IDENTIFIER's Notes Of Interest

PPQ National Identification Services Newsletter — December, 2013

What is ID Facilitation?

Message from Joe Cavey, NIS Assistant Director

On December 6, 2013, a message from the PPQ Deputy Administrator announced a plan to hire 30 pest identification positions beginning in January 2014. The new positions will include PPQ identifiers, national taxonomic specialists and positions under a new GS-11 Plant Health Safeguarding Specialist/Pest Identification position description. Discussions are under way with NAAE, affected SPHD's and CBP on what is the most significant employment incident affecting the pest identification function in 40 or more years. As Mr. El-Lissey stated in his message, we will announce the openings and locations in January. We will also share our hiring plans, as all positions will not be announced and opened at once.

This employment initiative is part of a larger effort named Identification Facilitation (IDF). The IDF project was developed to respond to increased demands on PPQ's pest identification system, especially regarding timeliness of processing cargo interceptions for identification. These demands result from increases over the past decade in cargo volume, numbers of pest interceptions, numbers of pest taxa intercepted, hours of cargo inspection operation and industry expectations. With support of the APHIS Administrator, the PPQ DA's office charged NIS and Field Operations (FO) managers with addressing the timeliness issues of pest identification processing. The resulting IDF plan was approved and supported with the addition of 30 new positions. In this article, I want to briefly introduce you to aspects of IDF other than staffing.

The IDF plan addresses potential issues of delay in the PPQ pest identification system by:

1. Investigating general and specific (to location) causes of delay to pest interception processing. (Note: pest interception processing is defined as the period from detection of the pest to the time the action decision is delivered to the broker, to include CBP and PPQ actions.)

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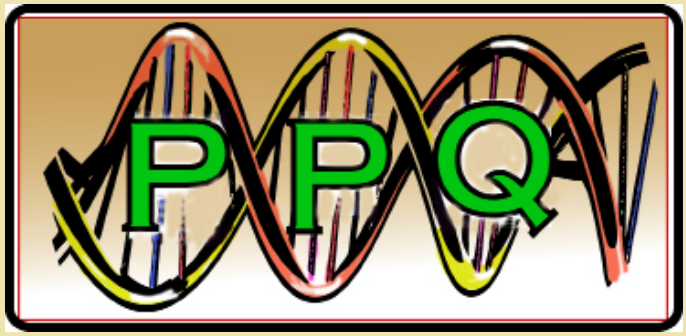
Good Progress Made by the PPQ Molecular Diagnostics Task Force

by Joel Floyd, NIS Domestic Diagnostics Coordinator



Thanks to the hard work of the PPQ Molecular Diagnostics Task Force (MDTF), we are closer to a goal of deploying molecular diagnostics for pest identification at ports of entry. In the coming year, there are three pilot projects planned to take place at Plant Inspections Stations (PIS) around the country. More on that later, but first let's catch everyone up on this significant effort.

While discussions began in early 2011, Mary Palm and Joe Cavey began in 2012 gathering personnel from various functions and levels in PPQ including identifiers, PIS supervisors, CPHST molecular biologists, Field Operations managers, taxonomic specialists,

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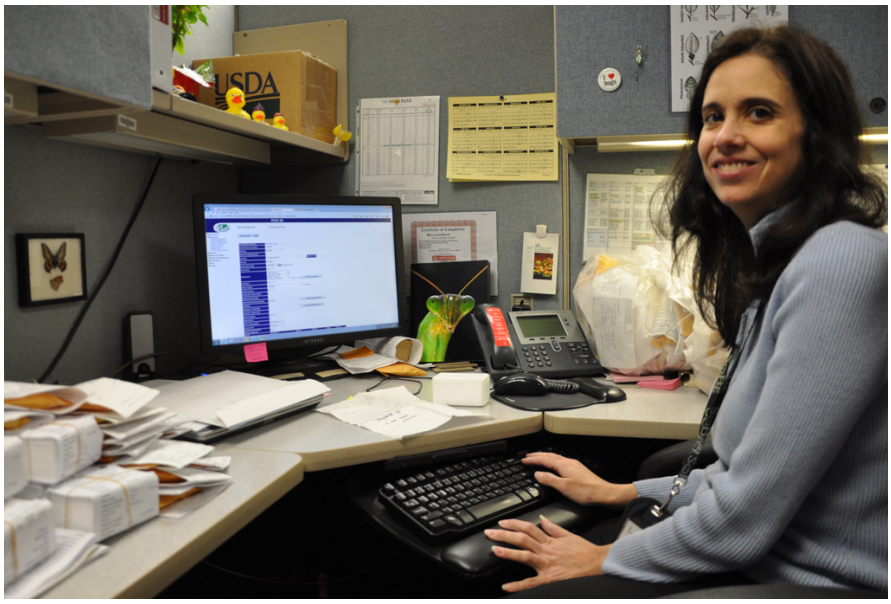
Many Happy Returns: A Big Thanks to Mary Joyce Burns, NIS' Program Assistant



Identifiers in entomology may have noticed more regular and increased numbers of specimens being returned from the ARS Systematic Entomology Laboratory (SEL) in the last year or so. This is thanks to the dedication and diligence of Mary Joyce Burns, our NIS Program Assistant who takes her job very seriously, and enjoys it too.

After the SEL specialists do their identifications / confirmations, the interceptions are then routed through the SEL Communications & Taxonomic Services Unit in Beltsville, MD where they are checked off and boxed up to be sent to Mary Joyce at NIS in Riverdale, MD. She checks the Pest ID data on each interception to make sure it is correct, and then divides them up according to work location to be returned for identifier's collections. This is no small task considering the mountains of interceptions submitted and processed from all ports by SEL weekly.

Mary Joyce does not see the job is not just simple mindless data checking and paper processing. To her, interceptions have become more than a Latin binomial and specimen in a vial or on a pin, which makes her job much more satisfying. She has actually become quite an insect enthusiast taking a little extra time to look at the more impressive insects that cross her desk. She sometimes shows NIS staff these interesting specimens and asks questions or reads up about the various kinds of insect pests. She even displays photos of insects in her cubicle, and has begun to take an interest in snails and slugs. Will nematodes be next?

