specimens

Some collecting methods produce dry material that is fragile. Dry samples can be shipped in vials or glassine envelopes, such as the ones that can be purchased here: http://www.bioquip.com/Search/default.asp. As with the alcohol samples, make sure the collection label is associated with the sample at all times. This method is usually used for larger insects and its downside is the higher chance of breakage during shipping. Additionally, dry samples are often covered in debris and sometimes difficult to identify.

Be sure that the samples are adequately packed for shipment to ensure safe transit to the identifier. If a soft envelope is used, wrap it in shipping bubble sheets; if a rigid cardboard box is used, pack it in such a way that the samples are restricted from moving in the container. Please include the accompanying documentation and tell the identifier before shipping. Remember to tell the identifier that samples are on the way, giving the approximate number and to include your contact information.

Documentation

Each trap sample/vial should have accompanying documentation along with it in the form of a completed PPQ form 391, Specimens for Determination. The form is fillable electronically and can be found here:

http://cals-cf.calsnet.arizona.edu/azpdn/labs/submission/PPQ_Form_391.pdf

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. Indicate the name of the person making any tentative identification before sending to an identifier. 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 trap type, lure used, and trap number on the form. Also, 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/vial with the accompanying form. This can be done with a label with the "Collection Number" in the vial or written on the envelope, etc.



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 PPO 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, PPO

901 W. State Street

Smith Hall, Purdue University

Lafavette, 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 PPO

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.govwith the overnight carrier tracking number.

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

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

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

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

Digital Images for Confirmation of Domestic Detections

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

Communications of Results

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

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

Data Entry

Cooperative Agricultural Pest Survey (CAPS)

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



Tortricidae Molecular Protocols

Contents

DNA Extraction using Qiagen DNeasy Blood and Tissue Kit (Gilligan, 2011)

Gene: Cytochrome oxidase I (COI) (Gilligan, 2011)

Gene: Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, and Dihydroorotase (CAD) (Gilligan, 2011)

Molecular sequencing protocols for Tortricidae

Tortricidae Molecular Protocol 1

T. M. Gilligan Colorado State University March 2011

DNA extraction using Qiagen DNeasy Blood & Tissue Kit:

- 1. Place 2-3 legs dry legs from moth in 1.5 ml tube
- 2. Crush legs with pestle
- 3. Add 180 µl Buffer ATL while washing off pestle
- 4. Add 20 µl proteinase K
- 5. Vortex and briefly spin down
- 6. Incubate at 56°C for 12-24 hours (overnight)

Note: following day

- 7. Vortex for 15 sec.
- 8. Add 200 µl Buffer AL, vortex
- 9. Add 200 μl 100% EtOH, vortex
- 10. Pipet mixture into spin column (650 μl)
- 11. Spin at 8000 RPM for 1 minute
- 12. Discard collection tube and flow through
- 13. Place column in new tube, add 500 µl Buffer AW1
- 14. Spin at 8000 RPM for 1 minute
- 15. Discard collection tube and flow through
- 16. Place column in new tube, add 500 µl Buffer AW2
- 17. Spin at 14,000 RPM for 3 minutes
- 18. Discard collection tube and flow through
- 19. Place column in clean 1.5 ml tube labeled "high"
- 20. Elute DNA with 100 µl Buffer AE, incubate for at least 1 minute
- 21. Spin at 8000 RPM for 1 minute
- 22. Place column in clean 1.5 ml tube labeled "low"
- 23. Elute DNA again with 100 µl Buffer AE, incubate for at least 1 minute
- 24. Spin at 8000 RPM for 1 minute

This protocol results in two extracts per sample (100 μ l each): a "high" concentration tube and "low" concentration tube. The "low" concentration extract is stored in -80°C as a back up and the "high" concentration extract is used for PCR.

At this point samples are ready for PCR.

Tortricidae Molecular Protocol 2

T. M. Gilligan Colorado State University March 2011

Gene: Cytochrome oxidase I (COI)

Length: Approximately 650-700 bp

Primers: 25 nmole DNA Oligo (IDT)

LCO-	GGT CAA CAA ATC ATA AAG ATA TTG
1490	G
HCO-	TAA ACT TCA GGG TGA CCA AAA AAT
2198	CA

PCR mix: TaKaRa Ex Taq Hot Start (RR006A)

PCR protocol:

1. Create master mix containing:

37.75	Water
μl	
5.00 µl	10X buffer
4.00 µl	dNTPs
1.00 µl	Primer LCO-1490
1.00 µl	Primer HCO-
	2198
0.25 μl	Taq

2. Add 49 µl of master mix to each PCR

tube

3. Add 1 µl DNA template for total of 50 µl per PCR reaction

PCR program:

T50/C39 (TODDCOI) – approximately 1 $^{1\!\!/_{\!\!2}}$ hours

- 1. 94°C / 3 min.
- 2. 39 cycles of:
 - a. 94°C / 20 sec.
 - b. 50°C / 20 sec.

- c. 72°C / 30 sec.
- 3. 72°C / 5 min.
- 4. Hold at 4°C

Run PCR product on normal gel (9 μ l DNA + 2 μ l dye) and note band intensity.

Clean successful amplifications using standard PCR cleanup protocols.

Samples are now ready for sequencing.

Tortricidae Molecular Protocol 3

T. M. Gilligan Colorado State University March 2011

Gene: Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)

This gene is very difficult to obtain consistently. The following primer combination amplified CAD across several genera in several tribes in both the Olethreutinae and Tortricinae. These primers are a mix from different labs and have different universal primer tails (M13 for the forward and T3 for the reverse). In theory they would work without the tails (or both with a M13 or T3/T7 combo), but this has not been tested and I have no desire to mess with this working protocol. So they are included below with the tails attached.

This primer combination results in multiple bands for most taxa, necessitating gel isolation after the initial PCR. Direct sequencing of the initial PCR product does <u>not</u> work.

Some samples that show very faint bands may be reamplified after the gel isolation, with varying results. It is recommended to gel isolate and attempt to sequence even very faint bands before attempting reamplification, as reamping faint bands usually fails. The reamplification protocol (Step 3) is included here but should not be relied upon for obtaining quality sequences.

Protocols for the entire process are included here and follow these general steps:

- 1. Initial PCR
- 2. Gel isolation and purification of PCR products
- 3. Reamplification of faint bands detected during step 2 (if necessary)

Length: Approximately 800 bp

Primers: 25 nmole DNA Oligo (IDT)

M13r-791F	CAG GAA ACA GCT ATG ACC TTY GAR GAR GCN
	TTY CAR AAR GC
T3-CAD1028R	ATT AAC CCT CAC TAA AGT TRT TNG GNA RYT
	GNC CNC CCA T

PCR mix: TaKaRa Ex Taq Hot Start (RR006A)

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