

**Phytoplasma Sample Submission for
Cooperative Agricultural Pest Survey (CAPS)
and Farm Bill Goal 1 Surveys**

Table 1: Phytoplasma National Survey Targets by Scientific Name, 16Sr Group, and Subgroup

Validly Published Scientific Name	Informally Proposed Scientific Name	Ribosomal Group	Subgroup	Common Name
<i>Candidatus</i> Phytoplasma australiense		16SrXII	Subgroup B (16SrXII- B)	Australian grapevine yellows
<i>Candidatus</i> Phytoplasma mali		16SrX	Subgroup A (16SrX-A)	Apple proliferation
<i>Candidatus</i> Phytoplasma phoenicium and related strains/subgroups		16SrIX	Subgroups B, D, F, and G (16SrIX-B, 16SrIX-D, 16SrIX-F, and 16SrIX-G)	Almond witches' broom
<i>Candidatus</i> Phytoplasma prunorum		16SrX	Subgroup F (16SrX-F)	European stone fruit yellows
<i>Candidatus</i> Phytoplasma solani		16SrXII	Subgroup A (16SrXII-A)	Bois noir; Stolbur
	<i>Candidatus</i> Phytoplasma palmae and related strains/subgroups	16SrIV	Subgroups A through F (16SrIV-A, etc.)	Palm lethal yellowing
	<i>Candidatus</i> Phytoplasma vitis	16SrV	Subgroups C and D (16SrV-C and 16SrV-D)	Flavescence dorée

Symptoms (what to look for)

Due to the nature of this type of pathogen, the survey will be a visual survey 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. **Do not** conclude that a plant with the symptoms described below is infected with a phytoplasma or with an exotic phytoplasma. Assume that the plant is suspect and take samples for further testing. If you have access to a camera, take a photograph of the symptomatic region(s) of the plant. Follow all

proper sanitation precautions to avoid spreading plant diseases. Use sterilized knives/cutters and clean aseptically between samples and prior to use on the next survey site.

As of now, we **are not** recommending vector sampling for early detection surveys of exotic phytoplasmas.

Characteristic symptoms of the phytoplasmas being targeted in CAPS/Farm Bill surveys can be found in the specific CPHST pest datasheet for each pest and are summarized below in Table 2.

Table 2: Symptoms of the Phytoplasma National Survey Targets

<i>Candidatus</i> Phytoplasma australiense - Australian grapevine yellows
<p>Symptoms vary depending on host – see full pest datasheet for a complete list and description of symptoms.</p> <p>Grapevine: Yellow (chlorotic) and downward curled leaves that fall prematurely; reddening may be seen in red cultivars. The chlorotic patches on affected leaves may become necrotic. Leaves of affected shoots can overlap one another. Shoots are stunted and unligified. Abortion of flowering bunches early in the season has been observed. Any time from flowering, bunches may shrivel and fall. Stems of affected shoots often take on a bluish hue. Only a few shoots on grapevine are usually affected, and inflorescence and fruit are generally only affected on symptomatic shoots. Later in the season, affected shoots tend to be green and rubbery. Be sure that each plant that is sampled exhibits shriveling of the fruiting cluster.</p> <p>Potato: In potato, upward rolling and purpling of the leaves has been observed. These symptoms appear similar to those of ‘zebra chip’, a disorder of potato recently found to be associated with <i>Candidatus</i> Liberibacter solanacearum in New Zealand and the United States.</p>

Candidatus Phytoplasma mali - Apple proliferation (AP)

Apple: In general, trees affected by the AP phytoplasma lack vigor. Trunk circumference and crown diameter are reduced in size compared to healthy trees. Shoots are thin, and the bark- which is sometimes fluted lengthwise- has a reddish-brown color. Necrotic areas appear on the bark and some branches may wither.

