United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

New Pest Response Guidelines

Helicoverpa armigera (Hübner)

Old World Bollworm

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First Edition Issued 2014

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Cover Image

Helicoverpa armigera larva and adults; Photo Credit: Gyorgy Csoka, Hungary Forest Research Institute, Bugwood.org.

Chapter

1

Introduction

Introduction

Use *New Pest Response Guidelines: Helicoverpa armigera* (Hübner); Old World Bollworm when designing a program to detect, monitor, control, contain or eradicate an outbreak of this pest in the United States and collaborating territories.

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

Any new detection may require the establishment of an incident command system to facilitate emergency management. This document is meant to provide the information necessary to launch a response to an *H. armigera* detection.

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

Users

The guidelines are intended as a field reference for the following users who have been assigned responsibilities for a plant health emergency involving *H. armigera*:

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

Contacts

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

- Academic entities with agricultural interests
- Agricultural interests in other countries
- Commercial interests
- Grower groups such as specific commodity or industry groups
- Land-grant universities and cooperative extension services
- National, state and local news media
- Other federal, state, county and municipal agricultural officials
- Public health agencies
- The public
- State and local law enforcement officials
- Tribal governments

Initiating an Emergency Pest Response Program

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

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported
2. The pest is examined and pre-identified by regional or area identifier
3. The pest's identity is confirmed by a national taxonomic authority recognized by the USDA–APHIS–PPQ National Identification System
4. Published New Pest Response Guidelines are consulted or a new NPAG is assembled to evaluate the pest
5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators and trading partners
6. A delimiting survey is conducted at the site of detection
7. An incident assessment team may be sent to evaluate the site

8. A recommendation is made, based on the assessment of surveys, other data and recommendation of the incident assessment team or the NPAG as follows:
   - **A.** Take no action
   - **B.** Regulate the pest
   - **C.** Contain the pest
   - **D.** Suppress the pest
   - **E.** Eradicate the pest
9. State departments of agriculture are consulted
10. If appropriate, a control strategy is selected
11. A PPQ Deputy Administrator authorizes a response
12. A command post is selected and the incident command system is implemented
13. State departments of agriculture cooperate with parallel actions using a unified command structure
14. Trace-back 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 surveys are conducted to evaluate program success
21. Pest monitoring surveys are conducted to evaluate program success
22. Programs are designed for eradication, containment or long-term use

Preventing an Infestation

Federal and state regulatory officials must conduct inspections and apply prescribed measures to ensure that pests do not spread within or between properties. Federal and state regulatory officials conducting inspections should follow the sanitation guidelines in the section *Preparation, Sanitization and Clean-Up* on page 6-1 before entering and upon leaving each property to prevent contamination.

Scope

The guidelines are divided into the following chapters:

1. *Introduction* on page 1-1
2. *Taxonomy* on page 2-1

3. *Identification* on page 3-1
4. *Biology* on page 4-1
5. *Damage* on page 5-1
6. *Survey Procedures* on page 5-1
7. *Regulatory Procedures* on page 7-1
8. *Control Procedures* on page 8-1
9. *Environmental Compliance* on page 9-1
10. *Pathways* on page 10-1

The guidelines also include appendices and a list of literature cited.

Authorities

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

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

Program Safety

The 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 Decision Making

The 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 the pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policies for pest response programs.

How to Use the Guidelines

The guidelines are 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 to coincide with the America National Standards Institute (ANSI) and are formatted as follows:

Example — Example provides an example of the topic.

Important — Important indicates information that is helpful.

CAUTION

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

DANGER

DANGEROUS indicates that people could easily be hurt or killed.

NOTICE

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

WARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

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

Lists

Bulleted lists indicate information listed in no particular order. Numbered lists indicate that information will be used in a particular order.

Disclaimers

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

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. At the bottom of the page is the year, edition, title and page number. PPQ–Pest Detection and Emergency Programs (PDEP) is the unit responsible for the content of the guidelines.

Footnotes

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 with the heading following 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 and tables are cross-referenced in the body of the guidelines and are highlighted in blue hypertext type.

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 represents the page, table or figure. This numbering scheme allows for identification and updating. Dashes are used in the 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, appendices, tables or index is updated. If no changes are made, then the transmittal number remains the unchanged. The transmittal number only changes when a new guidelines edition is issued or changes are made to the entire guidelines.

Acknowledgements

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

How to Cite the Guidelines

Cite the guidelines as follows:

U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2014. New Pest Response Guidelines: *Helicoverpa armigera* (Hübner) (Old World Bollworm). Washington, D.C.: Government Printing Office. http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

How to Find More Information

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

Chapter

2

Taxonomy

Given its host range and migratory potential, *H. armigera* is a serious pest with the potential for introduction into the United States. The scope of this document includes the following three sections relevant to an emergency response for an *H. armigera* incursion: *Identification*, *Survey* and *Control Procedures*.

Table 2-1 Classification of *H. armigera*

Rank	Taxon
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Family	Noctuidae
Genus	*Helicoverpa*
Species	*Helicoverpa armigera* Hübner

Synonyms

Heliothis armigera, *H. obsoleta*, *H. pulverosa*, *H. uniformis*, *H. armiger* (www.LepIntercept.org. Accessed 28 March 2014)

Common Names

Cotton bollworm, Old World bollworm, scarce bordered straw (www.LepIntercept.org. Accessed 28 March 2014)

Chapter 3

Identification

In October 2012, an *H. armigera* moth was caught in a cargo facility in Michigan (USDA New Pest Advisory Group (NPAG), 2012). A tentative identification was made based on the forewing color pattern and the dark marginal band on the hindwing. Dissection confirmed the specimen to be a female. The specimen's identity was subsequently confirmed as *H. armigera* based on a comparison with fresh dissections by Michael G. Pogue, research entomologist with the USDA Agricultural Research Service (ARS) Systematic Entomology Lab, Beltsville, MD (USDA New Pest Advisory Group (NPAG), 2012).

Helicoverpa armigera is among seventeen previously described species of the genus (Pedgley, 1985). In the continental U.S., *H. zea* is similar to *H. armigera* in its intact morphology and broad host range (Pogue, 2004). Another genus of the subfamily Heliothinae, *Heliothis*, is represented in the U.S. by *Heliothis virescens* (revised to *Chloridea virescens*) (Pogue, 2013), a pest whose host range overlaps those of *H. zea* and *H. armigera* (King, 1994).

Definitive species identification is based on the morphology of genitalia (Hardwick, 1965). Currently, intercepted eggs, larvae and pupae must be reared to the adult stage for dissection and examination of their genitalia to definitively distinguish *H. armigera* from other Heliothinae; or, specimens can be identified via molecular diagnosis (Passoa, 2014a).

Species Description/Morphology

Moths

The visible characteristics of *Helicoverpa* adults were described in terms of distribution of hair and scales, head features and wing characters (Pedgley, 1985).

Hardwick (1985) provided images of both male and female *H. armigera armigera* specimens. The wing features that may aid in screening noctuid moths for possible *H. armigera* are described by Brambila (2009a) in Figure 3-1.

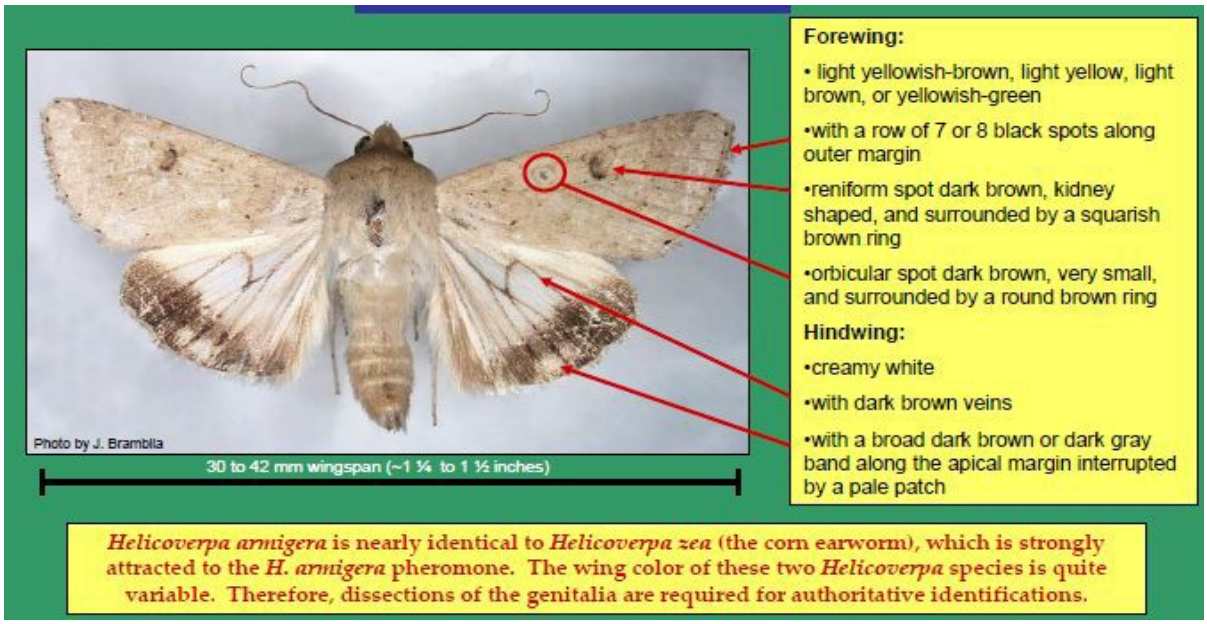

Figure 3-1 Forewing and hindwing features of *Helicoverpa armigera* (photo courtesy of J. Brambila, USDA-APHIS-PPQ)

The body length of adults is 18–19 mm (King, 1994). The vestiture (head and thorax) of fresh male specimens is olive and occasionally tinged with pink. The vestiture of fresh female specimens is orange brown, sometimes combined with dark brown or grey (Pedgley, 1985).

The abdomen of both sexes is greenish fawn or fawn combined with brown and sometimes pink. In males, the dorsum of the abdomen apex often bears a brown patch (Pedgley, 1985).

The wingspan of the subspecies *H. armigera armigera* and *H. armigera conferta* reportedly averages 35.1 ± 2.7 mm (Pedgley, 1985). Brambila (2009a) presents an *H. armigera* wingspan of 30–40 mm (Figure 3-1).

The forewings of males range in color from olive green to greenish fawn to grey green. Female forewings are brick red, reddish brown or dull orange brown (Pedgley, 1985) (Figure 3-2).

Figure 3-2 *Helicoverpa armigera* (photo courtesy of Paolo Mazzei, Bugwood.org)

The forewings are characterized by a brown, broad and irregular transverse band (Pedgley, 1985). The forewing margins feature 7 or 8 dark spots (Figure 3-1). Brambila's (2009a) photo shows reniform and orbicular marks that characterize *H. armigera* forewings (Figure 3-1).

The hindwings of both sexes are dull yellow or cream with a dark-brown outer marginal band. They feature a margin that is "yellowish" and a dark-brown ante-marginal border that contains at least one contrasting pale patch (Figure 3-1) (Pedgley, 1985). The specimen photographs published by Hardwick (1985) and Brambila (2009a) exhibit a distinct cross-vein on the hindwings (Figure 3-1). In Hardwick's photos, the cross-vein is most pronounced in the female moth (Pedgley, 1985).

Distinguishing *Helicoverpa* from *Heliothis*

In the U.S., the host ranges of *H. zea* and *H. virescens* overlap that of *H. armigera*. These three species must be distinguished in the event of a U.S. incursion by *H. armigera*. Hardwick (1985) distinguished the genera *Helicoverpa* and *Heliothis* based on male leg features and the morphological differences in their male and female genitalia. These characteristics are summarized in Table 3-1.

Table 3-1 Leg and genitalia features that distinguish genus *Helicoverpa* from *Heliothis* (Pedgley, 1985)

	Feature	*Helicoverpa*	*Heliothis*
Leg	specialized scales on fore femur	present	absent
Male genitalia—eversible vesica	relative vesica length	long	short
	degree of helicality	high	low
	scobinated bar at base of vesica	absent	present
	spines	present	absent
Female genitalia—appendix bursae	relative consistency	not leathery	leathery
	relative degree of coiling	present	none
	relative degree of dilation and constriction	present	none

Structures of the membranous eversible vesica of the aedeagus distinguish males of the *Heliothis* species from those of *Helicoverpa*. In contrast with *Helicoverpa* males, the vesica of *Heliothis* species is short; its degree of helicality is low; a scobinated bar is present near its base; and no spines are present on the vesica (Pedgley, 1985).

