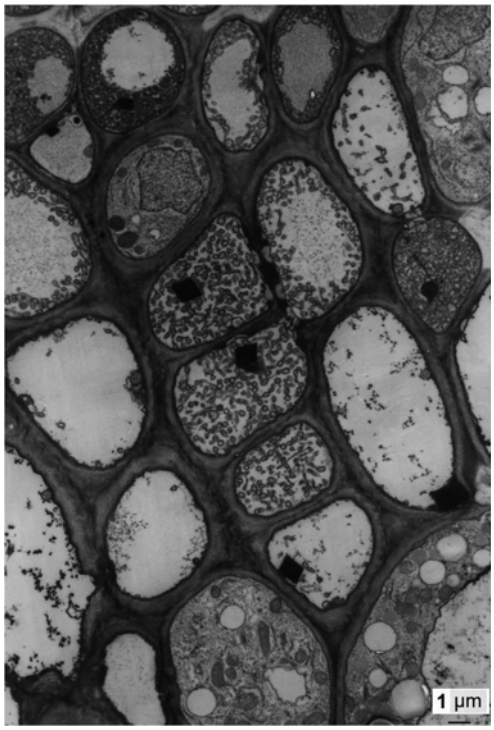
United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

New Pest Response Guidelines

Selected '*Candidatus* Phytoplasma spp.' of Apple, Grape and Peach



First Edition Issued 2012

Candidatus Phytoplasma

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Cover Images

Electron micrographs of cross section of sieve tubes colonized with phytoplasma cells, from Bertaccini and Duduk 2009.

Chapter

1

Introduction

Contents

Introduction

Use *New Pest Response Guidelines: Selected* Candidatus *Phytoplasma spp. of Apple, Grape and Peach*, when designing a program to detect, monitor, control, contain, or eradicate an outbreak of any of the selected phytoplasma species of apple, grape and peach in the United States and collaborating territories.

The United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the guidelines through discussion, meeting, or agreement with staff members at the USDA-Agricultural Research Service and advisors at universities.

Any new detection may require the establishment of an Incident Command System to facilitate emergency management. This document is meant to provide the necessary information to launch a response to a detection of any of the following phytoplasma species: *Candidatus* Phytoplasma mali (Apple proliferation), *Candidatus* Phytoplasma australiense (Australian grapevine yellows) and *Candidatus* Phytoplasma prunorum (European stone fruit yellows).

If any of these phytoplasmas are detected, PPQ personnel will produce a site-specific action plan based on the guidelines. As the program develops and new information becomes available, the guidelines will be updated.

Users

The guidelines is intended as a reference for the following users who have been assigned responsibilities for a plant health emergency for any of the selected *Candidatus* Phytoplasma spp. of apple, grape and peach:

- PPQ personnel
- Emergency response coordinators
- State agriculture department personnel
- Others concerned with developing local survey or control programs

Contacts

When an emergency pest response program for any of the selected *Candidatus* Phytoplasma spp. of apple, grape and peach has been implemented, the success of the program depends on the cooperation, assistance, and understanding of other involved groups. The appropriate liaisons and information officers should distribute news of the program's progress and developments to interested groups, including the following:

- Academic entities with agricultural interests
- Agricultural interests in other countries
- Commercial interests
- Grower groups such as specific commodity or industry groups
- Land-grant universities and Cooperative Extension Services
- National, State and local news media
- Other Federal, State, county, and municipal agricultural officials
- Public health agencies
- The public
- State and local law enforcement officials
- Tribal governments

Initiating an Emergency Pest Response Program

An emergency pest response program consists of detection and delimitation, and may be followed by programs in regulation, containment, eradication and control. The New Pest Advisory Group (NPAG) will evaluate the pest. After assessing the risk to U.S. plant health, and consulting with experts and regulatory personnel, NPAG will recommend a course of action to PPQ management.

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported
2. The pest is examined and pre-identified by regional or area identifier
3. The pest's identity is confirmed by a national taxonomic authority recognized by USDA–APHIS–PPQ-National Identification System
4. Published New Pest Response Guidelines are consulted or a new NPAG is assembled in order to evaluate the pest

