

United States Department of Agriculture

Animal and Plant Health Inspection Service

Cooperating State Departments of Agriculture

New Pest Response Guidelines

Cotton Seed Bug



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First edition issued 2010

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Cover Image

Cotton seed bug, *Oxycarenus hyalinipennis* (Julieta Brambila, Entomologist, USDA–APHIS–PPQ)



Introduction

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Purpose

Use *New Pest Response Guidelines: Cotton Seed Bug* as a guide when designing a program to detect, monitor, control, contain, or eradicate, an infestation of *Oxycarenus hyalinipennis*, the cotton seed bug (CSB). The United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the guidelines through discussion, consultation, or agreement with staff at USDA-Agricultural Research Service, and university advisors.

Any new detection may require the establishment of an Incident Command System to facilitate emergency management. The guidelines is meant to provide the necessary information to launch a response to a detection of the cotton seed bug.

If the cotton seed bug 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 can be updated.

Users

The guidelines is intended as a field reference for the following users who have been assigned responsibilities for a plant health emergency for the cotton seed bug:

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

Contacts

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

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

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 pests identity is confirmed by a national taxonomic authority recognized by USDA–APHIS–PPQ–National Identification System.
- **4.** Existing *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 an 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.

- **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 this pest does **not** spread within or between properties. The officials conducting inspections should follow the sanitation guidelines in *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
- 2. Pest Information
- **3.** Identification
- **4.** Survey Procedures
- **5.** Regulatory Procedures
- 6. Control Procedures
- 7. Environmental Regulations
- 8. Pathways

The guidelines also includes appendixes, 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.

What the Guidelines Does Not Cover

The guidelines does not cover the program for the cotton seed bug in the State of Florida. For further information on this topic, contact the Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Address

Florida Department of Agriculture and Consumer Services, Division of Plant Industry http://www.freshfromflorida.com/pi/

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
- National Environmental Policy Act

The following resources are related to all programs for the cotton seed bug:

- Screening Aid on page E-1, Field Screening Aid for the Cotton Seed Bug
- Submitting Survey Samples to Domestic and Other Identifier on page F-1
- ◆ Taxonomic Support for Surveys on page G-1

Program Safety

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

Support for Program Decisionmaking

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

Address

USDA–APHIS–PPQ–Center for Plant Health, Science, and Technology http://www.aphis.usda.gov/plant_health/cphst/index.shtml

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.

Address	Address indicates the person or agency to contact, along with their Web site address, email address, telephone number, or other means of contact.
Example	Example provides an example of the topic.
Source	Source indicates the location of information used for writing this section of the guidelines.
Important	IMPORTANT indicates helpful information.
	CAUTION indicates that people could possibly be endangered and slightly hurt.
NOTICE	NOTICE indicates a possibly dangerous situation where goods might be damaged.

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.

lf 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

 Table 1-1 How to Use Decision Tables

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.

Introduction



Pest Information

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Introduction

Use *Chapter 2 Pest Information* to learn more about the classification, history, host range, and biology of *Oxycarenus hyalinipennis*, the cotton seed bug (CSB) absent from the United States and collaborating territories.

Classification

Originally in the family Lygaeidae, *Oxycarenus* species are now classified in the family Oxycarenidae, superfamily Lygaeoidea (Henry, 1997).

Table 2-1 Classification of the Cotton Seed Bug

Phylum	Arthropoda
Class	Insecta
Order	Hemiptera
Family	Oxycarenidae
Genus	Oxycarenus
Full Name	Oxycarenus hyalinipennis (Costa)
Preferred Common Name	cotton seed bug
Synonyms	Aphanus hyalinipennis Costa, Aphanus tardus var. hyalipennis Costa 1847, Cymus cincticornis Walker 1870, Oxycarenus cruralis Stål 1856, Oxycarenus leucopterus Fieber 1852
Other Common Names	cottonseed bug, cotton stainer, dusky cotton bug, dusky cottonseed bug, dusky cotton stainer, Egyptian cottonseed bug, Kleine graue Baumwollwanze (German)

Historical Information

Native to Africa, the cotton seed bug is distributed in many areas of the world. Established in the Caribbean and moving northward toward the continental United States, it has been intercepted numerous times in the United States on various commodities, including those not known to be reproductive hosts.

In 2006, the USDA–APHIS–PPQ-Center for Plant Health Science and Technology (CPHST) analyzed the potential consequences associated with the introduction of the cotton seed bug into the United States, and determined that the bug was a high risk pest (PERAL, 2006). Based on host and climate availabilities and lack of competition from other members of the family Oxycarenidae in the United States, the cotton seed bug was found to have a high likelihood for establishment throughout the southern regions.

Dispersal potential of the cotton seed bug in the southern United States was also rated high due to local migratory behavior and the ability to seek refuge in various shelter locations until hosts become available. The potential economic impact of the establishment of the cotton seed bug in the United States was estimated to be high based on the current values of cotton, okra, and hibiscus, in addition to other malvaceous hosts. The potential for the cotton seed bug to affect some malvaceous hosts that are listed as federally threatened or endangered, and the likelihood that chemical control measures would be initiated, also resulted in a high risk rating for potential environmental impact.

Economic Impact

The cotton seed bug is a widespread pest of economic importance. As an important pest of cotton, hibiscus (including kenaf), and okra, the cotton seed bug presents a significant economic threat to the continental United States (Smith and Brambila, 2008; Sweet, 2000).

The cotton seed bug causes multiple types of injuries to cotton seed including reduction in the weight of seeds used for oil extraction and the manufacture of cake; damage to seeds used for sowing by causing non-germination and injuries to the embryo radicle and cotyledons (Kirkpatrick, 1923; Odhiambo, 1957); as well as reducing the value of cotton when insect bodies are crushed in the ginning process, staining the lint (Henry, 1983).

Feeding by large populations of the cotton seed bug can cause a significant decrease in cotton seed weight (up to 15 percent). The ability of seeds to germinate is also significantly reduced, potentially as much as 88 percent (Kirkpatrick, 1923). In 2009, the value of cottonseed produced in the United States was \$667 million; the seed was produced primarily in Texas, Georgia, California, Arkansas, and North Carolina (NASS, 2010).

Additional economic damage may result from a loss in marketability of various fruits that have been affected by feces or the toxic saliva of the cotton seed bug searching for moisture (Avidov and Harpaz, 1969; Sweet, 2000). Nakache and Klein (1992) reported damage to fruit of dates, figs, avocados, and persimmons due to contamination with a pungent odor. The cotton seed bug has caused damage to fruit of apricot, peach, persimmon, apple, pear, quince, and grapes (Avidov and Harpaz, 1969), in addition to other economically important plants listed in *Reported Hosts* on **page C-1**. Sucking by adults caused greasy spot with a light-colored gum exuding. Black feces contaminated the fruit (Avidov and Harpaz, 1969).

The cotton seed bug may also be a nuisance pest, congregating on walls of buildings, giving off an unpleasant odor, and may be attracted to lights at night (Nakache and Klein, 1992).

Ecological Range

The cotton seed bug is not known to be present in North America. The bug was found in Puerto Rico in January 2010, and on the islands of St. Croix and St. Thomas in the Virgin Islands in April 2010. In early 2010, it was found on Stock Island and Key West, Florida, on cotton (*Gossypium* sp.). Both populations in Florida are under eradication.

The cotton seed bug is distributed in the following areas (CABI, 2007):

Africa—Algeria, Angola, Benin, Botswana, Burkina Faso, Burundi, Congo Democratic Republic, Congo, Cote d'Ivoire, Egypt, Ethiopia, Ghana, Guinea, Kenya, Libya, Madagascar, Malawi, Mali, Mauritania, Morocco, Mozambique, Namibia, Niger, Nigeria, Rwanda, Sao Tome and Principe, Senegal, Somalia, South Africa, Sudan, Swaziland, Tanzania, Togo, Tunisia, Uganda, Zimbabwe

Asia—Bangladesh, Cambodia, China, India, Iran, Iraq, Israel, Laos, Myanmar, Pakistan, Philippines, Saudi Arabia, Sri Lanka, Syria, Thailand, Turkey, Vietnam, Yemen

Central America & Caribbean—Bahamas, Cayman Islands, Cuba (Ravelo, 1993), Hispaniola, Puerto Rico (Bowers, 2010), Saint Croix and Saint Thomas [US Virgin Islands] (Floyd, 2010), Turks and Caicos Islands (Slater and Baranowski, 1994)

Europe—Austria, Cyprus, France, Germany, Greece, Hungary, Italy, Portugal, Russia, Serbia and Montenegro, Spain

South America—Argentina, Bolivia, Brazil, Paraguay

Potential Distribution

The cotton seed bug could survive in many areas of the continental United States. Based on the reported global distribution, scientists estimated that the cotton seed bug could survive in plant hardiness zones 8–11, particularly in the cotton-growing areas of southeastern United States (Henry, 1983). It is estimated that 4–7 generations per year could occur in Florida. In *Figure 2-1*

on page 2-5, the availability of some economically important hosts, cotton and okra, was combined with the climatic suitability to estimate the risk of establishment of the cotton seed bug in the continental United States.

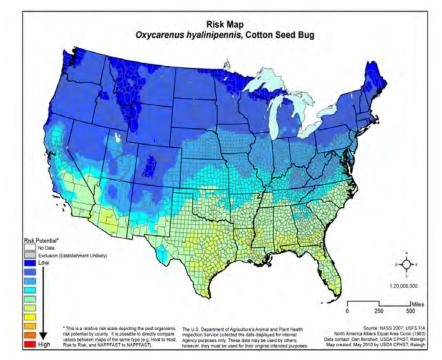


Figure 2-1 Risk Map of Establishment Potential of the Cotton Seed Bug in the Conterminous United States¹

1 The map was developed by J. Engle (USDA) using NAPPFAST (NCSU–APHIS Plant Pest Forecasting System). Data was collected from the USDA–NASS 2007 Agricultural Census for all classes of cotton and okra and 10 years of daily weather data. County-level distribution data of other malvaceous hosts is unavailable.