Characters of the female appendix bursae distinguish the *Heliothis* species from *Helicoverpa*. Unlike the appendix bursae of the *Helicoverpa* species, those of *Heliothis* females are "leathery" in consistency and exhibit no coiling, dilation or constriction (Pedgley, 1985).

Identification of *Helicoverpa* spp.

Hardwick distinguished the species of *Helicoverpa* using leg, wing and genitalic features as summarized in Table 3-2. The male genitalic characteristics can be used to distinguish *H. armigera* from *H. zea* (Brambila, 2009b; Pogue, 2004). Males of both species are trapped together using flight-intercept traps that utilize a pheromone lure attractive to both.

Table 3-2 Adult morphological characteristics distinguishing *Helicoverpa* species (Pedgley, 1985)

	Leg characteristics	Wing Characteristics	Genitalia
males and females	fore tibia: number, size and location of setae	wingspan forewing: color, shape, presence or absence of white sagittate apices on the veins hindwing: color	
males	hind tibia: number of setae		valve: ratio of length to width; orientation of dilation base of vesica: number and size of spines, orientation of spine apex basal pouch of vesica: number and size of diverticuli vesica: number of coils
females			appendix bursae: extent of sclerotization at the base; shape and degree of dilation of the apical terminus; length, density and distribution of the spicules on the lumen surface fundus bursae: location and orientation of small signum with respect to other signa

Method of Distinguishing Pheromone-Lure-Trapped *H. armigera* Males from *H. zea*

Brambila (2009b) presented a procedure for processing pheromone-lure-trapped males and distinguishing *H. armigera* from *H. zea* based on their genitalia. The method of clearing and examining the genitalia presented herein can also be used with light-trapped adults. Figures 3-3 to 3-5 present the diagnostic morphological structures.

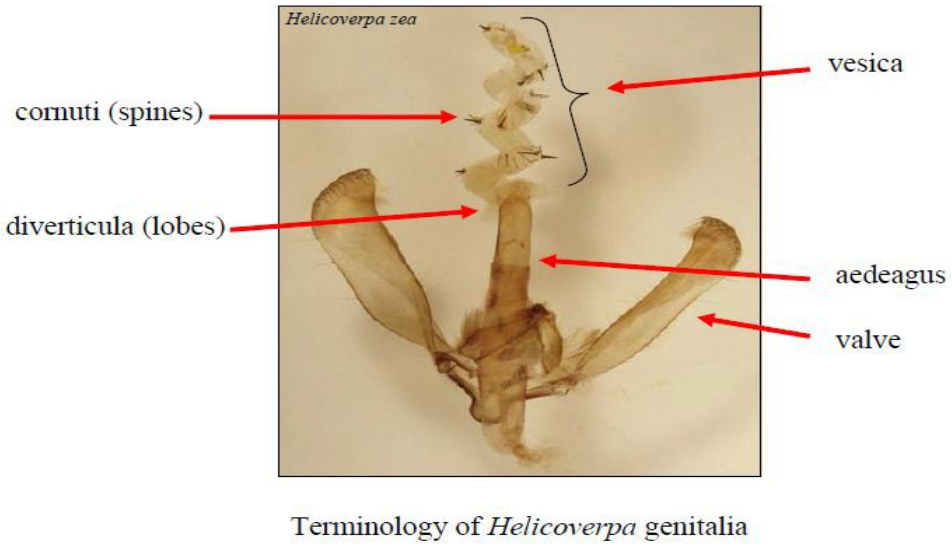

Figure 3-3 Components of the male *Helicoverpa* genitalia (image courtesy of J. Brambila, USDA-APHIS-PPQ). Appearance of the structures after dissection and excision from the specimen abdomen cleared in 10% KOH (Brambila, 2009b).

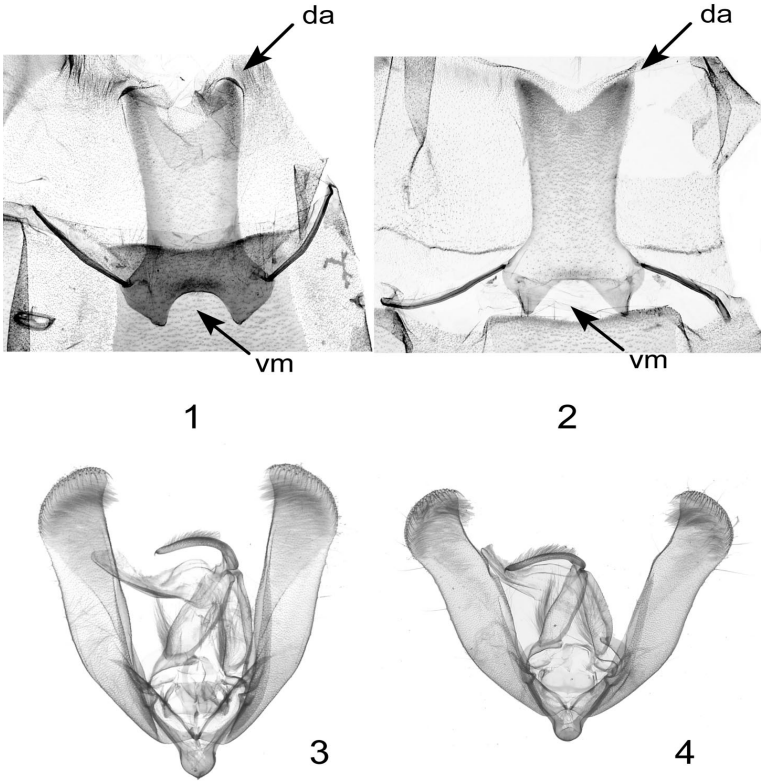

Figure 3-4 *Helicoverpa* abdominal sternite and male genital characteristics (image courtesy of Pogue (2004): "(1) Eighth sternite of *H. zea*; (2) eighth sternite of *H. armigera*; (3) male genitalia of *H. zea*; (4) male genitalia of *H. armigera*. da, distal apex; vm, ventral margin." Appearance of structures after dissection and excision from specimen abdomens cleared in 10% KOH (Brambila, 2009b).

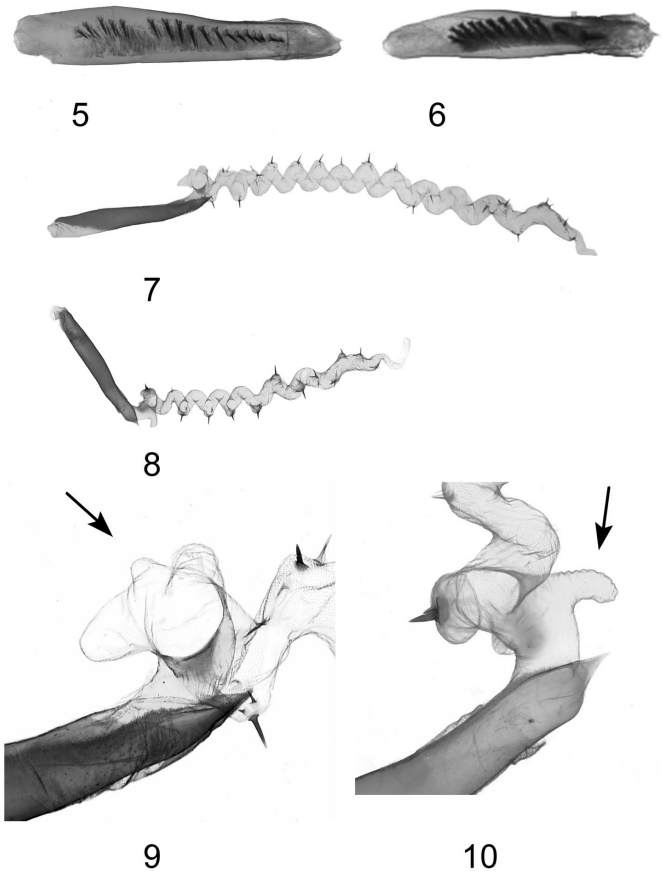

Figure 3-5 *Helicoverpa* male genitalia (image courtesy of Pogue (2004)): (5) Aedeagus of *H. zea* with uninflated vesica; (6) *H. armigera* aedeagus with uninflated vesica; (7) *H. zea* aedeagus with fully inflated vesica; (8) *H. armigera* aedeagus with fully inflated vesica; (9) three lobes at the base of *H. zea* vesica; (10) single lobe at the base of *H. armigera* vesica. Appearance of structures after dissection and excision from specimen abdomens cleared in 10% KOH (Brambila, 2009b).

Brambila's procedure (2009b) detaches the moth abdomen and places it in 10% potassium hydroxide (KOH) to clear the specimen of fats and proteins while retaining its chitinous structures including the integument and external and internal reproductive structures. The cleared specimen can be dissected to examine the valves (Figure 3-3), eighth abdominal sternite (Figure 3-4), aedeagus containing the uninflated (uneverted) vesica (Figure 3-5) and the everted vesica (Figures 3-3 and 3-5) for characteristics that distinguish *H. armigera* from *H. zea* (Figures 3-3 to 3-5). Brambila (2009b) suggests that *H. zea* specimens be available for practicing the procedure and to function as reference specimens during critical identification dissections.

Valves: Corona and Valve Length

The presence of a margin of curved, inward-directed spines located at the valve apex (corona) confirms that the moth is likely of the genus *Helicoverpa*.

Absence of such a corona indicates that the specimen is not *Helicoverpa* (Brambila, 2009b). A valve length of 4.9 mm or greater identifies a pheromone-lure trapped moth as *H. zea* (Pogue, 2004). A valve length below 4.9 mm qualifies the specimen for examination of the vesica to determine the number of cornuti (Pogue, 2004).

Eighth Abdominal Sternite: Shapes of Ventral Margin and Distal Apex

The margins of the eighth abdominal sternite of both species are moderately forked (Pogue, 2004). However, the distal apices of the sternite are more pointed in *H. armigera* and more rounded in *H. zea* (Figure 3-4); the ventral margin of the sternite is an apically flattened 'V' in *H. armigera* and U-shaped in *H. zea* (Pogue, 2004) (Figure 3-4).

Number of Cornuti Sets in the KOH-Cleared Aedeagus Containing the Uninflated Vesica

The spines of the uneverted vesica can be seen, aggregated as sets of discrete spines, through the sides of the KOH-cleared aedeagus (Brambila, 2009b) (Figure 3-5, views 5 and 6). Twelve or fewer sets indicates that the specimen "could be" *H. armigera*; more than 12 sets indicates that the specimen is "probably" *H. zea* (Brambila, 2009b). Few cornuti or an absence of spines suggests an aberrant or sterile specimen that is "considered to be" *H. zea* (Brambila, 2009b).

Inflated/Everted Vesica: Number of Basal Lobes, Vesica Length, Number of Coils and Number of Cornuti

Injecting alcohol into the base of the aedeagus inflates the vesica causing it to evert from the aedeagus (Brambila, 2009b) (Figures 3-3 and 3-5). Eversion of the vesica allows observation of the four vesica characteristics that distinguish *H. armigera* from *H. zea*: the basal lobes, vesica length, coils and cornuti. Diverticula are located at the base of the everted vesica at the distal end of the aedeagus: A single lobe characterizes *H. armigera*; three lobes characterize *H. zea* (Pogue, 2004) (Figure 3-5, views 9 and 10). The everted vesica has the form of a spiral or helical sac (Pogue, 2004) (Figure 3-3; Figure 3-5, views 7 and 8). The everted vesica of *H. armigera* is characterized by 6.5–8.5 coils distributed along its spiral length (Pogue, 2004) (Figure 3-5, view 8); the everted vesica of *H. zea* is much longer (Brambila, 2009b), with 8–11 coils (Pogue, 2004) (Figure 3-5, view 9). The shorter *H. armigera* vesica features fewer spines than the longer *H. zea* vesica (Pogue, 2004) (Figure 3-3; Figure 3-5, views 7 and 8).

Partially Everted Vesica: Number of Basal Lobes

Brambila (2009b) describes a procedure for the partial eversion of the vesica sufficient for microscopic examination of the basal lobes as previously described (Figure 3-5, views 9 and 10).

Eggs

Hardwick (1985) examined the eggs of *H. armigera* and five other *Helicoverpa* species. The egg shape is a sphere, flattened where the egg adheres to the plant tissue on which it has been deposited. As typically deposited, the micropyle is often at the apex of the egg. The eggs are characterized by radial ribbing (Pedgley, 1985) (Figure 3-6). Lateral carinae exist between the radial ribs of *H. zea* eggs (King, 1994).

