

CITRUS PEST DETECTION PROGRAM
CENTRAL CALIFORNIA TRISTEZA ERADICATION AGENCY

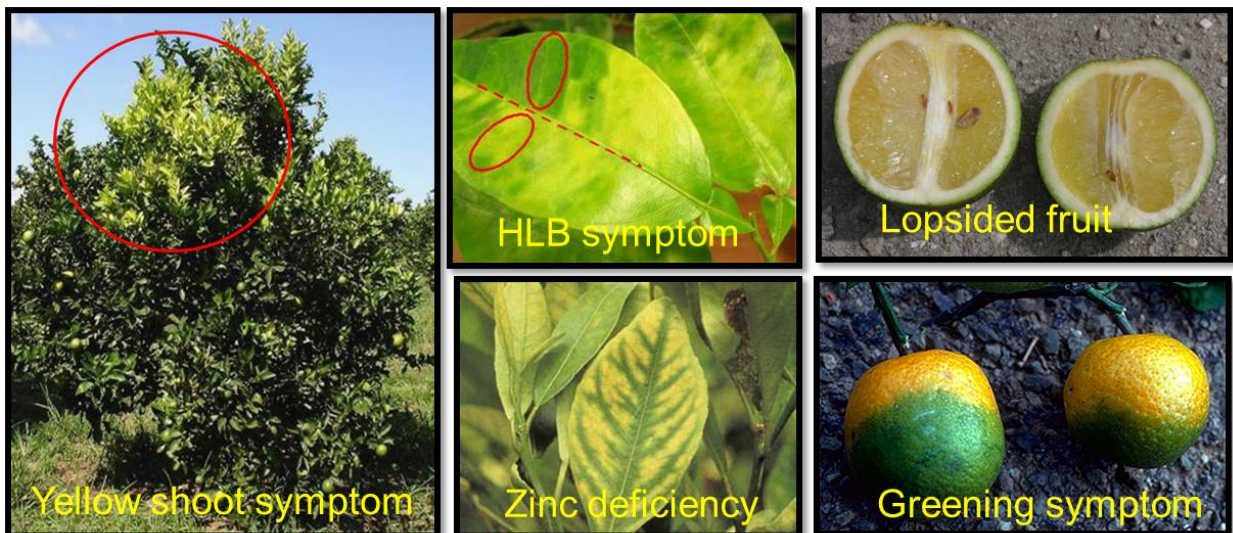
**Citrus Leaf Sample Collection Instructions
for Growers Submitting for HLB Testing**

The purpose of leaf sample collections is to conduct screening for the presence of potentially HLB-positive trees in citrus groves. The following instructions are provided to ensure that growers requesting testing follow 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) samples.

Note: All 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 must be reported by the Citrus Pest Detection Program laboratory to CDFA only.

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 select 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 not present, inspect the entire tree for other symptoms (leaf mottling, twisted leaf psyllid damage, vein corking). Visually divide the tree into cardinal

quadrants and collect three (3) leaves (symptomatic if possible, and each from a different branch) from each quadrant for a total of 12 leaves per tree (one sample).

Select young leaves of medium size, attempting to collect leaves one growth period old and as near to flush as possible. Include the entire leaf with the petiole attached. If the petiole breaks off, discard the leaf and select another. Select branches with symptomatic leaves, if possible, 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 only one tree.

4. If no symptoms are present, select 12 leaves (3 from each quadrant) from fully expanded current season flush.
5. If clippers are used, disinfect with alcohol after every sample collection.
6. Wipe or brush leaves to remove dust and debris. Thoroughly check the sample to ensure that there are no thorns and that all insects (especially ACP) and their life stages are removed. Separate instructions are available if there is a need to collect and test Asian citrus psyllid (ACP) samples.
7. Fold each sample (12 leaves from one tree) at the midrib. Wrap the folded bundle of leaves in a dry paper towel. Place the paper towel with leaf sample in a zip-lock plastic bag. Label the zip-lock bag with the unique sample identifier number, the date collected, the exact location of the host tree, address, cross street, city, and county. Then place the labelled zip-locked bag inside another zip-lock bag (double bagging).
8. Immediately place the double-bagged samples in an ice chest with blue ice packs to keep samples cool. It is essential to put protective material (i.e., a layer of newspapers) between the blue ice packs and the samples to ensure that the samples stay dry and do not get freezer burn. Keep the bags and ice-chest out of direct sunlight. Do not leave collection bags inside vehicles in summer heat.
9. Before sending the ice-chest/cooler with the samples, make sure there are no ACP with the leaf sample or inside the package. If any insect is found, the sample must be cleaned again.
10. Complete the packing list form provided with these instructions, to include all the sample identification information for the entire shipment.
11. Ship to:
Citrus Pest Detection Program
Attn: PCR Laboratory
22847 Road 140
Tulare, CA 93274-9367
Phone: (559)686-4973

