Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014 & FY 2015

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

Validly Published Scientific Name	Informally Proposed Scientific Name	Ribosomal Group	Subgroup	Common Name
'*Candidatus* Phytoplasma australiense'		Group 16SrXII	subgroup B **(16SrXII-B)**	Australian grapevine yellows phytoplasma
'*Candidatus* Phytoplasma mali'		Group 16SrX	subgroup A **(16SrX-A)**	Apple proliferation phytoplasma
'*Candidatus* Phytoplasma pini'		16SrXXI	subgroup A **(16SrXXI-A)**	Pine witches' broom phytoplasma
'*Candidatus* Phytoplasma prunorum'		16SrX	subgroup F **(16SrX-F)**	European stone fruit yellows phytoplasma
	'*Candidatus* Phytoplasma palmae' and related strains/subgroups	16SrIV	Subgroups A through F (**16SrIV-A**, etc.)	Palm lethal yellowing phytoplasma and others
	'*Candidatus* Phytoplasma vitis'	Group 16SrV	subgroups C and D (**16SrV-C and 16SrV-D)**	Flavescence dorée phytoplasma

What to look for?
Due to the nature of this type of pathogen, the survey will be a visual survey for symptoms of the disease caused by the phytoplasma. 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 a new property.

Right 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

Scientific/Common Name	Symptoms
'Candidatus Phytoplasma australiense' - Australian grapevine yellows phytoplasma	Symptoms vary depending on host – see full pest datasheet for all symptoms information. **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 unlignified. 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.** **Potato:** In potato, upward rolling and purpling of the leaves were observed. The symptoms appeared similar to those of 'zebra chip', a disorder of potato recently found to be associated with '*Candidatus* Liberibacter

	solanacearum' in New Zealand and the United States.
'*Candidatus* Phytoplasma mali'- Apple proliferation (AP) phytoplasma	**Apple:** Trees affected by the AP phytoplasma, in general, lack vigor. Trunk circumference and crown diameter are reduced 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 normal laterals of healthy trees arise nearer the base of the shoots. Leaves appear earlier than normal. Leaves of infected plants roll downward and become brittle; they are 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 rather short (an important symptom in nursery surveys). Leaf rosette 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 so that the larger ones are unable to develop (a fine hairy root system).

'Candidatus Phytoplasma pini' – Pine witches' broom phytoplasma	**Pine:** Yellowing, dwarfing/stunting, twisted needles ("form dense ball-like structures, prolific branching / proliferation of small shoots/twigs (i.e., witches' broom), and little leaves.
'Candidatus Phytoplasma prunorum' – European stone fruit yellows (ESFY) phytoplasma	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 palmae' and related strains/subgroups – Palm lethal yellowing phytoplasma and related subgroups	**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 accompanies 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. On 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 just 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 phytoplasma	**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.

Date 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).

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 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., Davis, R.E. and Helmick, E.E. 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.

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 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 (see Appendix A or use the fillable form 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 bring promptly 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:

A phytoplasma qPCR is being currently being evaluated by CPHST Beltsville in collaboration with Dr. Robert Davis (USDA-ARS) utilizing a range of phytoplasmas and host plants. Once evaluated, this test can be used by any laboratory with qPCR capacity for screening after they have attended a phytoplasma training session at CPHST Beltsville. A training session is scheduled to offered in March 2015 at the CPHST Beltsville lab.

Since the protocol isn't currently available, suspect symptomatic plant material should be sent to the **Clemson University**, or **Texas A & M University, or Craig Webb.** Email notification should occur prior to any samples being sent to any of the lab locations listed below. Be sure to include the packing tracking number and a completed PPQ 391 forms with the package

All samples should be sent to the following street address:

Curt Colburn
Clemson University
Molecular Plant Pathogen Detection (MPPD) laboratory
511 Westinghouse Rd.
Pendleton, SC 29670
Voice: 864-646-2133
Email: gcolbur@clemson.edu

Kevin Ong
Texas Plant Disease Diagnostic Lab
1500 Research Parkway, Suite A130
College Station, TX 77845
Voice: 979-845-8032 Fax: 979.845.6499
Email: kevo@tamu.edu

Craig Webb
Plant Pathologist - Domestic Identifier
USDA, APHIS, PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
Manhattan, Kansas 66506-5502
Office: (785) 532-134, Cell: (785) 633-9117, Fax: (785) 532-5692
Email: craig.a.webb@aphis.usda.gov

Non-Palm Samples:
All non-palm (*e.g.*, apple, grape, stone fruit, and pine) phytoplasma positive DNA should be sent to Dr. Robert Davis.

Each DNA must be labeled <u>exactly</u> 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. Nigel Harrison should receive all 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 <u>exactly</u> 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. Nigel A. Harrison
University of Florida
Plant Pathology Dept., FLREC
3205 College Avenue
Fort Lauderdale, FL 33314
Voice: 954-577-6321 Fax: 954-475-4125
Email: naha@ufl.edu

Appendix A: PPQ FORM 391

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. *See reverse for additional OMB information.*

FORM APPROVED
OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete Items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete Items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER
2. DATE — MO | DA | YR
3. SUBMITTING AGENCY — ☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER
6. ADDRESS OF SENDER
ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*
7. NAME AND ADDRESS OF PROPERTY OR OWNER
COUNTRY/ COUNTY

PURPOSE

8. REASON FOR IDENTIFICATION *("x" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)	E. ☐ Livestock, Domestic Animal Pest
B. ☐ Damaging Crops/Plants	F. ☐ Possible Immigrant *(Explain in* REMARKS)
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS)	G. ☐ Survey *(Explain in* REMARKS)
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
NAME OF HOST *(Scientific name when possible)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure and indicate* ☐ Number ☐ Percent*)*:

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

13. PLANT PARTS AFFECTED

☐ Leaves, Upper Surface	☐ Trunk/Bark	☐ Bulbs, Tubers, Corms	☐ Seeds
☐ Leaves, Lower Surface	☐ Branches	☐ Buds	
☐ Petiole	☐ Growing Tips	☐ Flowers	
☐ Stem	☐ Roots	☐ Fruits or Nuts	

PEST DATA

14. PEST DISTRIBUTION
☐ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION — **DETERMINED BY:**

24. DETERMINATION AND NOTES *(Not for Field Use)*

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

SIGNATURE
DATE

PPQ FORM 391 *Previous editions are obsolete.*
(AUG 02)

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

OMB Information

According to the Paperwork Reduction Act of 1995, no persons are 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 0579-0010. 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.

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	1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number **EXAMPLE** In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001 2. Enter the collection number
2	Enter date
3	Check block to indicate Agency submitting specimens for identification
4	Enter name of sender
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)
6	Enter address
7	Enter name and address of property owner
8A-8L	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	• Check appropriate block to indicate type of specimen • Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Enter X in block to indicate isolated or general plant symptoms
20	Enter X in appropriate block for weed density
21	Enter X in appropriate block for weed growth stage
22	Provide a brief explanation if Prompt or URGENT identification is requested
23	Enter a tentative determination if you made one
24	Leave blank

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

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