Figure 3-6 Recently oviposited *Helicoverpa zea* egg on cotton (photo courtesy of John Ruberson, University of Georgia, Bugwood.org)

The eggs of the six *Helicoverpa* species examined by Hardwick (1985) ranged in height from an average of 0.42 mm for *H. gelotopoeon* and *H. hawaiiensis* to 0.52 mm for *H. zea*. The mean diameter ranged from 0.50 mm for *H. gelotopoeon* to 0.59 mm for *H. zea* (Pedgley, 1985). Hardwick (1985) reports a mean diameter of 0.528 ± 0.027 mm and a mean height of 0.477 ± 0.039 mm for *H. armigera*. The eggs of the six species ranged in color from “greenish yellow” to “apple green” at deposition (Figure 3-6). When incubated at 25 °C, the eggs became “muddy yellow,” and a pink subequatorial band appeared 14–36 hours after deposition. Simultaneously or soon thereafter, the micropylar area also turned pink. During the subsequent 30–36 hours, the subequatorial band and micropylar spot darkened to red or “reddish brown.” The entire egg turned grey, with development of the larva within, 48–60 hours of deposition (Pedgley, 1985).

The species’ host range and oviposition behavior indicate that *H. armigera* eggs could be found associated with a broad range of plant matrices. *Helicoverpa*

armigera typically oviposits on florescent host plants—plants that have flowered, are flowering or will soon flower (Pedgley, 1985). The growing terminus of the plant—whether vegetative or flowering/fruiting—is the preferred site of oviposition; however, eggs are also deposited on older vegetation and fruiting structures (Pedgley, 1985).

Larvae

Hardwick (1985) described the larvae of six *Helicoverpa* species, distinguishing the species of antepenultimate, penultimate and ultimate stadia based on the head width; lengths of frons and coronal suture; the color of, angles formed by and distances between the thoracic and abdominal setae; the color of the spiracular rims and the area within them; and proleg crochet number.

The larval stage of *Helicoverpa* species persists through 5–7 instars, varying with the species, individuals, temperature and nutrition (Pedgley, 1985). Under controlled rearing conditions, 69% of the *H. armigera* larvae tested (n = 239) matured in six stadia, 30 percent in five, and 1 percent in seven stadia (Pedgley, 1985).

Czepak *et al.* (2013) noted three characteristics that distinguish *H. armigera* larvae from those of other Noctuidae in Brazil:

- Distinctly dark dorsal tubercles on the first abdominal segment of 4th instars form a "saddle-like" structure (Figure 3-7)
- The larva's cuticle is coriaceous
- When the larva is disturbed, it responds by curling downward such that the head contacts the first set of prolegs (Figure 3-9)

However, *H. virescens* (*Chloridea virescens*) larvae also have a saddle, and the cuticular spines may be more salient than a coriaceous integument texture; other species of *Helicoverpa* and *Heliothis* (*Chloridea*) also exhibit a downward curling of the head (Passoa, 2014c).

Overall Size and Color

Last-stage, prepupal *Helicoverpa* larvae, including *H. armigera*, were reportedly 35–52 mm long (King, 1994). The larval head and trunk colors as described by Hardwick are presented in Table 3-3.

Table 3-3 Head and trunk color of *H. armigera* larvae (Pedgley, 1985)

Instar	Head	Trunk
1	dark blackish brown	neonates are translucent or pale translucent greyish yellow or greyish green; late 1st instars are yellow
2	dark blackish brown	yellow to greyish cream
3	creamy fawn, fawn or light orange with medium- to dark-brown mottling that sometimes coalesces to become the predominant color	initially orange brown to medium chocolate brown becoming paler as stadium progresses
antepenultimate	light orange, fawn or cream; often with cream reticules or dark-brown mottles	brown with pale longitudinal lines
penultimate	orange to fawn, often with a greenish tone, cream reticules and brown mottles	brown; sometimes green with pale longitudinal lines
ultimate	light greyish green, light orange or fawn; often with dark-orange mottles and white reticules	shades of brown, orange, yellow or green with pale broken longitudinal lines

The intensity and hue of the prothoracic, suranal and proleg shields, the setal bases and the spiracular rims often vary from those of the head and trunk (Pedgley, 1985). A range in larval coloration is observable in Figures 3-8 through 3-10.

Figure 3-7 Late-instar *Helicoverpa armigera* on a soybean leaf in Brazil (photo courtesy of C. Czepak *et al.*)

Figure 3-8 Late-instar *Helicoverpa armigera* feeding inside a cotton boll in Brazil (photo courtesy of C. Czepak *et al.*)

Figure 3-9 Helicoverpa armigera larva (photo courtesy of G. Csoka, Hungary Forest Research Institute, Bugwood.org)

Figure 3-10 Late-instar *Helicoverpa armigera* feeding on a soybean pod (photo courtesy of C. Czepak *et al.*)

Figure 3-11 *Helicoverpa armigera* larva (photo courtesy of A. Guyonnet, Lépidoptères Poitou-Charentes, Bugwood.org)

Linear Markings

Hardwick (1985) and King (1994) described the larval markings: Longitudinal lines and bands contrast with the ground color of the integument of the larval trunk. During the 3rd to last instars, these lines and bands marking the trunk consist of the following:

- A narrow, dark median dorsal band consisting of paired lines or a single narrow band straddling the dorsal midline
- A subdorsal band on each side of the dorsal midline adjoining the median dorsal band; each subdorsal band is wider and paler than the median dorsal band
- A wide dark band on each side of the larva below the subdorsal band and above the spiracular band (Figure 3-7)

- The row of spiracles is apparent on a broad, solid white or yellowish spiracular band on each side of the larva below the supraspiracular band (Figure 3-9)

Integument Texture Based on Tubercles and Setae

Hardwick (1985) describes the skin thickenings that contribute to the integument texture of *Helicoverpa* late instars using a cobblestone analogy. In later instars, seta-bearing tubercles on *Helicoverpa* spp. abdominal segment 8 are free of cuticular microspines in the region between the seta at the tubercle center and the periphery. The integument of *Helicoverpa* last instars is described by King (1994) as having a "granular" appearance due to the density of dark single-seta-bearing tubercles.

Crochets

Helicoverpa proleg crochets are typically biordinal (Pedgley, 1985).

Passoa's Larval Heliothinae Key

Passoa (2014a) assembled and published a key for distinguishing the species of the Heliothinae subfamily encountered as larvae. The key applies to Heliothinae species of quarantine significance and is based on the following eight characteristics of larval morphology:

1. Distribution of spines across the larval integument
2. Orientation of the prothoracic L setae
3. Proleg crochets
4. Setal bases
5. Setal color
6. Setal bar
7. Mandibular retinaculum
8. Presence and distribution of spines on the dorsal setal bases of abdominal segments A1, A2 and A8

Of additional value to quarantine inspectors and identifiers, the key is also based on the larva's host plants and geographic origin (Passoa, 2014b).

Pupae

At the conclusion of the larval stage, the larva crawls or drops to the soil and burrows to a depth of 2.5–17.5 cm to pupate after the prepupal larva spins a loose web of silk around itself (King, 1994). King (1994) also reports that pupation occasionally occurs on soil or plant-tissue surfaces.

The pupae are obtect, with the developing appendages appressed to the body and

thus indistinguishable within the pupal case prior to adult emergence (Stehr, 1987) (Figure 3-12). The mass of a female noctuid pupa is typically greater than that of a male (King, 1994). Variations in pupa size are correlated with variations in food quality (King, 1994). *Helicoverpa armigera* pupae are typically light to dark brown or reddish brown, rounded at both ends, smooth textured and feature a posterior-tip cremaster in the form of two tapering parallel spines (King, 1994) (Figure 3-12).

Figure 3-12 *Helicoverpa armigera* pupa (photo courtesy of P. Mazzei, Bugwood.org)

The lengths of *H. armigera* pupae range from 14–22 mm, while the widths across the thorax range from 4.5–6.5 mm (King, 1994). Measuring lab-reared pupae, Neunzig (1960) reported lengths ranging from 17.0–26.0 mm for *H. zea* and 15.0–20.0 mm for *H. virescens* pupae. Widths across the thorax for the same strains ranged from 5.0–7.2 mm for *H. zea* and 3.3–5.0 mm for *H. virescens* (Neunzig, 1960).

Helicoverpa zea and *H. virescens* pupae can be distinguished based on spiracle size and maxillary palpae: *H. virescens* pupae have smaller spiracles than *H. zea* and lack the maxillary palpae sclerites that abut the eye pieces of *H. zea* pupae (Neunzig, 1960).

Potential Alternative Species—Identification Methods

Jia *et al.* (2007) examined the efficacy of near-infrared spectroscopy (NIRS) for distinguishing the egg, 1st instar, 2nd instar, 3rd instar and pupal stages of *H. virescens* from those of *H. zea*. After generating NIR reference spectra of both species at each stage, they were able to correctly identify the species in 98.5% of sampled eggs, at least 93.4% of 1st instars, at least 96.5% of 2nd instars, 97% of 3rd instars and 96.5% of pupae for which spectral data had been generated for the pupal head region (as distinct from 68.1% of pupae for which spectral data had

been generated for the pupal tail region). The challenges to developing this method include reference spectra that vary with the environment and diet of the reference specimens.

Monoclonal antibodies specific to *H. virescens* and *H. zea* eggs were identified and subsequently developed into a diagnostic kit capable of distinguishing the eggs of the two species as collected from cotton (Zeng, 1998, 1999), which suggests the possibility of developing similar technology to distinguish *H. armigera* eggs from those of other heliothines. Similarly, Trowell *et al.* (2000) developed monoclonal antibodies that reacted with a transport protein in the eggs and hemolymph of *H. armigera*. Based on this reaction specificity, they developed a kit to distinguish *H. armigera* eggs, 2nd instars and 3rd instars from those of *H. punctigera*.

Bailey *et al.* (2001) demonstrated the efficacy of a 24-hour feeding disruption bioassay to distinguish *H. virescens* neonates from *H. zea* neonates based on their relative susceptibility to a diagnostic dose of insecticide, which suggests the prospect of developing similar technology to distinguish *H. armigera* 1st instars from those of other *Helicoverpa* and *Heliothis* spp.

Potential DNA-Based Methods of Species Identification

Behere *et al.* (2008) used PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis to reveal species-specific differences in mitochondrial DNA (mtDNA) to distinguish *H. armigera* from *H. zea*, *H. punctigera* and *H. assulta*. They isolated genomic mtDNA from the eggs, larvae, pupae and adults of *H. armigera*, and from the larvae and adults of *H. zea*, *H. punctigera* and *H. assulta*. Behere *et al.* designed primers for use in the PCR-amplification of targeted partial DNA sequences containing two mtDNA genes: COI (cytochrome oxidase subunit I) and Cyt *b* (cytochrome *b*). The amplified DNA sequences were digested using two endonucleases: *BstZ*17I and *Hph*I. Species-to-species differences in the lengths of the resulting DNA fragments became evident as visually distinct DNA bands on agarose and polyacrylamide gels after electrophoresis. Although these researchers isolated mtDNA from all life stages—egg to adult—in the case of *H. armigera* and from larvae and adults only in the cases of *H. zea*, *H. punctigera* and *H. assulta,* they asserted the improbability that mtDNA from any stage of each species would yield different results; the full genes are constitutively expressed and code for the transmembrane proteins essential to aerobic respiration. Behere acknowledged that PCR-RFLP is more time-consuming than other methods but characterized it as reliable for distinguishing *H. armigera* and *H. zea* given both its consistent results for insect samples from five continents and the agreement between the RFLP results and DNA-sequence data for the studied individuals (Behere, 2013).

Norman B. Barr summarized the on-going Mission Laboratory effort to develop a reliable, robust and cost-effective DNA-based method to distinguish *H. armigera* from North American Heliothinae pests. Two DNA-based methods have been evaluated (a PCR-RFLP method and a DNA barcode method), and a third has been conceived (a real-time PCR method). Barr and colleagues tested the efficacy of the PCR-RFLP method developed by Behere *et al.* (2008) to distinguish *H. armigera* from *H. zea* and *H. virescens*. The method, characterized by a 1-day turn-around time, succeeded in distinguishing *H. armigera* and *H. zea* but was less effective for *H. virescens*. An optimization study of the PCR-RFLP method is in progress. Barr and colleagues also evaluated the use of a DNA barcoding method based on sequence data published for *H. armigera* and *H. zea* COIs. The method successfully diagnosed both species, but required 2–4 days. Barr and colleagues have recognized the value in exploring the feasibility of a real-time PCR method for species diagnosis. Real-time PCR methods have demonstrated efficacy in other applications and the potential for 1-hour species diagnosis (Barr, 2013).

