2025 Phytoplasma Sample Screening and Confirmation for CAPS and PPA 7721[1]

Collecting Samples

Symptoms (what to look for)

The survey method is visual inspection for symptoms of diseases caused by phytoplasmas. Only collect samples of plant tissues that have specific symptoms of the phytoplasma you are targeting. Symptoms alone are **not** diagnostic. Other plant pathogens and endemic phytoplasmas can cause similar symptoms. Assume that the plant is suspect and take samples for further testing. If possible, take a photograph of the symptomatic part(s) of the plant.

Characteristic symptoms of each phytoplasma survey target can be found in their respective Approved Methods pest pages and datasheets on the CAPS Resource and Collaboration website.

Data Collection

Collect and record data at each site. Data collected may include:

- County
- GPS coordinates and location description (closest cross streets, etc.)
- Owner/Grower's info (Name, mailing address, phone number, email)
- Acreage
- Variety/Cultivar
- History (previous cropping history, source of planting materials)
- Symptoms: Yes/No (foliar, blossom, etc.); briefly describe symptoms
- Date of sample collection
- Any other useful information

Sample Collection/Packaging/Submission

1. Collect at least 3 to 5 symptomatic leaves/needles (include the petiole if possible) from each suspected diseased plant (grape, stone fruit, apple, pine, etc.).

   Palm samples:
   From immature field-grown palms, collect symptomatic leaflets (pinnate species) or leaflet lamina and midvein tissues (palmate species) taken from the youngest leaf (*i.e.,* spear).

   For mature palms, collect stem borings.
   - Prior to sampling each palm, the drill bit should be flame sterilized using a portable propane torch and cooled by rinsing with water.
   - Stem samples are removed by boring a hole (4 to 6 inches in length) into the palm stem (trunk) using a portable electric drill and 5/16-inch diameter bit.
     - Begin sampling by drilling a shallow pilot hole in the lower stem to remove the outermost layer of pseudobark (discard these tissues).
     - Resume drilling incrementally through the pilot hole into the interior stem to the final depth of ~6 inches using a back-and-forth motion to dislodge shavings.
   - Allow shavings from the interior of the hole to fall into a clean sealable plastic bag positioned at a safe distance below the rim of the drilled hole.

[1] Cooperative Agricultural Pest Survey (CAPS) Program and Plant Protection Act Section 7721 Program (PPA 7721)

- Once the sampling is complete, the stem can be sealed (if necessary) by tapping a wooden dowel into the hole to prevent sap bleeding and to provide a barrier to invasion by pests (see Harrison et al., 2013 for more details).

Harrison, N. A., R. E. Davis, and E. E. Helmick. 2013. DNA extraction from arborescent monocots and how to deal with other challenging hosts. *In:* Dickinson, M and Hodgetts, J. (eds). Phytoplasma: Methods and Protocols, Humana Press, Springer NY. Pgs. 147-158.

Important: Follow all proper sanitation precautions to avoid spreading plant diseases.

2. Place all (at least 3 to 5) leaves/tissue from a given plant into a plastic bag and seal the bag. Do not put any extra moisture into the bag. If the leaves are wet, dry the excess moisture.

3. Label the sealed bag with the name of the host (including cultivar) and identifying code used in your records.

4. Place the sealed plastic bag into a second bag and seal the second bag. APHIS requires samples to be double-bagged.

5. DO NOT freeze the plant material. Instead, keep it cool by placing the samples into a cooler with a lid and freezer bags/cold packs.

6. Tape the box shut and package it for shipment. Include a PPQ form 391 completed for each sample from different plants and localities (i.e., each plant sample should have its own PPQ form 391). Retain a copy of each 391 for your records.

7. Send package by overnight delivery service or promptly take to the designated laboratory for screening. Notify the screening lab prior to mailing samples. Avoid shipping samples on Fridays since samples are not delivered on weekends.

Important: Surveyors must promptly submit all suspect phytoplasma samples for testing. If a sample tests positive for an exotic phytoplasma, the Plant Pathogen Confirmatory Diagnostics Laboratory (PPCDL) will need to obtain an official sample from the original host plant for confirmatory testing. This may not be possible if sample submissions are delayed.

Laboratory Analysis

Screening

Each sample is screened using approved DNA extraction and real-time PCR (qPCR) work instructions developed by the PPQ S&T PPCDL (formerly Beltsville lab). To request a copy of these protocols, email the PPCDL at APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov and use the subject line “Diagnostic protocol request”. Diagnostic laboratories with real-time PCR capacity can screen samples once they have completed the phytoplasma training given by the PPCDL or proficiency testing for DNA extraction and real-time PCR. Completion of annual proficiency testing (PT) for DNA extraction and real-time PCR managed by the Plant Pathogen Diagnostics Certification Program (PPDCP) is strongly recommended.

Numerous diagnostic labs that have completed the phytoplasma training and/or the proficiency testing may offer screening services for a fee. Diagnostic labs that meet these criteria are listed in the Appendix. You are strongly encouraged to use these labs.

We encourage you to communicate with your preferred sample screening lab prior to the start of your survey season. Contact the lab before sending them any samples. Also, send your samples for screening as soon as you collect them to allow surveyors enough time to revisit a location where a positive sample is found. Additional samples may be needed from the same plant or tree that tests positive for a phytoplasma for final confirmatory diagnostics.