Late growth of terminal buds in the autumn is usually the first noticeable symptom. A rosette of terminal leaves, which often become infected with powdery mildew, sometimes develops late in the season in place of the normal dormant bud. A more reliable symptom, however, is the premature development of axillary buds, which give rise to secondary shoots/shoot proliferation (witches' brooming). These abnormal secondary shoots are usually numerous near the apex of the main shoot, whereas normally lateral branches of healthy trees arise nearer the base of the shoots.

Leaves appear earlier than normal. Leaves of infected plants roll downward, become brittle, are abnormally finely and irregularly serrated, and are smaller than normal. They also tend to turn red in autumn in contrast to the yellow coloration of healthy plants. Summer leaves are chlorotic. Early defoliation may occur.

Stipules are abnormally enlarged (long) while petioles are noticeably shorter (an important symptom in nursery surveys). Leaf rosettes may appear on the shoot ends or the shoot tips may die (an important symptom in nursery surveys). Flowering is delayed, sometimes until late summer or autumn, but most blossoms on infected trees are normal. In some cases, flowers show numerous petals and the peduncles are abnormally long and thin. The calyx end and peduncular cavities are shallower and broader, giving the fruit a flattened appearance. Fruit fail to set and may stay on the tree for a long period. Fruit are reduced in size with incomplete coloration and poor flavor. Seeds and seed cavities are smaller.

Root weight is reduced; the fibrous root system of infected trees forms compact felt-like masses of short roots impeding the growth of larger roots (resulting in a fine hairy root system).

Candidatus Phytoplasma phoenicium and related strains/subgroups – Almond witches’ broom

Almond: Symptoms include early flowering, stunted growth, leaf rosetting (a disease symptom characterized by short, bunched growth habit due to shortened internodes and reduction in leaf size), dieback, off-season growth, proliferation of slender shoots, witches’ broom, and development of smaller pale green leaves. Witches’ broom symptoms arise mainly from the trunk or roots. The phytoplasma has also been detected in symptomless almond hosts.

Some almond cultivars are more susceptible to *Ca. P. phoenicium* than other cultivars.

Peach / Nectarine: Symptoms early in the growing season include premature flowering followed by the early development of buds. Symptoms later in the growing season include: shoot proliferation, smaller leaves with a pale green color, abnormal flowers (phylloidy), and witches’ broom symptoms in rare cases. Most infected trees do not set any fruit, but some trees bore a limited number of deformed fruits.

Candidatus Phytoplasma prunorum – European stone fruit yellows (ESFY)

Primarily a disease of apricot, Japanese plum, and peach.

Apricot/Japanese Plum: Generally typical ‘yellows’ symptoms accompanied by leaf roll followed by leaf reddening, reduction, or suppression of dormancy with the consequent risk of frost damage, severe and progressive necrosis, decline, and eventual death of the tree.

Peach: Early leaf reddening, severe upward longitudinal rolling of leaves, abnormal thickening and suberization of the midribs and primary veins, autumnal growth of latent buds which produce tiny chlorotic leaves and sometimes flowers, and early phylloptosis (leaf fall). The leaves also tend to be ‘more brittle’ than normal.

Candidatus Phytoplasma solani – Bois noir; Stolbur

Symptoms vary depending on host – see full pest datasheet for a complete list and description of symptoms.

Grapevine: Typical symptoms comprise discoloration of leaves including the veins, downcurling of the leaf blade, lack of or incomplete lignification of shoots that later turn black, abortion of fruit clusters or shriveling of the ripening fruit. Symptoms of bois noir remain restricted to parts of the infected vines for several years. In red grape cultivars, leaf reddening occurs. In white cultivars, yellow, necrotic veins occur. Shriveled grape clusters occur in both red and white grape cultivars.

Corn: Symptoms of maize redness include midrib, leaf, and stalk reddening, followed by desiccation of the entire plant, abnormal ear development, and incomplete kernel set. More severe disease is associated with early-planted fields and hot, dry summers.

