USDA APHIS PPQ Technical Working Group

Helicoverpa armigera- Old World Bollworm

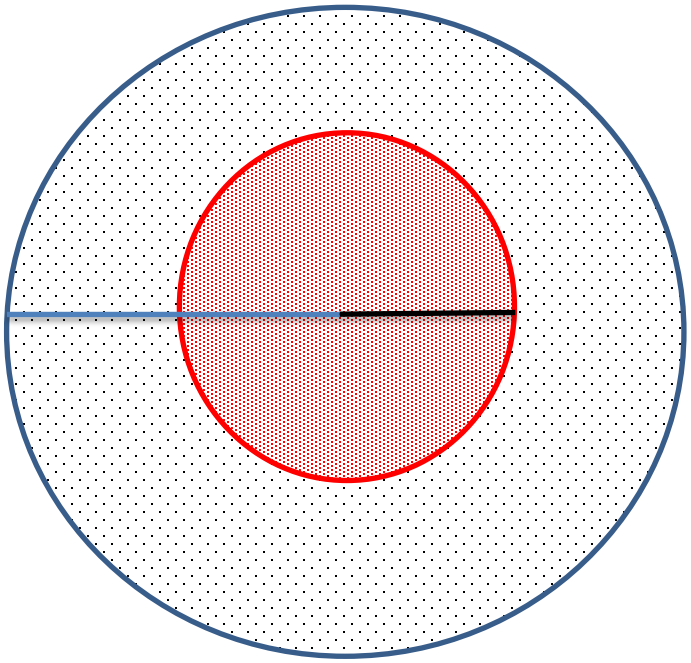
Purpose: The *Helicoverpa armigera* (Hubner), technical working group will address questions and provide scientifically based information to the emergency response activities as needed.

Situational Overview

On September 12, 2014 in San Germán, Puerto Rico, a single adult male *Helicoverpa armigera,* old world bollworm (OWB), was captured in a pheromone trap and subsequently positively identified by USDA taxonomists on September 26, 2014. There were three total pheromone traps in place on that farm, and the traps were placed in a bean field, as part of a PPQ Survey program conducted to detect OWB. As of December 5, 2014, 308 traps have been established for this survey, with additional detections in five different municipalities (San German, Lajas, Yauco, Guanica and Guayanilla) on five different hosts (beans, okra, sorghum, rice and pigeon pea).

Tentative Detection Survey Plan

Currently, PPQ is planning to establish approximately 3000 total traps to determine the distribution and level of potential infestation of OWB. In the area of initial detection, traps will be placed at a density of 25 traps per mi^2 for a radius of 1.44 mi within the core area of the delimitation; this would result in a core area of approximately 5 mi^2 and 125 traps installed. In the area surrounding the core area, a lower trap density area is recommended for an additional 1.24 mi in radius. This would equate to an additional area of roughly 10 mi^2 with a trap density of 9 traps per mi^2 for an additional 90 traps.



Inner delimiting area 1.24 mi radius circle ~5 mi^2-125 traps 25 traps per mi^2

Outer delimiting area 2.48 mi radius circle ~10 mi^2 90 traps-9 traps per mi^2

The single find and original low trapping density does not provide PPQ with a large amount of information about the potential source of infestation or the area of likely OWB infestation. In addition to the delimitation area, PPQ will also expand the trapping grid on the island. The island of Puerto Rico is approximately 3500 mi^2 in size. The roughly 2940 remaining traps (out of the available 3154) can be distributed in a generalized grid covering all or portions of the island. A few potential trapping schemes and options are as follows:

1) To sample the western third of the island (1161 mi^2), one trap would be placed every 0.4 mi^2 (subtracting the core area).

2) To sample the western half of the island (1742 mi^2), one trap would be placed every 0.59 mi^2.

3) To sample the entire island, (3485 mi^2) one trap would be placed every 1.19 mi^2.

4) It is also possible to focus the grid trapping area by areas of crop production and human movement, thus reducing sampling efforts in areas that are not easily accessible and may have lower host presence.

The following maps at the end of this document provide insight into some potential areas for trapping activity based on topography and some of the primary host production.

Questions related to trapping and survey:

1: Do you have any suggestions for improvement of the current trapping plan around the core detection area?

The group recommended that trapping occur in the vicinity of host material. Catches are dependent on what crops are nearby and should be placed near crops that are highly attractive, especially when the crop is flowering. Australia has found that the catch for this species is likely to be high when pigeon pea is present.

Question: What are the crops in the area (in immediate vicinity of finds)? And what are the seasonality/cropping patterns. Traps may have to be moved throughout the season depending on this information.

Farms tend to be fairly small (around 30 acres on average). Some of the seed production farms are larger. Pigeon pea is grown in the vicinity.

2: Do you have a recommendation for the gridded survey pattern?

A gridded pattern is not necessary, but wide distribution of the remaining traps through the island near host material would help to cover the largest area and increase the chance for detection. Make sure to check and change the lures often. Trapping should be in the vicinity of good host crops (like pigeon pea).

3: Is it necessary to place the pheromone traps in fields with host material, or is proximity to host material suitable?

Australia places traps in the vicinity of the host crops. This is mainly due to convenience/ease of access. It's easier to service the traps at the edge of the host crops vs. in the host crops. It is acceptable to place traps adjacent to crops. Pigeon pea seems to be commonly grown in the area and be a good crop to trap near. Pigeon pea is highly attractive and is considered a primary host. It's very attractive to adults when it's in flower; both males and females feed on it. Other good host options include corn, soybean, and tomato.

