ALLIANCE OF PEST CONTROL DISTRICTS
FOR CITRUS PESTS AND DISEASES
EST.1963

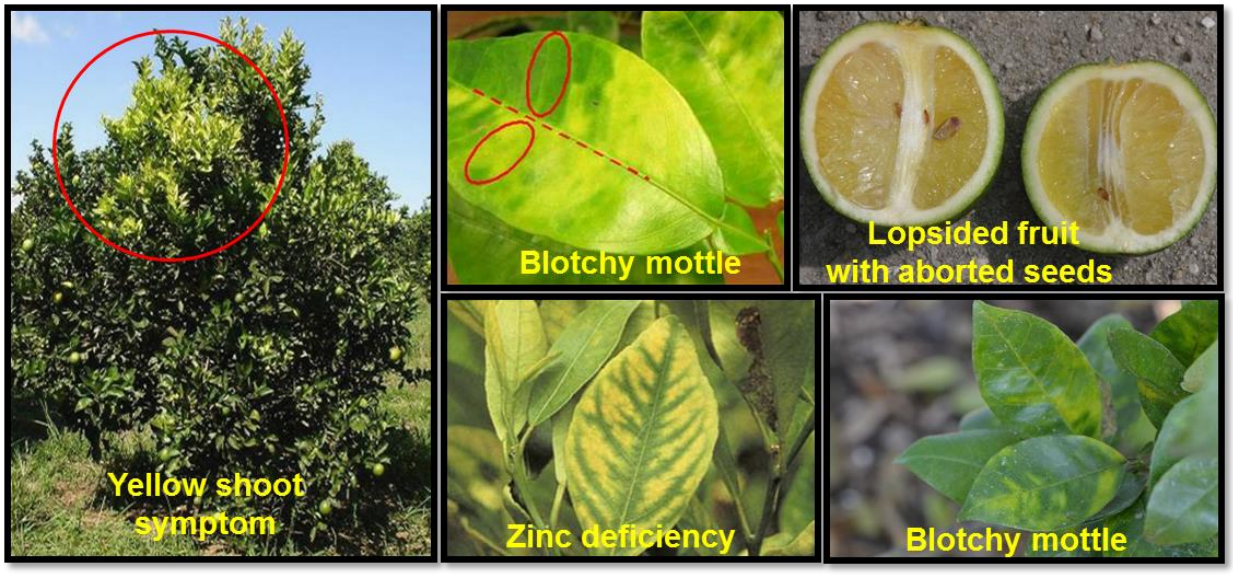
Leaf Sample Collection Instructions to Citrus Growers for HLB Testing

The purpose of leaf sample collections is to test for the presence of HLB-infected trees in citrus groves. The following instructions are provided to ensure that growers requesting testing follow the California Department of Food and Agriculture (CDFA) required leaf collection and handling protocols. Separate instructions are available if there is a need to collect and test Asian citrus psyllid (ACP), insect samples.

> ***Note:*** *All the samples submitted to the laboratory* ***must be*** *accompanied by the pertinent collection information. The laboratory is not authorized to test samples without the required collection information. Pursuant to CDFA Permit requirements, any HLB positive or "inconclusive" results will be reported directly to CDFA by the Alliance of Pest Control Districts laboratory.*

Leaf Sample Selection, Collection, Handling, and Shipping

1. Visually assess each tree to be sampled, looking for known HLB symptoms as pictured below. *Note: Due to the uneven distribution of the bacteria in the tree, it is best to search for and collect symptomatic leaves whenever possible.*



2. If yellow shoot symptoms are present, select 12 leaves from that branch. Select symptomatic leaves with the petiole attached. If the petiole breaks off, discard the leaf, and select another.

3. If yellow shoot symptoms are absent, inspect the entire tree for other symptoms, such as leaf mottling, twisted leaf psyllid damage, and vein thickening. Collect three leaves (symptomatic if possible, and each from a different branch) from each quadrant for a total of 16 leaves per tree (making it one sample).

   Select young leaves of medium size and attempt to collect leaves of one growth period/stage older or as near/closer to flush as possible. Include the entire leaf with the petiole attached. If the petiole breaks off, discard the leaf, and select another intact leaf. If possible, select branches with symptomatic leaves, preferably from the center (interior) part of the tree. In case of close plantings,

take extra care to ensure that each sample contains leaves from the intended tree only and does not get leaves from neighboring trees.

4. If no symptoms are present, select 16 leaves (4 from each quadrant) from the fully expanded current season flush.

5. If clippers are used, disinfect them with 10% bleach while sampling leaves in between the trees.

6. Wipe or brush leaves to remove dust, debris, and insects if any (such as whiteflies or aphids). Also, thoroughly check the sample to ensure that there are no thorns and no ACP adults with their life stages. This is an important step as ACP can have HLB bacteria, which could be accidentally released to a new place while opening the sample bag. Please request for separate instructions if you intend to test HLB on the Asian citrus psyllid (ACP).

7. For each sample, stack 16 leaves on top of each other and fold them at the midrib, as shown in the picture. You could use the rubber band to hold them in place, then wrap them with a dry paper towel. Finally, place this leaf sample in a zip-lock plastic bag. Then, label the zip-lock bag with the unique sample identifier number, the date collected, the exact location of the host tree, and the full address that includes cross street, city, and county. Now, place the labelled zip-locked bag inside another slightly puffed zip-lock bag (double bagging) to avoid any damage to samples during handling and shipping.

8. Immediately place the double-bagged samples in an ice chest with blue ice packs to keep samples cool and fresh until delivered to the lab. It is essential to put protective material (i.e., a layer of newspapers) in between the blue ice packs and the samples to ensure that the samples stay dry and do not get freezer burn. Please keep the sample bags with ice-chest out of direct sunlight and never leave those inside vehicles during the summer months.

9. Before sending the ice chest/cooler with the samples, please one more time make sure that there is no ACP with the leaf sample or inside the package. If any insect is found, the sample must be cleaned again.

10. Please complete the “Sample collection and submission form” provided here with all the needed information with a unique sample ID, and this should stay together with the sample bag being sent.

11. Please ship to:

Alliance of Pest Control Districts
Attn: PCR Laboratory
22847 Road 140
Tulare, CA 93274-9367
Phone: (559)686-4973

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SAMPLE COLLECTION and SUBMISSION FORM

Please complete items 1 – 6(a-f); add comments if necessary.

1. Name of Company/Grower:	2. Contact Person:
3. Address:	
4. Phone Number:	5. E-mail Address:

Sample Information (use additional sheet(s) if necessary):

6a. Grove Location (where samples were collected):

6b. Date Collected	6c. Variety	6d. Grove Age

6e. Unique Sample ID #	6f. Tree location (i.e., Block/Row/Tree#, GPS points); must be sufficient to relocate the specific tree sampled	6e. Unique Sample ID #	6f. Tree location (i.e., Block/Row/Tree#, GPS points); must be sufficient to relocate the specific tree sampled

6. Any additional comments:

APCD Laboratory Use Only

Received by:	Date Received:

Laboratory Notes / Comments:

Reminder: ***Pursuant to CDFA Permit requirements, any HLB positive or "inconclusive" results will be reported directly to CDFA by the Alliance of Pest Control Districts laboratory*****.**