Mary Joyce also has other duties but her first priority is always getting interceptions processed with accuracy and speed of delivery back to identifiers. There are some older stashes of interceptions she has discovered in NIS storage areas and has been trying to get those finally back out to the field as well. Summer help comes from student interns, but it is only for a couple months, so the vast majority of the responsibility falls on her. We at NIS are very appreciative of her hard work, attention to detail, and enthusiasm for the job she obviously takes great pleasure in. *JF*

National Identification Services Staff Roles/Responsibilities

APHIS Headquarters Riverdale, MD

Joe Cavey, Assistant Director
Administrative and quarantine action policies

Mary Palm, Supervisor
Administrative, policy, supervision, special initiatives

Joyce Cousins
Urgent process coordinator, pest categorizations, Pest ID technical support

Scott Neitch
Administrative and quarantine action policies, diagnostic reviews

Pete Touhey
Pest ID technical support, ARM technical liaison

Steve Bullington
Trend/pathway analysis

Indira Singh
Botany policy issues, HQ identifier orientation and advancement plan, pest interception training

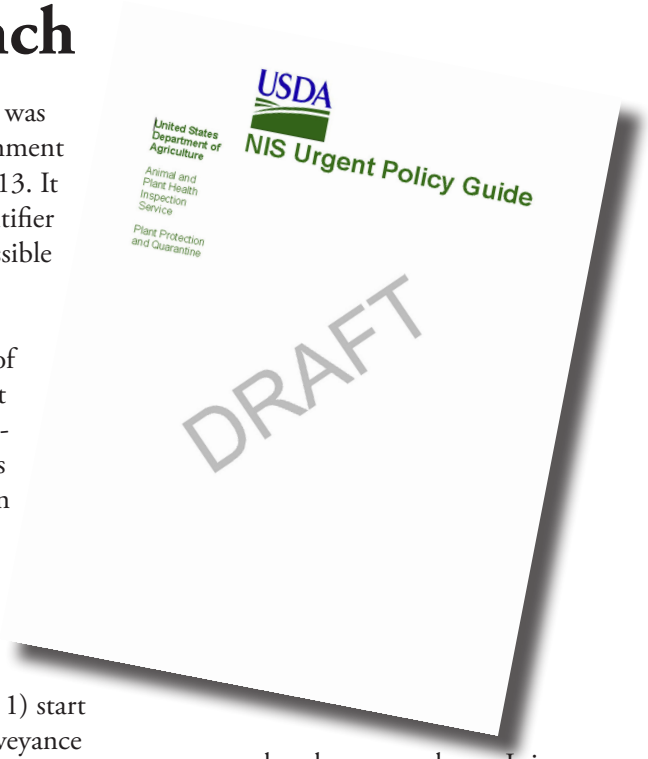
Tadd Dobbs
Identification authority (entomology), entomology identifier specialties

Mike Petrillo
CBP Liaison, cargo release authority

Joel Floyd
Domestic Diagnostics Coordinator, NIS website, *NIS INOI* editor

Mary Joyce Burns
Program Assistant, AQAS quality control & interception processing

NIS Urgent Policy Guide Launch



The process by which NIS makes decisions after urgent pests are identified by national specialists may seem to be somewhat of a mystery to identifiers. For years, NIS staff assigned to urgents have used a loose-leaf binder full of various instructions and policies to assist in making action determinations for urgents. Historic policies were handed down by ancestral staffers like sacred texts, some of which were lost or needed the likes of a Rosetta stone to decipher. The basis for some historical decision memos was not always there or well articulated. New policies were added to address a particular problem that arose, and the binder contents became more complicated to interpret every year.

In an effort to standardize the urgent process and make it more transparent, Bud Petit de Mange, formerly the director of the PPQ Manuals Unit, now retired, worked with Joyce Cousins and others to document our urgent process in a flowchart format, similar to other PPQ manuals. We had many meetings organized by Joyce to go over the flow and try to capture the various aspects correctly. When NIS staffer, Mike Petrillo returned to Riverdale, he started working on revising the entire Mexican Action Policy and then facilitated many more meetings on the overall organization and logic of the way the urgent manual would flow.

The final product of these efforts was sent out to you as a draft for comment by Joe Cavey in late October, 2013. It will be linked from the NIS Identifier Collaboration Intranet site, accessible only to PPQ.

While this guide is of most use, of course to NIS in Riverdale, but it will also come in handy to identifiers in making decisions on pests for which they have identification authority and during those times when NIS is shut down due to snow storms or other unpredictable eventualities.

A few caveats to using the guide: 1) start with Chapter 2 for all cargo/conveyance interceptions; 2) if you have a non-reportable organism, you should not be running it through the flow charts, (but there are exceptions, for example, ants intercepted in Hawaii); 3) the Mexican Action Policy no longer has species lists, so in most cases, be guided by the quarantine status in Pest ID (and remarks) when you have a species level identification. You will see there are no longer species list for mites, insects, mollusks, pathogens, or nematodes. (Seeds are still listed, i.e., FNWs). The one"list" is for actionable insect *genera* from Mexico with no known US species; and 4) please realize there is a hierarchy of these flow charts because one decision may supersede subsequent charts. It is suggested then, that you not go straight to the flow chart that has the taxa you are interested in, at least until you are more familiar with the overall structure of the guide.

Another nice advantage of the guide is that the policy documents are linked from many of the flow charts, for reference. Just click on the highlighted text, and the policy should pop up.

Take some time to familiarize yourself with the Urgent Policy Guide since, as identifiers, this is a key part of making decisions, in a consistent way, about the important work we do. *JF*

NIS Staff Roles/Responsibilities (continued)

See NIS Staff Directory

Philadelphia, PA

David Robinson
National Malacologist, identification authority (mollusks)

Program Manager (vacant)
Claireann Cook
Remote Pest Identification Program

Columbus, OH

Steven Passoa
National Lepidoptera Specialist

Beltsville, MD

Greg Evans
National Sternorrhyncha Specialist

John McKemy & Megan Romberg
National Mycologists
identification authority (plant pathology)

Rodney Young & David Bitzel
National Botanists
identification authority (botany)

Aaron Kennedy
Molecular Biologist, (mycology)

The New Nikon DS-Fi2 Camera and L3 Control Unit — First Impressions

by Patrick Haslem, Area Identifier, Entomology, Los Indios, Texas

We live in a technology-oriented society where many people go to great lengths to be the first to try out any new technological wizardry. But then there are some people (like my parents!) who need a little extra time to get used to changes in technology, and take longer to learn even simple tasks using it. I'm about in the middle of these two groups — I'm not afraid of new technology, nor am I hesitant to try it, but I usually require a little extra time to feel that I'm using a new product fluently. I was lucky enough to be part of the field evaluation team for the new Nikon DS-Fi2 Camera and L3 Control Unit that will be provided to the identifiers by the Remote Pest Identification Program (RPIP), starting later this fiscal year.

At first glance, the unit is quite similar to the previous generation Nikon DS-Fi1 Camera and L2 Control Unit. The dimensions are almost exactly the same; the screen is about the same size as well. Using the same amount of real-estate on the desktop, the camera will not cause any space deficiencies other than the usual "spaghetti ball" of cables connecting the terminals from the control unit to the camera, and the computer, plus the power plugs (now if Nikon could only invent a wireless electrical plug connection . . . !).