Hosts

The primary economic host plants of concern in the United States include cotton (*Gossypium*), hibiscus (*Hibiscus*) and okra (*Abelmoschus esculentus*) (Kirkpatrick, 1923; Sweet, 2000). The preferred reproductive host is cotton.

In order for the cotton seed bug to breed, seeds of a plant within the order Malvales must be present. Breeding occurs in opened Malvales seeds, either ripe or those that have been affected by, for example, a bollworm. The cotton seed bug has been observed on many plants; however, the bugs are known to drink fluids from almost any leaf, so the status of these records outside the order Malvales is unclear. Some may be incidental findings, and others may be records of the insect sucking juices from various fruits in search of moisture.

Host plants within the order Malvales, on which the cotton seed bug has been observed growing and laying eggs, are listed in *Table 2-2* on page 2-6. Additional reports of plants outside of the order Malvales, or on which reproduction has **not** been shown to occur, were listed in *Table C-1* on page C-2.

Genus	Species	Common Name
Abelmoschus	esculentus ²	okra
	moschatus ³	musk okra
Abutilon	crispum	bladdermallow
	fruticosum	Texas Indian mallow
	guineense	
	indicum	monkeybush
	mauritianum	
	spp.	Indian mallow
	thompsonii	
	venosum	
Althaea	rosea	hollyhock
	spp.	marshmallow
Cola (Sterculiaceae)	sp.	
Corchorus	sp.	
Dombeya	sp.	
Eriodendron	sp.	
Gossypium	barbadense	Creole cotton
	hirsutum	upland cotton
	spp.	cotton
Hibiscus	cannabinus	brown indianhemp
	micranthus	
	mutabilis	dixie rosemallow
	sabdariffa	roselle
	sp.	
	tiliaceus	sea hibiscus
	trionum	flower of an hour
	vitifolius	tropical rose mallow

Table 2-2 Reported Host Plants, in the Order Malvales, of the Cotton Seed Bug¹ (continued)

Genus	Species	Common Name
Malva	parviflora	cheeseweed mallow
	pusilla ⁴	low mallow
	sp.	mallow
	sylvestris	high mallow
Malvastrum	corchorifolium	false mallow
	coromandelianum	threelobe false mallow
	sp.	false mallow
Pavonia	spinifer	
Sida	acuta	common wireweed
	cordifolia	
	mollis	
	rhombifolia	Cuban jute
	sp.	fanpetals
Sphaeralcea	miniata	Latin globemallow
	sp.	globemallow
	umbellata	
Sterculia (Sterculiaceae)	diversifolia	
Thespesia	populnea	portia tree
Triumfetta (Tiliaceae)	sp.	burbark
Urena	lobata	caesarweed
Wissadula	amplissima	big yellow velvetleaf

Table 2-2 Reported Host Plants, in the Order Malvales, of the Cotton Seed Bug¹ (continued)

1 Adu-Mensah and Kumar, 1977; Ananthakrishan et al., 1982; Dimetry, 1971; Kirkpatrick, 1923; Rajashekhargouda et al., 1983; Ram and Chopra, 1984; Slater and Baranowski, 1994

2 Synonym Hibiscus esculetus.

3 Synonym Hibiscus abelmoschus.

4 Synonym Malva rotundifolia.

Life Cycle

Kirkpatrick (1923) performed in-depth research on the biology of the cotton seed bug, and is the source of the life cycle information cited here, except where otherwise noted. The life cycle data described in this section pertains to cotton. Variations on other hosts pertain primarily to the timing of breeding. Movement among host plants within the order Malvales as seeds become available allows for a longer breeding season than breeding only on cotton.

Eggs

Eggs of the cotton seed bug are oval shaped, measure 0.28 by 0.95 mm, longitudinally striated, with a pale yellow to pink color (Henry, 1983). The eggs are generally laid in the lint, close to the seed. Later in the season, eggs may be found between the calyx and the base of green bolls, between two contiguous lobes of the epicalyx, or in holes made by boll worms in green bolls. Each female lays up to 110 eggs, either singly or in groups. The incubation period generally lasts from 4 to 8 days (Kirkpatrick, 1923; Sweet, 2000).

Nymphs

Nymphs have a pinkish or orange abdomen (Brambila, 2010). There are five nymphal stages which last 14–20 days in warm weather. The insects pierce ripe or almost ripe seeds with their setae, and suck juices out. Feeding on seeds within the order Malvales is required by the nymphs for survival and to complete their development. When dew is present on the cotton plant, nymphs can be found drinking it from the bolls or leaves nearby. When dew is unavailable, they may seek moisture by piercing leaves (Kirkpatrick, 1923).

Adults

Adults are 4.0–4.3 mm long, generally with a brown or black thorax, head, antennae, and femora and translucent white wings (Smith and Brambila, 2008). Adults congregate in bolls and begin feeding on seeds as soon as the bolls open; mating occurs soon afterwards. Three to four generations occur per year. At the end of the breeding season, adults enter diapause, leave the cotton fields, and fly to various shelter locations for overwintering. Resting locations vary, and may include obscure locations such as the crevices between strands of barbed wire, tree trunks, among the roots of grasses at the edges of fields or canal banks, or on leaves of plants not known to be hosts (Kirkpatrick, 1923).

The cotton seed bug adult generally aggregates in large groups, and can be found resting on nearly any surface. During the quiescent period, the cotton seed bug generally prefers cryptic locations such as on tree trunks, on the undersides of both living and dead leaves, pods of leguminous plants, cracks in telephone poles or wooden posts and fences, under bark, in old nests of *Polistes*, in crevices between strands of barbed wire, on dried flower heads, among roots of grasses, under sheath-leaves of maize and sugarcane, in stored cotton, in artificial traps such as old sacks on poles or in hedges near cotton fields, or in dry grass or leaf litter (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923; Smith and Brambila, 2008).

Behavior

The cotton seed bug begins feeding, mating, and laying eggs when mature seeds of its host plants become available. Both adults and nymphs suck oil from seeds. After winter, resting adults leave their shelters, move to young cotton or other Malvales host plants, and wait for the bolls to open. Females lay eggs in the cotton lint and feed on the seeds. At the end of the breeding season, the cotton seed bug undergoes aestivation, and finds a shelter in which to rest for the winter. Overwintering adults are not completely inactive, but do not feed or mate until Malvales seeds are available again. Seeds of various host plants are available at different periods throughout the year, maintaining the cotton seed bug populations (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923; Sweet, 2000). In Hissar, India, the cotton seed bug was observed breeding on Gossypium spp. from August to November, and migrating to Thespesia, Dombeya, and Abutilon when the cotton was harvested (Ram and Chopra, 1984). The cotton seed bug nymphs and adults may also be found searching for water by sucking leaves or young stems (Odhiambo, 1957), or fruits (Avidov and Harpaz, 1969) of hosts outside of the order Malvales.

Environmental Impact

Reproductive hosts of the cotton seed bug include *Abutilon* spp. and *Hibiscus* spp., some of which are listed as federally threatened or endangered, though they are currently only known to occur in Hawaii (USFWS, 2010). Chemical control programs may be initiated in the event of an introduction of the cotton seed bug in the United States, which may negatively impact non-target pests and the environment. Wild cotton (*Gossypium hirsutum*) is listed by the State of Florida as an endangered/protected plant (NRCS, 2010).

Pest Information



Identification

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Introduction

Use *Chapter 3 Identification* as a guide to recognizing the cotton seed bug, *Oxycarenus hyalinipennis* (CSB). 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 preliminary identification and screening of suspected cotton seed bug. Before survey and control activities are initiated in the United States, an authority recognized by USDA–APHIS–PPQ-National Identification Services must confirm the presence of this pest. Submit specimens to the USDA-National Identification Services (NIS).

In the field, surveyors should use the *Screening Aid* on **page E-1** to sort specimens. Final identification by an expert will require dissection and examination of the adult male sexual structures. Refer to the images of the cotton seed bug in the Appendix *Images* on **page D-1**.

Reporting

Forward reports of positive identifications by National specialists to PPQ-National Identification Service (NIS) in Riverdale, Maryland, according to Agency protocol. The 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.

Characteristics

Use the following characteristics to identify eggs, nymphs, and adults, of the cotton seed bug.

Eggs

Sweet (2000) collected information to develop the following description of cotton seed bug eggs:

The egg is 0.29 mm wide by 0.97 mm long, slender, subcylindrical, with 25 longitudinal ribs or corrugations. The anterior end is broadly rounded, and bears six chorionic processes; the posterior is distinctly pointed. During development, the eggs change from straw yellow to orange as seen through the transparent chorion.

Nymphs

Sweet (2000) collected information to develop the following description of cotton seed bug nymphs:

The nymphs are orange-red on hatching and later have a dark red abdomen that has a greenish tint. In the aggregate when in a feeding swarm, the nymphs are very conspicuous. The investiture is of the peculiar glandular peglike hairs characteristic of the Oxycarenidae. There are two abdominal scent glands located between terga 4 to 5, and 5 to 6. The orifices are close together. Kirkpatrick (1923) measured the average instar lengths as first, 1.2; second, 1.53; third, 2.25; fourth, 3.28; fifth, 4.27 mm.

Adults

Refer to Figure 3-2 on page 3-4.

Henry (1983) provided the following general description of adult cotton seed bugs:

Newly emerged individuals pale pink but rapidly turning black. Length of male about 3.8 mm; female 4.3 mm. Male abdomen terminates in round lobe, while female is truncate. The insects have three tarsal joints and a pair of ocelli. Second antennal segment usually in part pale yellow. Hemelytra hyaline and usually whitish; clavus, base of corium, and costal vein more opaque than rest.¹

A technical description was developed by Samy (1969):

General color black and white. Characterized by the light brown coloration of basal two-thirds and basal third of second and third antennal segments respectively, the light ochreous clavus, the triangular shape of dorsal opening of pygophore, and presence of a lobe on blade of each paramere.

Head black; rostrum black with first segment and joints brownish, third segment extending beyond mid-coxae, fourth segment reaching third of abdominal segment; antennae brownish-black, basal two-thirds of second segment and basal third of third segment light yellowish-brown.