Question: Does pigeon pea become unattractive at any point?

This would be a good option for trapping near because this crop is known to repeatedly flower. It's very attractive to adults when it's in flower. Pigeon pea is also attractive to females at the pre-flowering stage and will lay eggs the plants at this stage. This characteristic may vary depending on the variety grown.

Indeterminate varieties of pigeon peas are reported to be grown in Puerto Rico, flowering of the plant is currently occurring.

Question: Is *Bt* cotton grown in Puerto Rico? Single or double gene?

The amount and type of cotton grown on Puerto Rico is not known at this moment. This will need to be determined as the management of resistance to the *Bacillus thuringiensis* (Bt) crops of all kinds (soybean, cotton and corn) will become more important as additional information on OWB in Puerto Rico is gathered.

Question: In current IPM management practices, what is the number of pheromone traps per hectare used for monitoring of OWB populations?

Traps shouldn't be any closer than 50m to each other so they do not interfere with one another. It would be better if they were no closer than 100 to 150m apart.

General comments

Puerto Rico has been trapping for this species since February of this year. They have submitted about 80 samples so far. However, the trapping density across the island was fairly low. Traps were flooded with *H. zea*, a native. *Helicoverpa armigera* cannot be easily distinguished from the native.

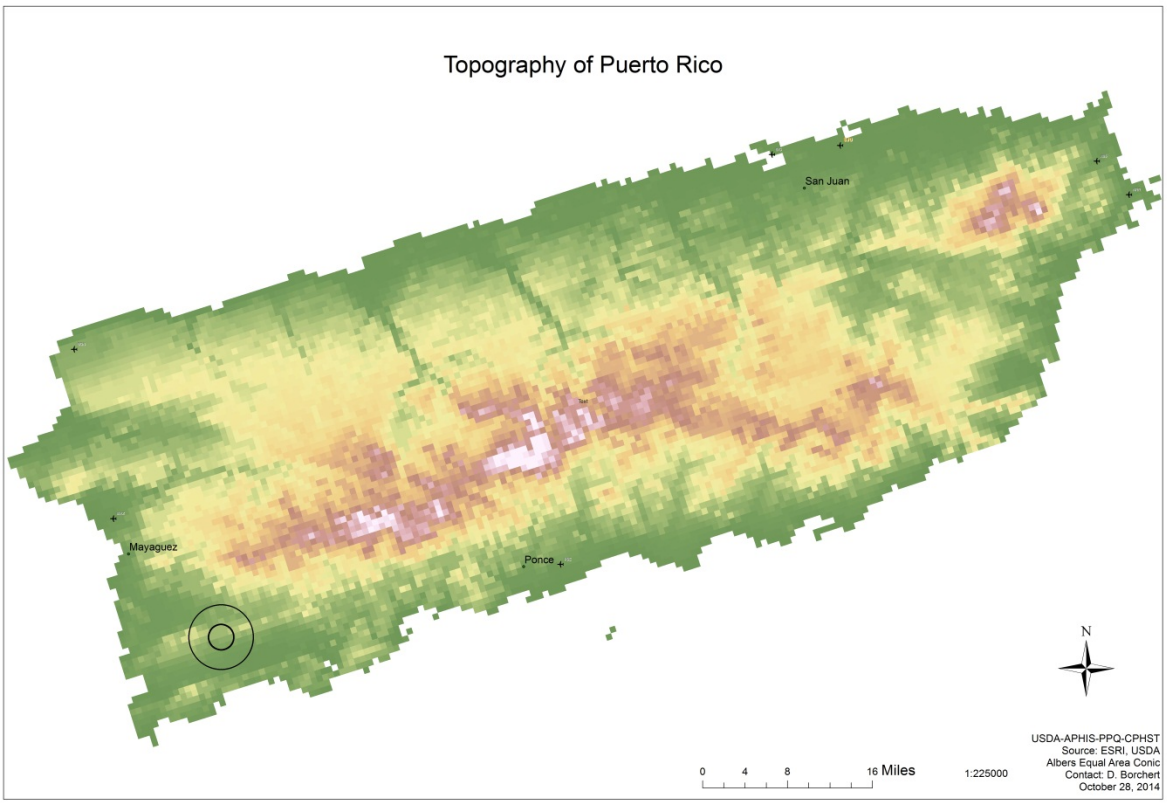
The first positive was identified by morphology and confirmed by molecular diagnostics, looking at the gene region, and sequencing. All of the subsequent identifications have been based on morphological characters.

There is some evidence that there may be hybridization going on between the populations based on molecular observations of OWB in Brazil.