If you are unable to find a lab to process your samples after contacting the labs in the Appendix, you may contact CAPS Science Support (stcaps@usda.gov) to see if additional screening options exist.

Confirmation

Negative Results: Communicate negative results to submitter by email and copy the USDA-APHIS-PPQ Domestic Diagnostics Coordinator at: PPQ.Domestic.Diagnostic.Coordinator@usda.gov

Positive Results: All phytoplasma positive sample DNA, regardless of the screening laboratory used, should be forwarded, with the original copy of the completed PPQ form 391, for confirmation to PPCDL unless otherwise instructed.[2] A Diagnostic Request (DR) should be submitted through ARM (your SPHD office can submit the request) prior to sending the samples.

Prior to sending samples for PPCDL confirmation, email the PPCDL at APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov and include the number of samples you are sending, a DR form, a PDF file of your completed PPQ Form 391, and the package tracking number in your message.

PPCDL shipping address:

Sample Diagnostics
USDA-APHIS-PPQ-S&T PPCDL
Bldg. 580, BARC-East
9901 Powder Mill Rd, Laurel, MD 20708
Phone 301-313-9292 or 301-313-9208
APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov

Upon completion, test results are reported to the Domestic Diagnostics Coordinator. If necessary (e.g., inconclusive test results or poor DNA sample quality), PPCDL may request a new plant/tissue sample to be collected for official confirmatory testing.
PPCDL has permits to receive the infected material sent overnight in a properly secured crushproof container with the original copy of the completed PPQ form 391.

[2] The X-disease phytoplasma group (16SrIII) is common in Pennsylvania, which has its own process for routing and submitting 16SrIII phytoplasma positives. In addition, palm phytoplasmas occur in Texas and Florida, and these states have their own process for routing and reporting 16SrIV phytoplasma positives.

Visit the APHIS website "Request Official Confirmation of Preliminary Pest Identifications of Domestic Samples" for more information about the official confirmation process.

Contact S&T CAPS Support if you have any questions: STCAPS@usda.gov

Appendix: State or NPDN diagnostic laboratories that have completed phytoplasma training or extraction and real-time PCR proficiency testing from the PPCDL that may accept suspect phytoplasma samples.

This list of labs was accurate at the time of publication (June 2025) but is subject to change without notice. If using a lab from this list, **please contact them prior to sending samples**. Be sure to include the shipment tracking number and a completed PPQ form 391 with the package.

State	Laboratory	Contact	Proficiency Testing	Phytoplasma Training
AL	Auburn University Plant Diagnostic Lab	Kassie Connor connekn@auburn.edu (334) 844-5507	Yes	Yes
AZ	University of Arizona School of Plant Sciences	Alex Hu epp@email.arizona.edu (520) 626-6287		Yes
CA	CA Dept. of Food & Agriculture Plant Pest Diagnostics Lab	Sebastian Albu sebastian.albu@cdfa.ca.gov (916) 738-6723	Yes	Yes
CO	Colorado State University	Matias Reynoso csuplantlab@colostate.edu (970) 491-6950		Yes
FL	University of Florida Plant Diagnostic Center	Carrie Harmon clharmon@ufl.edu (352) 392-1795	Yes	Yes
LA	Louisiana State University Plant Diagnostic Center	Raj Singh RSingh@agcenter.lsu.edu (225) 578-4562		Yes
MI	MDARD – Plant Pathology Laboratory	Stefanie Rhodes RhodesS2@michigan.gov (517) 449-7843	Yes	
MI	Michigan State University Plant & Pest Diagnostic Lab	Jan Byrne byrnejm@msu.edu (517) 355-3504	Yes	Yes
MN	Minnesota Department of Agriculture Plant and Seed Analysis Lab	Sara Bratsch Sara.Bratsch@state.mn.us Diandra.Viner@state.mn.us (651) 201-6404		Yes
NY	Cornell University	Karen Snover-Clift kls13@cornell.edu 607-227-0397	Yes	Yes
OK	Oklahoma State University	Jennifer Olson sickplants@okstate.edu (405) 744-9961		Yes
OR	Oregon Department of Agriculture	Elisabeth Savory Elizabeth.Savory@oda.oregon.gov 503.986.4570	Yes	Yes

PA	Pennsylvania Department of Agriculture	Katya Nikolaeva enikolaeva@pa.gov (717) 705-5857	Yes	Yes
PR	University of Puerto Rico	Consuelo Estevez de Jensen consuelo.estevez@upr.edu (787)-837-3905		Yes
SC	Clemson Molecular Plant Detection Lab	Curt Colburn gcolbur@clemson.edu (864) 646-2133		Yes
TX	Texas A&M Plant Disease Diagnostic Lab – College Station	Jake Ueckert jueckert@tamu.edu 979-845-2802	Yes	Yes
VA	VA Tech	Lina Rodriguez Salamanca clinic@mail.cals.vt.edu 540-231-8020 or 540-231-6530		Yes
WA	WA State Dept of Ag	Telissa Wilson Twilson@agr.wa.gov 360-664-8974 or 360-972-0918	Yes	Yes
WV	West Virginia University Plant Diagnostics Clinic	M. Mahfuz Rahman MM.Rahman@mail.wvu.edu (304) 293-8838		Yes
WI	Wisconsin DATCP Plant Industry Laboratory	Samantha Fieweger Sam.fieweger@wisconsin.gov (608) 733-0463	Yes	Yes