Potato: Symptoms in potato include reddening and upward rolling of leaflets, reduced size of leaves, shortened internodes, and aerial tuber formation. Plants grown from infected tubers give rise to normal or spindly sprouts (hair-sprouting). Where normal sprouts arise, symptoms are first apparent about 60 to 80 days after sowing, as a yellowing and rolling of the leaves. This is followed by production of aerial stolons and tubers in different parts of the stems close to the axils.

Tomato: Leaves that develop before infection become greenish-yellow, especially at the margins, which may roll upward. Newly formed leaves become more yellow and are smaller. Stems become thin at the apex as growth ceases, but stems enlarge at infection sites as a result of abnormal phloem formation. This abnormal phloem appears as a greenish, water-soaked band 1 to 2mm wide, which extends towards the xylem. Lateral shoots develop, giving the plant a bushy aspect. Flower buds assume an abnormally erect position; the sepals, whose veins develop a violet color, remain completely joined and the calyx is enlarged and cyst-like ("big bud"). Flowers, if already formed when infection occurs, become similarly erect and may be sterile, and petals are greenish instead of yellow. Flower distortion is common, and petals of young flowers become totally dwarfed and green (virescent). Peduncles are thicker than normal. Fruit development is arrested following infection. Green fruits formed prior to infection become solid, dry and ripen very slowly. Necrosis occurs at the embryonic center in younger fruits. Pedicels of fruits are thicker than in healthy plants, in spite of the relatively small fruit size.

Candidatus Phytoplasma palmae and related strains/subgroups – Palm lethal yellowing

Palm: For mature palm species, the earliest visible symptom is a premature shedding of most or all fruit regardless of developmental stage. Fruit that are shed from coconut often develop a blackened or water-soaked appearance at the calyx end. Necrosis of newly emergent inflorescences occurs concurrently with or follows fruit drop. Flower spikelets, which are normally light yellow to creamy white in color, appear partially or totally blackened.

Fruit and flower symptoms are followed shortly thereafter by foliar discoloration. In the Atlantic tall coconut ecotype, leaves turn a golden yellow color. Discoloration begins on the lowermost (oldest) leaves and progresses towards successively younger leaves in the upper part of the crown. Discolored leaves typically remain turgid for some time before turning brown, drying and hanging downward around the stem for a few days before falling to the ground. The newest unopened leaf (spear) collapses once foliar discoloration is advanced. Death of the apical meristem occurs at this stage, after which the remaining crown withers and topples away, leaving nothing more than a bare trunk standing.

While premature fruit drop and inflorescence necrosis are common to all palms with lethal yellowing, leaves turn reddish brown rather than yellow on many coconut ecotypes and most other palm species. On date palms, death of the spear leaf and underlying apical meristem occurs shortly after leaves first begin to discolor. Most affected palms die within 3 to 5 months after the onset of symptoms.

Candidatus Phytoplasma vitis - Flavescence dorée

Grapevine: Leaves turn yellow or red depending on the cultivar. They roll downward and become brittle. The interveinal areas of leaves may become necrotic.

Shoots show incomplete lignification, and rows of black pustules develop on the green bark along the diseased branches; they are thin, rubbery, and hang pendulously. During winter they blacken and die. The inflorescences dry out and fall off. Fruit setting is reduced. In later infections, bunches are irregular and berries become shriveled. They have a significantly lower sugar content and higher acidity compared to healthy grapes. **Be sure that each plant that is sampled exhibits shriveling of the fruiting cluster.**

Data Collection

Collect and record data at each site.

Data collected may include:

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

Sample Collection/Packaging/Submission:

1. Collect 3 to 5 symptomatic leaves/needles (include petiole if possible) from each suspected diseased plant (grape, stone fruit, apple, pine). Follow all proper sanitation precautions to avoid spreading plant diseases.

Palm samples from immature field-grown palms with symptoms suggestive of phytoplasma disease should be received as freshly harvested leaflets (pinnate species) or leaflet lamina and midvein tissues (palmate species) taken from the youngest leaf (*i.e.*, spear).

