Harvesting a Standard Disease identification sample

With most palm diseases, the lab can identify the issue by grinding up and culturing a sample of live roots, a spear leaf or a symptomatic frond. The decision as to what part of the palm to test is decided by the prognosis. The quality of the results will be governed by the quality of the samples and by the way they are stored before getting to the lab. When harvesting a sample, sampling personnel can closely adhere to the instructions below performing sample harvesting steps in the order in which they are set forth.

Rule #1: Always clean your trimming or digging tolls with alcohol or bleach before starting and in between each palm (*if multiple palms are to be sampled*).

Rule #2: Always store samples in a cooler or refrigerator (*not a freezer*) until they can be shipped to the lab. The fresher the sample, the more reliable the results so harvesting and overnighting to the lab during the same day is preferable.

Procedure – Frond samples

1. Use a suitably sized, new paper bag to contain/store the sample.
2. Write the site name, variety name and date on the sample bag.
3. Sanitize the tool you will use to harvest the sample by misting it with isopropyl alcohol or straight bleach and then thoroughly wiping it off.
4. **For a Frond tissue sample**. Clean your clippers as in (1) above and then obtain a sample from the youngest, central spear leaf (frond) of the palm or, if symptomatic fronds are in evidence, from a symptomatic frond. For symptomatic frond samplings, harvest the sample section from the point where green tissue intersects necrotic tissue capturing some of both the green and brown. A sample that is all brown (*100% dead, necrotic tissue*) will usually return unreliable results, you need some of the green as well. If harvesting a spear leaf (*youngest, central most frond*) your sample should be taken by removing one of the youngest, un-opened spear leafs and then clipping 6” off the bottom and placing it into a paper bag. Paper bags are preferred for frond tissue samples but you need to pay attention to storage before shipment to the lab because the sample may dry down if refrigerated for too long. As a rule, if stored in paper under refrigeration for 96 hours (*4 days*) or longer, harvest a new sample.
5. Once you have acquired the samples, store them in a small cooler with a bit of ice in your truck. You are not trying to get them real cold and you don’t want to, you are just trying to avoid allowing them to get hot. If you leave them sitting out on the seat of the

truck and its hot out, the heat may damage the sample and cause the results to be unreliable.

6. Over-night your samples to a University or private Agricultural lab requesting disease identification.

For a Root Sample: Using a sanitized digging tool, dig down at the base of the palm and obtain moist, white roots. You need approximately 2 ounces of root tissue. Bury the roots in some of the soil from the site in the sample bag. This will help to keep them fresh and moist until they get to the lab. For root/soil samples use a plastic or wax lined paper bag. Dried out or fully dead roots will return unreliable results so be sure to get live roots and be cognizant of how long they are stored under refrigeration before being shipped to the lab.

The palm looks sick but the Lab report does not demonstrate the presence of disease – What is going on?

If you have an obviously sick/failing palm tree and the lab report comes back indicating that no disease is present, consider that the palm may be infected by a Phytoplasma disease (*Phytoplasma disease <u>will not show up</u> in a standard disease identification culture - see below)* and/or the sample you sent in did not contain the pathogen that is harming the palm. It is also very important to recognize that disease isn't the only issue that can harm the palm. Cultural and environmental scenario related issues can cause problems as can nutritional deficiencies. Further, it is important to recognize that where a disease is identified in your sample, there is almost always a base cause (Cultural, environmental, site specific etc.) that you need to identify and correct. Simply using a disease control agent to target and eliminate the pathogen identified will only result in a short term solution. If the condition that enabled the establishment & proliferation of the disease is left un-corrected the disease is sure to return sooner or later.

Instructions for harvesting a Phytoplasma test sample.

Phytoplasma diseases (*Lethal Yellowing Disease and Texas Phoenix Palm Decline*) cannot be detected in a Standard Disease Identification *(culture*) test. The procedure for harvesting a sample for a Phytoplasma disease test varies considerably from that which you will employ when harvesting a Basic Disease identification sample. With Phytoplasma tests, the test itself is called a nested PCR/DNA extraction test. This is a specialized test that can be performed by either the University of Florida in Ft Lauderdale or Gainesville (both campuses have the capacity to run PCR/DNA) and by the Agri Life Lab at Texas A&M University.