Pronotum blackish-brown with anterior and posterior margins brown to light brown, lateral margins slightly sinuate, prosternum black, posterior margin whitish; scutellum black; meso- and metasterna black; acetabula white; femora black; fore femora with four spines; fore tibiae light brown; mid and hind tibiae dark brown, annulated with yellowish-white; tarsi light brown, third segment darker; corium yellowish-white; exocorium yellowish-white with spot at apex small, light brown; clavus light ochre with three rows of pores slightly distinct; membrane hyaline.

Abdomen dorsally reddish-brown; connexivum dark brown; venter dull brown with median area lighter, posteriorly blackish.

Male genitalia: Pygophore with dorsal opening triangular with narrow pointed angle; posterior edge straight without notch; subgenital plates in posterodorsal view transversly rectangular with median notch. Aedeagus moderately long; vesica without lobe; vesical wings long; vesical sclerite straight, long. Parameres long; blade in same plane as body, long, tapering; with a lobe; shank small, with two nodules (one less distinct).²

¹ The Caribbean population of the cotton seed bug, including the individuals recently detected in Florida, is brownish rather than black.

² The coloration of the Caribbean populations of the cotton seed bug is dark brown, rather than black, but final identification based on the male genitalia concludes that these insects are *Oxycarenus hyalinipennis*.

Similar Species

Species similar to the cotton seed bug include other species of *Oxycarenus*, none of which are currently known to occur in the United States. Other species of North American Oxycarenidae are **not** known to feed on Malvales host plants (Sweet, 2000).

A similar oxycarenid known to occur in the United States is the introduced *Metopoplax ditomoides* (Costa) (*Figure 3-3* on page 3-5). It is currently present in Oregon (Lattin and Wetherill, 2002). The anterior end of the head is rounded rather than acute.



Figure 3-2 Adult Cotton Seed Bug (Natasha Wright, Division of Plant Industry, Florida Department of Agriculture and Consumer Services. Last accessed June 14, 2010: http://www.ipmimages.org/browse/detail.cfm?imgnum=5190058)



Figure 3-3 Adult *Metopoplax ditomoides* (Jeffrey Lotz, Florida Department of Agriculture and Consumer Services. Last accessed July 27, 2010: <u>http://www.insectimages.org/browse/detail.cfm?imgnum=5385256</u>)

Identification

Survey Procedures

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Introduction

Use *Chapter 4 Survey Procedures* as a guide when conducting a survey for cotton seed bug, *Oxycarenus hyalinipennis* (CSB).

Survey Types

Plant regulatory officials will conduct detection, delimiting, and monitoring surveys, for the cotton seed bug. Conduct a detection survey to ascertain the presence or absence of cotton seed bug in an area where it is not known to occur. After a new U.S. detection, or when a detection in a new area is confirmed, conduct a delimiting survey to define the extent of an infestation. Conduct a monitoring survey to determine the success of control or mitigation activities conducted against a pest. Visual inspection is the only survey method available.

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.** Obtain permission from the landowner before entering a property.
- **2.** Before starting a survey, determine if there have been recent pesticide applications that would make it unsafe to inspect the property. 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.
- **3.** Conduct the survey at the proper time. The cotton seed bug is more active when cotton has mature bolls, or when mature seeds of the order Malvales are available; however, large populations may also be found congregating near malvaceous plants year round. Based upon the pests reported global distribution, it is estimated that the cotton seed bug may establish in plant hardiness zones 8–11. General survey efforts should focus on months when seeds of host plants are available, especially when cotton bolls are open. Refer to *Potential Distribution* on **page 2-4** for further information.
- **4.** Determine if quarantines for other pests, of cotton or other crops, are in effect for the area being surveyed. Comply with any and all quarantine requirements.
- **5.** When visiting the cotton field, nurseries, or landscape planting to conduct surveys or to take samples, everyone must take strict measures to prevent contamination by the cotton seed bug or other pests between properties during inspections.

Before entering a new property, make certain that clothing and footwear are clean and free of pests and soil to avoid moving soilborne pests and arthropods from one property to another. Wash hands. Change clothes if clothing is covered with bugs.

- **6.** Gather together all supplies.
- **7.** Mark the cotton plant 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 or entomologist familiar with the cotton seed bug and the symptoms of its presence.

Detection Survey

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

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

Refer to *Authorities* on **page 3-1** for information on screening, examination, and reporting, of the cotton seed bug.

Procedure

Use the following tools singly or in any combination to detect the cotton seed bug:

- **1.** Focus on high risk areas where the cotton seed bug is most likely to be found. Refer to *Targeted Survey* on **page 4-6** for further information.
- **2.** Establish regular sites to inspect along your normal surveying route. Refer to *Sentinel Sites* on **page 4-6** for further information.

3. Check plants for the presence of pests and their damage. Refer *Visual Inspection* on **page 4-7** for further information

Delimiting Survey After Initial U.S. Detection

If the cotton seed bug is detected, conduct a delimiting survey in the area to determine the distribution of the pest. In large areas, locating the source of an infestation could be difficult. The cotton seed bug tends to aggregate on host plant material within the order Malvales (primarily cotton), though large populations may also be detected resting on nearly any other surface nearby. Cotton seed bugs are capable of short flight which may be aided by wind (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923).

Procedure

- **1.** Follow the guidelines in *Traceback and Trace-Forward Investigation* on **page 4-4** to determine surveying priorities and define the potential for dissemination.
- **2.** Once cotton seed bugs have been confirmed in an area, continue to survey in nearby areas in order to determine the full extent of the infestation. Cotton seed bugs are capable of short flights, aided by wind.
- **3.** Inspect continually larger areas, particularly where malvaceous hosts are known to occur.
- **4.** Conduct the most intensive surveys around the known positive detections, and any discovered through traceback and trace-forward investigations, if possible.

Traceback and Trace-Forward Investigation

Traceback and trace-forward investigations help surveyors to determine priorities for delimiting survey activities after an initial U.S. detection. Use traceback investigations to determine the source of an infestation. Conduct trace-forward investigations to define the potential dissemination through means of natural and artificial spread (commercial or private distribution of infested plant material). Once a positive detection is confirmed, conduct investigations to determine the extent of the infestation or suspect areas in which to conduct further investigations. Infestations of cotton seed bug may go undetected if populations are small and breeding bugs are within the cotton lint, or resting on nearby plants. The cotton seed bug does not damage the cotton plant itself, outside of sucking juices from the seeds (Sweet, 2000).

The cotton seed bug may arrive with cotton seed or as a hitchhiker on nearly any commodity, regardless of its host status. The importation of cotton is regulated by Title 7 of the Code of Federal Regulations (CFR) 319.8 (Foreign Cotton and Covers).

Domestic movement of agricultural commodities from Puerto Rico to the mainland is regulated by quarantine Title 7 CFR 318 (Fruits and Vegetable, 318.13; Cotton, 318.47), and Title 7 CFR 330.105 (Bare Rooted Plants or Plant Cuttings).

However, the cotton seed bug may enter as a hitchhiker pest in passenger baggage.

For nursery stock, a list of facilities associated with nursery stock infested with cotton seed bug will be compiled by State-level cooperators.



The list of facilities associated with nursery stock infested with cotton seed bug 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 these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited. Each State is only authorized to see locations within their State and sharing of confidential business information may be restricted between State and Federal entities. Check the privacy laws with the State Plant Health Director (SPHD) 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 cotton seed bug-infested material. Speak to the growers or farm managers and obtain proper permission before entering private property. If any sales or distribution has occurred from infested nursery during the previous 6 months, then check nursery records to obtain names and addresses for all sales or distribution sites.

Monitoring Survey

If the cotton seed bug is detected in the United States a Technical Working Group will be assembled by the CPHST National Science Program Leader, to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments on the pest population.

Targeted Survey

Conduct targeted surveys in areas where introduction of the cotton seed bug may be considered more likely. For example, in Florida this may include cultivated or wild cotton fields in southern Florida closer to the Caribbean islands where it is currently known to be established. Areas with regular traffic from countries with known infestations that may carry hitchhiker bugs should also be targeted for regular surveys. Surveyors should work with their State-level cooperators to identify the best locations for targeted surveys.

Sentinel Sites

Sentinel sites are locations that are regularly inspected along the surveyors' normal route. The sites are selected based on their ease of access, the large number of cotton or other malvaceous plants found at those locations, and the reduced likelihood that those plants would be removed or destroyed by the next survey period.

For example, in Florida the Department of Agriculture and Consumer Services, Division of Plant Industry has selected and established multiple sentinel sites throughout the State. Sentinel sites were chosen in pathways that were easily accessible (parks with open admission, roadsides, etc) and had several host plants for inspection. Selected host plants included cotton, okra, and kenaf. All sites should be surveyed extensively after hurricanes and tropical storms.

Once the sentinel site is established, the surveyor should re-inspect the site on a regular basis (bimonthly or monthly) as permitted by the person's regular survey schedule. Any nymphs or adults should be processed as described. GIS can be use to map the sentinel site locations to help visualize an even coverage, particularly in high risk areas.

Rainy weather has been shown to significantly decrease population levels of adult and immature cotton seed bugs (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923; Odhiambo, 1957). Large populations of cotton seed bug are most likely to be easily detected during dry periods.

Visual Inspection

This section contains instructions for inspecting plants for infestations of the cotton seed bug.

Procedure

1. Inspect cotton plants, other potential malvaceous host plants, and nearby resting places for aggregations of the cotton seed bug.

Refer to *Hosts* on **page 2-5** for further information on which host plants to inspect. Refer to *Symptoms* on **page 4-8** for the symptoms to look for, and to the images of the cotton seed bug in *Images* on **page D-1**, and *Screening Aid* on **page E-1**.

2.



Do not remove live insects from the survey sites.

If during this life cycle of the CSB:	Then:
Breeding period, when cotton seed bolls have opened	 At each survey site, randomly select twenty cotton plants, and remove five bolls from each plant using pruning shears. Put the bolls into a plastic bag and spray them with a small amount of isopropyl alcohol using a hand spray bottle. If the boll is infested, bugs will immediately swarm out. If so, spray
	additional alcohol to kill the bugs, seal the collection by double-bagging it, and store in an ice chest.
Quiescent period, when seeds of host plants are unavailable	For detection purposes, surveying during the quiescent period is not recommended. Refer to <i>During the Quiescent Period,</i> <i>when Seeds of Host Plants are</i> <i>Unavailable</i> on page 4-9 for further information.