The most noticeable difference between the two units will be familiar to those keeping up with the ever-evolving cell phone industry — it has a touch screen (see Figure 1). In my opinion, this feature will be popular among identifiers who have their cameras/ scopes located on the same desk as their workstations. If configured properly, the identifier could view the image that is being captured by the camera on one of the computer monitors, and, if the unit is easily within reach, he or she could click the large camera button with a finger rather than having to use the mouse required by the L2 . In my case, the unit sits further back on the desk than my short arms can reach, so I elected to keep the mouse (which, by the way, plugs in at the same exact USB port as on the L2).

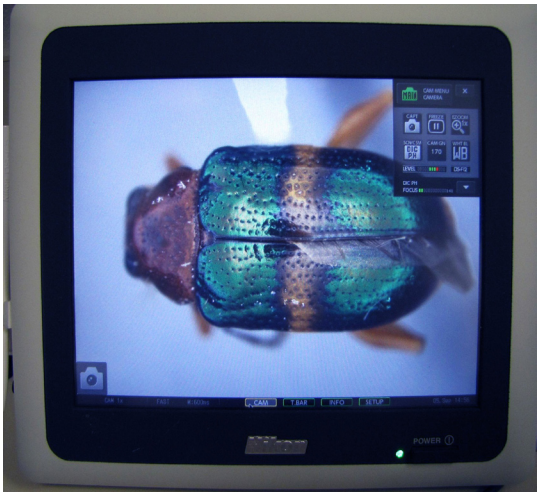


Figure 1. The camera menu on the monitor of the DS-L3 can be displayed either on the top right or the top left of the screen.

Physical setup is the same with the L3 as it was with the L2. No additional cables are needed, and when you, the identifier receive the camera and control unit and place the two side by side, transferring each cable one at a time from the old to the new units should make setup simple. In fact, if you have the cable from the L2 zip-tied or bundled with other cables, it is possible to use all but the camera connection cable from the L2 with the L3. Another helpful tool is the quick setup guide provided by Nikon (with a few extra tips provided in a setup guide by RPIP). These give a pictorial step by step process for setting up the new unit.

Okay, now for the meat and potatoes of the new unit. This is where the L3 and L2 differ, and in some cases, differ significantly — especially for the less tech-savvy identifiers. The first thing I noticed was that the menus have been redesigned and many of the menu functions familiar from the L2 have been relocated in the L3. The best suggestion I can make for becoming familiar with the new design is to allot some time for just "playing" with the unit when you first receive it. Work through the menus one at a time until they become familiar. This will take time and patience but is well worth the effort. Most of the identifiers who field tested the new units felt very comfortable with the new design within a couple weeks.

The Main Menus

The L3 has four main menus accessible from the image screen CAM (Camera), T.BAR (Toolbar), INFO, and SETUP (see Figure 2). The Camera menu includes functions like white balancing (one of the golden rules of digital imaging), zooming, camera gain, scene selection, etc. Clicking the CAM button once places the menu in the top-right of the screen, clicking it again places it in the top left. This is helpful if you are examining a specimen and need to see the setup menu but when it is open it obscures part of the specimen. By simply clicking the CAM button, you can then move it to a different location for a more convenient view. The T.BAR menu includes functions such as distance measuring, color, erasing etc. Clicking the button once places the menu in the top of the screen, clicking it again places the menu in the lower 1/3rd of the screen, and clicking the X closes the menu. The INFO menu shows the histogram and related information about the image as it is being viewed on the screen. Finally the SETUP menu has all of the options for changing the format, size and quality of acquiring the image.

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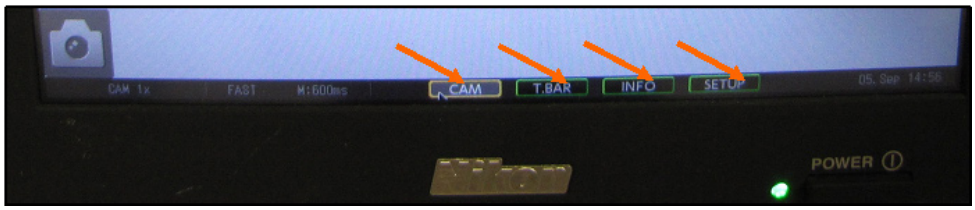

Figure 2. The main menu buttons on the monitor of the DS-L3, including CAM, T.BAR, INFO, and SETUP.

The Format Function — Still Important!

One function that is critical for maintaining your memory card in good working order is FORMAT. Formatting requires a few extra steps with the new unit (see Figure 3). You must first select the SETUP menu, then click on the "Main Menu" button, then click on the "File" button. There, you will find the Format button, on the lower left of the screen.

Adjusting to the New Camera and Control Unit

To become proficient using the new camera system it's helpful to practice using the different settings of the different menus. One way to do this is by taking two sets of images, one using the default settings on the camera, then changing a setting, recapturing, and comparing the images to find out which has a better appearance for what you're trying to show in your image.

When my camera arrived, the settings were not the same as my L2. I spent some time "tweaking" my settings to get the image size, quality, and format that I wanted. Also, my camera had a tendency to over-emphasize the yellows / reds of my subjects. I found I could correct this by carefully adjusting a few settings within my camera unit. By tweaking the Hue ("purity" of the color), Chroma (color intensity), and Camera Gain red channel (sensitivity of camera to light in the red/yellow range), I did achieve an accurate color rendering. It's important that identifiers are aware of what the settings mean so its possible to adjust them for the best image possible.

It's worth skimming through Chapter 8 of the full Nikon Manual that comes with the control unit, as this chapter discusses custom settings and what they do. To manipulate these settings I first had to change the camera setting from auto to manual.

Another very important feature of the new system is that the camera can be used as an independent unit (with images recorded on the CF card, as the current cameras) or can be controlled by Nikon Elements software (with the images captured directly to the computer workstation hard drive).

The L3 is a very sophisticated camera system that will bring years of imaging support to the identifier community. With a little experimentation identifiers can get the settings just right for many of their commonly-encountered specimens. The touch screen function will be a welcome update by many, and this interface drives the Nikon system into the current technological times that many of us have found a most welcome development.

Figure 3:. Formatting the data card requires a few more steps using the DS-L3 than the L2.