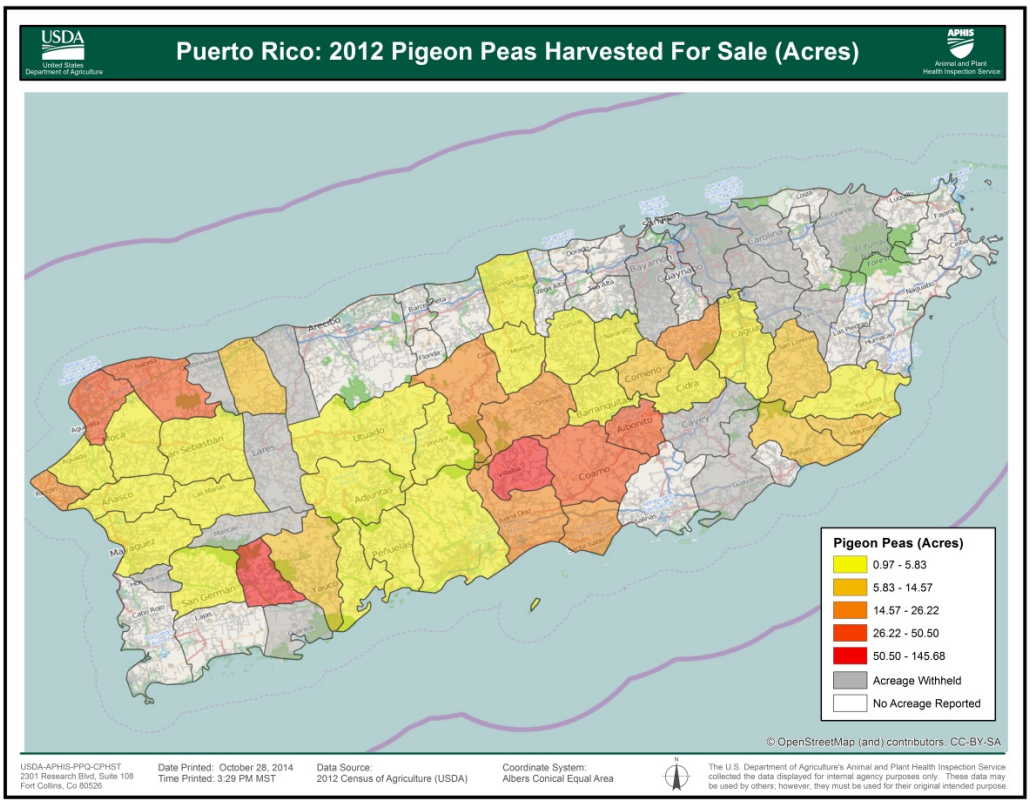
Question: Are they trapping in Florida? What about other islands in the Caribbean?

This species has been on the CAPS list for several years, so states are aware of it. Florida did survey for this species in 2014 through CAPS.

We're not sure if other islands are surveying for this species. USDA believes that OWB may be present in the Dominican Republic; however, we do not have official confirmation from their government. We are working closely with our Caribbean NPPO counterparts to increase our knowledge of where OWB may be in the Caribbean Basin and in other South and Central American countries. We know that OWB is present throughout Brazil, spreading from Southern to Northern Brazil from 2008 to 2012.



The map above depicts the rough location of the initial detection of OWB in Puerto Rico and the core area as proposed. A variety of commodities are grown in Puerto Rico, due largely to the island topography, climate and precipitation patterns. The growing areas and relative amounts of production for four primary hosts (pigeon peas, soybean, corn and tomato) are found below. Out of the potential primary hosts, these four hosts have been found in the highest amount of acreage in Puerto Rico. Additional host maps are being developed.


USDA
United States Department of Agriculture
Puerto Rico: 2012 Pigeon Peas Harvested For Sale (Acres)
APHIS
Animal and Plant Health Inspection Service
Pigeon Peas (Acres)
0.97 - 5.83
5.83 - 14.57
14.57 - 26.22
26.22 - 50.50
50.50 - 145.68
Acreage Withheld
No Acreage Reported
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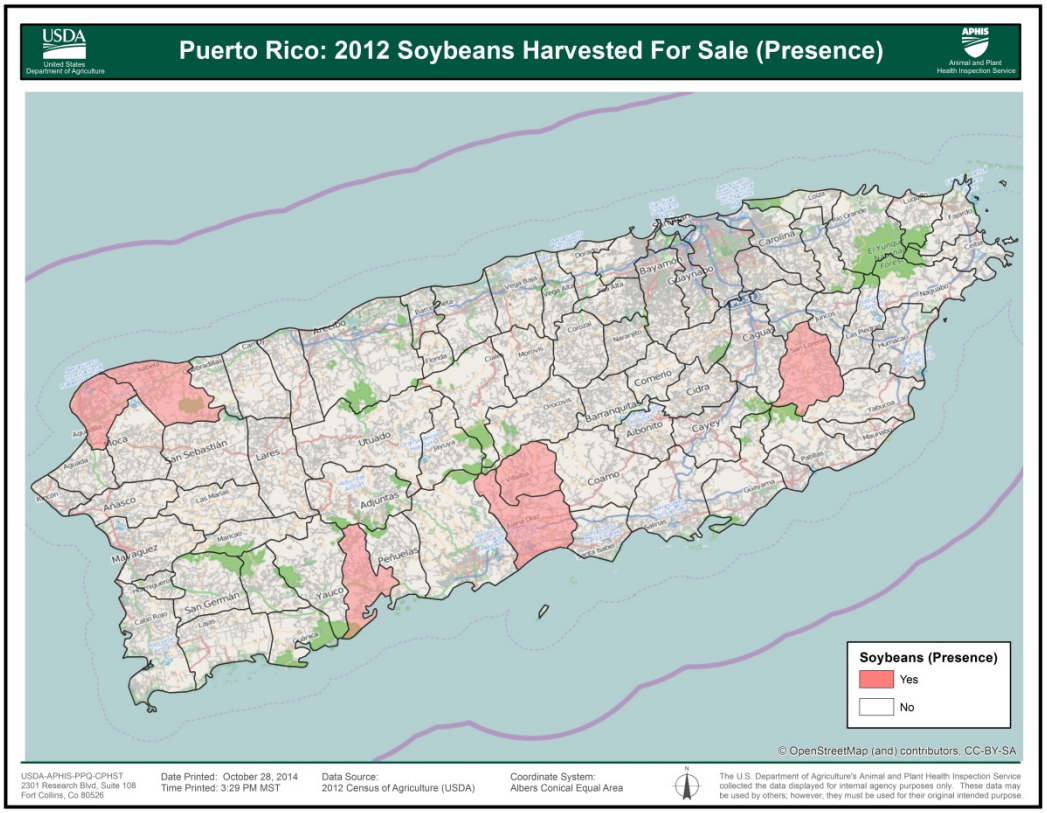
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Date Printed: October 28, 2014
Time Printed: 3:29 PM MST
Data Source:
2012 Census of Agriculture (USDA)
Coordinate System:
Albers Conical Equal Area
The U.S. Department of Agriculture's Animal and Plant Health Inspection Service collected the data displayed for internal agency purposes only. These data may be used by others; however, they must be used for their original intended purpose.


