Sample Preparation and Submission to the Plant Pest Diagnostics Branch Entomology Laboratory









PPDB Meadowview Facility

There are five laboratories at the Plant Pest Diagnostics Center (PPDC).

To avoid delays in identification, please be sure to submit samples to the appropriate lab.

Samples submitted to an incorrect lab will be subject to delays to determine the appropriate lab for sample analysis.



Botany



Plant Pathology



Entomology



Nematology



Seed Science

Sample Collection Basics

- Don't be stingy, more is better.
 Please send multiple specimens whenever possible.
- For insects, collect as many individuals as possible, including multiple life stages if present. Often, males are needed to provide a species level determination due to the need for genitalia dissection. Multiple life stages can provide better evidence for ID.
- Except for certain sap-sucking insects and leaf-miners (which require the associated plant material), insects must be submitted as specimens, not with pieces of plant material or embedded in fruit.







Sample Preparation Basics

- If submitting insect samples in alcohol, only put alcohol in stoppered vials and plastic nalgene bottles. Do not put alcohol in containers with metal screw top lids as they will leak and cause ink and label problems. Do not submit plant samples, including seeds, in alcohol.
- Kill insects ASAP or keep them out of sun until killed.
 Exposure to direct sunlight can cause color fading and/or shriveling.
- Keep specimens out of direct sunlight, and cool and dry. This will help preserve the sample for lab analysis.
- Don't cram and jam. Use a jar, bag, or envelope of appropriate size. Specimens damaged in transit will be difficult to identify, and delay the identification. Use a second container if needed rather than try to fit a sample into one small container.







Sample Preparation Basics

• Never send rotten, liquefied plant parts to any of the 5 labs.



Chopped up pieces of fruit.



Liquefied mango.

Sample Preparation Basics

 Properly affix PDR labels to sample vials such that PDR numbers are clearly visible along the length of the vials and not over the vial and lid..

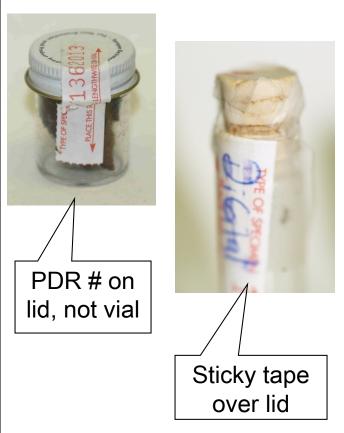
CORRECT





- PDR # on vial, not on lid
- label lengthwise on vial

INCORRECT

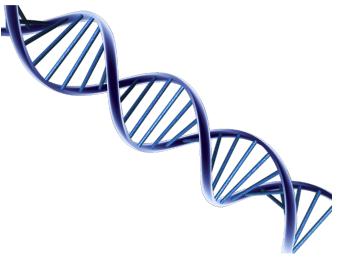




Specific Examples: Moths and Butterflies

- Adults submit in a large dry vial. Do not submit in alcohol.
- Larvae (caterpillars) should be prepared in the following way: place larvae directly into 75% alcohol. If there are large and/or many specimens put them in a higher concentration up to 95% alcohol to prevent too much dilution. There is no need to boil specimens any longer.





Specific Examples: Sap-Sucking Insects (scales, whiteflies, thrips, etc.)

- Submit sap-sucking insects still attached to plant material (fruit skin, twig, leaf) on which they are feeding. Pulling them off damages them. For fruit samples avoid including fruit pulp, which will degrade specimen. Preserve in alcohol.
- Plant fragments must fit easily into a vial without bending or forcing. Bigger plant parts stiffen up in alcohol, and there will be difficulty getting them out of the vial without damaging specimens.

• If sending in a dry sample, use a pen to indicate the specimen to be identified (draw arrow to it or circle around it). Use crumpled dry tissue to hold sample in

position within the vial.









Specific Examples: Sap-Sucking Insects (psyllids, whiteflies, etc.)

 Whiteflies - submit puparia only, still on leaves. Adults and earlier larvae usually cannot be identified. Preserve specimens in alcohol.







Psyllids - submit adults/larvae preserved in alcohol. Adult males are most useful.
 For efficient identification, it is critical to provide host-plant names on PDR.





Specific Examples: Sap-Sucking Insects (scales, mealybugs, aphids, etc.)

Mealybugs and scale insects – submit medium-sized and large adult specimens
preserved in alcohol. Immatures are very difficult to identify and will usually
be rated 'Q' when found on material originating from outside California.