Using DNA to reveal introduction pathways for fruit flies: *Identifiers' contribution to comparing interception results from ports of entry with fly captures in the US*

By Norman Barr and Raul Ruiz-Arce, CPHST Mission Laboratory, Edinburg, Texas

To protect U.S. agriculture from exotic fruit flies (Tephritidae), APHIS PPQ in concert with DHS Customs and Border Protection institutes pest exclusion activities such as passenger baggage inspection at ports of entry. Despite our joint efforts, exotic pests are occasionally detected in the U.S. and sometimes result in additional surveillance and/or eradication costs to the agency. Is it possible to reduce these events? We believe so. One way to enhance our exclusion operations is to identify likely pathways by which flies enter the U.S. and shut them down. This change could be accomplished by directing our attention to higher risk pathways and targeting increased inspections of problem pathways or outreach activities along those pathways, such as traveler education.

A major obstacle to identifying high risk pathways is collecting and analyzing the right information. Even though shipping and host records are important for examining trends from interception data, identification of interceptions to species is also needed. Since fruit flies are usually intercepted as larvae and not as adults at ports, making a definitive identification to species level based on morphology is not possible.

The PPQ Mission Lab in south Texas is continually exploring better DNA methods to help identify pest species and support pathway analysis. For example, in cooperation with the PIS' in Florida, Texas, and Puerto Rico, the Mission Lab conducted a pilot study to identify intercepted *Anastrepha* larvae from November 2010 to May 2011. That work demonstrated PPQ's ability to analyze port intercepted material using standard operating procedures and confirmed the identity of important pest species.

In addition to using DNA to identify an interception to species, it is possible to determine its likely geographic origin as well. This, however, often requires a thorough understanding of genetic diversity across a species' known distribution and the development of species-specific protocols. Despite the investment, these tools significantly improve our confidence and resolution of pathways.

The Mission Lab is currently completing the development of methods for pathway analyses of two important fruit fly pests: the Mediterranean fruit fly, *Ceratitis capitata* and oriental fruit flies, *Bactrocera* spp. For both diagnostic tools, genetic profiles of fly captures occurring in the US are compared to potential source populations in Hawaii and other regions. This will help identify likely starting points in the pathways.

The Mediterranean fruit fly method distinguishes among likely pathways of flies based on worldwide collections. This method is currently being tested using historical Mediterranean fruit fly captures from California and Florida. The oriental fruit fly method has been used in determining whether Hawaii is a possible source of flies captured on the mainland U.S. This project was developed through the cooperation of PPQ S&T, PPQ Hawaii FF Exclusion and Detection Program, USDA-ARS Pacific Basin Agricultural Research Center, University of Hawaii and the California Department of Food and Agriculture. This method was successfully applied in 2013 to analyze recent California fly captures.

Apart from the host, it is also important to know the origin of specimens intercepted at ports of entry. If these intercepted flies share genetic profiles with the captures in the U.S., then perhaps they share a common pathway. However, if the port intercepted material and fly captures occurring in the US have distinct genetic profiles, then they may have different pathways.

illustration by Joel Floyd

In 2012, NIS requested that PPQ entomology identifiers submit all suspect Mediterranean and oriental fruit fly interceptions to the Mission Lab. These specimens include all tephritid larvae that matched the morphological type of these two genera. This material is currently being stored in ultra-cold freezers to preserve DNA and its analysis will commence in FY14. These interceptions represent a valuable resource in pathway analysis because they provide spatial and temporal samples for comparison with flies captured over the past few years. Our lab continues to work with identifiers to collect fly material for analyses in future years. Once validated, our goals include working closely with identifiers and state cooperators to ensure that these methods and reference data sets provide end users informative diagnostic tools.

Exposing the true invasion pathway of a pest is a complicated task. It requires careful consideration of complex information and strong collaborations among identifiers, scientists, and risk analysts within and outside PPQ. Our ongoing fruit fly projects at the Mission Lab are good examples of how members of different PPQ functional areas are working together to address programmatic goals.

Task Force: Molecular Diagnostics at PIS'

(continued from page 1)

and NIS policy managers to form the MDTF. Their task was to take a broad look at the potential for using molecular diagnostics at port of entry field locations to make identifications on cargo interceptions or to gather pathway information (see the Dec. 2012 *Identifier Notes of Interest*, page 6).

First, the three working groups examined in detail 1) the breadth of pest organisms which this technology could be applied to; 2) possible criteria for developing and using molecular diagnostics for Agriculture Quarantine Inspection (AQI) purposes; and 3) currently available molecular assays including finding out the extent to which other country's national plant protection organizations use molecular diagnosis for making regulatory decisions.

Preliminary discussions were held with the PPQ Management Team, and an operational plan was developed at their request. Fulfilling aspects of the Operational Plan is the effort that the group has been working on since the Spring of 2013. The highest priority pests were then determined and grouped according to the type of tests. The MDTF identified representative test types, each of which could be used for a range of pests. The test types chosen represent different aspects of what molecular diagnostic techniques can do to assist PPQ in the goal of accurate, timely, and reliable identifications as well as pathway data gathering.

After much telephone collaboration of the working groups, the test types finally chosen for pilot studies are Polymerase Chain Reaction (PCR) and DNA sequence analysis for fungi (using *Colletotrichum*), for insects (using thrips), and detection assays such as immunetest strips and a new technology called CANARY (using *Ralstonia solanacearum*, a bacterial plant pathogen). The MDTF conducted in-depth analyses of the logistics, resources needed, policy implications, and other impacts for each of the representative test types. These analyses were developed to provide PPQ leadership the framework that they need to determine strategic resource allocation for using molecular techniques to detect and identify pests at ports.

A template was developed and used for each analysis in order to cover all the aspects, impacts, and implications of the representative test type being analyzed. For each pilot, each detailed implementation plan included: hosts, specificity, logistics, policy implications, costs of equipment, supplies, physical space needed, training and personnel requirements, interactions with CBP, trade implications, benefit to PPQ, needed collaborations, and other considerations. The very detailed plans were presented to the PPQ Management Team in August, and after a lively discussion, they approved each of the pilots for immediate implementation .We are moving forward in purchasing supplies and planning for training at the various work locations.

The three pilots were chosen because they represent a variety of molecular diagnostics technologies and each are real-world questions or needs for PPQ interception diagnostics and identification. The main objectives revolve around the feasibility of doing this kind of work at PIS', and the potential information gained from each of these pilots represent secondary objectives. The pilots as planned are the following (click on the pilot title for more details):

***Colletotrichum* Sequencing Pilot:** This pilot project will take place at the San Francisco PIS, and focuses on DNA sequencing of interceptions of fungal pathogens in the genus *Colletotrichum*. The pilot lead, Aaron Kennedy, molecular biologist with NIS in Beltsville, has been working on sequencing various intercepted fungi in genera that are difficult to identify to species using morphology. Other members of this group participating at the San Francisco PIS are Supervisor Art Berlowitz, plant pathology identifier Fengru Zhang, and technicians Shana Gallant and Kyle Beucke. This pilot will sequence interceptions of *Colletotrichum* from San Francisco and other California ports of entry to ascertain what species are coming in on various hosts. *C. gloeosporioides* is currently considered non-reportable morphologically, but is actually a complex of twenty-two species when looked at with DNA analysis. Some sequencing data for *Colletotrichum* have already been analyzed from port interceptions and determined at NIS Beltsville, but this pilot seeks to gather more data and answer the question if some of these species should actually be considered reportable/actionable.