Chapter 4

Biology

Life Cycle

The number of generations per year and seasonal abundance of *H. armigera* are influenced by the temperature, humidity, host sequence and host suitability (Fitt, 1989; King, 1994). The seasonal abundance of all life stages at a given location is affected by weather, predators, parasites, host-plant factors, emigration and immigration (Fitt, 1989). Survival of the pupal stage is also affected by soil factors (Fitt, 1989; King, 1994). In temperate regions, often fewer than 50 percent of pupae survive overwintering, but the high fecundity of the survivors (and possibly immigration) enables populations to increase to damaging densities by the 3rd or 4th generation (Fitt, 1989).

The developmental threshold temperature for eggs is 10.6 °C, and that for the life cycle from larva to adult is 11.0 °C (Venette *et al.*, 2003). Adult survival, fertility and fecundity are reduced with prolonged exposure to temperatures above 35 °C (Fitt, 1989). In subtropical and temperate regions, 3–5 generations occur per year. Discrete generations become indistinct as the generations overlap after the 1st or 2nd spring generation. In the tropics, where climate and host vegetation allow continuous breeding, a generation is complete in 28–30 days (Fitt, 1989).

Adult emergence from pupal cells in the soil occurs from dusk to midnight (King, 1994). Emerged adults climb nearby vertical surfaces for wing drying. Dispersal to hosts suitable for adult feeding and oviposition occurs for distances up to 10 km (Fitt, 1989). Long-distance migration, up to 1,000 km, is prompted by a lack of suitable hosts near the emergence sites.

Female calling (release of sex pheromones to attract males) occurs 2–5 nights after emergence (King, 1994). Calling and mating peak between midnight and sunrise. The pre-oviposition period lasts 1–4 days. The first eggs are thus oviposited on host plants 4–5 nights after emergence, with peak oviposition occurring on the 9th night after emergence (King, 1994).

Laboratory studies determined the average female lifespan was 15.7 ± 6.4 days (Pedgley, 1985), with a reproductive period of 8–10 days (Fitt, 1989). Fecundity

is influenced by temperature, humidity and nutrition during both the larval and adult stages (Fitt, 1989). In a laboratory study, an average of 1,702 ± 1,057 eggs per female was produced, with a maximum of 4,394 eggs (Pedgley, 1985). Modeling of the field fecundity yielded estimates of 500–3,000 eggs per female (Fitt, 1989).

Oviposition occurs singly, with the female selecting preferred host plants at or near the flowering or fruiting stage (King, 1994). Nocturnal ovipositing alternates with nectar feeding.

In laboratory studies at 25 °C, the eggs hatched within 66 hours of deposition (Pedgley, 1985). Neonate larvae feed on their egg shells before feeding on plant tissue (King, 1994). Larvae move to preferred host tissues, such as flowers and fruiting structures, but will also feed on vegetative tissues. Larvae often molt in full sunlight on the upper surfaces of leaves and feed wholly or partially concealed in flowers and fruit (King, 1994).

In laboratory studies, the number of larval instars ranged from 5–7 (Pedgley, 1985). The duration of the larval stage varies with temperature, available host species and host quality (King, 1994). In laboratory studies at 25 °C, the average duration of the larval stage, from egg hatch to cessation of feeding, was 15.4 days (Pedgley, 1985).

Upon completion of larval development, feeding ceases, and the larva leaves the host plant to burrow into nearby soil to pupate (Pedgley, 1985). Pupation occurs at depths of 2.5–17.5 cm (King, 1994). Diapausing pupae pupate at greater depths than non-diapausing pupae (Pedgley, 1985). The prepupal stage from cessation of feeding to formation of the pupa averaged three days. In laboratory studies, duration of the non-diapausing pupal period averaged 12.7 ± 1.1 days for females and 13.7 ± 1.1 days for males (Pedgley, 1985). In other studies, the pupal duration for non-diapausing pupae varied with temperature, ranging from 6 days at 35 °C to more than 30 days at 15 °C (King, 1994). Diapause in the pupal stage is induced when the larvae are exposed to temperatures and photoperiods that adversely affect the host availability and insect development. Diapausing pupae pupate for several months until favorable conditions resume (King, 1994). Winter diapause is induced at temperatures of 19–23 °C and photoperiods of 11.5–12.5 hours (Fitt, 1989). Summer diapause has been induced in *H. virescens* at temperatures above 32 °C. In an *H. armigera* sample in low-temperature-induced diapause, the hibernation of 36% of the insects was protracted by a temperature of 35 °C (Fitt, 1989).

Chapter 5

Damage

Signs and Symptoms

Helicoverpa armigera is polyphagous with a feeding-host range that includes 180 species of wild and cultivated plants in more than 45 families (Venette *et al.*, 2003). Adults feed on a broad range of nectar sources (King, 1994). Larvae feed on a wide range of plant tissues but primarily on the growing points and flowering and fruiting structures of host plants (Fitt, 1989). Saleem and Yunus (1982) reported that larvae fed on the stems, leaves, flowering structures and fruiting structures of maize and tomato. On cotton, chickpea, tobacco and okra, larval feeding was observed on the leaves, flowering and fruiting structures. However, on tomato, the larvae reportedly prefer leaves to fruit (Venette *et al.*, 2003).

The feeding habits of larvae include penetration and burrowing into buds, flowers and fruit. Feeding on cotton bolls, maize cobs, chickpea pods, tomato fruit, okra pods and tobacco seed capsules was cryptic or semi-cryptic (Saleem and Yunus, 1982). Feeding on cotton bolls was accompanied by fungal and bacterial infection, and feeding on maize cobs was associated with fungal infection (Saleem and Yunus, 1982). Thus, in addition to inspecting for all life stages of the insect, all host-plant material—especially upper leaves, buds, flowers and fruit—requires visual inspection for signs and symptoms of feeding, including entry holes, frass and associated microbial infection. Evidence of larval penetration requires follow up with dissections to ascertain the presence or absence of pupae in addition to larvae (Venette *et al.*, 2003).

Impacts

Environmental

According to Pimentel *et al.* (2001), the environmental damage associated with approximately 4,500 introduced arthropod species in the United States costs approximately $2.137 billion annually. In the event of an *H. armigera* introduction, the impact to the environment would come both from direct insect feeding and control measures.

Economic

Helicoverpa armigera is polyphagous, feeding on a wide range of plant hosts. The major host families include the Gramineae (or Poaceae), which include maize, wheat and other small grains, rice, sorghum and sugarcane; Malvaceae, which include cotton, okra and cacao; Leguminosae, which include peas, beans and forage legumes; Solanaceae, which include potatoes, tomatoes, bell peppers and tobacco; and Compositae, which include sunflower, artichokes and chrysanthemum (King, 1994). In most places where it occurs, *H. armigera* is a severe economic pest (Venette *et al.*, 2003).

Chapter 6

Survey Procedures

Survey Objectives

Plant regulatory officials will conduct detection, delimiting and monitoring surveys for *H. armigera*. Detections surveys will be conducted to ascertain the presence or absence of *H. armigera* in an area in which it is not known to occur. After a new detection in the United States, or when detection in a new area is confirmed, a delimiting survey should be conducted to define the extent and geographic location of the pest. In addition, when a control procedure is applied, its effectiveness should be measured via a monitoring survey.

Surveys aim to detect, delimit and monitor via two methods: trapping female-seeking male adults in-flight and inspecting host plants for eggs and feeding larvae. The adult, egg and larval specimens acquired in the surveys must be identified to confirm the target species. The challenge for surveyors in the U.S. is to distinguish *H. armigera* from the indigenous *H. zea* and *H. virescens*. Additionally, detection surveys must be designed to account for the possibility that trapped adults can represent both those emerging from local pupae and immigrants representing distant populations (Fitt, 1989).

Detection surveys will be used to ascertain the presence of *H. armigera* in an area from which it had previously been absent. Subsequent to detection, delimiting surveys will define the geographic distribution of the detected pest and thereby the location for eradication efforts. Follow-up monitoring surveys in a delimited treatment area will measure the effectiveness of such efforts to eradicate or otherwise manage *H. armigera* populations.

Preparation, Sanitization and Clean-Up

This section provides information that will aid personnel in preparing 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. Prior to beginning a survey, determine whether there have been recent pesticide applications that would render it unsafe to inspect the plants and leaf litter. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
2. Conduct the survey during a time when infestations should be apparent. General surveys should focus on months in which host plants are easily accessible, during active growing phases and when symptoms are known to be expressed.
3. Obtain permission from the landowner prior to entering a property.
4. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with any and all quarantine requirements.
5. When visiting the area to conduct surveys or take samples, everyone must take strict measures to prevent contamination by *H. armigera* or other pests between properties during inspections.
6. Prior to entering a new property, ensure that clothing and footwear are clean and free of pests, soil and litter to avoid moving soil-borne pests and arthropods from one property to another.
7. Wash hands with approved antimicrobial soap. If not using an antimicrobial soap, wash hands with regular soap and warm water to remove soil and debris. Then, use an alcohol-based antimicrobial lotion with an equivalent of 60% ethyl alcohol. If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, antimicrobial lotions are less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.
8. Gather all supplies. Confirm that equipment and tools are clean and sanitized. When taking plant samples, disinfest tools with a 5% bleach solution or other approved sanitizing solution to avoid spreading diseases or other pests. A brief spray to runoff or immersion of the cutting portion of the tool in a 5% solution of sodium hypochlorite (bleach) and allowing the tool to air-dry is effective for inactivating plant pathogens and preventing their spread.
9. Flag the plant or sampled location whenever possible and draw a map of the immediate area, indicating reference points so that the areas can be found in the future if necessary. **Do not** rely solely on the flagging or other markers to re-locate a site as they may be removed. Record the GPS coordinates for each sampled area so that the area or plant may be re-sampled if necessary. The re-sampling period for this pest can be short based on environmental conditions.

10. Survey strike teams should consist of an experienced survey specialist or plant pathologist familiar with *H. armigera* and the symptoms the insect causes.

Survey Methods

Surveys are conducted via the flight-intercept trapping of adult males and by visual inspection of host-plant vegetation, floral structures and fruit for eggs, larvae and pupae.

Trapping of *Helicoverpa* Adult Males

CAPS-Approved Trapping Method

As of November 2013, *H. armigera* survey was a topic of the commodity-based survey guidelines (CSG) and the commodity-based survey references (CSR) for maize, cotton, small grains and soybeans published to support the Cooperative Agricultural Pest Survey (CAPS) program (USDA Cooperative Agricultural Pest Survey (CAPS), 2013). The CAPS-approved method for trapping female-seeking male *H. armigera* moths combines a sex pheromone lure with a flight-interception trap. Light traps have been used for male and female *H. armigera* in Australia (Baker *et al.*, 2011) and in China (Yang *et al.*, 2013). Lunar cycles may affect adult activity and reduce trap catches (Fitt, 1989; King, 1994).

Pheromone Lure

The pheromone lure, "*Helicoverpa armigera* lure," consists of three solutions combined and added to a rubber septum: Z11-16Ald, Z9-16Ald and butylated hydroxytoluene (USDA Cooperative Agricultural Pest Survey (CAPS), 2013). The pheromone-solution-impregnated rubber septum is added to a trap; the pheromone volatilizes and disperses downwind; and adult males follow the pheromone plume upwind to its source. The lure effectively attracts male *H. armigera* moths for up to 28 days. However, hot, dry weather may reduce the efficacy and usefulness of the lure to two weeks (USDA Cooperative Agricultural Pest Survey (CAPS), 2013). While the lure does not attract *H. virescens*, it does attract *H. zea* in addition to the targeted *H. armigera,* complicating its utility for surveys in the continental U.S. (Pogue, 2004).

The *Helicoverpa armigera* lure is available from several vendors. Venette *et al.* (2003) summarized the work of others who combined (Z)-11-hexadecenal and (Z)-9-hexadecenal in a 97:3 ratio. A 1-mg dose of this

combination added to a rubber septum was more effective in attracting male *H. armigera* moths than 0.75 or 1.25 mg/septum.

Trap Type

The CAPS (2013)-approved method of trapping *H. armigera* male moths specifies the addition of a *Helicoverpa armigera* lure to one of three trap types:

- Plastic bucket trap (Brambila *et al.*, 2010)
- Scentry® Heliothis trap
- Texas (Hartstack) trap (Hartstack *et al.*, 1979)

Both *H. armigera* and *H. zea* males are attracted to the current pheromone lure widely used with traps in the U.S. (Pogue, 2004).