For mature palms, tissue samples can be removed as 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 (10 to 15 cm in length) into the palm stem (trunk) using a portable electric drill and 5/16 in. (ca. 7.8 mm) 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 ~15 cm using a back and forth motion to dislodge shavings.
- Tissue borings from the stem are collected directly into a clean sealable plastic bag.
- 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.

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 as to the host cultivar, and identifying code to be used in your records.
4. Place the sealed plastic bag into a second bag, and seal the second bag. This will give a double-bagged sample, which is required by APHIS.
5. DO NOT freeze the leaves. Instead, keep the leaves cool by placing the samples into a styrofoam box with lid and add freezer bags/cold packs. Tape the box shut and package it for shipment.
6. Include a PPQ Form 391 (Appendix A, use the [fillable form](http://www.aphis.usda.gov/library/forms/pdf/PPQ_Form_391.pdf) available at 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).
7. Send by overnight delivery service or promptly take to the designated laboratory for analysis. The package should be shipped on a Monday or Tuesday so that there will be time to process the samples upon arrival and the package will not sit in the delivery service over a weekend.
8. Laboratory Analysis:

Screening:

A phytoplasma qPCR has been evaluated by CPHST Beltsville in collaboration with Dr. Robert Davis (USDA-ARS) utilizing a range of phytoplasmas and host plants. This test can be used by any laboratory with qPCR capacity for screening after they have attended a phytoplasma training session at CPHST Beltsville. Several diagnostic labs received this training in recent years. They may offer sample screening services for a fee. A listing of the labs that have completed the training and have indicated that they are willing to process samples from other areas are available in Appendix B*. You are strongly encouraged to utilize these labs.

***Note:** This list of labs was accurate at the time of publication 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 packing tracking number and a completed PPQ 391 forms with the package.

Previously, suspect plant samples could be sent to Clemson University or Texas A&M University for screening for the cost of shipping only. However, the cooperative agreements with these two universities were not renewed. The agreement with Clemson expired June 30, 2016, and the agreement with Texas A&M expired August 31, 2017. Screening is still offered by both labs, but submitters will be charged a fee for each sample. The lab at Texas A&M may offer a bulk discount for a high volume of CAPS survey samples.

If you are unable to find a lab to process your samples after contacting the labs in Appendix B, you may contact Dr. Craig Webb for assistance. However, he should only be contacted as a last resort for phytoplasma screening:

Craig Webb

Plant Pathologist - Domestic Identifier
USDA, APHIS, PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
Manhattan, Kansas 66506-5502
Voice: (785) 532-134, Cell: (785) 633-9117, Fax: (785) 532-5692
Email: PPQ.FO.KS.Manhattan.Lab@aphis.usda.gov

Confirmation:

All phytoplasma positive DNA samples should follow the approved protocol below regardless of screening laboratory.

Non-Palm Samples:

All non-palm (*e.g.*, apple, grape, stone fruit, and pine) phytoplasma positive DNA should be sent to Dr. Robert Davis, with the exception of phytoplasma positive fruit trees or grapevines from Pennsylvania. The X-disease phytoplasma group (16SrIII) is common in Pennsylvania, and this state has its own process for routing and submitting 16SrIII phytoplasma positives. The state will continue to forward finds on new host plants or samples that are not straightforward in their identification.

Each DNA must be labeled exactly the same as the leaf/tissue sample from which the DNA was extracted. The PPQ form 391 should also be sent with the sample.

Dr. Robert Davis
USDA-Agricultural Research Service
Molecular Plant Pathology Laboratory
Bldg 004, Room 220 /221
10300 Baltimore Avenue
Beltsville, MD, 20705
Voice: 301-504-5745 or -6290
Fax: 301-504-5449
Email: robert.davis@ars.usda.gov

Palm Samples:

Dr. Brian Bahder should receive all suspect palm phytoplasma positive samples with the exception of palm phytoplasma samples collected from Texas and Florida with the authorization of the [State Plant Regulatory Official \(SPRO\)](#) of the state of origin. Since palm phytoplasmas (Group 16SrIV) are known to occur in Texas (16SrIV-D) and Florida (16SrIV-A, D, and F), these states have their own process for routing and reporting 16SrIV phytoplasma positives.