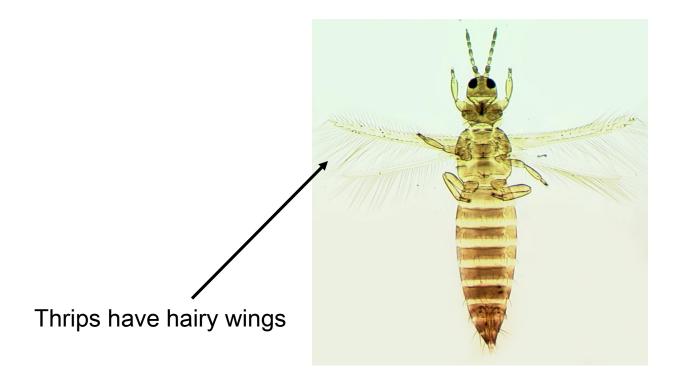
 Aphids - include large (adult) specimens, especially wingless individuals preserved in alcohol. It is very important to provide the host-plant name.





Specific Examples: Sap-Sucking Insects (thrips, etc.)

 Thrips tend to hide in cracks and crevices of plants. Collect plant pieces into alcohol and note in the PDR remarks section, "thrips seen". For infested foliage, preserve infested leaves in alcohol or beat foliage on a white surface to dislodge insects; collect insects with a brush wetted with alcohol, preserve in alcohol. Winged adults are important for identification.



Specific Examples: Mites

 Mites – Males (usually smaller, skinnier and fewer in number than females) are needed for identification, but are uncommon. To ensure capturing at least one male, collect at least 20+ specimens. Do not collect mites on tape. Send specimens preserved in alcohol.





Specific Examples: Snails and Slugs

- For rush identifications, kill mollusks in alcohol. If there will be a delay in submission, drown mollusks in water in a sealed vial. Within 24 hours, blot and transfer dead specimens to alcohol before shipping. Do not ship mollusks in water.
- Killing mollusks in alcohol makes them difficult to identify because they contract.
- Never put snails in plastic bags; they get crushed easily.
- Never place snails in the same vial with other specimens (mites, insects).









Sample Submission to PPDC

Important Guidelines - Entomology

- Do <u>Not</u> submit live insect specimens!
- State on PDR if insect was dead or alive when you found it.
 - > State <u>ALIVE</u> if it was moving before you killed it.
 - State <u>DEAD</u> if it was already dead when you found it.
- State on PDR if insect eggs were dead or alive when you found them.
 - Poke eggs before placing in alcohol, if eggs move or "bounce back" from the poke then they are alive.
 - ➤ State <u>ALIVE</u> when eggs are fluid filled.
 - ➤ State <u>DEAD</u> when eggs are dry and/or shriveled in appearance.



Sample Submission to PPDC – PDR Basics

- Enter PDR information electronically into all relevant fields.
- Submit a PDR hard copy with specimen(s) as part of the "sample".
- List all host plant data whenever possible on the PDR.
- List the origin of the specimens. This is particularly helpful for samples originating outside of California.
- List the suspect pest species, if known, to identify in the "suspect" field on the PDR.
- Always indicate the Program Code (e.g., QPHYT)
- Provide any additional information that you think is
 important in the remarks section. This may include what part of the plant the
 pest was located on, the type of feeding damage that was visible, etc.
- For Service Samples, **ALWAYS** indicate "Service Sample (99)" for the Activity Code.
- For Service Samples, **ALWAYS** fill in the Billing Address in the fields for "Quarantine shipper/broker"



Sample Submission to PPDC - PDR Specifics

Important Guidelines - Entomology

- 1 PDR = 1 insect species (suspect pest) on a single host plant. There can be multiple individuals of the same insect species on the same PDR and in the same vial.
 [See Plant Pest Diagnostics Advisory 01-2015 and the PDR Cloning Manual for more details (linked on Price List web page)].
- **NEVER** put multiple different insect species on the same PDR!
 - > This creates logistical problems here at the lab.
 - > This delays getting the official identification and rating back to you.
 - > RUSH samples, even if one or more species is determined as A or Q rated, cannot be entered until all others have been identified.
 - > More than one scientist is often needed with multiple species.
 - One or more specimens may require DNA analysis.
- ALWAYS submit the actual insect specimen(s) with a PDR slip.
 - Final identification is only made after examining the actual specimen.
 - ➤ Digital photographs should not be sent to the lab instead of the actual specimen(s). These will not get identifications.
 - Associated photographs of a host plant or the infestation can be printed and submitted along with the actual specimen(s) and PDR slip.