Trap Placement: Spatial Distribution

At the initiation of moth emergence and population build-up, Kant *et al.* (1999), working in India, determined an optimal distance between traps of 50 m. They also detected no difference in moth catches between traps separated by distances of 25 m, 50 m and 100 m once the rate of population increase had stabilized. Sidde Gowda *et al.* (2002) validated an integrated pest management (IPM) program to protect pigeonpea, *Cajanus cajan*, crops from *H. armigera*; a pheromone trap density of 5 traps/ha was used to monitor the population in this program.

Kant *et al.* (1999) examined the efficacy of three heights for pheromone traps used to monitor moth flights in chickpeas (variety Radhey) in India and determined that traps set 1.8 m above soil level (1 m above the crop canopy) produced more catches than those set at 0.6 m and 1.2 m. Baker *et al.* (2011), working in Australia, reported placing pheromone traps for *H. armigera* 1.5 m above the ground.

Trap Placement: Temporal Distribution

The prospect of an introduction via escapees from regulated points of entry is enhanced both by the availability of non-cultivated wild hosts and by the ability of the escapees to enter pupal diapause until conditions that favor host-plant availability and all developmental life stages of the insect resume. Placement, maintenance and monitoring of flight-interception traps for *H. armigera* adults should coincide with the availability of host plants and with the emergence and flights of adult males. At subtropical and temperate latitudes in the continental U.S., monitoring can be initiated and sustained throughout the growing season while weather conditions that favor host-plant

growth and insect development persist. Transient conditions unfavorable to host plants and insects can result in diapause or down-wind migration for distances reaching 1,000 km (Pedgley, 1985) and thus require flexibility in trapping-season planning.

Aestivation allows pupae to survive conditions unfavorable to host plants such as hot, dry soil conditions (Fitt, 1989). In *H. virescens*, aestivation has been induced when temperatures exceed 32 °C (Fitt, 1989). In an *H. armigera* sample in low-temperature-induced diapause, the hibernation of 36% of the insects was protracted by a temperature of 35 °C (Fitt, 1989).

Hosts

Helicoverpa armigera is polyphagous, feeding on a wide range of plant hosts. The major host families include the Gramineae (or Poaceae), which include maize, wheat and other small grains, rice, sorghum and sugarcane; Malvaceae, which include cotton, okra and cacao; Leguminosae, which include peas, beans and forage legumes; Solanaceae, which include potatoes, tomatoes, bell peppers and tobacco; and Compositae, which include sunflower, artichokes and chrysanthemum (King, 1994). Venette *et al.* (2003) compiled an extensive list of crop and non-crop host species.

Venette *et al.* (2003) summarized the available studies describing *H. armigera* preference with respect to host plants for oviposition and larval development. Three different laboratory studies ranked the host preference as follows:

- pigeon pea > maize > sorghum > red ambadi > cowpea > marigold
- tobacco + maize + sunflower > soybean + cotton + alfalfa > cabbage + pigweed + linseed
- maize ≫ cowpea

Insect Emergence: Temperature

Diapausing *H. armigera* pupae resume development at a temperature threshold of 17 °C; for non-diapausing *H. armigera*, the threshold temperature is 12–13.5 °C (Fitt, 1989). Twine (1978) examined the effect of six constant temperatures on the development time of larvae fed an artificial diet and maintained in constant darkness. The temperatures ranged from 13.1–38.4 °C with a developmental threshold temperature of 11 °C. The maximum rate of development occurred at 33.9 °C. The optimal survival temperature for larvae (minimal larval mortality) was 24 °C. The optimal survival temperature for pupae (minimal pupal mortality) was 27 °C (Twine, 1978). Venette *et al.* (2003) reported a minimum developmental threshold temperature for a

complete life cycle of 11.0 °C.

Insect Emergence: Moisture

Helicoverpa armigera pupate in pupal cells formed at a soil depth of 2.5–17.5 cm (King, 1994). Weather conditions that render the soil environment unfavorable to pupae can delay or arrest moth emergence. Pupae die in waterlogged soil (King, 1994); thus, soil texture and depth to the water table can combine with rain events to kill pupae. Pupal mortality is greater in cold wet soil than in cold dry soil (Fitt, 1989). Soil compaction can also adversely affect pupal development and survival (Fitt, 1989).

Insect Emergence: Spring Emergence of Overwintering Pupae

In the Southern Hemisphere, studies of *H. armigera* overwintering pupal diapause were conducted in southeastern Australia and southern Africa (Fitt, 1989). Crop sources of the *H. armigera* overwintering generation were cotton and maize. Among the populations studied, diapause initiation began proximal to the autumnal equinox (March) and extended into May; spring emergence of the overwintered moths occurred proximal to the vernal equinox (September) and extended to October. Baker *et al.* (2011) reported a peak in trap catching for three generations of *H. armigera* present in a wide variety of crop- and non-crop hosts in Australia from summer to early fall.

Adult females emerge from pupal cells in the soil one day earlier than males (Pedgley, 1985). When Kant *et al.* (1999) monitored pheromone-trap moth captures in chickpeas in India, they found adult males responsive from 6:00 P.M.–6:00 A.M. and reported that 82.3% of the male moths were trapped between 11:00 P.M. and 4:00 A.M. Thus, surveyors may undertake overnight trapping.

Trace-Back and Trace-Forward Investigations

Trace-back and trace-forward investigations aid in prioritizing delimiting survey activities after an initial detection. Trace-back investigations attempt to determine the source of the infestation. Trace-forward investigations attempt to define further potential dissemination through natural and artificial spread (commercial or private distribution of infested plant material) or the movement of farm implements and equipment form one field to the next. Once a positive detection is confirmed, efforts should be made to determine the extent of the infestation or potentially infected areas in which to conduct further investigations.

Homeowner Properties

For positive detections on homeowner properties, ask the owner of the infested material to determine its point of origin (nursery, neighbors, *etc.*) and any possible sites of further distribution.

Nursery Properties

For nursery hosts, a list of facilities associated with potentially infested nursery stock from those testing positive for *H. armigera* will be compiled. These lists will be distributed by the state to the field offices and are not to be shared with individuals outside the USDA–APHIS–PPQ regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is **prohibited**.

Each state is only authorized to see locations within their state, and sharing of confidential business information may be restricted between state and federal entities. Check the privacy laws with the State Plant Health Director for the state.

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received *H. armigera*-infested material. Speak to the growers or farm managers and obtain proper permission prior to entering private property.

Several actions should occur immediately upon confirmation that a nursery sample is positive for *H. armigera*:

- Check nursery records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from infested nursery during the previous 6 months).
- Evaluate the infestation, the location within the nursery and severity.
- Check nursery records to identify potential sources of the infestation including sources of seed outside the nursery.

Visual Inspection of Host Plants for Eggs and Larvae

Helicoverpa armigera is polyphagous with a feeding-host range that includes 180 species of wild and cultivated plants in more than 45 families (Venette *et al.*, 2003). Adults feed on a broad range of nectar sources (King, 1994). Larvae feed on a wide range of plant tissues but primarily on the growing points and flowering and fruiting structures of host plants (Fitt, 1989). Saleem and Yunus (1982) reported that larvae fed on the stems, leaves, flowering structures and fruiting structures of maize and tomato. On cotton, chickpea, tobacco and okra, larval feeding was observed on the leaves, flowering structures and fruiting structures.

However, on tomato, the larvae reportedly prefer leaves to fruit (Venette *et al.*, 2003).

The feeding habits of larvae include penetration and burrowing into buds, flowers and fruit. Feeding on cotton bolls, maize cobs, chickpea pods, tomato fruit, okra pods and tobacco seed capsules was cryptic or semi-cryptic (Saleem and Yunus, 1982). Feeding on cotton bolls was accompanied by fungal and bacterial infection, and feeding on maize cobs was associated with fungal infection (Saleem and Yunus, 1982). Thus, in addition to inspecting for all life stages of the insect, all host-plant material—especially upper leaves, buds, flowers and fruit—requires visual inspection for signs and symptoms of feeding, including entry holes, frass and associated microbial infection. Evidence of larval penetration requires follow up with dissections to ascertain the presence or absence of pupae in addition to larvae (Venette *et al.*, 2003).

Sentinel Sites

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

Targeted Surveys

Conduct regular targeted surveys at nurseries and in areas with regular traffic from countries with known infestations.

Survey Records

Records should be maintained for each survey site. Survey records and data recording formats should be consistent to standardize information collection. These records should include details regarding when the pest was found and when it was not.

If automated field collection devices are used, such as the Integrated Plant Health Information System (IPHIS), ensure that all surveyors are trained in the

technology before beginning the survey. Use the appropriate IPHIS templates for this pest. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders prior to working in the field. Upon survey conclusion, all survey data should be entered into a designated state or national pest database.

Data Collection

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

- Date of collection or observations
- Collector's name
- Grower's field identification numbers
- GPS coordinates
- Host plant species and specific crop plant variety, if applicable
- History of machinery usage
- Observations of symptoms
- Other relevant information

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

1. Mark the location
2. Obtain samples of infested plant parts and flag the location within the field
3. Notify the state or PPQ inspector
4. Place the samples from the infected plant inside two resealable plastic bags
5. Label the sealed bags with the following information:
   - **A.** Date
   - **B.** Name of person responsible
   - **C.** Location of sample collection
6. Keep bagged samples cool or refrigerated until the inspector arrives
7. **Do not** freeze the samples

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize outbreaks that could be associated with *H. armigera* and similar pests.

Chapter 7

Regulatory Procedures

Introduction

Use *Chapter 7 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against *H. armigera*. After a new detection in the United States, or when detection in a new area is confirmed, conduct a delimiting survey to define the geographic location where infested plants are present. Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness.

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to people interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in line with labeling restrictions. During all field visits, ensure that proper sanitation procedures are followed.

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 state measures are inadequate, intrastate regulatory action can be taken 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 obtain permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property without owner permission. PPQ prefers to work with the state to facilitate access when permission is denied, however each state government has varying authorities regarding entering private property.

A general Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas in which 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 Native American 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 Native American tribal governments about actions that may have substantial direct effects on tribes. Whether an action is substantial and direct is determined by the tribes. Effects are not limited to tribal land boundaries (reservations) and may include effects on off-reservation land or resources which tribes customarily use or even effects on historic or sacred sites in states where tribes no longer exist.

Consultation is a specialized form of communication and coordination between the federal and tribal governments. Consultation must be conducted early in the development of a regulatory action to ensure that tribes have opportunity to identify resources that may be affected by the action and to recommend the best ways to take actions on tribal lands or affecting tribal resources. Communication

with tribal leadership follows special communication protocols. For more information, contact PPQ's Tribal Liaison.

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.

Overview of Regulatory Program after Detection

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

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

Record-Keeping

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

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

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

Issuing an Emergency Action Notification

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

Establishing a Federal Regulatory Area or Action

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

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

Regulatory Records

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

Use of Chemicals

The PPQ *Treatment Manual* and the guidelines identify the authorized chemicals and describe the methods and rates of application and any special instructions. For further information refer to *Control Procedures* on page 8-1. Agreement by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for this pest.

Chapter

8

Control Procedures

Overview of Emergency Programs

Plant Protection and Quarantine (PPQ) develops and makes control measures available to involved states. Environmental Protection Agency (EPA)-approved treatments will be recommended when available. If selected treatments are not labeled for use against the organism or in a particular environment, PPQ's FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) 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, as amended. The PPQ FIFRA coordinator and pesticide-use coordinators are also available upon request to work with the EPA to expedite approval of a product that may not be registered in the United States, or to obtain labeling for a new use. Refer to *Resources* on page A-1 for information on contacting the coordinator.

In the current USDA PPQ treatment manual, *H. armigera* is identified as a pest of lettuce from Spain, which is treated with methyl bromide at normal atmospheric pressure (NAP) (USDA, 2014). In the event of an incursion, an effort to eradicate *H. armigera* before it can disperse and establish may require the use of insecticide sprays.

Cotton receives the highest quantity of insecticide to control *H. armigera* (Jouβen *et al.*, 2012). This section identifies insecticides used to control *H. armigera* in cotton in China (Table 8-1), Spain (Table 8-2), Cameroon (Table 8-3), India (Table 8-4) and Australia (Table 8-5). Table 8-5 summarizes these insecticide options and identifies those already labeled to control *H. zea* and *H. virescens* in U.S. (NC) cotton. Table 8-5 also includes the Insecticide Resistance Action Committee (IRAC) (2011) mode of action associated with the identified insecticide.