Each DNA must be labeled exactly the same as the leaf/tissue sample from which the DNA was extracted. The PPQ form 391 should also be sent with the sample.

Dr. Brian Bahder

University of Florida
Entomology and Nematology Dept.,
FLREC
3205 College Avenue
Fort Lauderdale, FL 33314, USA
Voice: 954-577-6300
bbahder@ufl.edu

Contact Daniel Mackesy if you have any questions:

Daniel Mackesy
Biological Science Technician
USDA-APHIS-PPQ-CPHST
2301 Research Blvd. Suite 108
Fort Collins, CO 80526
Voice: 970-490-4494
Daniel.Z.Mackesy@aphis.usda.gov

Appendix A: PPQ FORM 391

http://www.aphis.usda.gov/library/forms/pdf/PPQ_Form_391.pdf

<p>According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0377. The time required to complete this information collection is estimated to average .25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.</p>		<p>This report is authorized by law (7 U.S.C. 147a). While you are not required to respond, your cooperation is needed to make an accurate record of plant pest conditions.</p>		<p>OMB APPROVED 0579-0010 EXP. DATE 02/2017</p>		
<p>UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE</p>		<p>Instructions: Type information requested. Block 1 – assign a number for each collection using your own numbering convention or use the following example by beginning with the year, followed by the collector's initials and the collector's number. Example: 14-JD-001.</p>		<p>LOT NUMBER</p>		
<p>SPECIMENS FOR DETERMINATION</p>						
<p>1. COLLECTION NUMBER</p>		<p>2A. DATE - SUBMISSION MONTH DAY YEAR</p>		<p>2B. DATE - COLLECTION MONTH DAY YEAR</p>		
				<p>3. SUBMITTING AGENCY <input type="checkbox"/> State Cooperator <input type="checkbox"/> University <input type="checkbox"/> APHIS PPO <input type="checkbox"/> Other:</p>		
SUBMITTER AND ORIGIN	4A. NAME OF SUBMITTER		4B. NAME OF COLLECTOR		INTERCEPTION SITE	
	5. ADDRESS OF SUBMITTER					
	6. TYPE OF PROPERTY (FARM, RESIDENCE, NURSERY, ETC.)					
	7. NAME AND ADDRESS OF PROPERTY OWNER					
5. ADDRESS OF SUBMITTER		ZIP		CITY COUNTY STATE		
EMAIL ADDRESS OF SUBMITTER				LATITUDE LONGITUDE		
PURPOSE	8. REASON FOR IDENTIFICATION ("X" all applicable items)					
	A. Biological Control (Target Pest Name _____)		E. Export Certification			
	B. Damaging Crops/Plants		F. Targeted Survey (Pest Name _____)			
	C. Suspected Pest of Regulatory Concern (Explain in REMARKS)		G. Smuggling Interdiction/Trade Compliance (SITC)			
	D. Stored Product Pest		H. Other (Explain in REMARKS)			
9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".						
HOST DATA	10. HOST INFORMATION			11. QUANTITY OF HOST		
	NAME OF HOST (Scientific name and name of cultivar if appropriate)			NUMBER OF ACRES/PLANTS		
				Plant affected (insert figure and indicate) <input type="checkbox"/> Number: <input type="checkbox"/> Percent:		
12. PLANT DISTRIBUTION		13. PLANT PARTS AFFECTED				
<input type="checkbox"/> Limited		<input type="checkbox"/> Leaves, Upper Surface		<input type="checkbox"/> Trunk/Bark		
<input type="checkbox"/> Scattered		<input type="checkbox"/> Leaves, Lower Surface		<input type="checkbox"/> Bulbs, Tubers, Coms		
<input type="checkbox"/> Widespread		<input type="checkbox"/> Petiole		<input type="checkbox"/> Buds		
		<input type="checkbox"/> Stem		<input type="checkbox"/> Flowers		
		<input type="checkbox"/> Growing Tips		<input type="checkbox"/> Fruits or Nuts		
		<input type="checkbox"/> Roots				
PEST DATA	14. PEST DISTRIBUTION		15. <input type="checkbox"/> INSECTS <input type="checkbox"/> NEMATODES <input type="checkbox"/> MOLLUSKS			
	<input type="checkbox"/> FEW		NUMBER SUBMITTED		CAST SKINS	
	<input type="checkbox"/> COMMON		LARVAE		EGGS	
	<input type="checkbox"/> ABUNDANT		PUPAE		NYMPHS	
<input type="checkbox"/> EXTREME		ADULTS		JUVS.		
		ALIVE		CYSTS		
		DEAD				
16. SAMPLING METHOD		17. TYPE OF TRAP AND LURE		18. TRAP NUMBER		
19. REMARKS						
<p>METHOD <input type="checkbox"/> MORPHOLOGY <input type="checkbox"/> SYMPTOM <input type="checkbox"/> CULTURE <input type="checkbox"/> SEROLOGICAL <input type="checkbox"/> PCR <input type="checkbox"/> SEQUENCING</p>						
20. TENTATIVE DETERMINATION		DETERMINED BY		POSITION AND AFFILIATION		
21 FINAL DETERMINATION AND NOTES (Not for Field Use)						
<p>METHOD <input type="checkbox"/> MORPHOLOGY <input type="checkbox"/> SYMPTOM <input type="checkbox"/> CULTURE <input type="checkbox"/> SEROLOGICAL <input type="checkbox"/> PCR <input type="checkbox"/> SEQUENCING</p>						
PRINT NAME (Person Making Final Determination)		DISPOSITION OF SPECIMEN/SAMPLE				
		<input type="checkbox"/> Returned <input type="checkbox"/> Retained for Collection/Stored <input type="checkbox"/> Destroyed <input type="checkbox"/> Transferred to:				
SIGNATURE		DATE		LAB CONFORMATION NUMBER		
				DATE RECEIVED		