Thrips Sequencing Pilot: The thrips pilot will take place at the Miami PIS with Cheryl O'Donnell, now an identifier in San Diego, as the pilot lead working with CPHST Mission molecular biologist, Norman Barr. The pilot builds on work already begun by Identifier Tom Skarlinksi, in cooperation with a researcher at the University of California at Riverside, has been looking at identifications of immature thrips, primarily *Franklinella* spp., frequently intercepted on cut flowers from South America. Tom found some morphological differences in larve based on re-examination after molecular identification. The molecular identifications will help PPQ determine if immature thrips are actionable or not, thus potentially reducing the numbers of fumigations required.

Sequence data from interceptions and laboratory methods will be developed

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Molecular Diagnostics Task Force
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at UC Riverside and shared with the Miami PIS over time, to eventually be able to implement the procedures on their own. Besides Tom, other members of the Miami PIS team involved include PIS Supervisor Pedro Milan, the new Plant Pathology Identifier, Weston Msikita, and biological technicians. Ultimately, the information gathered from immature thrips sequencing will help inform the designation of consistent morphological characters that can be used for species level identifications.

***Ralstonia solanacearum* CANARY Pilot:** The two PIS' were chosen for this pilot are Atlanta, GA and Linden, NJ. because of their volume of geranium, or *Pelargonium*, plant imports from countries not part of the off-shore certification program. This host show no symptoms while harboring the bacterial pathogen, *Ralstonia solanacearum,* race 3, biovar 2 which can also infect potato, tomato, and eggplant among others. It is a serious quarantine pathogen also on the Select Agent list.

The pilot lead is Laurene Levy, who with CPHST Beltsville molecular biologists, Zhaowei Liu, Gang Wei, and Jinbo Wang have been working on the CANARY technology for a number of years. The test is a serological assay designed to detect *R. solanacearum* (but not the race/biovar) from cuttings imported through the PIS'. The PIS participants include Atlanta Plant Health Safeguarding Officer, Cesar Cardona, Linden PIS Supervisor Randy Cadet and others. The purpose of the pilot is to see if this technology can be implemented effectively at a PIS, if it can detect *R. solanacearum* in plant imports and blind tested positive controls, and how it compares with immunostrip technology, currently used on some imported *Pelargonium* plants.

IDP Facilitation
(continued from page 1)

2. Revising PPQ policies, procedures and staffing to facilitate ID processing.

Delays may result from local circumstances, such as the absence of a PPQ identifier or lack of sufficient numbers of inspectors at the site. Overarching issues may cause delays at multiple port of entry locations. For example, delays in identifications of Prompt and Routine interceptions made at the national level can slow issuance of ID Authority which, in turn, affects cargo clearance time adversely. NIS and FO managers are working with CBP and you to identify and resolve these issues.

The IDF plan includes a number of internal policy and procedural changes that can help facilitate time for identification without sacrificing credibility. While PPQ may find that we do not need to implement all of the changes listed in the IDF plan, some were identified as critical some time ago. Revision of our ID Authority policies constitutes one of these areas. In January, NIS plans to implement revisions of IDA, especially for non-reportable groups and for Provisional IDA. Changes will expand conferred entomology and botany IDA, primarily. Another potential objective involves modifying submission procedures for botany interceptions to increase use of digital imaging. PPQ may also look into how we might maximize the contributions of technicians involved with processing interceptions and the use of digital imaging, in general. And finally, discussions with you are planned for the IDF project. NIS and FO will contact identifiers at most ports to discuss local pest interception processing efficiency. We want to hear your assessment and ideas for improving the process at your location and within your area of coverage.

The PPQ identifier position was developed and first staffed more than 45 years ago, specifically to facilitate the clearance of imported cargo at a time when overnight mail service did not exist. Since then, a small identifier cadre has grown, with expanding international trade and domestic programs, and will soon exceed 100 in number. You, the current identifier cadre, efficiently clear 85-95% of cargo shipments held for intercepted pests through commendable and consistent performance. But, as cargo volume and clearance responsibilities continue to increase, this task becomes more difficult. The IDF project and associated hiring plan will be implemented to help PPQ meet current and future timeliness requirements for pest interception processing while maintaining the credibility of pest identifications.

Welcome Recently Hired New Identifiers

Plant Pathology

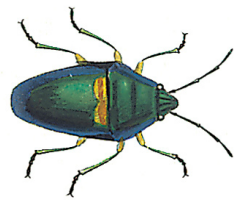
Portland. OR	Jinya "Jack" Qiu
Miami, FL	Weston Msikita

Entomology

Miami, FL	Alexander Cunningham
Buffalo, NY	Carla Coots

Recent Newly Relocated Plant Inspection Stations:

Atlanta, GA
Los Angeles, CA
Nogales, AZ



Entomology Specialists Star in Instructional Videos for Identifiers

by Joel Floyd

Video products of a 2012 Farm Bill suggestion are starting to appear on a computer monitor near you. Due to the difficulties with getting travel funds and organizing pest identification workshops, an alternative approach is being tried by video taping training and posting them on the web.

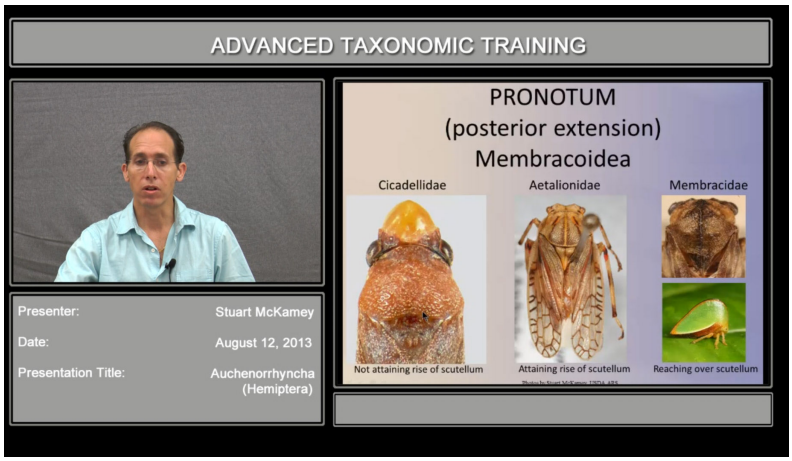
ARS-SEL Research Leader Gary Miller in one of the instructional videos on how to mount insects on slides.