USDA
United States Department of Agriculture
Puerto Rico: 2012 Soybeans Harvested For Sale (Presence)
APHIS
Animal and Plant Health Inspection Service
Soybeans (Presence)
Yes
No
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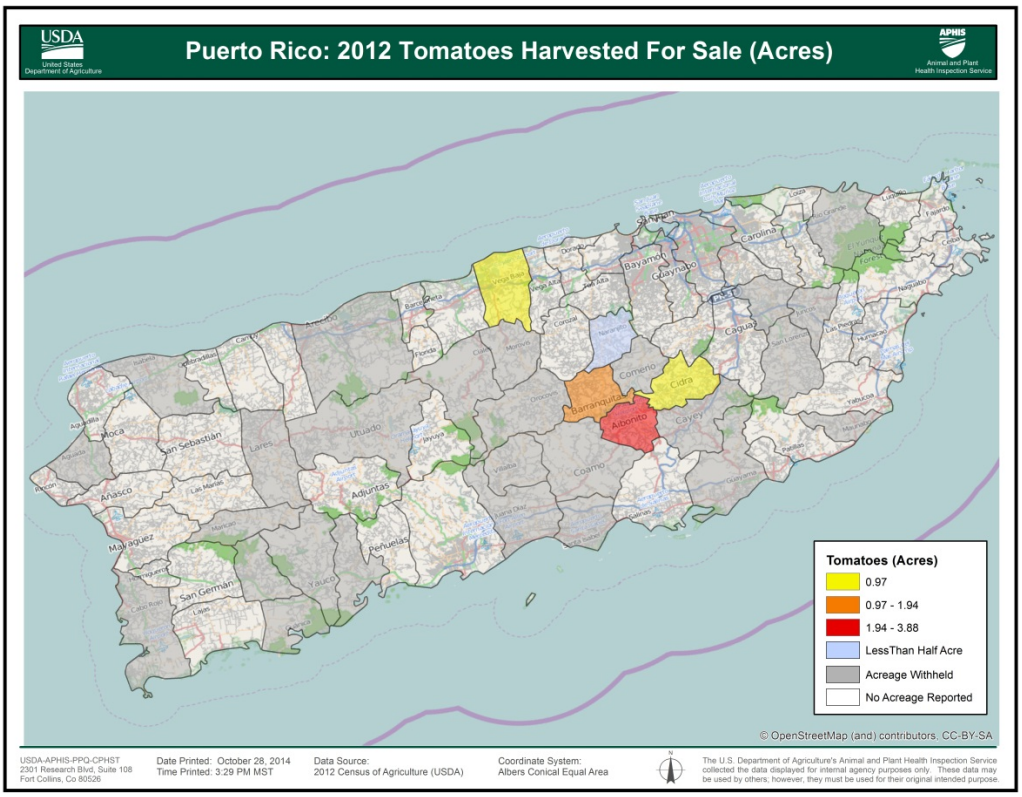
USDA
United States Department of Agriculture
Puerto Rico: 2012 Tomatoes Harvested For Sale (Acres)
APHIS
Animal and Plant Health Inspection Service
Tomatoes (Acres)
0.97
0.97 - 1.94
1.94 - 3.88
LessThan Half Acre
Acreage Withheld
No Acreage Reported
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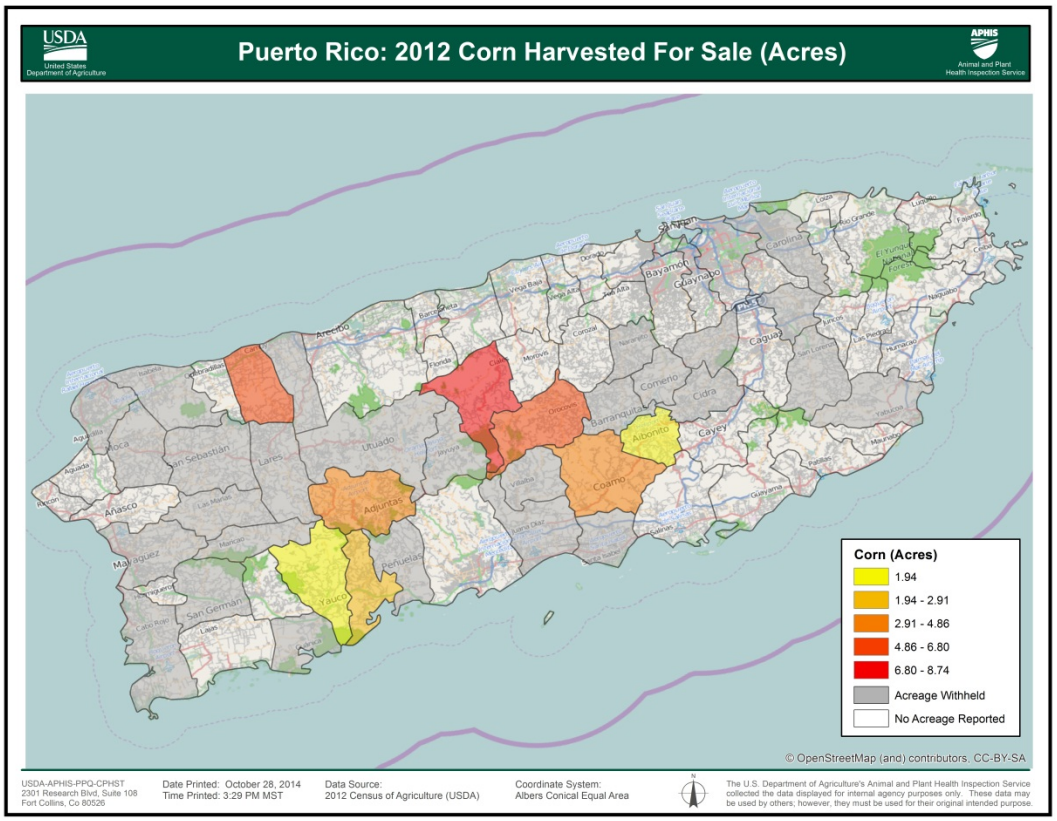
USDA
United States Department of Agriculture
Puerto Rico: 2012 Corn Harvested For Sale (Acres)
APHIS
Animal and Plant Health Inspection Service
Corn (Acres)
1.94
1.94 - 2.91
2.91 - 4.86
4.86 - 6.80
6.80 - 8.74
Acreage Withheld
No Acreage Reported
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Current questions of interest to be posed to the OWB TWG.