- **3.** Record the presence or absence of the cotton seed bug for each sample. Work with your State-level cooperators to identify the best location for the storage of records.
- 4. Collect samples of lygaeid insects while inspecting potential host plants.
- Follow the instructions described in *Processing Samples* on page 4-10 when preparing specimens. Submit specimens and plant material to the proper authority. Refer to *Submitting Survey Samples to Domestic and Other Identifier* on page F-1 and *Taxonomic Support for Surveys* on page G-1 for further information.
- **6.** If the cotton seed bug is detected in an area, a Technical Working Group for this pest will be assembled by the CPHST National Science Program Leader. The Group will provide further guidance concerning additional surveys.

Symptoms

Cotton and Other Malvales

Aggregations of large populations may be detected visually on host plants, or on resting locations as described below.

The cotton seed bug does not cause visible damage to cotton or other Malvales host plants outside of sucking oil from mature seeds (Sweet, 2000). Cotton seed shows no external signs of damage due to the cotton seed bug (Kirkpatrick, 1923). Internally, seeds are shriveled and discolored (Kirkpatrick, 1923).

Other Host Plants

The cotton seed bug damages the fruit of apricot, peach, persimmon, apple, pear, quince, grapes, dates, figs, and avocados, in addition to other economically important plants included in the sections *Hosts* on **page 2-5** and *Reported Hosts* on **page C-1**. The quality of fruits may be affected by feces, pungent odors, or the toxic saliva of the cotton seed bug searching for moisture (Avidov and Harpaz, 1969; Nakache and Klein, 1992; Sweet, 2000).

Other Locations

The cotton seed bug may also aggregate on the walls of buildings (Nakache and Klein, 1992).

Survey Timing

During the Breeding Period, when Seed Bolls are Open

This is the best time to survey for the cotton seed bug. The cotton seed bug can be detected visually on open cotton bolls (Derksen et al., 2010). In cotton, the cotton seed bug is most frequently found in the cotton bolls, and will emerge when the boll is agitated. During this time, the vast majority of the bugs will be found within the bolls, and only occasionally in leaf litter or on the leaves of cotton plants (Smith and Brambila, 2008).

During the Quiescent Period, when Seeds of Host Plants are Unavailable

For detection purposes, surveying during the quiescent period is not recommended, due to the cryptic nature of the cotton seed bug. Surveys conducted during the quiescent period may be useful for determining the efficacy of eradication efforts.

The cotton seed bug has been observed on tree trunks, on the undersides of both living and dead leaves, pods of leguminous plants, cracks in telephone poles or wooden posts and fences, under bark, in old nests of *Polistes*, in crevices between strands of barbed wire, on dried flower heads, among roots of grasses, underneath sheath-leaves of maize and sugarcane, in stored cotton, or in artificial traps such as old sacks on poles or in hedges near cotton fields (Kirkpatrick, 1923). It could also be found in leaf litter beneath cotton plants, or occasionally on the leaves (Smith and Brambila, 2008), in dry fruit pods, under tree bark, between planks of wooden structures, or in dry grass and leaf litter (Adu-Mensah and Kumar, 1977).

Trees appear to be a preferred resting location of the cotton seed bug. Rough barked trees are more attractive than smooth barked trees. The cotton seed bug was rarely observed on willows or poplars. Very few bugs were observed on date-palms and mulberries. The more common trees included various species of *Ficus*, *Acacia*, and some *Eucalyptus*. Colonies on the trees may be detected near the ground, up to a height of 6–7 meters (Kirkpatrick, 1923).

Sweep-netting of weeds between cotton rows, or along field edges is not recommended except in cases where there is a high likelihood that the pest is present. It may be useful to sweep remaining vegetation after removing infested host material. Kirkpatrick (1923) found significant numbers of the cotton seed bug when old sacking was tied to bushes during the winter quiescent period.

Traps

UV-light traps are not recommended for surveying for the cotton seed bug except in cases where there is a need to confirm eradication or enhance detection of a known population. UV-light traps are not pest specific, and consequently are cumbersome and time-consuming for sampling and identification purposes. In addition, it is unclear whether or not UV-light traps would be an effective monitoring tool for the cotton seed bug.

Kirkpatrick (1923) demonstrated positive phototropism in laboratory experiments; however, when Kirkpatrick placed light traps at night in the direct path that the cotton seed bug was known to use between a tree and nearby field where they were coming from, no individuals were captured. It was concluded that the cotton seed bug did not migrate at night, and was not attracted to light at night.

Conversely, Nakache and Klein (1992) noted that the cotton seed bug was strongly attracted to light at night in Israel. Additional research regarding the efficacy of UV-light traps is needed.

Processing Samples

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

Preparation

- **1.** Remove bolls from the plants using pruning shears.
- **2.** Immediately seal the bolls in resealable plastic bags, and store in an ice chest.
- **3.** Preserve the cotton seed bugs in 70 per cent isopropyl alcohol.
- **4.** Send the preserved for identification and preservation.

Shipping

Call the laboratory prior to shipping the samples via overnight delivery service. Refer to *Submitting Survey Samples to Domestic and Other Identifier* on **page F-1** and *Taxonomic Support for Surveys* on **page G-1** for instructions and contact information.

Data Collection and Survey Records

Surveyors should work with their State-level cooperators to identify the best location for the storage of records.

Records should be kept for each survey site. Recording negative results in a survey is just as important as recording positive detections since it helps to define an area of infestation. Survey records and data-recording formats should be consistent, to allow for standardized collection of information.

Use a system of data collection that efficiently tracks the suspect samples at various stages in the confirmation process. If available, use pre-programmed hand-held units with GPS capability to tag each sample.

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 the cotton seed bug. Large aggregations of cotton seed bug may occur on nearly any surface, including trunks, leaves, and fruits of non-host plants.



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 cotton seed bug, *Oxycarenus hyalinipennis* (CSB).

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in accordance with labeling restrictions. During all field visits, ensure that proper sanitation procedures are followed as outlined in *Survey Procedures* on **page 4-1**.

Regulatory Actions and Authorities

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

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides the authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under State authority. State departments of agriculture normally work in conjunction with Federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the States measures are inadequate, USDA can take intrastate regulatory action provided that the governor of the State has been consulted and a notice has been published in the *Federal Register*. If intrastate action cannot or will not be taken by a State, PPQ may find it necessary to quarantine an entire State.

PPQ works in conjunction with State departments of agriculture to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property in the absence of owner permission. PPQ prefers to work with the State to facilitate access when permission is denied, however each State government has varying authorities regarding entering private property. A General Memorandum of Understanding (MOU) exists between PPQ and each State that specifies various areas where PPQ and the State department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected State.

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 current 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 an 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.

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 more information on contacting the officials.

If you need the following:	Then contact:
Additional information concerning communication with Tribal leadership	USDA-PPQ Tribal Liaison
To determine if there are federally-recognized Tribes in a State	State Plant Health Director (SPHD)
To determine if there are sacred or historic sites in an area	State Historic Preservation Officer (SHPO)
Clarification of information	State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO)

Table 5-1 Regulatory Consultation for Tribal Governments

Overview of Regulatory Program After Detection

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

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

Refer to *Forms* on **page B-1** for an example of the PPQ 523 Emergency Action Notification form and instructions for completing it.

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 locate suspect plants, and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout, 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, cotton seed bug cultural control measures, or plant waste material disposal.

Any regulatory treatments used to control the cotton seed bug, 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 database(s) in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

Use of Chemicals

The PPQ *Treatment Manual* and the guidelines identify the authorized chemicals, and describe the methods and rates of application, and any special application instructions. For further information refer to *Control Procedures* on **page 6-1**. Concurrence by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended unless it is specifically labeled for the cotton seed bug. Refer to the PPQ *Treatment Manual* for further information.

Address

PPQ Treatment Manual http://www.aphis.usda.gov/import export/plants/manuals/ online manuals.shtml



Control Procedures

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Introduction

Use *Chapter 6 Control* to learn more about controlling an infestation of the cotton seed bug, *Oxycarenus hyalinipennis* (CSB).

Overview of Emergency Programs

APHIS-PPQ develops and makes control measures available to involved States. Environmental Protection Agency-approved treatments will be recommended when available. If selected treatments are not labeled for use against the organism or in a particular environment, PPQs 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 expedite approval of a product that may not be registered in the United States or to obtain labeling for a new use-site. The PPQ FIFRA Coordinator is available for guidance pertaining to pesticide use and registration. For contact information, refer to *Resources* on **page A-1**.

Treatment Options

Consider the treatment options described within this chapter when taking action to eradicate, contain, or suppress the cotton seed bug. There are various chemical control measures available for use against the cotton seed bug, although it has been found that geographic races are developing based on resistance to insecticides used in various regions of the world (Sweet, 2000).

Biological control using parasites and predators for the cotton seed bug is not generally a practical control tactic (Sweet, 2000). Sabarinathan et al. (2007) found that the spiders *Argiope pulchella* and *Cyrtophora cicatrosa* were effective predators against the cotton seed bug, and have potential as biological control agents. Additional research would be required.

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

Treatments can include any combination of the following options:

- ♦ Sanitation
- ◆ Application of insecticides
- Other cultural control methods

Refer to *Resources* on **page** A-1 for information on contacting the FIFRA Coordinator.

Eradication

Eradication, the elimination of a pest from an area through phytosanitary measures, is the first priority to consider with the introduction of a new pest. Eradication may be feasible when the following conditions exist:

- Pest population is confined to a small area
- Pest population density is low
- Detection occurs soon after the introduction

If an infestation of the cotton seed bug is discovered that is apparently limited in distribution, 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 of the find, and treatment of the soil and surrounding vegetation with an approved pesticide after removal of the infested plants.

Treatment Area

The treatment area for the cotton seed bug has not been defined.