When harvesting a Phytoplasma test sample you should follow the instructions below very carefully and in the order in which they are set forth.

To harvest this sample you will need:

A. A battery or cord powered drill with (1) normal sized 1/2" drill bit and (1) 18" long ½ inch drill bit.
B. Isopropyl Alcohol and a clean dry rag.
C. A hand held propane torch.
D. Brand new zip lock bag.
E. A wood dowel that matches the caliper of your drill bit.
F. Push pins
G. Permanent marker

Rule #1 Never let anything at all (<u>especially your fingers</u>) touch the inside of the plastic bag or the 18" drill bit after heat sterilizing. If you accidently do, start over with a new bag and/or re-sterilize the drill bit. Any foreign DNA inside the bag or on the drill bit may ruin the test so if anything at all touches the inside of the sample bag or the sterilized drill bit(other than the sample) you need to start over.

<u>Procedure</u>

1. Write the site name and Palm variety name on the outside of a new zip lock bag using a permanent marker.
2. Clean the drill bit that you will use to harvest the sample by misting it with alcohol wiping it off with the clean rag.
3. Use the normal sized drill bit to drill into the trunk approximately 1" to get past and clear away the woody exterior petiole layer at a point that is approximately 18- 24" above ground level. Once this is done, switch to the 18" drill bit.
4. Using the push pins, pin the inside lip of the sample bag to the trunk being very careful not to allow your fingers to touch the inside of the bag.
5. Using the hand held propane torch, run the flame over all surfaces of the 18" drill bit being sure to heat the surface well exposing every surface to the flame. Your goal is to sterilize the drill bit so once it is done, do not lay it on the ground or you will have to do it again.
6. After allowing the drill bit to cool, place the tip of the bit into the pre-started hole and drill into the trunk of the palm down to a depth of 12-14". Have your co-worker hold the tacked on bag open (remember, no fingers inside!) and catch the internal tissue coming out on the drill bit in the zip lock bag.

7. Once you have approximately 1/2 ounce of the drilled out internal tissue, carefully zip the bag closed squeezing the air out as you go until the bag is sealed. Here again, any physical contact with inside of the bag or with the sample tissue will ruin the test, if it happens, start over.
8. Once you have acquired the sample (one sample per palm is all you need) store it in a small cooler with a bit of ice in your truck. Here again, the goal is not to thoroughly chill the sample, you are simply avoiding allowing it to get hot. If you leave it sitting out on the seat of the truck and it's hot out, the heat may ruin the sample so don't let it happen.
9. Keep the sample refrigerated until it can be overnighted to the lab requesting DNA Extraction testing for Phytoplasma disease.

Lab contact(s) information for Basic Disease identification testing and/or for DNA Extraction/Phytoplasma disease testing:

AgriLife - Texas Plant Disease Diagnostic Lab
1500 Research Parkway, Suite A130
College Station, TX 77845
(979)845-8032

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Ft. Lauderdale Research & Education Center- University of Florida
3205 College Ave.
Davie, FL 33314
Phone: (954) 577-6300

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UF Plant Diagnostic Center – University of Florida
Bldg 1291, 2570 Hull Rd
Gainesville, FL 32611
(352)392-1795

If you are unable to nail down what is going on with your palms and feel that you need professional advice, you can arrange to have me visit the site by contacting our offices at (800) 753-5127 or you can send an email to sickpalms@datepalm.com Please make sure to include your telephone contact information when emailing us and we will get back to you during normal business hours. If your scenario is an emergency, you will want to do your best to determine what your issues are on your own. My time is allocated over 5 states and is severely restricted. Scheduling site visits/diagnosis & treatment instruction usually requires at least some notice. The length of notice required depends on the location of the site and my schedule at the time the request is received.