5: What do you consider to be the primary hosts of OWB? What are the secondary hosts?

The experts did not go into extensive listing of hosts for OWB, as there are already numerous ones; however, they indicated that the hosts listed in the literature are good hosts. A few hosts were mentioned specifically and were prioritized as: pigeon pea, corn, soybean and tomato (in that order). The program should also be concerned with weedy hosts as populations can build up on non-crop hosts.

OWB will move from different host plants at different times of the year. Adults can be trapped some distance from where they were feeding. There is a list from Brazil that looks at weeds that this species can feed on. The number of weedy hosts is astounding. They can use these non-crop hosts during the off season.

6: What is the average and maximum flight distance for OWB?

When ovipositing, it's not unheard of for females to move 5-10km (dependent on landscape and hosts available). They can also migrate very far and can be aided by wind (100's km is possible).

7: What are the effective pesticides used for control of OWB?

A list of insecticides used in Australia for control of OWB in cotton has been provided and amended with a tentative list of registered crops in the United States. Brazil has reported that the diamides, spinosads and some organophosphates were effective for control of OWB. Control of this pest is necessary during the early stages of larval development.

8: What are effective alternatives to pesticides for OWB: i.e. mating disruption, biological control, sterile insect technique…?

Nuclear Polyhedrosis Virus is currently being sprayed in Brazil and has been used previously in Australia (although it's not used much now). However, this option would not be for eradication, but only to help manage populations. *Trichogramma pretiosum* has been fairly effective in the tropics for management in conjunction with Bt cotton.

9: What is the current known distribution of OWB and potential new areas that may be infested?

The literature on distribution is fairly accurate. OWB is likely to be established in all of the states of Brazil, but it has not been reported from Rio de Janeiro, Rio Grande del Norte and Amazonas. It may also be in Venezuela, but there is no official confirmation on this. It is not currently known to be in Mexico. USDA believes it may be in the Dominican Republic, but there is no official confirmation on this.

10: Are there rapid screens to determine if the OWB captured are resistant to various insecticides available?

There is a screen for synthetic pyrethroids (SP) resistance (p450). Also, there are screens to detect Bt resistance as well.

There is some work being completed on the *H. armigera* genome. It will be coming out soon for publication. This work also includes *H. zea*.

11: What are the recommended trap servicing intervals for traps, lures and kill strips (Appendix 1 contains the current trapping recommendations)?

In Australia, traps are checked weekly and lures are changed every month. The kill strips are changed every two months. Because Puerto Rico is in the tropics, the lures and kill strips will likely break down more quickly. It is recommended that the program perform trap checking and servicing more frequently.

12: In your region, which hosts/commodities have suffered the most damage/yield loss, etc. from OWB? Why (difficult to control in specific crop, etc.)?

This is highly variable and depends on the season. The damage seen in Brazil has been more dramatic, with heavy damage on soybeans being observed and sometimes the plants are eaten to the ground. This is all dependent on the OWB population levels. It doesn't seem to do too much damage to corn; they are usually cannibalistic on this host and damage the end of the corn ear. However, corn can increase the population levels in the area.

13**.** Is sorghum grown in Puerto Rico? This would be a great monitoring host.

Sorghum is reported to be produced in Puerto Rico, but production levels and extent of production is not known.

14. How long should a program continue past the date of a last detection?

The participants on the Technical Working Group expressed the opinion that it will be very hard if not impossible to eradicate this pest. There likely won't be a last detection. The program may need to look at hybridizations between *H. armigera* and *H. zea* at low levels as the group has seen this in some instances in Brazil. If you get to zero, you should continue the program for at least two months (around 800 DD/2 generations/2 months). The opinion of the TWG was that eradication was unlikely, even with the small number of moths captured at the time.