Sanitation

Carry out sanitation in nurseries, gardens, landscapes, fields, and other establishments where hosts are present within the core and buffer areas. Depending on the circumstances and equipment available, use the following techniques.

Burning

Burning old cotton stalks with bolls may limit future damage by the cotton seed bug (Odhiambo, 1957).

Field Sanitation

Removal of all weeds or alternative malvaceous host plants near cotton fields is recommended (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923). Destruction or removal of crop residues after harvest may reduce the population size of the cotton seed bug (Atwal, 1976).

Insecticides

Various chemical control measures are available for use against the cotton seed bug, though it has been found that geographic races are developing based on resistance to insecticides used in various regions of the world (Sweet, 2000). Effective application of insecticides for control of the cotton seed bug may be difficult due to the tendency of the bug to aggregate in many different areas on or near host plants, and to hide within cotton bolls.

In other areas of its distribution, effective control of the cotton seed bug has been achieved through the use of a combination of chemicals with both contact and systemic properties (Smith and Brambila, 2008). Some chemical controls are recommended to be applied as ULV (ultra low volume) sprays applied aerially in early morning while the insects are less active (Smith and Brambila, 2008). Sprays or dusts may be applied when the bugs are seen on newly opened bolls (Hill, 1983).

Ibrahim et al. (1993) tested four insect growth regulator/insecticide mixtures and four synthetic pyrethroids in cotton fields and found that all tested insecticides effectively reduced adult and nymph population levels of cotton seed bug by more than 80 per cent.

In Australia, control of swarms of the similar species *Oxycarenus luctuosus* is difficult because while the chemicals may kill the pests it comes into contact with, it does not restrict the movement of additional pests from nearby alternative host plants. Chemical control was most effective to reduce the numbers of pests on crops, minimizing damage (Chin et al., 2009).

Ripper and George (1965) cautioned against insecticide use as it may spoil the lint in the open bolls lowering the grade of cotton, as well as result in chemical residues in the cotton seed oil. Refer to *Table 6-1* on page 6-5 for a list of insecticides available.



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

Chemical Name	Reference	Registration for Cotton
Chlorpyrifos-ethyl/ hexaflumuron	Ibrahim et al., 1993	Yes/No ²
Dimethoate	Ikisan, 2000	Yes ³
Biphenthrin	lbrahim et al., 1993	Yes
Endosulfan	Sweet, 2000	Yes
Fluvalinate	lbrahim et al., 1993	No ⁴
Malathion	Sweet, 2000	Yes
Metasystox	Ikisan, 2000	No
Methamidophos ⁵	Ibrahim et al., 1993	Yes
Methomyl/diflubenzuron	Ibrahim et al., 1993	Yes/Yes ⁶
Neem	Khan and Ahmed, 2000	No

Table 6-1 Insecticides Available for Use Against the Cotton Seed Bug¹

1 Many of the chemicals are approved for a wide variety of crops and plants. The cotton seed bug is frequently found on fruits, leaves, or young stems of a wide variety of plants. For this reason, we focused on determining the specific registration of chemicals on the primary host.

- 2 Chlorpyrifos-ethyl has been registered for use on cotton. Hexaflumuron has **not** been registered for use on cotton.
- 3 The chemical has been registered for use on cotton.
- 4 The chemical has **not** been registered for use on cotton.
- 5 Listed in Ibrahim et al. (1993) as methamidophos/triflumuron. Triflumuron is not approved for use in the United States.
- 6 Both methomyl and diflubenzuron have been registered for use on cotton.

Treatment of Cotton Seed

Cottonseed treatments T203-f-1 to f-4 (methyl bromide or phosphine treatments) in the PPQ *Treatment Manual* (PPQ, 2010) are reported to be effective against the cotton seed bug in trade of cottonseed (Komm, 2010).

Address

PPQ Treatment Manual http://www.aphis.usda.gov/import_export/plants/manuals/ online_manuals.shtml

Suppression

Pest management includes steps taken to either contain or suppress a pest population. Damage attributed to the cotton seed bug is most effectively managed with the cultural controls described below. According to some older literature, the most effective cultural control tactic was the removal of hibernation sites, weed shelters, and alternative malvaceous hosts near cotton fields (Adu-Mensah and Kumar, 1977; Kirkpatrick, 1923). Damage to cotton seed may be limited by picking earlier in the season and at shorter intervals, reducing the exposure time of the seeds to attack by the cotton seed bug (Kirkpatrick, 1923; Odhiambo, 1957). Removal of all infested plants, and subsequent elimination of residual populations is more likely to effectively suppress populations of the cotton seed bug.

Damage to cotton seed significantly increases when the bolls are affected by a bollworm (Kirkpatrick, 1923). Control of bollworms may reduce early damage to the cotton seed.

Many recommended cultural control practices suggested for controlling the cotton seed bug, such as drying the cotton in the sun or bagging the cotton (Kirkpatrick, 1923; Odhiambo, 1957; Ripper and George, 1965) are infeasible in modern cotton cultivation. Seed cotton is stored in large compacted modules after harvest but before being sent for ginning (Cotton Counts, 2010), which should be covered to prevent further infestation (Pearson, 1958). In addition, the first two steps of the ginning process dry the cotton and send it through cleaning equipment to remove foreign matter (Cotton Counts, 2010) which may reduce staining to the ginned cotton.

Summary

The most effective control program for suppression of the cotton seed bug 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 a cotton 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 Compliance

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Introduction

Use *Chapter 7 Environmental Compliance* as a guide to environmental regulations when conducting a program against cotton seed bug, *Oxycarenus hyalinipennis* (CSB).

Overview

A key element in designing a program or an emergency response is consultation with Environmental Services (ES), a unit of APHIS' Policy and Program Development Staff (PPD). PPD–ES prepares environmental documentation such as Environmental Impact Statements (EIS) and Environmental Assessments (EA) to aid in program operational decisions, as well as endangered species consultation. PPD–ES also coordinates pesticide registration and approvals for APHIS pest control and eradication programs, ensuring that registrations and approvals meet program needs and conform to pesticide use requirements.

Refer to the appendix *Resources* on **page A-1** for contact information for staff in PPD-Environmental Services and EDP-Environmental Compliance.

National Environmental Policy Act

Agencies should prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) concurrently and integrated with environmental impact analyses, surveys, and studies required by the Fish and Wildlife Coordination Act, National Historic Preservation Act of 1966, Endangered Species Act, and other laws and executive orders. Environmental documents prepared to comply with other Acts also may be incorporated into National Environmental Policy Act (NEPA) documents as part of the NEPA process.

Categorical Exclusion

Categorical Exclusions (CE) are categories 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 generally required.

USDA–APHIS managers are encouraged to use categorical exclusions where appropriate to reduce paperwork and speed up decision making. Proposed actions are subject to sufficient environmental review to determine whether they fall within the broadly defined categories. Each time a specific categorical exclusion is used, the required review must be done. An EA may be prepared for proposed actions otherwise excluded when the manager determines that the action may have potential to significantly affect the environment or an EA would be helpful in planning or decision-making.

Environmental Impact Statement

An Environmental Impact Statement (EIS) is a detailed statement that must be included in every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment. The primary purpose of an EIS is to serve as an action-forcing device to ensure that the policies and goals defined in the National Environmental Policy Act (NEPA) are infused into the ongoing programs and actions of the Federal government. Generally, EIS's are prepared when Federal agencies recognize that their actions have the potential for significant environmental effects (adverse or beneficial), or when an Environmental Assessment leads to a finding of potentially significant impact.

APHIS prepares EIS's for administrative proceedings that establish broad scale significant impact-generating strategies, methods, or techniques, such as large-scale aerial pesticide applications. This can include contingency or emergency strategies that are comprehensive in scope or long-range plans with potential for significant environmental impact. APHIS also prepares programmatic EIS's to examine strategies and options for dealing with issues with important implications for the maintenance and enhancement of environmental quality.

Environmental Assessment

An Environmental Assessment (EA) is a concise public document that briefly provides sufficient evidence and analysis for determining whether to prepare an Environmental Impact Statement (EIS) or a Finding of No Significant Impact (FONSI). An EA aids an agency's compliance with the National Environmental Policy Act (NEPA) when no EIS is necessary and facilitates the preparation of an EIS when necessary. Generally, an EA leads to a FONSI or an EIS, but it could also lead to abandonment of a proposed action.

The content of an EA must include brief discussions of the need, alternatives, and potential environmental impacts of the proposal, and a list of agencies and persons consulted.

Environmental Monitoring

APHIS–PPQ requests assistance from EDP-Environmental Services (EDP–ES) before PPQ personnel or funding are used for control operations. Additionally, program staff should consult with PPQ–EDP-Environmental Compliance (EDP–Environmental Compliance) staff to determine if an environmental monitoring plan is required for the operation. State, regional, and national program managers will determine which counties need to be treated. Refer to *Resources* on **page A-1** for contact information for staff members in EDP-Environmental Services and EDP-Environmental Compliance.

Biological Assessment

A biological assessment is an analysis of the effects that a Federal agency action may have on listed or proposed endangered or threatened species and designated critical habitat. The Endangered Species Act (ESA) requires this analysis if the proposed action may affect a listed species. In such a case, consultation with the U.S. Fish and Wildlife Service (FWS) or the National Marine Fisheries Service (NMFS) is required. Federal agencies are required to ensure that any action authorized, funded, or carried out, is not likely to jeopardize listed species or result in adverse modification of designated critical habitat.



Pathways

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Introduction

Use *Chapter 8 Pathways* as a source of information on the pathways of introduction of the cotton seed bug, *Oxycarenus hyalinipennis* (CSB) in the United States. The cotton seed bug moves easily in trade, even with commodities that are not known as hosts. The cotton seed bug may also enter the continental United States through natural movement from nearby islands in the Caribbean.

Natural Movement

The cotton seed bug may be introduced into the continental United States through natural movement. In recent years, it has been observed steadily extending its distribution northward through the Caribbean (Smith and Brambila, 2008), though there have been no definitive studies conducted on the specific mode of spread. The cotton seed bug has been documented flying short distances, but may also be aided by wind. The occurrence of hurricanes or tropical storms may aid the spread of the cotton seed bug from Caribbean islands to the continental United States.