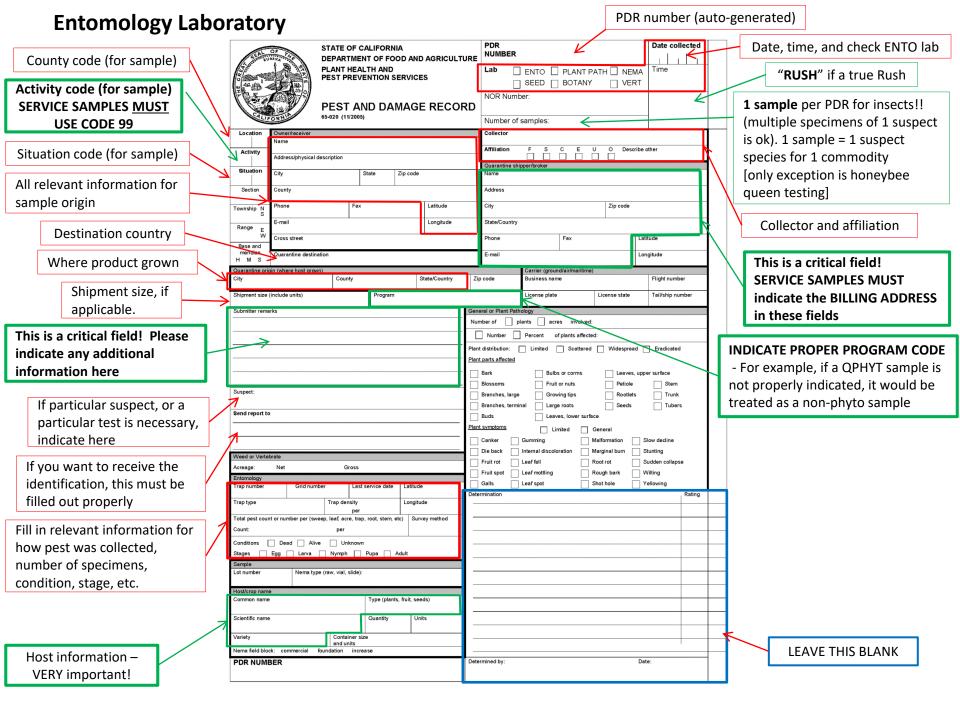
Sample Submission to PPDC - PDR Specifics

Important Guidelines – Entomology – Honeybee queen certification

- Samples of honeybees for queen certification do not follow the 1 PDR = 1 insect species (suspect pest) rule (they are not suspect pests, they are part of a testing program to certify queens)
- Guidelines specific to honeybees!
 - > 1 sample consists of 1-3 workers per hive (i.e., per queen)
 - ➤ 1 PDR may contain 1-40 samples (i.e., for testing of 1-40 queens/hives) from a single apiary
 - > Test results are reported per sample (i.e., per hive/queen)

Sample Submission to PPDC - PDR Specifics

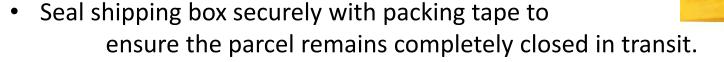
- The following PDR example form will walk you through the relevant fields that should be included when completing a PDR slip for a sample submission to the PPDC Entomology Laboratory.
- Properly completed PDR slips are essential to providing you with an accurate and timely identification for the plant pest or disease you are submitting.
- Provide as much relevant information as possible. The more information that is provided the higher the likelihood of a specific identification. The less information the less likelihood of receiving a specific identification.
- Please be sure to provide us with the appropriate contact information for how you want to receive the identification.
- If you are submitting a service sample, please provide us with the correct billing information in the "Quarantine shipper/broker" fields for invoicing purposes.



Sample Submission to PPDC - Sending a Parcel

- Use plenty of shipping peanuts (not shredded paper) or other packing material around the sample to adequately buffer the vial/container. Use at least 1-2" of packing material around all sides of sample container.
- Be sure to have the complete address of lab on shipping label.

CDFA - Plant Pest Diagnostics Center c/o Entomology Laboratory 3294 Meadowview Rd. Sacramento, CA 95832



- Always use a box to send samples through the mail. Glass vials in envelopes tend to be crushed along with the sample inside them.
- Send samples early in the week. Samples sent later may sit at post office over a weekend. Hold late samples and send on Monday.
- *** Assume a parcel will have a rough ride to the lab and package the sample to survive drops and bumps along the way.

Sample Submission Checklist

- 1. Sample collected and prepared using specific lab guidelines for each of the 5 different laboratories.
- 2. PDR completed with all relevant and necessary information (host, origin, etc.), and submitted along with corresponding sample.
- 3. Sample packaged according to safe shipment guidelines. All sample vials or bags clearly labelled with corresponding PDR numbers.
- 4. Sample addressed and sent to appropriate PPDB lab.
- 5. If any problems arise, inform appropriate on-site supervisor or call one of the designated lab contacts.

PPDB Designated Lab Contact List

Entomology Laboratory

Lab Supervisor: Dr. Shaun Winterton (916-738-6686)

PDR Sample Tracking/Queries:

Obie Sage (916-738-6683)

Jacqueline Airoso (916-738-6673)

Environmental Program Manager II (Branch Chief)

Dr. Umesh Kodira (916-738-6664)

Environmental Program Manager I (Botany & Entomology)

Dr. Stephen Gaimari (916-738-6671)

Environmental Program Manager I (Nematology, Plant Pathology & Seed Science)

Dr. Cheryl Blomquist (916-738-6707)