The following are several reasons for the inability to eradicate OWB:

The large crop and weed host range of OWB provides many suitable hosts and areas for the pest to survive and establish; the ability of OWB to develop through multiple generations per year allows the pest to build up large populations; the inability to easily distinguish OWB from *H. zea* and the ability of OWB to move long distances will make future re-introductions on periodic basis probable. The combinations of these factors all make eradication an unlikely option.

Action Item: USDA is requesting about 50 to 100 *H. armigera* samples. These will be provided to port inspectors/identifiers to assist them in knowing what to look for. These should not be in alcohol. They do not need to be pinned.

Technical Working Group members

PPQ-CPHST:

Dan Borchert (TWG Chair-entomologist), Russ Bulluck, Dave Lance (survey, trapping and lure)

Lisa Jackson, Talitha Molet (CAPS), Alison Neeley (Economic Impact), Norman Barr (Molecular Diagnostics)

Jaap van Kretschmar (prepared NPRG), Godshen Pallipparambil (NPRG), Gary Cave (NPRG), Paul Sutton (GIS Support)

PPQ- FO:

Brian Kopper (CAPS Survey Support)

PPQ- NIS:

Joel Floyd, Julieta Brambila (Insect identification and Survey), Steven Passoa -larval identification

USDA-ARS Identifier

Paul Goldstein (confirmation)

Molecular Identification-

Tom Walsh, Weetek Tay -CISRO Australia

Expertise in Control

Sharon Downes –CISRO Australia

Geoff Baker- CISRO Australia

Myron (Meron) Zalucki- University of Queensland- Australia

Cecilia Czepak- Universidade Federal de Goias/Goiânia/GO/Brasil

Appendix 1

Plastic Bucket Trap Protocol

(Revised October 10, 2014)

The plastic bucket trap is a long-lasting insect trap used in conjunction with a lure to monitor or detect various species of moths. The plastic bucket trap is the preferred trap for some moth species as it is able to catch large numbers of moths without damaging some of their identifying characters. The trap has four parts: 1) lid, 2) lure basket with cap, 3) funnel, and 4) bucket.

The trap is available in various color combinations. For PPQ programs, the trap consists of a green lid, yellow funnel, and white bucket. Figure 1 is a photograph of a trap cut in half.

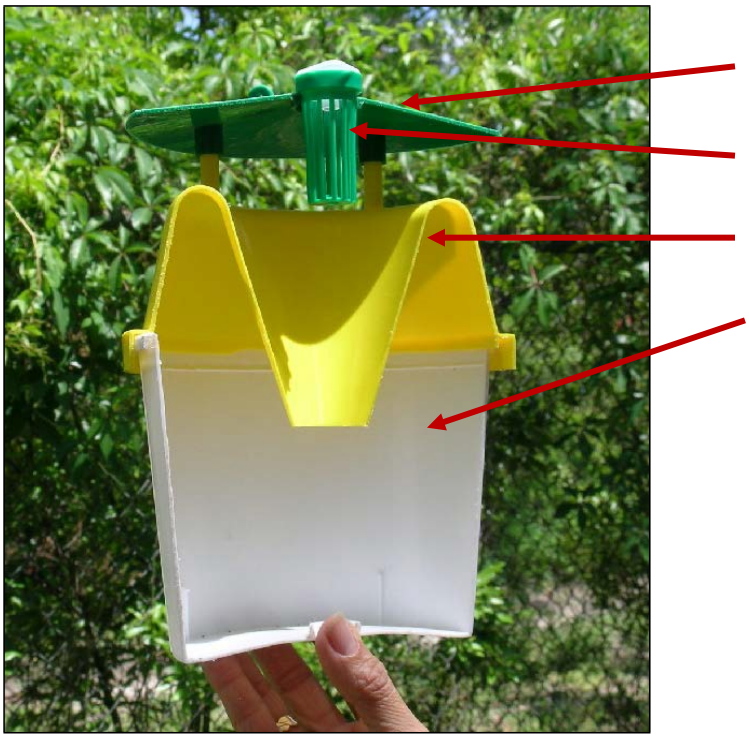


Fig. 1. Plastic bucket trap cut in half to show its interior.

Follow the steps below to prepare the bucket traps for use in the field.

1. Pheromone

The synthetic pheromone is embedded in a laminate, (a small rubberized square as in Fig. 2) or a septum (similar to a pencil eraser as in Fig. 3) dispenser. Use gloves when handling lures and unwrap the lure from its packaging.

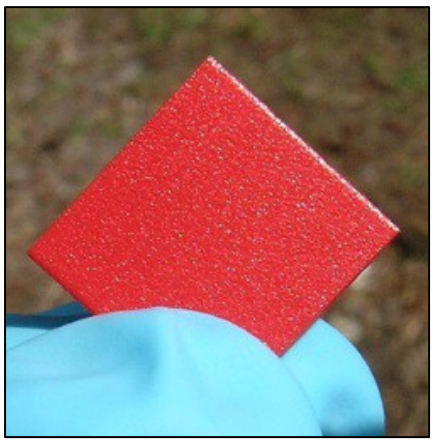
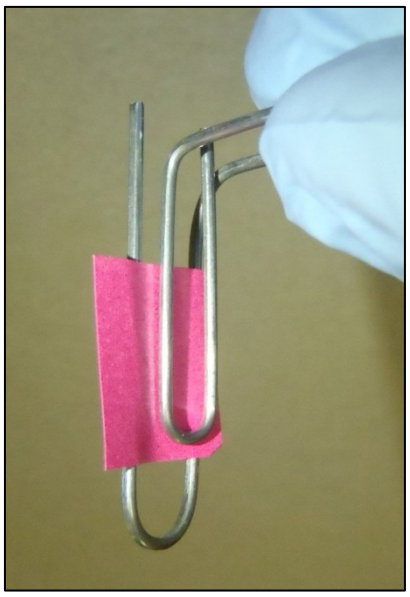


Fig. 2. Laminate lure.