The first of these are 8 videos (click here) on techniques for mounting soft-bodied insects on microscope slides, including one on mailing them. These were initiated by Gary Miller, SEL Aphididae Specialist, and now Research Leader at the USDA-ARS Systematic Entomology Laboratory (SEL). Also included is a video on mounting mites, with Ron Ochoa. They are now posted on our NIS Identifier Collaboration Intranet site. The videographer and editor is Arthur Tracy, who coincidentally is the son of Area Identifier Bob Tracy at Linden, New Jersey.

The second group of videos are still in the editing process at the University of Florida and include lectures, student questions, and keying out exercises. The first group includes Aleyrodidae, Aphididae, and Coccoidea conducted by John Dooley and Greg Evans (PPQ), Gary Miller (SEL), Susan Halbert and Ian Stocks (Florida Division of Plant Industry). The second group included Auchenorrhyncha (Fulgoroidea, Membracoidea, Cercopoidea), and Pentaomoidea taught by SEL's Stuart McKamey, Charles Bartlett of the University of Delaware, and Joe Eger of Dow Agrosciences. We only had small groups of students who included a few area identifiers who have these taxa as specialties.

PPQ Specialist Greg Evans showing insect morphology during filming of a taxonomic instrutional video in Gainsville, FL. Photo courtesy of Julieta Brambila

Thanks much goes to Amanda Hodges, Director, Doctor of Plant Medicine program at University of Florida, Gainseville for organizing this major effort, with help from Stephen McLean and Stephanie Stocks. These videos should be ready in early 2014.



Screen shot of SEL Specialist Stuart McKamey from edited presentation on Auchenorrhyncha taxonomy.

Entomologist Joe Eger during filming of video on Pentatoidea keying out instructions. Photo courtesy of Stephanie Stocks

imageID

by Deena Walters, PPQ Identification Technology Program (ITP) Cooperator at Colorado State University, Ft. Collins, Colorado

About a year ago, the Identification Technology Program (ITP) in Fort Collins, Colorado received Farm Bill funding to begin work on an image database and website based on pest images taken over the years by Identifiers. We call the project imageID. It is being developed in cooperation with Dr. Mark Simmons of Colorado State University and Joe LaForest of the University of Georgia.

The purpose of the website imageID is to give all identifiers access to each other's images, but particularly those images of diagnostic value. Diagnostic images are those that aid in the identification process because they show relevant characters and character states. Typically, no one image is going to allow someone to identify an unknown pest all the way down to species. So, what we are doing is collecting and displaying suites of images that, when taken together, point an identifier in the right direction or, at the very least, narrow down the possible taxon identifications.

What's trustworthy?

Although imageID began as a way to let identifiers share their images, plans for the project have since evolved into what we see as an identification aid offering more than just the identifiers' images. As many of you know, there are innumerable images on the internet of various plant pests. Unfortunately, there is also considerable variation in the quality of those images for identification purposes. For example, images need to be clear, sufficiently large to see desired details, and perhaps most importantly, they need to be correctly identified. The latest trend at some of the websites featuring pest images is to say whether or not the identification of the pest(s) in an image has been verified by an expert. At Encyclopedia of Life (EOL), they use the phrase "trusted" to indicate that an image has been so verified.

A large group of images that automatically have been verified are those of type specimens. A type specimen is the specimen that a taxon name was originally based on. So, the identification of images of these specimens are inherently correct, although sometimes labelled with an outdated synonym given the ever-changing nature of plant and animal nomenclature. In any case, various organizations, including the MCZ Type Database @ Harvard Entomology, are offering digital images of the type specimens in their collections.

My point is that there are many images on the internet that *are* of value for pest identification. The trick is to weed through all the junk and find those accurately-identified images that represent the taxa in the PestID system. Instead of identifiers having to this on their own, we are creating a searchable website containing such images.

Examples of Digital Images Taken of SFO Botany Collection
Deena Walters, June 2013

Figure caption: Florets of *Setaria italica* (Poaceae), the first two with sterile lemma still attached.

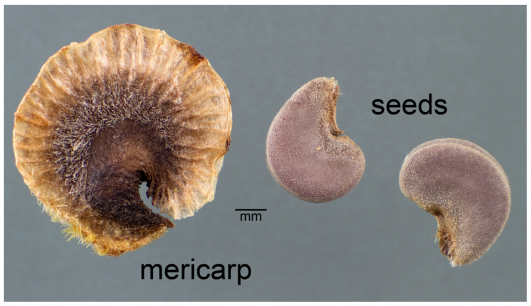


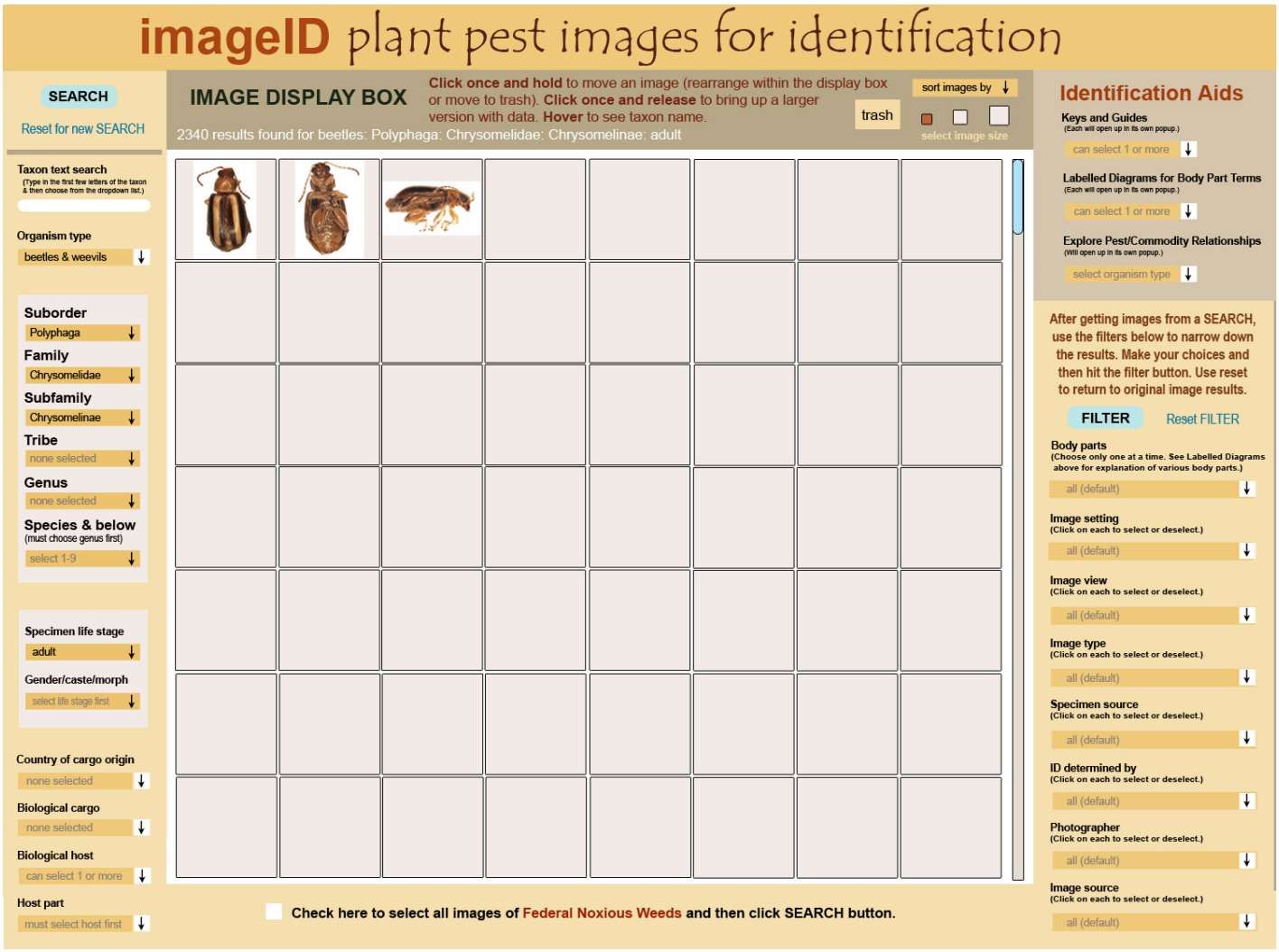
Figure caption: Mericarp and two seeds of *Alcea rosea* (Malvaceae).