Commerce

The cotton seed bug moves easily in trade, even with commodities that are not known as hosts (CAPS, 2007; Henry, 1983). The cotton seed bug has been intercepted 570 times at U.S. ports-of-entry since 1984, primarily on cut

flowers or fruit for consumption. A few interceptions have also been recorded on plants for propagation. Seventy percent of those interceptions have occurred since 2000. The cotton seed bug has been found to move in both baggage and commercial cargo shipments. Sixty-six percent of the interceptions were recorded in permit cargo, and 26 percent were recorded in baggage. Eighty-four percent of the interceptions occurred on plants not known as hosts (PestID, 2010).

Given the frequency of interceptions on non-host material, it would be extremely difficult to effectively restrict the importation of this species in trade. Inspection is especially important in reducing the risk of moving the cotton seed bug in commerce.

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

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Glossary

Use the *Glossary* to find the meaning of specialized words, abbreviations, acronyms, and terms used by USDA–APHIS–PPQ–Emergency and Domestic Programs.

acute. pointed or triangular **APHIS**. Animal and Plant Health Inspection Service AQAS. Agricultural Quarantine Activity System, a Web database accessible from any USDA-APHIS computer backtracking. tracing the possible movement of infested materials to determine the extent of the infestation CFR. Code of Federal Regulations confirmed detection. positive identification of a submitted specimen containment control. application of phytosanitary measures in and around an infested area to prevent spread of a pest conterminous. having a boundary in common control. suppression, containment or eradication of a pest population **CPHST.** Center for Plant Health Science and Technology day degree. measure of physiological time using the accumulation of heat units (degrees) above an insects developmental threshold for a 24-hour period delimiting survey. survey to determine the extent of the infestation in an area after the target species has been detected detection. collection of any life stage of the target pest detection survey. survey conducted in a susceptible area not known to be infested with the target pest developmental thresholds. minimum and/or maximum temperatures that support physiological development of a species dispersal, active. spread of an organism by its own method of locomotion (e.g., walking, flight, etc.) dispersal, passive. spread of an organism aided by other than its own method of locomotion (e.g., wind, water, man, etc.) diurnal. active during the day EAN. Emergency Action Notification **EPA.** United States Environmental Protection Agency eradication. application of phytosanitary measures to eliminate a pest from an area before it becomes too large in area or numbers for current technology entomopathogen. pathogen that induces illness in insects exotic species. pest species not native to or historically resident in North

America

FIFRA. Federal Insecticide, Fungicide, and Rodenticide Act **FONSI.** Finding of No Significant Impact

fumigation. application of an approved fumigant, such as methyl bromide, as a treatment

generation. period of time for the pest to complete all stages of development predicated on the basis of biological information

GPS. global positioning system

ground spray. using ground spray equipment to apply pesticide to the ground, selected resting places, or host vegetation in a target infested area

host. plant species, substrate, debris, or other food reproduction of the target pest

host collecting. collection and retention of infested host material for the purposes of determining characteristics of a pest's use of the host; also known as holding

ICS. Incident Command System

indigenous. native

ISIS. Integrated Survey Information System

infestation. collection of one or more target pests from an area

malvaceous. belonging to the Malvaceae, a family of plants that includes mallow, cotton, okra, althaea, and abutilon

management. application of selected phytosanitary measures in and around an infested area to keep an invading population in check when other means of eradication of the population would fail

monitoring. using interdependent visual or trapping surveys in an area where treatment has been applied to evaluate the effectiveness of the application; also known as evaluation survey

NAPIS. National Agricultural Pest Information System

natural enemies. living organisms found in a natural community that kill, weaken or inhibit the biological potential of a pest species

nonmigratory. species in which the individuals typically do not move far from the area of their birthplace

parasites. organism living on the host at one or multiple life stages; may kill or debilitate the host

pathogen. agent, usually microbial, that induces illness

pathway. means by which plant pests are introduced

PestID. database containing all the information recorded from the PPQ Form 309 Pest Interception Record

phenology. timing of recurrent biological events

plant hardiness zones. planting zones are defined in the USDA Plant

Hardiness Zone Map which is maintained by the U.S. National Arboretum **PPQ.** USDA-Plant Protection and Quarantine

predator. free-living organisms that consume substantial numbers of prey **regulated articles.** all known or suspected hosts of a confirmed infestation of an exotic species including soil and any other suspected product or article

regulatory inspection. visual examination of host material, containers, and transport soil treatment. application of an approved insecticide to the soil of nursery or within the drip line of host plants **SPHD.** State Plant Health Director SPRO. State Plant Regulatory Official suppression. application of phytosanitary measures in an infected area to reduce pest populations sweep net. survey method in which a mesh net suspended around a hoop is swept through the air or around vegetation to collect insects **TESS**. Threatened and Endangered Species System trap survey. determining the presence or absence of a pest by the use of traps placed in a predetermined pattern and serviced on a given schedule true host. capable of sustaining reproduction (e.g., in the order Malvales) **USDA**. United States Department of Agriculture **USFWS**. United States Fish and Wildlife Service visual survey. examining hosts, substrate, or hiding places for eggs, larvae, adults, or visible damage

Glossary



Resources

Use the information in *Appendix A Resources* to contact the regulatory staff mentioned in the guidelines.

Table A-1 Cotton Seed Bug Resources

USDA-PPQ Function	Contact Information
Environmental Services Coordinator	USDA–APHIS-Policy and Program Development 4700 River Road Riverdale, MD 20737 Telephone: (301) 734-5861
Environmental Compliance Coordinator	USDA–APHIS–PPQ–Emergency and Domestic Programs 4700 River Road Riverdale, MD 20737 Telephone: (301) 734-7175
Tribal Liaison	USDA–APHIS–PPQ National Program Manager for Native American Program Delivery and Tribal Liaison 14082 S. Poston Place Tucson, AZ 85736 Telephone: (520) 822-5440
State Plant Health Directors (SPHDs)	State Plant Health Director (SPHD) http://www.nationalplantboard.org/member/
State Historic Preservation Officers (SHPOs)	National Register of Historic Places State Historic Preservation Officers http://www.nps.gov/history/nr/shpolist.htm
State and Territory Plant Regulatory Officials (SPROs)	Postentry Quarantine Manual for State Inspectors http://www.aphis.usda.gov/import_export/plants/ manuals/online_manuals.shtml

Resources



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Determination B-5
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Instructions for Completing PPQ Form 523, Emergency Action
Notification B-9

	This report is authorized by law (7 your cooperation is needed to mak	U.S.C. 147a). While y	you are no of plant pe	ot request co	uired to ndition	o respon	nd	See	e reverse for addition	al OMB inform		M APPROVED
	ANIMAL AND PLANT HEALTH INSPECTION SERVICE			Instructions: Type or print information requested. Press hard and print when handwritten. Item 1 - assign number for each collection beginnin year, followed by collector's initials and collector's number. Example (c John J. Dingle): 83-JJD-001.				l print legibly ginning with	FOR II LOT NO.	IBIII USE		
	Pes			Pest Data Section – Complete Items 14, 15 and 16 or 19 or 20 and 2 applicable. Complete Items 17 and 18 if a trap was used.				d 21 as	PRIORITY			
	1. COLLECTION NUMBER			2. DATE 3. SUBMITTING AGENCY								
	MO			D	A	YR	State PPQ Other					
SIN				5. T	5. TYPE OF PROPERTY (Farm, Feedmill, Nursery, etc.)							
SENDER AND ORIGIN	6. ADDRESS OF SENDER					NTERCEPTION SITE	7. N	IAM	E AND ADDRESS O	F PROPERTY	OR OWNER	
DER.						ERCE						
SEN		ZIP				_					COUNTRY/ COUNTY	
	A. Biological Control (Target		SON FOR I	IDENT	FIFICAT	'ION ("x".	ALL App E.	olica	<i>able Items)</i> Livestock, Dome	stic Animal F	Pest	
OSE	B. Damaging Crops/Plants		,				F.		Possible Immigra			
PURPOSE	C. Suspected Pest of Regula	atory Concern (Explain	n in REMA	RKS))		G.		Survey (Explain		<i>,</i>	
₽.	D. Stored Product Pest						H.		Other (Explain in			
	 IF PROMPT OR URGENT IDENTIFI 10. 	HOST INFORMATION	D, PLEASE	PRO	VIDE A	BRIEF E	XPLAN	ATIC		NS". QUANTITY OF	HOST	
	NAME OF HOST (Scientific name when								R OF /PLANTS		FECTED (Inser	t figure and
HOST DATA							/10/				Percent):	
	12. PLANT DISTRIBUTION					13. PLA	ANT PAP	RTS	AFFECTED		,	
		се			Seeds							
Ŧ		Leaves, Lower Surfa					Buds					
		Petiole Stem			Growin Roots	ig rips			Flowers			
	14. PEST DISTRIBUTION		ISECTS] NEM	ΑΤΟ	DDES		MOLLUSKS	
		NUMBER JBMITTED LARVAE	PUPA	AE	ADU	LTS	CAST	SKI	NS EGGS	NYMPHS	JUVS.	CYSTS
ATA	ABUNDANT	ALIVE										
PEST DATA	16. SAMPLING METHOD	DEAD 17. TYPE	OF TRAP	AND L	URE				18. TRAP NU	JMBER		
Ē	19. PLANT PATHOLOGY – PLANT SY		describe syr	mptom	ıs)							
	20. WEED DENSITY	RAL	21. WEE	D GRC	OWTH S	STAGE						
	EFEW SPOTTY	GENERAL	SEEI	DLIN	G [VEGE	TATIV	E		FRUITING	MATURE	-
	22. NEWANG											
	23. TENTATIVE DETERMINATION											
	24. DETERMINATION AND NOTES (A	Vot for Field Use)									BIII USE	
										DATER	ECEIVED	
										NO.		
										LABEL	D	
	PREPARED DATE ACCEPTED											
										DATE A	CCEPTED	
	SIGNATURE		—	DA	TE					RR		
	PPQ FORM 391 Previous editio (AUG 02)	ons are obsolete.										
Т٢		nust be dissemin:	ated as f	ollov	vs.							
	This is a 6-Part form. Copies must be disseminated as follows:											
	PART 4 – INTERMEDIATE IDENT	FIFIER PART !	5 – INTER	MED	IATE II	DENTIF	IER		PART 6 – RET	AINED BY SU	JBMITTER	

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

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

- 1. Send Original along with the sample to your Area Identifier.
- 2. Retain and file a copy for your records.