Fig. 3. Septum lure.

If you are using a laminate lure, you may attach the lure to a small paper clip and fold the clip so that the lure does not fall out of the basket (Figures 4 and 5).



If you are using a septum lure, place the septum inside the lure basket (Fig. 6). Cover the basket with the lid and insert the basket through the circular opening on the center of the lid (Fig. 7). If the cap no longer snaps snuggly into the trap lid opening, secure it with a piece of tape.

Fig. 6. Septum lure inside lure basket.

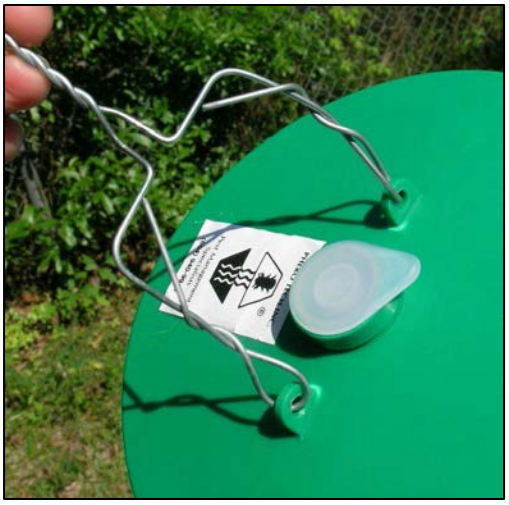
Fig. 7. Lure basket with cap inserted through center of lid.

When not in use, the lures should be stored in a freezer not used for food or drinks. Refer to the pheromone MSDS documents for storage and safety information.

It is important to label the lure baskets with the name of the moth species. To avoid cross-contamination from lure residue, lure baskets should not be used for different species in different survey seasons.

2. Handle

Attach a wire handle to the lid through its two loops, as shown in the photos below (Figs. 8 and 9). A wire handle is usually included with each purchased trap. If a handle is not included, is lost, or is damaged and needs to be replaced, make one with a 12-inch long wire or use a string, but the latter does not last as long as the wire.



Figs. 8 and 9. Wire handle attached to trap's lid.

3. Trap Modifications to Improve Moth Specimen Quality

There are two trap modifications that have been used to improve moth specimen quality. If trapping will occur during potentially rainy conditions, then one of the two methods should be used. Recently, some U.S. researchers have found that the sponge method is easier to use and allows the insecticidal strips to kill the moths more quickly. The holes drilled into the bottom of the trap may allow the insecticide in the strips to escape from the trap, making them less effective at killing the moths. Surveyors are encouraged to use whichever method provides the highest quality moth specimens in their State's unique climatic conditions

3a. Sponge

Place a dry cellulose sponge in the bottom of the trap, as shown in Fig. 10. The sponge will absorb rainwater (except for extremely heavy amounts) that may enter the trap, keeping the moths somewhat dry. Sponges should be purchased locally. Purchase sponges without additives such as antibiotics, fragrances, etc.

Fig. 10. Cellulose sponge (yellow) and insecticidal strip (red) inside the trap.

Fig. 11. Bucket with four drilled hole

3b. Drain holes and wire screen

Alternatively, the bottom part of the trap, the bucket, requires two modifications. Drill two to four drain holes in the bottom (see Fig. 11, above) to allow excess rainwater to drain. If water remains in the trap, the killing agent (the insecticide) can spoil; in addition, the trapped insects may decay, making identification impossible.

Then, add a wire screen slightly larger than the bucket bottom's inside diameter (Figs. 12 and 13). The screen keeps the insecticidal strip(s) and the moths from getting too wet from rainwater accumulated in the trap. Prepare a cardboard template for long term use. Cut the wire mesh with metal-cutting scissors.

Figs. 12 and 13. Metal wire screen inside the bucket.

4. Insecticidal strips

Insecticidal strips (Fig. 14) are used in the traps to kill the moths that enter the bucket. The active ingredient in the strips is Dichlorvos, also known as DDVP and Vapona. Rain, wind, high heat, or an abundance of captured moths may reduce the potency of the insecticidal strips. In addition, large numbers of non-target moths can enter the trap and pile up over the strip, decreasing the strip's effectiveness. The manufacturers of insecticidal strips list an effectiveness of 12 weeks.

Fig. 14. Insecticidal strip.

The CAPS program has listed a conservative length of effectiveness of 8 weeks, which can be used as a starting point for all states. Note, in established surveys, you may continue to use the change interval that works best for your state and its unique climate.

When conducting a survey for a new target in your state that uses the plastic bucket trap, plan to use one insecticidal strip per bucket and replace the strip at 8 week intervals for the duration of the survey. Once you have conducted the survey over a complete survey season, you may use your experience to determine how often to change the strips for this target in any subsequent surveys. For instance, you may observe few non-target moths in the traps or that the strips last longer in your cooler climate. In either of these cases, you can extend the change interval of the strips. If you find that moths are badly damaged (indicating they were not killed quickly), you should change the strips more frequently.