Control of *H. armigera* in Cotton in China

Yang *et al.* (2013) tested the efficacy of three insecticides against the F1 progeny of field-collected individuals in 16 populations from cotton-growing regions of northern and northwestern China. Prior to the adoption of Bt cotton, *H. armigera* in these regions had exhibited resistance to phoxim (organophosphate) and fenvalerate (pyrethroid). Their results demonstrated the efficacy of emamectin benzoate over phoxim, which was more effective than fenvalerate (Table 8-1). The results suggested reversion of phoxim resistance but stability of fenvalerate resistance related to selection pressure since the adoption of Bt cotton. Emamectin benzoate is U.S. EPA registered and is labeled for use to protect U.S. cotton from beet armyworm, *Spodoptera exigua*; soybean looper, *Chrysodeixis includens* and cabbage looper, *Trichoplusia ni* (Bacheler and Reisig, 2013).

Table 8-1 Susceptibility/efficiency ranking based on the topical testing of F1 *H. armigera* 3rd instars representing 16 field populations sampled from cotton-growing regions of China (Yang *et al.*, 2013)[1]

	Emamectin benzoate >	Phoxim >	Fenvalerate
RR range	1.3–2.1	0.7–8.9	3–830
LD50 range	0.825–1.325 ng/larva	0.046–0.554 µg/larva	0.042–11.658 µg/larva
Insecticide class	avermectin	organophosphate	pyrethroid
IRAC (2011) MoA class	6	1B	3
MoA	Cl channel activators (muscle & nerve action)	acetylcholinesterase (AChE) inhibitors (nerve action)	Na channel modulators (nerve action)

[1]LD50 = Lethal Dose 50% = amount of toxicant applied to test subjects that killed 50% of test subjects; RR = resistance ratio = LD50 of the subject population/LD50 of the susceptible reference strain; IRAC = Insecticide Resistance Action Committee; MoA = mode of action

Control of *H. armigera* in Cotton in Spain

Avilla and González-Zamora (2010) measured *H. armigera* resistance to insecticides included in an IPM system implemented to control *H. armigera* in cotton grown in the Guadalquivir River valley of southern Spain. They tested *H. armigera* populations in response to the failure of endosulfan and methomyl to protect cotton grown in southern Spain in 2003. The chemical control portion of the IPM program rotated the two most frequently used insecticides—endosulfan and methomyl—with pyrethroids, organophosphates and other carbamates. The results of their tests on the F1 progeny of insects collected from the field in 2004 ranked the relative efficacy as follows: chlorpyrifos > lambda-cyhalothrin > methomyl > endosulfan (Table 8-2). Chlorpyrifos is U.S. EPA registered and labeled to protect U.S. cotton from

fall armyworm, *S. frugiperda* (Bacheler and Reisig, 2013). Based on their results, Avilla and González-Zamora (2010) concluded that the levels of resistance to the tested insecticides ranged from low (for methomyl, chlorpyrifos and lambda-cyhalothrin) to moderate (for endosulfan). They reasoned that these levels were too low for resistance to these insecticides to have caused field failure in 2003.

Table 8-2 Susceptibility/efficacy ranking based on results of topical testing of 3rd instars representing *H. armigera* from cotton in southern Spain (Avilla and González-Zamora, 2010)

	Chlorpyrifos >	Lambda-cyhalothrin >	Methomyl >	Endosulfan
RF	1.9	4.0	6.0	11.4
LD50 for Sept. 2004 strain	0.23 µg/larva	0.08 µg/larva	0.48 µg/larva	2.86 µg/larva
Insecticide class	organophosphate	pyrethroid	carbamate	cyclodiene organochlorine
IRAC (2011) MoA class	1B	3	1A	2A
Activity	Acetylcholinesterase (AChE) inhibitors (nerve action)	Na channel modulators (nerve action)	Acetylcholinesterase (AChE) inhibitors (nerve action)	GABA-gated Cl channel antagonists (nerve action)

[1] RF = Resistance Factor = LD50 for Sept. 2004 field-collected *H. armigera*/LD50 for 1999 laboratory strain; IRAC = Insecticide Resistance Action Committee; MoA = mode of action.

Control of *H. armigera* in Cotton in Cameroon

In response to widespread organophosphate, pyrethroid and organochlorine resistance, Brévault *et al.* (2009) examined the initial activity and the post-simulated-rainfall persistence of activity of six insecticides for controlling *H. armigera* and two other Noctuidae species that compose a complex of cotton bollworms in Sub-Saharan Africa. Based on its activity against all larval stages (1st–5th instar) soon after application and its low persistence in the environment, Brévault *et al.* (2009) recommended indoxacarb for *H. armigera* outbreaks. Test results for the six insecticides are summarized in Table 8-3. Indoxacarb is U.S. EPA registered and used to protect U.S. cotton from *H. zea* and *H. virescens* (Bacheler and Reisig, 2013).

Table 8-3 Efficacy of insecticides tested against *H. armigera* in Cameroon (Brévault *et al.*, 2009). Mortality results from leaf-disk bioassay testing of F1 and F2 generations of *H. armigera* from northern Cameroon.[1]

Insecticide	Avg. % 1st instar mortality 48 HAI	Avg. % 5th instar mortality 48 HAI	Avg. post-rainfall persistence (days)	Chemical class	IRAC MoA class (2011)
thiodicarb	98.6 a	96.5	17.2	carbamate	1A
indoxacarb	86.2 c	94.5	3.7	oxadiazine	22
endosulfan	93.3 b	92.5	5.2	cyclodiene organochlorine	2A
emamectin benzoate	97.3 a	89.4	10.6	avermectin	6
spinosad	96.7 a	61.3	8.9	spinosyn	5
cypermethrin + profenofos	93.8 b	55.2	2.7	pyrethroid + organophosphate	3 + 1B

[1]HAI = hours after infesting leaf disks from cotton plants to which insecticides were applied (note: leaves were collected the same day insecticide application*); avg. post-rainfall persistence was measured in days after simulated rainfall at which larval mortality dropped below 50%; IRAC MoA = Insecticide Resistance Action Committee mode of action [* the time that insecticide-treated leaves were collected was deduced by the author using the following information: 1. 1st instars were the *H. armigera* test insects used for the persistence test (Brévault *et al.*, 2009); 2. the 1st instar mortalities align with those in Brévault *et al.* (2009)].

Control of *H. armigera* in Cotton in India

Chaturvedi (2007) tested the insecticide susceptibility of *H. armigera* after instances in which pyrethroid failed to protect cotton grown in central and southern India. The test insects were the F1 progeny of *H. armigera* collected from 14 sites in four cotton-growing states of India. The results for seven insecticides are presented in Table 8-4. Based on RF (resistance factor) ranges, the populations exhibited highest susceptibility to chlorpyrifos, methomyl and monocrotophos. Chlorpyrifos is labeled to protect U.S. cotton from fall armyworm, *S. frugiperda* (Bacheler and Reisig, 2013); methomyl is labeled for use against *H. zea* and *H. virescens* in U.S. cotton (Bacheler and Reisig, 2013).

Table 8-4 Assay results for 14 populations of *H. armigera* collected from cotton, potato, pigeonpea, chickpea or sunflower in India (Chaturvedi, 2007). Test insects were 1st, 3rd and 5th instars; mortality observed at 48 and 72 hours.[1]

Insecticide	Chemical class	MoA category	LD50 range	RF range
chlorpyrifos	organophosphate	1B	1.01–35.24	1–38
methomyl	carbamate	1A	0.31–18.51	1–49
monocrotophos	organophosphate	1B	1.12–35.31	2–50
endosulfan	cyclodiene organochlorine	2	4.71–30.01	12–79
quinalphos	organophosphate	1B	2.37–40.01	11–182
fenvalerate	pyrethroid	3	4.91–113.21	11–245
cypermethrin	pyrethroid	3	15.01–285.3	48–919

[1] MoA Category = IRAC (Insecticide Resistance Action Committee (IRAC), 2011) mode of action categories; RF = resistance factor = LD50 of field s strain/LD50 of susceptible strain; note: It is unclear whether reported results are based on 48- or 72-hour observations.

Control of *H. armigera* in Cotton in Australia

Control of *H. armigera* in Australian cotton is guided by IPM and IRMS (insecticide resistance management strategy) programs (The Australian Cotton Industry Development & Delivery Team, 2012). The synthetic insecticides available for use against eggs and larvae in Australian cotton are included in Table 8-5. The Cotton Pest Management Guide (2012) lists insecticides from the following classes: carbamates, pyrethroids, avermectins, spray-formulations of Btk, triazapentadienes, oxadiazines, anthranilic diamides and two biopesticides, a semiochemical mixable with insecticides, a desiccant, paraffinic oil and a synergist.

The lowest levels of resistance are reported for avermectins, indoxacarb, chlorantraniliprole and Bt sprays. Widespread *H. armigera* resistance was reported for carbamates and pyrethroids; however, their use is included based on appropriate thresholds, developmental stage (egg, larval instar) and insect exposure to application. Magnet®, a plant-volatile-based moth attractant, has been combined with methomyl and thiodicarb to produce a *Helicoverpa* moth attracticide (Downes *et al.*, 2010; Gregg *et al.*, 2010) and is included in the Cotton Pest Management Guide (2012) as a control option. Magnet® can potentially be combined with other insecticides to attract and kill adult *Helicoverpa* (Downes *et al.*, 2010; Gregg *et al.*, 2010).

The avermectins include emamectin benzoate, which is U.S. EPA registered and labeled for use to protect U.S. cotton from beet armyworm, *S. exigua*; soybean looper, *C. includes,* and, cabbage looper, *T. ni* (Bacheler and Reisig, 2013). Carbamates, pyrethroids, spray-formulations of Btk, oxadiazines and anthranilic diamides are U.S. EPA registered and used to protect U.S. cotton from *H. zea* and *H. virescens* (Bacheler and Reisig, 2013).

Table 8-5 U.S. EPA-registered synthetic chemical insecticides and formulated Bt spores used to control *H. armigera* in cotton grown in China,[2] Spain,[3] Cameroon,[4] India[5] and Australia[6] and/or to control *H. zea* and *H. virescens* in U.S. (NC)[1] cotton

IRAC (2011) MoA	Insecticide class	Insecticide name
1A	carbamate	methomyl,[1,4,6] thiodicarb[1,3,5,6]
1B	organophosphate	chlorpyrifos,[3,5] profenofos[4]
2A	cyclodiene organochlorines	endosulfan[3,4,5]
3	pyrethroid and pyrethrin	bifenthrin,[1,6] cyfluthrin,[1,6] beta-cyfluthrin,[6] gamma- cyhalothrin,[1,6] lambda-cyhalothrin,[1,3,6] cypermethrin,[1,4,5,]6 alpha-cypemethrin,[6] deltamethrin,[6] esfenvalerate,[1,6] fenvalerate[2,5]
5	spinosyn	spinosad[1,4]
6	avermectin	emamectin benzoate[2,4,6]
11	*Bacillus thuringiensis* subsp. *kurstaki*	formulated bacterial spores[1,6]
19	triazapentadiene	amitraz (ovicide)[6]
22	oxadiazine	indoxacarb[1,4,6]
28	diamides	chlorantraniliprole[1,6]

IRAC MoA = Insecticide Resistance Action Committee mode of action; [1] (Bacheler and Reisig, 2013); [2] (Yang *et al.*, 2013); [3] (Avilla and González-Zamora, 2010); [4] (Brévault *et al.*, 2009); [5] (Chaturvedi, 2007); [6] (The Australian Cotton Industry Development & Delivery Team, 2012)

Chapter

9

Environmental Compliance

Overview

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

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

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

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

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decision maker before taking action and to tell the public of the decision. Actions that are excluded from this examination, that normally require an environmental assessment and environmental impact statements, are codified in APHIS' NEPA implementing procedures located in 7 CFR 372.5.

The three types of NEPA documentation are categorical exclusions, environmental assessments and environmental impact statements.

Categorical Exclusion

Categorical exclusions (CEs) are classes of actions that do not significantly affect the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have been built into the actions themselves (7CFR 372.5(c)).

Environmental Assessment

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

Environmental Impact Statement

If a major federal action may significantly affect the quality of the human environment (adverse or beneficial) or the proposed action may result in public controversy, then prepare an environmental impact statement (EIS).

Endangered Species Act

The Endangered Species Act (ESA) is a statute requiring that programs consider their potential effects on federally protected species. The ESA requires programs to identify protected species and their habitats in or near program areas and to document how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife

Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act

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

Clean Water Act

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

Tribal Consultation

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

National Historic Preservation Act

The statute requires that programs consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local state historic preservation offices. Documentation under this act involves preparing an inventory of the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may need public involvement and comment before the start of program activities.