Revealing relationships

During our interactions with identifiers over the past year, sometimes in person and also by way of e-mail and questionnaires, we've been given a clearer picture as to the functionalities that identifiers would like to see in imageID. Since some pests are relatively specific when it comes to association with a particular crop import, especially when originating from a particular part of the globe, we have begun an analysis of the years of data in the PestID system as it relates to region/commodity/pest associations. Results of these analyses will be incorporated into the website in a way that is useful for identifiers. For example, a search on the pest group "Coleoptera," the commodity "mango," and the region of origin as "Central American & the Caribbean" would bring up a note saying that ". . . 95% of past Coleoptera interceptions have been of the pest species *Stenochetus mangiferae*."

Size Matters

Other concerns expressed by identifiers that we are addressing include: 1) the inclusion of synonyms and their direct links in the database; 2) coding of images based on life stage and body parts so that an identifier can focus on a narrowed group of images (this is called "filtering"); 3) including labelled organism outlines for various pest groups since body part names can vary across these groups; and 4) having a few organism-specific characters than can be used for filtering images. With respect to the latter, I've been



working with Charles Brodel on some basic distinguishing Coleoptera characters that could reduce a larger selection of images to those more likely to be of use to an identifier trying to identify a particular unknown. It can be as simple as selecting a size category, e.g., beetles < 3 mm long VS. beetles 3-13 mm long VS. beetles 13-20 mm long VS. beetles > 20 mm long. We also plan to offer at the website pertinent screening aids that have been produced by various experts over the years.

The first identifier collection of images I evaluated belonged to retired identifier Bill Carlson. More recently, we've received images from several identifiers, including Greg Bartman, Patrick Marquez, Alan Smith-Pardo, Phil Johnson, John Dooley, David McCoy, Shannon Jarmin, Liana Maller, and Rodney Young. The plan in the future will be to put out a "call" for images of particular pest taxa, instead of trying to choose images from a particular person's collection. Late this summer, we put out a request for images of various Coleoptera taxa, along with instructions on how to transfer these images to our FTP site.

In addition to using images created by the identifiers, various other scientific experts, and reliable sources on the internet, we have also begun a program of taking high-quality images of intercepted pests that have survived well in storage over the years. These pests are mostly plant propagules such as seeds or fruits. Our trial program with Shannon Jarmin at SFO has gone very well. She sent us close to 100 intercepted seeds and fruits last spring. We've taken detailed images of this material, focusing on characters important for identification, for which we often provide labelling on the images as well as detailed image descriptions.

What's ahead?

Currently, we are developing the imageID website from several angles, all emphasizing the usefulness of images to the pest identification process. This is the end of year one of what was proposed as a three-year project. However, we hope to have a beta-version online for testing in June of 2014. We will let you know when the website becomes available. In the meantime, any suggestions anyone may have are certainly welcome and can be sent to me at : deena.s.walters@aphis.usda.gov.

Identifying Beetle Larvae from Wood Packing Material

Hannah Nadel, CPHST Otis Laboratory, Buzzards Bay, MA and Peter Reagel, Xavier University, Cincinnati, OH

After establishment of Asian longhorned beetle and emerald ash borer in North America, International Standards for Phytosanitary Measures #15 (ISPM-15) for solid wood packing materials (WPM) were adopted in 2005 to reduce risk of further introductions of tree and timber pests. WPM is treated before export with heat or methyl bromide fumigation at certified facilities and marked with an IPPC stamp to signal compliance. But live wood borers are still routinely intercepted in marked WPM at US ports, indicating ineffective phytosanitary treatment or counterfeit marking.

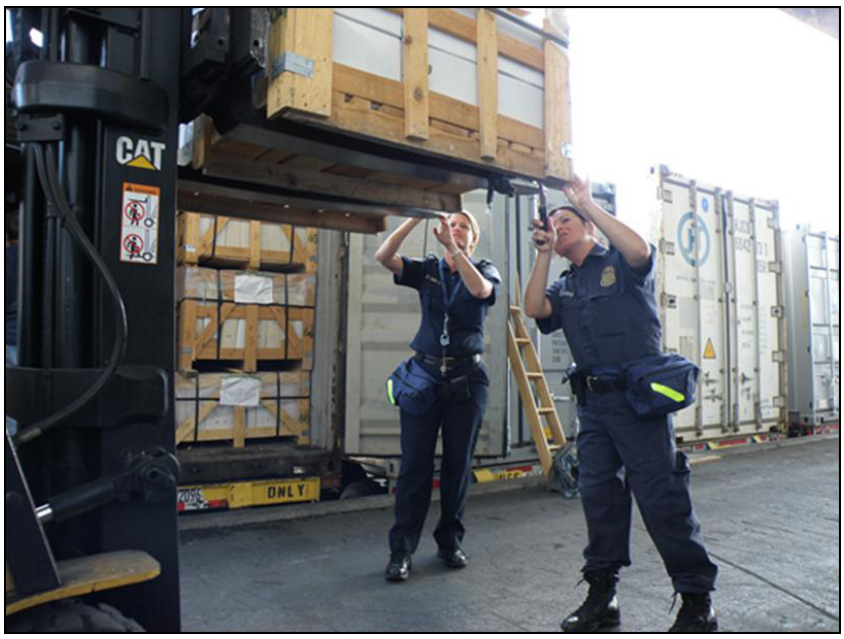


Figure 1. CBP agriculture specialists examine wood packing material at Miami seaport. (All photos courtesy of Hannah Nadel)