The most important thing to keep in mind is that these strips need to deliver a quick death to the captured moths. The longer the moths survive within the trap, the more damaged their bodies become, which can negatively impact the identification process.
The insecticidal strip may be arranged in the trap by one of two ways: 1) the strip can be placed in the bottom of the trap; 2) the strip can be stapled to strings and hung inside of the trap (if large trap catches are experienced).

The strip should be handled with gloves. Refer to the MSDS document for this product for safety information. Store unopened strips in a freezer that is not used for food or drink.

5. Label the trap

Attach a rain-proof printed label (see Fig. 15) or handwrite a note with a water-proof black marker on the bucket trap. It should indicate that the trap belongs to a state or a PPQ program. Include a phone number in case someone has concerns or questions about the trap.

Figs. 15 and 16. Label on the trap's lid (Fig. 15). Trap set away from foliage, in open field (Fig. 16).

6. Placement of traps

The traps function best when placed in the open, away from foliage, as illustrated on Fig. 16. When hung under foliage, the 3-dimensional shape of the pheromone plume (chemical in the air) is disrupted and the effectiveness of the trap is reduced. Hang the traps from such places as greenhouse roofs or in the open using metal rods (see Fig. 16) or other materials.

7. Trap servicing

In the field, transfer the caught moths to labeled re-sealable plastic bags and store them in a cooler (Figs. 17 and 18). Place them in a freezer overnight to kill any surviving specimens.

Figs. 17 and 18. Moths placed in a ziploc bag and stored in a cooler.

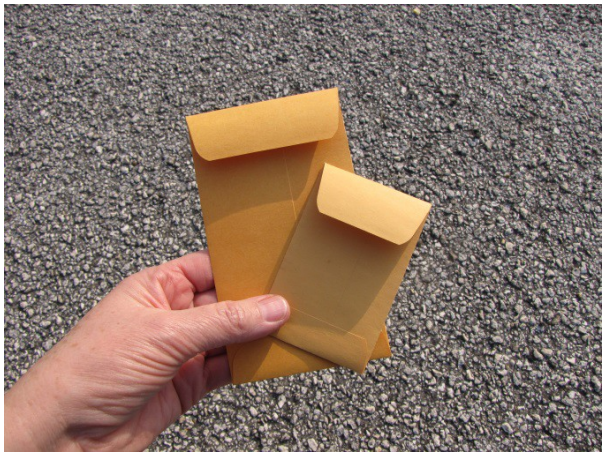
8. Sample submission

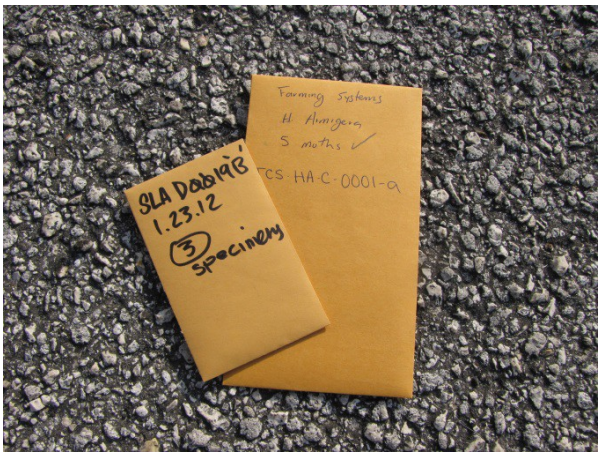
Prior to shipping, screen the samples. Remove any moths vastly different from the target and all other arthropods (beetles, flies, spiders). Write the approximate number of moths being submitted on PPQ Form 391.

Moths may be shipped by one of the following methods:

1) Small envelopes: Place the moth specimens in an envelope. Preferably, simply fold the envelope flap. **Please do not use staples, tape, glue, or water to seal the gummed flap.** Write the sample code on the outside of the envelope. The envelopes keep the specimens dry and they arrive in good condition. **This is the preferred method.**

   A range of sizes of envelopes may be used. The ideal envelopes are sized 3 ½ x 6 ½ inches and are commonly referred to as "coin envelopes" in office supply stores (Figs. 19 and 20). Smaller or larger envelopes are acceptable as well.





Figs. 19 and 20. Examples of small envelopes for specimens.

2) Re-sealable plastic bags: Place an absorbent paper, such as a piece of a paper towel, inside each plastic bag to reduce moisture and to pad the specimens for their protection.

3) Small boxes: Place the moths in small paper boxes with a folded paper towel placed over the specimen for padding.

For any of the above methods, the specimens should be well padded inside a box to prevent the specimens from being crushed or otherwise damaged. If longer-term storing is necessary, freezing works best, but refrigeration is acceptable as well.

9. Trap maintenance and storage

The general recommendation for maintenance of the plastic bucket traps is to wash them occasionally with soap and water to keep them clean, and to store them indoors, or at least protected from sun, rain and dust. Keep the wire handle and the wire screen in good repair. The traps can be used multiple times and for multiple species since the chemicals degrade quickly in outdoor conditions. These traps usually last more than five years.

Acknowledgments

This protocol has been designed to aid in the detection of exotic moths of concern by giving instructions on how to use generic plastic bucket traps.

All photos were taken by J. Brambila; trapping materials were supplied by R. Meagher. These instructions are primarily based on work by R. Meagher. This aid was revised on October 10, 2014 by Julieta Brambila, Lisa Jackson, Robert L. Meagher, Douglas Restom Gaskill (USDA/APHIS/PPQ), and Andrew Derksen (USDA/APHIS/PPQ).