Coastal Zone Management Act

The statute requires coordination with states in which programs may impact coastal zone management plans. Federal activities that may affect coastal resources are evaluated through a process called federal consistency. This process affords the public, local governments, tribes and state agencies an opportunity to review the federal action. The federal consistency process is administered individually by states with coastal zone management plans.

Environmental Justice

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

Protection of Children

The executive order requires federal agencies to identify, assess and address environmental health and safety risks that may affect children. If such a risk is identified, measures must be described and carried out to minimize such risks.

Chapter

10

Pathways

Old World bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae) is a widely distributed caterpillar pest of a wide range of crops with pest status in Australia, New Zealand, Africa, Asia, Europe and the islands of the Atlantic and Pacific Oceans (Jouβen *et al.*, 2012; Pedgley, 1985). This polyphagous species' host range includes maize, tomato, peanuts, sorghum, chickpea, pigeonpea, sunflower and okra (King, 1994).The species is the target of almost 30% of all pesticides used worldwide and has developed resistance to the widest range of insecticides of any insect targeted, with populations having demonstrated resistance to organochlorines, organophosphates, carbamates, pyrethroids, spinosad and Bt toxins (Jouβen *et al.*, 2012).

Potential for *Helicoverpa armigera* Incursion into the U.S.

Interceptions

From June 1984 to August 2013, 965 *H. armigera* interceptions were reported at U.S. ports of entry on various import categories (PIN (Port Interception Network) database 309 accessed by Gary L. Cave, 12 August 2013). Details of these interceptions are summarized in Tables 9-1 to 9-4.

Table 9-1 Insect stages of *H. armigera* interceptions at U.S. ports of entry from 6/3/1983–8/6/2013

Insect stage	Number of insects intercepted
adult	43
egg	2
larva	1330
pupa	5
total	**1380**

Table 9-2 Locations of 965 *H. armigera* interceptions at U.S. ports of entry from 6/3/1983–8/6/2013

Location	Number of interceptions
permit cargo	740
baggage	146
general cargo	50
stores	14
holds	7
quarters	3
mail	1
miscellaneous	4
total	**965**

Table 9-3 *H. armigera* interceptions at U.S. ports of entry from 6/3/1983–8/6/2013

Import category	Number of interceptions
cut flower	646
leaf	129
fruit	105
seed	11
stem	11
plant	4
wood product	3
cutting	1
uncategorized	55
total	**965**

Table 9-4 Origin of 965 *H. armigera* interceptions at U.S. ports of entry from 6/3/1983–8/6/2013

Origin	Number of interceptions
Netherlands	290
Israel	221
India	73
Kenya	45
Italy	33
Palestinian Territory	30
Zimbabwe	27
France	26
Spain	25
New Zealand, South Africa	19
Japan	16
Jordan	13
Morocco	12
Nigeria, Tanzania	9
Turkey	8
South Korea	7
Thailand	6
Portugal, United Kingdom of Great Britain and Northern Ireland	5
Germany, Senegal	4
Cape Verde, Ghana, Greece, Pakistan	3
Albania, Bosnia and Herzegovina, Bulgaria, Egypt, Gambia, Mali, Togo, Unknown, Yugoslavia, Zambia	2
Eritrea, Ethiopia, Guam, Hong Kong, Iran, Iraq, Lebanon, Malaysia, Malta, Mexico, Moldova, Mozambique, Philippines, Romania, Syrian Arab Republic, Trinidad and Tobago, Tunisia, Ukraine, United Arab Emirates, West Pacific Country Unknown	1
total	**965**

Dispersal Potential

Helicoverpa armigera is characterized by three categories of movement: short range, long range and migratory (Fitt, 1989). The timing and extent of the movement align with temporal and spatial variations in the quality and quantity of food plants available to support development and reproduction. An additional factor in the movement of migratory magnitude is the coincidence of weather (wind and temperature), which facilitates such movement (Fitt, 1989).

Short-range movement accommodates appetitive behaviors (eluding predators, feeding, mating and ovipositing) and occurs within or immediately above the host canopy. The distances traversed are generally limited to less than or equal to 1,000 m within a habitat and occur up to 2 hours after dusk (Fitt, 1989).

Long-range flight generally occurs at altitudes reaching 10 m above the host canopy and covers distances of 1–10 km, typically downwind. These flights occur between crops and between sites of emergence, feeding and oviposition (Fitt, 1989).

Migratory flights result in displacements of hundreds of kilometers downwind and occur over several nocturnal hours above the flight boundary layer at altitudes of 1–2 km (Fitt, 1989). Pedgley (1985) reported the downwind migration of *H. armigera* from North Africa and southern Europe to Britain and northern Europe over distances reaching 1,000 km. In eastern China, *H. armigera* flew between 192 and 451 km during nocturnal migratory flights that lasted 8–11 hours (Feng *et al.*, 2009).

Establishment Potential

In January 2013, *H. armigera* was first detected in Western-Hemisphere farm fields, feeding on Brazilian cotton and soybeans (Czepak *et al.*, 2013; Tay *et al.*, 2013). A 2001 USDA PERAL risk assessment concluded that based on the climate, crops and wild hosts *H. armigera* could potentially become established in every state of the continental U.S. (Fowler and Lakin, 2001).

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Appendix

A

Resources

Use *Appendix A Resources* to find the Website addresses, street addresses and telephone numbers for the resources mentioned in the guidelines.

Table A-1 Resources

Resource	Contact Information
Center for Plant Health, Science and Technology (USDA-APHIS-PPQ-CPHST)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst
Pest Detection and Emergency Programs, Emergency Management (USDA-APHIS-PPQ-PDEP-EM)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport?1dmy&urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases
PPQ Treatment Manual	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst
Plant, Organism and Soil Permits (APHIS-PPQ)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_import%2Fsa_permits%2Fct_plant_health_permits
National Program Manager for Native American Program Delivery and Tribal Liaison (USDA-APHIS-PPQ)	14082 S. Poston Place Tucson, AZ 85736 Telephone: (520) 822-5440 http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/tribalrelations/sa_tribal_consultation/!ut/p/a0/04_Sj9CPykssy0xPLMnMz0vMAfGjzOJNPC2MjIwNjDwNTHyMDBwNnMKMDZxDDQ2NDfQLsh0VAb0Q-SQ!/
Biological Control Coordinator (USDA-APHIS-CPHST)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fsa_cphst%2Fct_abcu
FIFRA Coordinator (USDA-APHIS-PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: 301-851-2243
Environmental Compliance Coordinator (USDA-APHIS-PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: 301-851-2345 http://www.aphis.usda.gov/wps/portal/banner/help?urile=wcm%3apath%3a%2FAPHIS_Content_Library%2FSA_Our_Focus%2FSA_Plant_Health%2FSA_Domestic_Pests_And_Diseases%2FSA_EMT
PPQ Forms	http://www.aphis.usda.gov/wps/portal/aphis/resources/forms

list of State Plant Health Directors (SPHD)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_plant_health%2Fsa_program_overview%2Fct_sphd
list of State Plant Regulatory Officials (SPRO)	http://nationalplantboard.org/member/index.html
National Climatic Center, Database Administration	Box 34 Federal Building (151 Patton Ave) Asheville, NC 28801-5001 http://www.ncdc.noaa.gov/oa/ncdc.html
CAPS Survey Manual	http://caps.ceris.purdue.edu/
GenBank®	http://www.ncbi.nlm.nih.gov/
iPhyClassifier	http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi

Appendix

B

Forms

PPQ Form 391, Specimens for Determination **B-2**

PPQ 523 Emergency Action Notification **B-6**

PPQ Form 391, Specimens for Determination

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

FORM APPROVED
OMB NO. 0579-0010

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

SPECIMENS FOR DETERMINATION

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

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER
2. DATE — MO | DA | YR
3. SUBMITTING AGENCY — ☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER
6. ADDRESS OF SENDER
ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*
7. NAME AND ADDRESS OF PROPERTY OR OWNER
COUNTRY/ COUNTY

PURPOSE

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

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

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

HOST DATA

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

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

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

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

PEST DATA

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

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

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

16. SAMPLING METHOD
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

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

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

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

22. REMARKS

23. TENTATIVE DETERMINATION

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

SIGNATURE
DATE

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

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

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

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

PPQ Form 391, Specimens for Determination (cont.)

OMB Information

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

Instructions

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

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

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

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

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

Purpose

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

Instructions

Follow the instructions on page B-3. Inspectors must provide all relevant collection information with samples. This information should be shared within both the state and the regional office program contact. If a sample tracking database is available at the time of detection, please enter the collection information in the system as quickly as possible.

Distribution

Distribute PPQ Form 391 as follows:

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

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

Block	Description	Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: 2-letter state code-5-digit sample number (survey identification number in parentheses); example: PA-1234 (0402010001) 2. CONTINUE consecutive numbering for each subsequent collection 3. ENTER the collection number
2	DATE	ENTER the date of the collection
3	SUBMITTING AGENCY	PLACE an X in the PPQ block
4	NAME OF SENDER	ENTER the sender's or collector's name
5	TYPE OF PROPERTY	ENTER the type of property from which 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 from which the specimen was collected
8A–8H	REASONS FOR IDENTIFICATION	PLACE an X in the correct block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE GIVE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE BLANK; ENTER remarks in *Block 22*
10	HOST INFORMATION, NAME OF HOST	If known, ENTER the scientific name of the host
11	QUANTITY OF HOST	If applicable, ENTER the number of acres planted with the host
12	PLANT DISTRIBUTION	PLACE an X in the applicable box
13	PLANT PARTS AFFECTED	PLACE an X in the applicable box
14	PEST DISTRIBUTION: FEW/COMMON/ABUNDANT/ EXTREME	PLACE an X in the appropriate block
15	INSECTS/NEMATODES/ MOLLUSKS	PLACE an X in the applicable box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage
16	SAMPLING METHOD	ENTER the type of sample
17	TYPE OF TRAP AND LURE	ENTER the type of sample
18	TRAP NUMBER	ENTER the sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check the appropriate box; otherwise LEAVE BLANK
20	WEED DENSITY	If applicable, check the appropriate box; otherwise LEAVE BLANK
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 and 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; to be completed by the official identifier

PPQ 523 Emergency Action Notification

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

FORM APPROVED - OMB NO. 0579-0102

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

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION
2. DATE ISSUED
3. NAME AND QUANTITY OF ARTICLE(S)
4. LOCATION OF ARTICLES
5. DESTINATION OF ARTICLES
6. SHIPPER
7. NAME OF CARRIER
8. SHIPMENT ID NO.(S)
9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. FAX NO.

SS NO. TAX ID NO.

10. PORT OF LADING
11. DATE OF ARRIVAL
12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)
12a. PEST ID NO.
12b. DATE INTERCEPTED
13. COUNTRY OF ORIGIN
14. GROWER NO.
15. FOREIGN CERTIFICATE NO.
15a. PLACE ISSUED
15b. DATE

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

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

16. ACTION REQUIRED

☐ TREATMENT:

☐ RE-EXPORTATION:

☐ DESTRUCTION:

☐ OTHER:

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

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

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE:

DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER:

DATE:

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

Figure B-3 Example of PPQ 523 Emergency Action Notification

Purpose

Issue a PPQ 523 Emergency Action Notification (EAN) to hold all host plant material at facilities that house the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines that 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 is made to destroy plants, or in the case of submitted samples, once positive confirmation is received, the same EAN that placed plants on hold also documents any actions taken, such as destruction and disinfection. More action may be warranted if other fields test positive for this pest.

Instructions

If plant lots or shipments are held as separate units, issue separate EANs for each unit of suspected and associated plant material. The EANs are issued under the authority of the Plant Protection Act of 2000 (state 7 USC 7701-7758). States are advised to issue their own hold orders parallel to the EAN to prevent intrastate movement of plant material.

When using an EAN to hold articles, the EAN language must 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 the areas disinfested. Include language that these actions will occur 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, use the same EAN to document any disposal, destruction and disinfestation orders resulting from the investigations or testing.

Appendix C

How to Submit Plant Samples

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.

Sorting Trap Samples

When a trap is serviced, sorting is critical. Debris and non-target insect orders must be sorted from the trap material. The taxonomic level of sorting will depend on the expertise available and can be confirmed with the identifier.

Screening Trap Samples

Screening is a process of eliminating non-target families, genera or 'look-alikes' of the surveyed species. Consult the CAPS website for screening aids for particular groups. When in doubt, however, forward the specimens to the identifier/taxonomist. The use of these aids should be coupled with training from identifiers and/or experienced screeners prior to their use. These aids can be found at the following Website: https://caps.ceris.purdue.edu/screening_aids.