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

Purpose

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

Instructions

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

Address

Fillable PPQ Form 391 http://cals-cf.calsnet.arizona.edu/azpdn/labs/submission/ PPQ Form 391.pdf

Distribution

Distribute PPQ Form 391 as follows:

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

	Determination	
Block		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 PROVIDE 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 HOS	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 forDetermination

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

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

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE		SERIAL NO.			
		1. PPQ LOCATION	2. DATE ISSUED		
3. NAME AND QUANTITY OF ARTICLE(S)		4. LOCATION OF ARTICLES			
		5. DESTINATION OF ARTICLES			
6. SHIPPER		7. NAME OF CARRIER			
		8. SHIPMENT ID NO.(S)			
. OWNER/CONSIGNEE OF ARTICLES		10. PORT OF LADING	11. DATE OF ARRIVAL		
. OWNER/CONSIGNEE OF ARTICLES			Th. DATE OF ANNIVAL		
Name:		12. ID OF PEST(S), NOXIOUS WEED	S, OR ARTICLE(S)		
Address:					
		12a. PEST ID NO.	12b. DATE INTERCEPTED		
		—			
		13. COUNTRY OF ORIGIN	14. GROWER NO.		
PHONE NO.	FAX NO.	15. FOREIGN CERTIFICATE NO.			
SS NO.	TAX ID NO.				
Act (7 USC 8303 through 8306), you and he pest(s), noxious weeds, and or an neasures shall be in accordance with the	ne Plant Protection Act (7 USC 7711, 771 re hereby notified, as owner or agent of th tide(s) specified in Item 12, in a mannen he action specified in Item 16 and shall be	15a. PLACE ISSUED 1, and 7714) and Sections 10404 through the owner of said carrier, premises, and/or a r satisfactory to and under the supervisio completed within the time specified in Item HEREIN DESIGNATED MUST NOT BE	articles, to apply remedial measures n of an Agriculture Officer. Reme n 17.		
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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

Figure B-3 Example of PPQ 523 Emergency Action Notification

Purpose

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

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

Instructions

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

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

Follow the instructions in *Table B-2* on page B-9 when completing PPQ 523 for the cotton seed bug. Find additional instructions for completing, using, and distributing the form in the PPQ *Manual for Agricultural Clearance*.

Address

PPQ Manual for Agricultural Clearance http://www.aphis.usda.gov/import_export/plants/manuals/ online_manuals.shtml

Table B-2 Instructions for Completing PPQ Form 523, Emergency Activ	on
Notification	

Block		Instructions
1	COLLECTION NUMBER	ENTER the name and location of the nearest PPQ office
2	DATE	ENTER the date of the collection
3	PPQ LOCATION	ENTER the host scientific name and cultivar
4	LOCATION OF ARTICLES	ENTER the location of the article (premise location, pier, dock, container yard, hold space, etc.)
6	SHIPPER	ENTER the plant material source if known
7	NAME OF CARRIER	LEAVE blank unless that information is known
8	SHIPMENT ID NO.	LEAVE blank unless that information is known
12	ID OF PEST	To place plant material on a property on "Hold", enter "suspect <i>Oxycarenus hyalinipennis</i> , cotton seed bug"; the authority under which actions are taken is The Plant Protection Act of 2000, Statute 7 USC 7701-7758
16	ACTION REQUIRED	ENTER the following text: "All host plants of <i>Oxycarenus hyalinipennis</i> , the cotton seed bug (CSB), are prohibited from movement from the property pending further notification by USDA–APHIS–PPQ and/or the State department of agriculture. No other plant material may leave the property until further evaluations can be made. After further investigations are conducted on the listed plants and other host material, if a positive detection is confirmed on the property, plant material will be treated/destroyed under supervision, with approved methods in accordance with USDA and State policies. Any additional hosts of the cotton seed bug on the property are subject to Federal and State quarantine requirements prior to movement from the property."

Forms



Reported Hosts

Appendix C, Reported Hosts is a list of host records of the cotton seed bug that have been reported in the literature. The cotton seed bug requires the availability of seeds within the order Malvales for growth and reproduction; however, many authors have reported the cotton seed bug on many additional host plants, though the true host status of these plants is unknown. Many may be merely incidental records of cotton seed bug resting or hitchhiking on various plants, or could also be records of the bug piercing fruits, leaves, or young stems, in search of moisture.

Host plants within the order Malvales, on which the cotton seed bug has been observed growing and laying eggs, are listed in *Table 2-2* on page 2-6.

(continued)		
Scientific Name	Plant Family	Common Name
Asclepias spp.	Asclepiadaceae	Silkweed
Bauhinia variegata	Fabaceae	Mountain ebony
Bougainvillea glabra	Nyctaginaceae	Paperflower
Brachychiton sp.	Sterculiaceae	Bottletree
Brassica juncea	Brassicaceae	Indian mustard
Cajanus cajan	Fabaceae	Pigeon pea
Callicarpa sp.	Lamiaceae	Beautyberry
Cassia siamea	Fabaceae	Kassod tree
Ceiba sp. ²	Bombacaceae	Ceiba
Celosia cristata	Amaranthaceae	Cockscomb
Cestrum diurnum	Solanaceae	Day jessamine
Chrysanthemum sp.	Asteraceae	Chrysanthemum
Citrus sp.	Rutaceae	Citrus
Crossandra sp.	Acanthaceae	Crossandra
<i>Cydonia</i> sp.	Rosaceae	Cydonia
<i>Dahlia</i> sp.	Asteraceae	Dahlia
Dalbergia sissoo	Fabaceae	Indian rosewood
Diospyros sp.	Ebenaceae	Persimmon
Eranthemum pulchellum	Acanthaceae	Blue-sage
Ficus carica	Moraceae	Fig
Ficus racemosa	Moraceae	Clustertree
<i>Gmelina</i> sp.	Lamiaceae	Beechwood
Citrus sp.	Rutaceae	Citrus
Crossandra sp.	Acanthaceae	Crossandra
<i>Cydonia</i> sp.	Rosaceae	Cydonia
<i>Dahlia</i> sp.	Asteraceae	Dahlia
Dalbergia sissoo	Fabaceae	Indian rosewood
Diospyros sp.	Ebenaceae	Persimmon
Eranthemum pulchellum	Acanthaceae	Blue-sage
Ficus carica	Moraceae	Fig
Ficus racemosa	Moraceae	Clustertree
<i>Gmelina</i> sp.	Lamiaceae	Beechwood
Grewia subinaequalis	Tiliaceae	Raisin bush
Helianthus annuus	Asteraceae	Sunflower
Helichrysum sp.	Asteraceae	Strawflower
lxora barbata	Rubiaceae	Bearded ixora
Jacquemontia pentanthos	Convolvulaceae	Skyblue clustervine

Table C-1 Reports in the Literature of Cotton Seed Bug on Various Host Plants¹ (continued)

Scientific Name	Plant Family	Common Name
Jasminum multiflorum	Oleaceae	Star jasmine
Lagerstroemia indica	Lythraceae	Crapemyrtle
Lantana camara	Verbenaceae	Lantana
Malus sp.	Rosaceae	Apple
Mangifera indica	Anacardiaceae	Mango
Nerium indicum	Apocynaceae	Indian oleander
Pennisetum glaucum	Poaceae	Pearl millet
Persea americana	Lauraceae	Avocado
Phoenix dactylifera	Arecaceae	Date-palm
Plumeria rubra	Apocynaceae	Templetree
Poinsettia pulcherrima	Euphorbiaceae	Poinsettia
Prosopis juliflora	Fabaceae	Mesquite
Prunus persica	Rosaceae	Peach
Prunus spp.	Rosaceae	Stone fruit
Psidium guajava	Myrtaceae	Guava
<i>Pyrus</i> sp.	Rosaceae	Pear
Ricinus communis	Euphorbiaceae	Castor bean
Saccharum officinarum	Poaceae	Sugarcane
Solanum melongena	Solanaceae	Eggplant
Solanum nigrum	Solanaceae	Black nightshade
Spondias mangifera (=S. pinnata)	Anacardiaceae	Indian hog plum
Tabernaemontana divaricata	Apocynaceae	Pinwheelflower
Tecoma stans	Bignoniaceae	Yellow trumpetbush
Thevetia peruviana	Apocynaceae	Luckynut
Tithonia sp.	Asteraceae	Mexican sunflower
Triticum vulgare	Poaceae	Wheat
Vigna sinensis (=V. unguiculata?)	Fabaceae	Blackeyed pear
Vigna unguiculata	Fabaceae	Cowpea
<i>Vitis</i> sp.	Vitaceae	Grape
Withania somnifera	Solanaceae	Withania
Zea mays	Poaceae	Maize

Table C-1 Reports in the Literature of Cotton Seed Bug on Various Host Plants¹ (continued)

1 The true host status of these reports has not been tested or verified.

2 The only reference to the cotton seed bug on *Ceiba* sp. is an alternative host in Hill (2008). The true host status of *Ceiba* sp. is unclear, even though it is in the order Malvales.