PPQ Form 391
AUG 2014

Previous editions are obsolete.

INSTRUCTIONS

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS
1	<p>1. Assign a number for each collection using your own numbering convention or use the following example by beginning with the year, followed by the collector's initials and the collector's number.</p> <p style="text-align: center;">EXAMPLE</p> <div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;"> <p>In 2014, Brian K. Long collected his first specimen of the year for determination. His first collection number is 14-BLK-001</p> </div> <p>2. Enter the collection number</p>
2A-2B	Enter dates
3	Check block to indicate Agency submitting specimens for identification
4A	Enter name of submitter
4B	Enter name of collector
5	Enter address of submitter
6	Enter type of property specimen obtained from (farm, nursery, residence, etc.)
7	Enter name and address of property owner
8A-8H	Check all appropriate blocks
9	Leave Blank
10	Enter scientific name of host, if possible
11	Enter quantity of host and plants affected
12	Check block to indicate distribution of plant
13	Check appropriate blocks to indicate plant parts affected
14	Check block to indicate pest distribution
15	<input type="checkbox"/> Check appropriate block to indicate type of specimen <input type="checkbox"/> Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Provide a brief explanation if Prompt or URGENT identification is requested
20	Enter a tentative determination and who made it
21	Leave blank

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

1. Send Original along with the sample to your Area Identifier or for national confirmation.
2. Retain and file a copy for your records.

Appendix B: Diagnostic Laboratories that have taken required phytoplasma training at CPHST Beltsville and are willing to accept samples from other states.

