2023 Phytoplasma Sample Screening and Confirmation for CAPS and PPA 7721[1]

Collecting Samples

Symptoms (what to look for)

Due to the nature of this type of pathogen, the survey is visual inspection for symptoms of the diseases caused by phytoplasmas. Only collect samples of plant tissues that have the specific symptoms of the phytoplasma that 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 (https://caps.ceris.purdue.edu/).

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

   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).

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

    - 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.
- 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 (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. Tape the box shut and package it for shipment.
6. Include a PPQ form 391 (http://www.aphis.usda.gov/library/forms/pdf/PPQ_Form_391.pdf) 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. 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 Beltsville Lab. Numerous diagnostic labs have completed the training and 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 plan ahead with the lab that you intend to use for sample screening prior to the start of your survey. Contact the lab each time 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 Steve Bullington, 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]

Prior to sending samples, email the PPCDL at APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov and Dr. Stefano Costanzo at Stefano.Costanzo@usda.gov. Include number of samples you are sending, a PDF file of your completed PPQ Form 391, and the package tracking number.

Beltsville Lab shipping address:

Sample Diagnostics
USDA-APHIS-PPQ-S&T PPCDL
Bldg. 580, BARC-East
9901 Powder Mill Rd, Laurel, MD 20708
Phone 301-313-9211 or 301-313-9200
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 the permits to receive the infected material sent overnight in a properly secured crushproof container with the original copy of the completed PPQ form 391.

Visit the APHIS website "Request Official Confirmation of Preliminary Pest Identifications of Domestic Samples" for more information about the official confirmation process: https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/request-official-confirmation-preliminary-pest-id.

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

[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.

Appendix: State diagnostic laboratories accepting samples.

This list of labs was accurate at the time of publication (May, 2022) 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	Address	Contact
Alabama	Auburn University Plant Diagnostic Lab	961 South Donahue Dr. Auburn, AL 36849	Kassie Connor connekn@auburn.edu (334) 844-5507
Arizona	University of Arizona School of Plant Sciences	1140 E. South Campus Dr. Room 303, Forbes Tucson, AZ 85721	Alex Hu epp@email.arizona.edu (520) 626-6287
California	CDFA Plant Pest Diagnostics Lab	3294 Meadowview Rd. Sacramento, CA 95832	Sebastian Albu sebastian.albu@cdfa.ca.gov (916) 738-6723
Florida	University of Florida Plant Diagnostic Center	2570 Hull Rd., Bldg. 1291 Gainesville, FL 32611	Carrie Harmon clharmon@ufl.edu (352) 392-1795
Iowa	Iowa State University Plant and Insect Diagnostic Clinic	ATTN: Lina Rodriguez-Salamanaca 2445 ATRB 2213 Pammel Dr. Ames, IA 50011	Lina Rodriguez Salamanca pidc@iastate.edu (515) 294-0581
Louisiana	Louisiana State University Plant Diagnostic Center	Plant Diagnostic Center 302 Life Sciences Building LSU Campus Baton Rouge, LA 70803	Raj Singh RSingh@agcenter.lsu.edu (225) 578-4562
Michigan	MDARD- Plant Pathology Laboratory	Attn: Elizabeth Dorman P.O. Box 30017 Lansing, MI 48909	Elizabeth Dorman dormane@michigan.gov (517) 449-8491
Michigan	Michigan State University Plant & Pest Diagnostic Lab	578 Wilson Rd. East Lansing, MI. 48824	Jan Byrne byrnejm@msu.edu (517) 355-3504
Minnesota	Minnesota Department of Agriculture Plant and Seed Analysis Lab	Attn: Sara Bratsch (PSAU) 601 Robert Street North Saint Paul, MN 55155	Sara Bratsch Sara.Bratsch@state.mn.us Diandra.Viner@state.mn.us (651) 201-6404
North Dakota	North Dakota State University Plant Diagnostic Laboratory	**(USPS)** Dept. 7660, PO Box 6050 Fargo, ND 58108-6050 **(NON-USPS)** 1402 Albrecht Blvd. Walster Hall, 306 Fargo, ND 58102	Presley Mosher presley.mosher@ndsu.edu (701) 231-7854

Oklahoma	Oklahoma State University	Plant Disease and Insect Diagnostic Lab 127 Noble Research Center Oklahoma State University Stillwater, OK 74078	Jennifer Olson (405) 744-9961 sickplants@okstate.edu
Oregon	Oregon Department of Agriculture	635 Capitol Street NE Salem, OR 97301	Elisabeth Savory
Pennsylvania	Pennsylvania Department of Agriculture	2301 N. Cameron St. Harrisburg, PA 17110	Katya Nikolaeva enikolaeva@pa.gov (717) 705-5857
Puerto Rico	University of Puerto Rico	Car 510 Km 3.2 Bo Sabana LLana Juana Diaz, PR 00795	Consuelo Estevez de Jensen consuelo.estevez@upr.edu (787)-837-3905
South Carolina	Clemson Molecular Plant Detection Lab	511 Westinghouse Rd. Pendleton, SC 29670	Curt Colburn gcolbur@clemson.edu (864) 646-2133
Texas	Texas A&M Plant Disease Diagnostic Lab	1500 Research Parkway Suite A130 College Station, TX 77845	Jake Ueckert jueckert@tamu.edu (979) 845-8032
Washington	WSDA Plant Pathology & Molecular Diagnostics Lab	3939 Cleveland Ave SE Tumwater, WA 98501	Nathan Chambers nchambers@agr.wa.gov (360) 664-8974
West Virginia	West Virginia University Plant Diagnostics Clinic	G102 South Agricultural Sciences Building P.O. Box 6108 Morgantown, WV. 53718	M. Mahfuz Rahman MM.Rahman@mail.wvu.edu (304) 293-8838
Wisconsin	Wisconsin DATCP Plant Industry Laboratory	2601 Agriculture Drive STE 150 Madison, WI 53718	Samantha Fieweger Sam.fieweger@wisconsin.gov (608) 224-4601