Storage

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

Packaging and Shipping

Ensure specimens are dead prior to shipping by either placing them in a vial of alcohol or placing dry specimens in the freezer for at least 1 day. 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 method of collection determine how the specimens are handled, preserved and shipped to the identifier. In general, mites, insect larvae, soft- and hard-bodied adult insects can be transferred to vials of 75–90% ethanol (EtOH) or an equivalent such as isopropyl alcohol. At times, Lindgren funnel trap samples containing bark beetles may also contain rainwater. To prevent later decay, drain off all liquid and replace with alcohol. For more guidance regarding these samples please follow the procedures in the newly revised *Guidelines for Submitting Wood Borer and Bark Beetle (WBBB) Specimens for Identification* found at the following Website: http://caps.ceris.purdue.edu/taxonomic_services/wbbb_sample_submission.

Vials should contain samples from a single trap and a printed or hand-written label with the associated collection number that can be found in the top right corner of form 391. Please use a writing utensil that is not alcohol soluble such as a Micron® pen or a pencil. Samples from multiple traps **must not** be combined in a single vial to preserve the locality-associated data. Vials can be returned to field personnel upon request.

If the mail or freight forwarder takes issue with sending specimens in alcohol, the majority of the liquid can be decanted from the vial, which should then be sealed tightly in the container immediately prior to shipping. Notify the identifier that the vials will require the alcohol be replaced as soon as they are received. If shipped quickly, the specimens should not dry out if the vial is properly sealed.

Sticky Trap Samples

Due to their fragile appendages, scales on wings, *etc.*, adult Lepidoptera, 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 without the sticky surfaces touching and secured loosely with a rubber band for shipping. Inserting a few Styrofoam peanuts on trap surfaces away from insects will cushion and prevent the sticky surfaces from adhering during shipment to taxonomists (see Figure C-1). **Do not** simply fold traps flat or cover traps with transparent wrap (or other material) to avoid seriously damaging or pulling apart specimens rendering identification difficult or impossible.

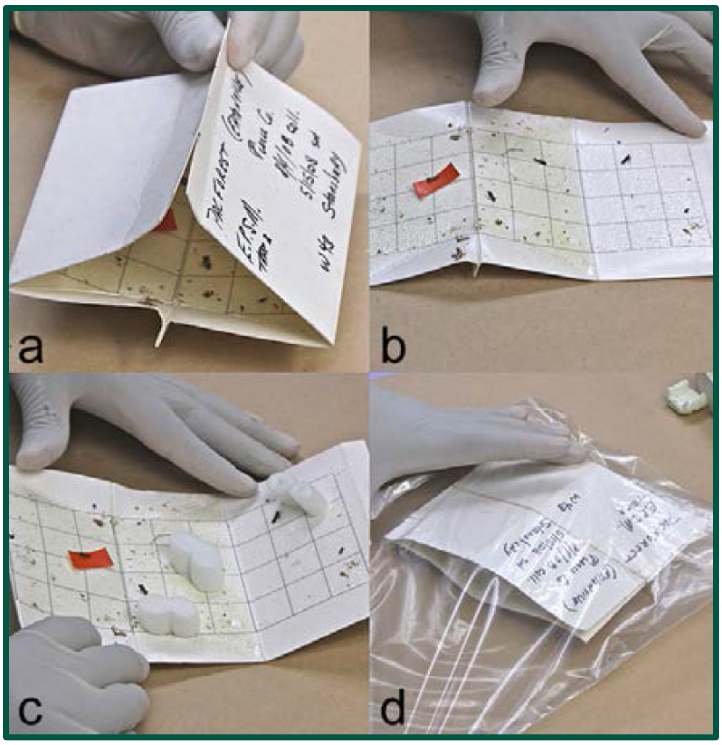

Figure C-1 Recommended method for packing sticky traps: (a) open and (b) unfold trap; (c) place 2–4 packing peanuts in areas of trap with no moths; (d) fold trap, secure with rubber band and place in a plastic bag

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. Maintain space around the specimen for pinning and future manipulation. For multiple traps, place several foam peanuts between sticky surfaces (arranged around suspect specimens) to prevent surfaces from sticking to one another. **Do not** simply fold traps flat or cover traps with transparent wrap (or other material) as this will seriously damage or pull apart the specimens rendering identification difficult or impossible.

Dry Specimens

Some collection methods produce dry material that is **fragile** (**Note:** bark beetle/wood borer samples collected in Lindgren funnel traps should not be sent dry. Follow the guidelines listed in the specific protocol described in *Liquids*). Dry samples can be shipped in vials or glassine envelopes. As with the alcohol samples, make sure the collection label is associated with the sample at all times. This method is typically used for larger insects, but has a greater risk of breakage during shipping. Additionally, dry samples are often covered with debris and sometimes difficult to identify.

Ensure that samples are adequately packed 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, samples should be packed so that movement within the container is restricted. Please include the accompanying documentation and notify the identifier prior to shipping. Remember to inform the identifier that samples are on the way, providing the approximate number and your contact information.

Documentation

Each trap sample/vial should be documented in and accompanied by its own completed PPQ form 391, *Specimens for Determination*. You should maintain a partially pre-filled electronic copy of this form on your computer with your address and other information to save time. Indicate the name of the person making any tentative identification prior to sending to an identifier. Please ensure all applicable fields are completed and that the bottom field (block 24, *Determination and Notes*) is left blank for completion by the identifier. Include the phone number and/or e-mail address of the submitter. Other documentation in the form of notes, images, *etc.* can be included if useful to the determination. A method for cross-referencing the sample/vial with the accompanying form is critical. For example, write the collection number on both Form 391 and the envelope containing the sample.

Appendix

D

Taxonomic Support for Surveys

Background

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

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

On 13 June 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02, which established the role of PPQ NIS as the point of contact for all domestically detected confirmations and communications regarding introduced plant pests. The position of Domestic Diagnostics Coordinator (DDC) was established to administer the policy and coordinate domestic diagnostics for the NIS. This position was filled in October of 2007 by Joel Floyd (USDA, APHIS, PPQ-PSPI, NIS 4700 River Rd., Unit 52, Riverdale, MD 20737, phone (301) 851-2115, fax (301) 734-5276, e-mail: joel.p.floyd@aphis.usda.gov). Any questions regarding sample routing or communication of results can be directed to the PPQ Survey Field Operations Manager (Brian Kopper: phone (919) 855-7318; e-mail, brian.j.kopper@aphis.usda.gov) or the Domestic Diagnostics Coordinator

Taxonomic Support and Survey Activity

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

Fortunately most states have, or have access to, good taxonomic support. Taxonomic support should be considered in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the state often require supplies, develop training materials or hire technicians to meet their screening and identification needs. When considering whether to survey for a particular pest during a given year, consider the challenges of taxonomic support.

Sorting and Screening

For survey activities, the proper sorting and screening of samples prior to examination by an identifier will result in improved turn-around times for identification.

Sorting

Sorting is the first level of activity to ensure samples submitted are of the correct target group for the pests being surveyed. Select those plant samples that are symptomatic if appropriate. A minimum level of sorting is expected of surveyors depending on the target group, training, experience or demonstrated ability.

Screening

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

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

Resources for Sorting, Screening and Identification

Sorting, screening and identification resources and aids useful to CAPS and PPQ surveys are best developed by taxonomists knowledgeable in the taxa that include the target pests and the established or native organisms in the same group that are likely in the samples and can be confused with the target. These aids are often regionally based and can be in the form of dichotomous keys, picture guides or reference collections. The NIS encourages the development of these resources, and when aids are complete, posts them in the CAPS Website for the benefit of

others. If local screening aids are developed, please notify Joel Floyd, the Domestic Diagnostics Coordinator, as to their availability. Please see the following Website for some available screening aids: https://caps.ceris.purdue.edu/node/34.

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a state is inadequate for a particular survey, other entities may assist including PPQ identifiers, universities and state departments of agriculture from other states and independent institutions. Check with the PPQ regional CAPS coordinators regarding the availability of taxonomic assistance.

Universities and State Departments of Agriculture

Depending on the taxonomic group, a few cases involve two entities that 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 several regional hub laboratories that can provide service identifications of plant pests in their respective regions. PPQ currently has arrangements with two state departments of agriculture (Oregon and Washington) and one university (Mississippi State University) through Farm Bill funding to provide taxonomic services to other states should they desire it. Contact your CAPS NOM for more information.

Independent Institutions

The Raleigh PPQ Field Operations office has set up multi-state arrangements for the 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 to 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 during PPQ-conducted surveys and occasionally those from CAPS surveys. They can also enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to their being forwarded for confirmation by an NIS-recognized authority. The list of PPQ port identifiers and their areas of coverage can be found on the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

PPQ Domestic Identifiers

PPQ has a limited number of domestic identifiers normally stationed at universities who are primarily responsible for survey samples. Domestic identifiers can handle unscreened or partially screened samples with prior arrangement through the PPQ CAPS NOM. They can also act 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. In addition, these identifiers can enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to forwarding the sample for confirmation by an NIS-recognized authority.

Bobby 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
e-mail: robert.c.brown@aphis.usda.gov

Specialty: Forest pests (Coleoptera, Hymenoptera)

Area of coverage: Primarily northeast and Midwest U.S.

Julieta Brambila
Domestic Entomology Identifier
USDA–APHIS–PPQ
P.O. Box 147100
Gainesville, FL 32614-7100
Phone: (352) 395-4792
e-mail: julieta.brambila@aphis.usda.gov

Specialty: Adult Lepidoptera, Heteroptera

Area of coverage: Primarily eastern U.S.

Kira Metz
Domestic Entomology Identifier
USDA–APHIS–PPQ
Minnie Belle Heep 216D
2475 TAMU
College Station, TX 77843
Phone: (979) 450-5492
e-mail: kira.zhaurova@aphis.usda.gov

Specialty: Lepidoptera, Coleoptera

Area of coverage: Primarily western/southern U.S.

ATTENTION SAMPLE SUBMITTERS: When sending domestic samples to domestic identifiers, you must notify them first by e-mail or phone that you plan to send samples, describing what type and how many. Once notification has been sent, forward an e-mail to them with a tracking number for the express carrier through whom the samples were forwarded. If you plan to send a domestic sample to a national specialist, notify the CAPS NOM or the National Domestic Diagnostics Coordinator prior to sending the sample.

Final Confirmations

If identifiers or laboratories at the state, university or institution level suspect the detection of 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 place the specimen into the PPQ system. They will then send the specimen to the NIS-recognized authority for that taxonomic group. In some cases, domestic identifiers can make final confirmation depending on their ID authority, accreditation and proficiency testing.

State-level taxonomists, who are reasonably certain that they have a new United States record, CAPS target or 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 to the NIS-recognized authority for confirmation, please complete a PPQ form 391 with the tentative determination. In addition, fax a copy of the completed PPQ Form 391 to 'Attention: Domestic Diagnostics Coordinator' at (301) 851-2115, or send a PDF file in an e-mail to aphis-ppq.nis.urgents@aphis.usda.gov with the overnight carrier tracking number.

The addresses of the NIS-recognized authorities to which suspect specimens are to be sent can be found at the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

Only use the 'Urgent' listings for suspected new United States or state records of a significant pest, and the 'Prompt' listings for all others.

When the specimen is forwarded to a specialist for final confirmation, use an overnight carrier, insure proper and secure packaging and include a hard copy of the PPQ form 391 marked 'Urgent' or 'Prompt' as previously described.

Please contact Joel Floyd, the Domestic Diagnostics Coordinator if you have questions regarding a particular sample routing at (301) 851-2115, or joel.p.floyd@aphis.usda.gov.

Digital Images for Confirmation of Domestic Detections

For the aforementioned confirmations, send specimens, not digital images. For

entry into the National Agricultural Pest Information System (NAPIS), digital imaging confirmations can be used for new county records of widespread pests by state taxonomists or identifiers with their prior approval. These scientists always have the prerogative to request that the specimens be sent. Pests with PPQ regulatory programs may require specimens to be sent to SEL for new county records depending on the species.

Communication of Results

If no suspect CAPS target, program pests or new detections are found, communication of these identification results can be sent by the domestic identifiers or taxonomists at other institutions directly back to the submitter. The information can be presented in a spreadsheet, in a hardcopy of PPQ form 391 or other informal means labelled with the species 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 the NIS-recognized authorities, positive or negative, are communicated by the NIS to the PPQ Emergency and Domestic Programs (EDP) staff at PPQ headquarters. The 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, identifier and/or submitter of the specimen or sample.

Data Entry in NAPIS

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. When in doubt, contact the PPQ Domestic Survey Coordinator.