<p>Alabama</p> <p>Kassie Conner Plant Diagnostic Lab 961 South Donahue Drive Auburn, AL 36849 Phone: 334-844-5507 connekn@auburn.edu</p>	<p>Pennsylvania</p> <p>Ekaterina (Katya) Nikolaeva PA Department of Agriculture 2301 N. Cameron St. Harrisburg, PA 17110 Phone: 717-705-5857 Fax: 717-705-6518 enikolaeva@pa.gov</p>
<p>Florida</p> <p>University of Florida Plant Diagnostic Center Attention: Carrie Harmon, Director, and Sladana Bec, Lab Manager 2570 Hull Rd., Bldg. 1291 Gainesville, FL 32611 Phone: 352-392-1795 Fax: 352-392-1922 pd@ifas.ufl.edu; clharmon@ufl.edu ; sladanabec@ufl.edu</p>	<p>Puerto Rico (Puerto Rico samples only)</p> <p>Consuelo Estevez de Jensen University of Puerto Rico, Juana Diaz Car 510 Km 3.2 Bo Sabana LLana Juana Diaz, PR 00795 Phone: 787-837-3905 consuelo.estevez@upr.edu</p> <p>Note: This lab was damaged by Hurricane Maria. Please double check before sending in samples.</p>
<p>Iowa</p> <p>Lina Rodriguez-Salamanca/ Laura Jesse Plant & Insect Diagnostic Clinic clinic.ipm.iastate.edu</p> <p>Phone 515-294-0581, 515-520-2441 pidc@iastate.edu</p>	<p>South Carolina</p> <p>Curt Colburn Clemson University Molecular Plant Pathogen Detection (MPPD) laboratory 511 Westinghouse Rd. Pendleton, SC 29670 Phone: 864-646-2133 gcolbur@clemson.edu</p>
<p>Michigan</p> <p>Jan Byrne Diagnostic Services Plant Pathologist Michigan State University 578 Wilson Road East Lansing, MI 48824-6469 Phone: 517-355-3504 byrnejm@msu.edu</p>	<p>Texas*</p> <p>Kevin Ong Texas Plant Disease Diagnostic Lab 1500 Research Parkway, Suite A130 College Station, TX 77845 Phone: 979-845-8032 Fax: 979-845-6499 kevo@tamu.edu</p> <p>*A bulk discount may be available for CAPS samples</p>

<p>Michigan (continued)</p> <p>MDARD-Plant Pathology Laboratory Attn: Elizabeth Dorman Phone: 517-337-5083 dormane@michigan.gov</p>	
<p>North Dakota</p> <p>Jesse Ostrander Physical Location: Main Campus - 206 Waldron Hall Billing/USPS Mailing Address: NDSU Plant Diagnostic Lab Dept 7660, PO Box 6050 Fargo, North Dakota 58108-6050</p> <p>Shipping Address (NON-USPS): NDSU Plant Diagnostic Lab 306 Walster Hall Fargo ND 58102 Phone: 701-231-7854 Fax: 701-231-7851 jesse.ostrander@ndsu.edu Lab Web: www.ag.ndsu.nodak.edu/diaglab</p>	<p>Washington</p> <p>Rachel Bomberger Plant Pest Diagnostic Clinic Department of Plant Pathology Washington State University 100 Diary Rd/P.O. Box 646430 Pullman, WA 99164-6430 Office: 509-335-0619 Clinic: 509-335-3292 rachel.bomberger@wsu.edu plant.clinic@wsu.edu</p> <p>Washington (continued)</p> <p>Nathan Chambers WSDA Plant Pathology & Molecular Diagnostics Lab 3939 Cleveland Ave SE Tumwater, WA 98501 Phone: 360-664-8974 nchambers@agr.wa.gov</p>
<p>Ohio</p> <p>Colette Gabriel Plant and Pest Diagnostic Clinic - Phytoplasma 8995 E. Main St. Bldg. 23 Reynoldsburg, OH 43068-3399 Phone: 614-752-2329 gabriel.89@osu.edu Lab web: https://ppdc.osu.edu/home</p>	