

Palm Trunk Sampling for DNA Extraction and Phytoplasma Detection

(Lethal Yellowing and Texas Phoenix Palm Decline)

Carlos Oropeza¹, Ivan Cordova¹, Maria Narvaez¹ and Nigel Harrison²

¹ Centro de Investigación Científica de Yucatán, A. P. 87, Mérida 97310, Yucatán, MÉXICO.

² University of Florida, Fort Lauderdale Res. & Ed. Ctr., 3205 College Avenue, Fort Lauderdale, FL 33314, USA.

Materials and Tools

- A portable electric drill and 6½ inch long x 5/16 inch diameter drill bit (longer is better for *Phoenix* palms). Alternatively, a carpenter's brace and a brace bit (e.g., ½ inch diameter).
- Clean, self-sealable plastic freezer bags (e.g., Ziploc bags).
- Squirt bottle containing water.
- Golf tees to fill the hole made by the drill bit, or wooden dowels 5 inch long x ½ inch diameter for the hole left by brace bit. Hammer to put golf tee or dowel in hole.
- Portable propane torch.

Procedure – Sample prior to OTC injections!

(1) Obtaining the trunk sample (figure at end of document)

- Begin by flame sterilizing the drill bit by running and twisting it slowly through the propane torch flame to cook any debris or DNA that might be adhering to its surface (Fig. 1a).
- Cool the bit using a liberal squirt of water from a squirt bottle (Fig. 1b).
- Bore a hole into the trunk of the palm. Anywhere on the trunk is fine but the lower the better for cosmetic considerations. The interior wood shavings (at least 3 grams; enough to fill a film canister) removed from the hole (Fig. 1c) should be collected into a sealable plastic bag (Fig. 1d) (do not use paper bags). Avoid any hand contact with the shavings since this tissue is the sample used for DNA extraction.
- For *Phoenix* palms, be sure to drill past the old leaf bases to obtain trunk tissue.
- Shavings that appear noticeably discolored (reddish-brown) due to interior trunk decay are much less reliable as samples than non-discolored samples. Such samples should be avoided, if possible; as should sampling dead palms.
- Label bags with useful details (e.g., palm name, date and location) so you can match results with the palm sample.

(2) Surface sterilization of the drill bit (to prevent DNA cross-contamination)

- At the end of the operation, the boring bit is first rinsed with water to remove debris.
- The bit is then flame-sterilized with the propane torch and then cooled again with another squirt of water (Figs. 1a and 1b).
- This operation must be carried out before boring a hole in another plant trunk to avoid cross-contaminating tissue samples.

(3) Sealing the hole

- Insert a golf tee or wooden dowel into the sample hole and tap it flush to the trunk with a hammer (Fig. 1e).
- This should seal the hole and prevent copious sap bleeding while preventing penetration of pests and or other unwanted potential pathogens.

(4) Handling and shipping of samples

- Once the trunk sample is obtained (Fig. 1d), keep it chilled (e.g., ice chest with ice or blue ice). Cooling will prevent the samples from discoloring during transportation from the collection site to where they will be stored prior to shipment.
- DO NOT FREEZE the samples. Ship within 24 hours of collection.
- When the samples are ready for shipment, send them by **overnight courier** (e.g., FedEx) still in their original sealable plastic bags. Do not ship samples on Friday as there is no Saturday delivery to university addresses. No ice is necessary for shipping.
- **Cost for each sample is \$75.** Go to the websites for each clinic location below for the required form. If paying by credit card, include required information on the form.

(5) Ship to either the Plant Diagnostic Clinic in Gainesville or the Tropical Research and Education Center in Homestead. These are the only two labs that accept these samples.

UF Plant Diagnostic Center
2570 Hull Rd, Bldg 1291
Gainesville, FL 32611-0830
352.392.1795

<http://plantpath.ifas.ufl.edu/extension/plant-diagnostic-center/>

Tropical Research and Education Center
18905 SW 280st
Homestead, Fl 33031
786.217.9275

<http://www.plantclinic.org/>



Figure 1. Trunk sampling for phytoplasma detection by molecular techniques. Boring a hole to obtain interior trunk tissues from which DNA will be extracted (c). The shavings are collected into a polybag avoiding hand contact with the sample (d). Rinsing of the boring bit after flame sterilization at the end of the operation to avoid contamination in a subsequent sampling operation (a & b).