Reported Hosts



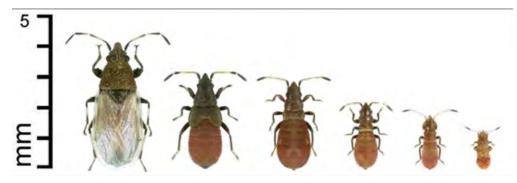


Figure D-1 Adult and five nymphal instars of cotton seed bug (Natasha Wright, Division of Plant Industry, Florida Department of Agriculture and Consumer Services)



Figure D-2 Adult cotton seed bug (Natasha Wright, Division of Plant Industry, Florida Department of Agriculture and Consumer Services. <u>http://www.ipmimages.org/browse/detail.cfm?imgnum=5190058</u>)



Figure D-3 Adult cotton seed bug (Julieta Brambila, USDA-APHIS-PPQ)



Figure D-4 Male genital capsule of adult cotton seed bug with triangular dorsal opening (Julieta Brambila, USDA–APHIS–PPQ)



Figure D-5 Cotton Seed Bug Male Capsule Attached to the Body (Julieta Brambila, USDA-APHIS-PPQ, 2010)

Images



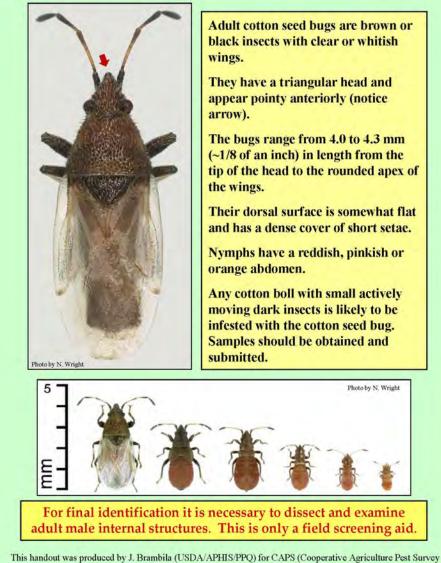
Screening Aid

1/2011-1 Emergency and Domestic Programs



Field Screening Aid for the Cotton Seed Bug Oxycarenus hyalinipennis (Hemiptera)





This handout was produced by J. Brambila (USDA/APHIS/PPQ) for CAPS (Cooperative Agriculture Pest Survey program). Natasha Wright (Division of Plant Industry, Florida Department of Agriculture and Consumer Services) provided the photographs. Susan Halbert and Leroy Whilby (both with DPI-FDACS) reviewed this screening aid. June 2010

Figure E-6 Field Screening Aid for the Cotton Seed Bug (Julieta Brambila, USDA–APHIS–PPQ, 2010)



Submitting Survey Samples to Domestic and Other Identifier

Procedures for Submitting Survey Samples to Domestic and Other Identifiers

A. 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.

Submission requirements for insects are:

- Sorting trap samples: Trapping initiative is most commonly associated with a
 pest survey program, such as Wood Boring and Bark Beetles (WBBB), see <u>Bark
 Beetle Submission Protocol</u> 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.
- 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: <u>http://pest.ceris.purdue.edu/caps/screening.php</u>
- 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 prior to shipping. This can be accomplished by placing them in a vial of alcohol or place 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% 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 very 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, the majority of liquid can be decanted off from the vial and then sealed tightly in the container just prior to shipping. Notify 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.

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Figure F-1 Procedures for Submitting Survey Samples to Domestic and Other Identifiers, page 1

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. 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 very 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, it should be wrapped 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 notify the identifier prior to shipping. Remember to inform 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/PPO_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 identifications prior to 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 crossreference 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.

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Figure F-2 Procedures for Submitting Survey Samples to Domestic and Other Identifiers, page 2

B. PLANT SAMPLES FOR PLANT PATHOLOGY ANALYSIS

1. Sampling

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

2. Storing

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

3. Documentation

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

4. Packing

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

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

5. Shipping

The Identifier Laboratory should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded, what types of samples they are (e.g. SOD-suspect Camellia leaves), when the samples will be shipped, and the package tracking number.

Label the shipping box as 'URGENT' and send via overnight express courier (FedEx, UPS, Airborne, DHL, etc) to the appropriate Identifier.

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Figure F-3 Procedures for Submitting Survey Samples to Domestic and Other Identifiers, page 3

C. MOLLUSKS

1. Specimen Handling

When collecting live samples, specimens should be placed directly into water making sure that no air bubble remains inside. Seal for 24 hours or until drowned, then transfer to 70 percent ethyl alcohol. Replace the water with a 70-80 percent alcohol solution after the snail has extended from the shell or when the slug is fully extended. Label the container with the appropriate information. After handling slug samples, hands should be washed in hot soapy water, and rinsed in alcohol or a standard disinfectant.

2. Labeling & Documenting Samples

Collection information is vital and should be completed immediately after a collection is made. Write directly on the collection container or on a paper label placed inside the vial using a pencil or with alcohol-proof ink. Complete PPQ form 391, *Specimens for Determination*. Write the date, collector's name, collector's contact information, and location including any transect and plot numbers. If multiple vial samples are collected from a location, assign individual sample numbers. When transferring the specimens to alcohol, ensure the label accompanies the sample.

3. Sample Submission Procedures

Sort samples:

As such, it is important to sort out the debris and non-target pests. The taxonomic level of sorting will depend on the expertise available on hand and can be confirmed with the identifier.

Screen Target Pests:

Utilize local resources. Some states may have taxonomic support, access local training aids or identification guides.

Packaging and Shipping:

Ensure specimens are dead prior to shipping. Use a sturdy cardboard box or heavy styrofoam container so that the samples are not damaged during shipping and handling. When shipping large vials, carefully wrap vials with adequate packing material so that if breakage occurs during transit, the alcohol will not leak outside the shipping box. It is recommended that vials be wrapped in ziptype bags.

4. Identification

The Identifier should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded and when the samples will be shipped.

Reporting results are "positive" or "negative." Identifications usually take 2-3 weeks. However, identification time may take longer based on identifier's current workload or the volume of samples submit.

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Figure F-4 Procedures for Submitting Survey Samples to Domestic and Other Identifiers, page 4

Taxonomic Support for Surveys

Background

On June 13, 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02 which established the role of the PPQ National Identification Services as the point of contact for all domestic plant pest confirmations and communications. The National Identification Services (NIS) coordinates the identification of plant pests in support of USDA's regulatory programs. Accurate and timely identifications provide the foundation for quarantine action decisions and are essential in the effort to safeguard U.S. agricultural and natural resources.

National Identification Services employs and collaborates with scientists specializing in 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. For further information, contact the Domestic Diagnostics Coordinator.

Address

Joel Floyd, Domestic Diagnostics Coordinator USDA-APHIS-PPQ-PSPI-NIS 4700 River Rd., Unit 52 Riverdale, MD 20737 Telephone: (301) 734-4396 Fax: (301) 734-5276 Email: 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 access to good taxonomic support within their borders. Taxonomic support should be accounted for in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the State often may require supplies, develop training materials, or need to hire technicians to meet the needs of screening and identification. Moreover, when considering whether to survey for a particular pest it is advisable to consider the challenges of taxonomic support.

Sorting and Screening

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

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

Screening is a higher level of discrimination of samples. 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. The degree of appropriate screening is dependent on the target group, and the training, experience, and demonstrated ability, of the screener.

Check individual survey protocols to determine if samples should be sorted, screened 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 the USDA–Cooperative Agricultural Pest Survey (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, posts them on the CAPS Web site. If local screening aids are developed, please notify the Domestic Diagnostics Coordinator. Visit the CAPS Website to view the screening aids available.

Address	Joel Floyd, Domestic Diagnostics Coordinator USDA–APHIS–PPQ–PSPI–NIS 4700 River Rd., Unit 52 Riverdale, MD 20737 Phone: 301-734-4396 Fax: 301-734-5276 Email: joel.p.floyd@aphis.usda.gov
Address	CAPS Screening Aids Web site: <u>http://pest.ceris.purdue.edu/caps/screening.php</u>

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a State is not adequate for a particular survey other entities may assist, including PPQ identifiers, universities, State departments of agriculture, 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 the 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 PPQ Eastern Region 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 access our PestID database, and 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 PestID 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.

Specialty	Area of Coverage	Identifier's Name/Address	Telephone/Fax/Email
forest pests (Coleoptera, Hymenoptera)	Eastern Region	Robert Brown Domestic Entomology Identifier USDA–APHIS–PPQ 901 W. State Street Smith Hall Purdue University West Lafayette, IN 47907-2089	Phone: 765-496-9673 Fax: 765-494-0420 robert.c.brown@aphis.usda.gov
adult Lepidoptera, Hemiptera	Eastern Region	Julieta Brambila Domestic Entomology Identifier USDA–APHIS–PPQ P.O. Box 147100 Gainesville, FL 32614-7100	Phone: 352-372-3505 ext. 438, 182 Fax: 352-334-1729 julieta.bramila@aphis.usda.gov
To be determined	Western Region	Kira Zhaurova Domestic Entomology Identifier USDA–APHIS–PPQ Minnie Belle Heep 216D 2475 TAMU College Station, TX 77843	Phone: 979-450-5492 kira.zhaurova@aphis.usda.gov
molecular diagnostics (citrus greening, <i>Phytophthora</i> <i>ramorum</i> , bacteriology, cyst nematode screening)	Eastern Region	Grace O'Keefe Domestic Plant Pathology Identifier USDA–APHIS–PPQ 105 Buckhout Lab The Pennsylvania State University University Park, PA 16802	Phone: 814-865-9896 Cell: 814-450-7186 Fax: 814-863-8265 grace.okeefe@aphis.usda.gov
molecular diagnostics (citrus greening, <i>Phytophthora</i> <i>ramorum</i> , cyst nematode screening)	Western Region	Craig A. Webb, Ph.D. Domestic Plant Pathology Identifier USDA–APHIS–PPQ Department of Plant Pathology Kansas State University 4024 Throckmorton Plant Sciences Manhattan, KS 66506-5502	Cell: 785-633-9117 Phone: 785-532-1349 Fax: 785-532-5692 craig.a.webb@aphis.usda.gov

Table G-1 PPQ Domestic Identifiers

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. See the Manual for Agricultural Clearance, Appendix G, Table G-1-1 for those identifiers. They will then send it to the NIS-recognized authority for that taxonomic group.

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Address
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Manual for Agricultural Clearance Appendix G: Table G-1-1 http://www.aphis.usda.gov/import_export/plants/manuals/ports/mac.shtml

State level taxonomists, who are reasonably sure they have a new U.S. 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 U.S. 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.

Communication 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.

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