Because most wood boring insects are intercepted as larvae, which are at best difficult to identify while vessels and cargo are awaiting clearance, they generally trigger re-export based on family level identification. However, valuable regulatory and pathway risk information is lost when larvae remain unidentified. Documentation of consistent treatment failure for identified high risk species can be used to direct phytosanitary research toward improving treatment efficacy for those species, and pathway data can be used to further streamline targeted inspection of WPM from treatment facilities consistently exporting higher-risk species, in addition to shedding light on the validity of treatment marks. Currently, unidentified larvae may be sent to the USDA-ARS Systematic Entomology Laboratory (SEL), where systematic knowledge for identification below the family or subfamily level is usually limited or unknown.

Figure 2. At the CPHST Otis Laboratory under containment, infested host wood is held in metal mesh bags and checked periodically for adult emergence.

In 2012, with funding from AQI and cooperation with Assistant Professor Ann Ray at Xavier University, Cincinnati, OH, the CPHST Otis Laboratory in Buzzards Bay, MA, took up the challenge to improve identification capacity for intercepted larval wood borers. Two complementary approaches are used: larval rearing to the identifiable adult stage and expansion of DNA barcode databases from identified adults. Once barcode sequences from identified adults are submitted to database libraries GenBank and BOLD, they can generally be used to identify conspecific or congeneric barcodes submitted earlier or later in time, and from any life stage.

Project scope was limited to the Cerambycidae and Buprestidae because Otis Laboratory has the potential to rear members of these families on artificial diet, if necessary. The laboratory is in a unique position to carry out this project not only because of its rearing capacity under PPQ permit and containment, but because it houses a DNA laboratory and expertise in phytosanitary wood treatment. After a six-month pilot study in Seattle coordinated with entomology identifier Emilie Bess and CBP agriculture supervisor Alisha Beckham, the handling, shipping, and rearing protocols were developed and the project was expanded to include Long Beach, CA, Detroit/Romulus, MI, and Houston, Laredo, and Pharr/Hidalgo, TX.

continued on next page

Figure 3. A cerambycid larva that left its host wood and is being reared on artificial diet. This larva was intercepted at Long Beach with tile cargo from Italy.

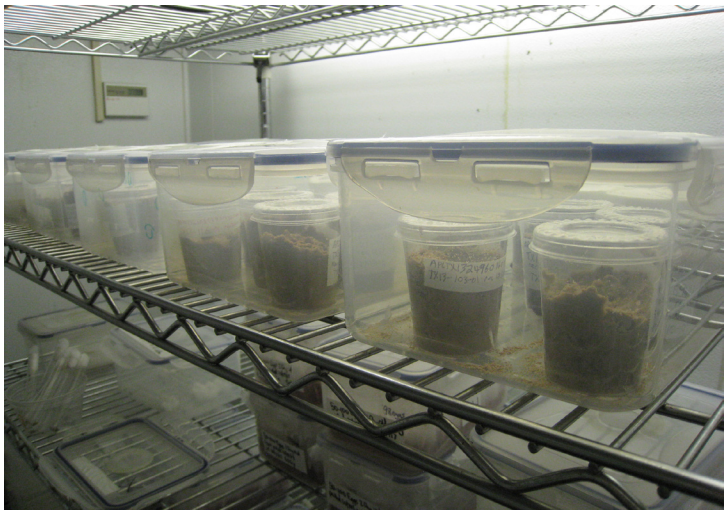


Figure 4. Larvae are reared singly in cups of artificial diet under controlled conditions with containment.

In October 2013, the ports of Miami and Port Everglades, FL, also signed on. The work is carried out by a large team. CBP agriculture specialists (Fig. 1) bring live cerambycid and buprestid larvae and ideally up to 6 inches of host wood to the PPQ identifiers, along with the completed PPQ form 309 and IPPC stamp information. Action status at the port is carried out as usual. A kit supplied by Otis Laboratory is used to ship the insects overnight to the Otis Insect Containment Facility where they are reared, preferably in the tunneled host wood (Fig.2), but in artificial diet if host wood is not supplied (Figs. 3,4). Reared adult tissue is retained for DNA barcoding at Otis Laboratory, and pinned specimens (Fig. 5) are sent to SEL for identification or confirmation by morphology. Tissue from larvae that die during transit or rearing is also retained at Otis Laboratory for DNA barcoding and the sequences are compared with others in databases for potential conspecific or congeneric matches.

To date about 345 intercepted cerambycid and buprestid larvae from 19 countries were received by Otis Laboratory; of these, over 140 cerambycid and 13 buprestid larvae appeared healthy (if visible); the rest died in transit, during rearing, or were hidden in wood. Rearing is still in progress for about 80 larvae. Of 160 dead cerambycid larvae, 75% of them were identified to 16 species and 11 genera, while the remainder matched no barcode even at the genus level in the databases. About 70 cerambycid adults were reared, of which more than 30 examined so far at SEL were identified to 14 species in 10 genera. Combined larval and adult identifications total 19 species and 14 genera.

Notable cerambycid interceptions include known pests such as Asian longhorned beetle, *Anoplophora glabripennis* (5 interceptions), and velvet longhorned beetle, *Trichoferus campestris* (6 interceptions), although several other species have pest potential. To date, 35 buprestids were received but none was reared to adult, and DNA barcoding has just begun. Overall, early project results are promising. Risk pathways are beginning to emerge that should result in targeted inspection of wood coming from particular treatment facilities and treatment types. For example, the Asian longhorned beetles arrived on separate vessels but in WPM bearing the IPPC mark of a single wood treatment facility in China and indicating treatment with methyl bromide.

The next phase of the project will also include wood identification at Pennsylvania State University to further examine risk factors. The project is expected to continue on its present course through 2014 and will likely be extended beyond, with future tabling of options to shift the focus to particular commodities and other pest taxa.

Figure 5. Asian longhorn beetle reared from a larva intercepted in Seattle (note that two legs were removed for DNA analysis).

Plant Quarantine Inspectors, Port of Philadelphia, 1926
Photo courtesy of the APHIS Library

Entomological Workers at the US National Museum, Washington DC, 1925
Photo courtesy of the Smithsonian Instituion Archives