5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators, and trading partners
6. A delimiting survey is conducted at the site of detection
7. An Incident Assessment Team may be sent to evaluate the site
8. A recommendation is made, based on the assessment of surveys, other data, and recommendation of the Incident Assessment Team or the NPAG, as follows:
   - **A.** Take no action
   - **B.** Regulate the pest
   - **C.** Contain the pest
   - **D.** Suppress the pest
   - **E.** Eradicate the pest
9. State Departments of Agriculture are consulted
10. If appropriate, a control strategy is selected
11. A PPQ Deputy Administrator authorizes a response
12. A command post is selected and the Incident Command System is implemented
13. State departments of agriculture cooperate with parallel actions using a Unified Command structure
14. Traceback and trace-forward investigations are conducted
15. Field identification procedures are standardized
16. Data reporting is standardized
17. Regulatory actions are taken
18. Environmental Assessments are completed as necessary
19. Treatment is applied for required pest generational time
20. Environmental monitoring is conducted, if appropriate
21. Pest monitoring surveys are conducted to evaluate program success
22. Programs are designed for eradication, containment, or long-term use

Preventing an Infestation

Federal and State regulatory officials must conduct inspections and apply prescribed measures to ensure that pests do not spread within or between properties. Federal and State regulatory officials conducting inspections should follow the sanitation guidelines in the section *Survey Procedures* on page 4-1 before entering and upon leaving each property to prevent contamination.

Scope

The guidelines is divided into the following chapters:

1. *Introduction* on page 1-1
2. *Pest Information* on page 2-1
3. *Identification* on page 3-1
4. *Survey Procedures* on page 4-1
5. *Regulatory Procedures* on page 5-1
6. *Control Procedures* on page 6-1
7. *Environmental Compliance* on page 7-1
8. *Pathways* on page 8-1

The guidelines also includes appendixes, a references section, a glossary, and an index.

The Introduction contains basic information about the guidelines. This chapter includes the guideline's purpose, scope, users, and application; a list of related documents that provide the authority for the guidelines content; directions about how to use the guidelines; and the conventions (unfamiliar or unique symbols and highlighting) that appear throughout the guidelines.

Authorities

The regulatory authority for taking the actions listed in the guidelines is contained in the following authorities:

- Plant Protection Act of 2000 (Statute 7 USC 7701-7758)
- Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments

- Fish and Wildlife Coordination Act
- National Historic Preservation Act of 1966
- Endangered Species Act
- Endangered and Threatened Plants (50 CFR 17.12)
- National Environmental Policy Act

Program Safety

Safety of the public and program personnel is a priority in pre-program planning and training and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decisionmaking

USDA–APHIS–PPQ-Center for Plant Health, Science and Technology (CPHST) provides technical support to emergency pest response program directors about risk assessments, survey methods, control strategies, regulatory treatments, and other aspects of pest response programs. PPQ managers meet with State departments of agriculture in developing guidelines and policies for pest response programs.

How to Use the Guidelines

The guidelines is a portable electronic document that is updated periodically. Download the current version from its source, and then use Adobe Reader® to view it on your computer screen. You can print the guidelines for convenience. However, links and navigational tools are only functional when the document is viewed in Adobe Reader®. Remember that printed copies of the guidelines are obsolete once a new version has been issued.

Conventions

Conventions are established by custom and are widely recognized and accepted. Conventions used in the guidelines are listed in this section.

Advisories

Advisories are used throughout the guidelines to bring important information to your attention. Please carefully review each advisory. The definitions have been updated so that they coincide with the America National Standards Institute (ANSI) and are in the format shown below.

EXAMPLE Example provides an example of the topic.

Important Important indicates information that is helpful.

⚠ CAUTION

CAUTION indicates that people could possibly be endangered and slightly hurt.

⚠ DANGER

DANGEROUS indicates that people could easily be hurt or killed.

NOTICE

NOTICE indicates a possibly dangerous situation where goods might be damaged.

⚠ WARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

Boldfaced type is used to highlight negative or important words. These words are: never, not, do not, other than, prohibited.

Lists

Bulleted lists indicate that there is no order to the information being listed. Numbered lists indicate that information will be used in a particular order.

Disclaimers

All disclaimers are located on the unnumbered page that follows the cover.

Table of Contents

Every chapter has a table of contents that lists the heading titles at the beginning to help facilitate finding information.

Control Data

Information placed at the top and bottom of each page helps users keep track of where they are in the guidelines. At the top of the page is the chapter and first-level heading. At the bottom of the page is the month, year, title, and page number. PPQ–EDP-Emergency Programs is the unit responsible for the content of the guidelines.

Change Bar

A vertical black change bar in the left margin is used to indicate a change in the guidelines. Change bars from the previous update are deleted when the chapter or appendix is revised.

Decision Tables

Decision tables are used throughout the guidelines. The first and middle columns in each table represent conditions, and the last column represents the action to take after all conditions listed for that row are considered. Begin with the column headings and move left-to-right, and if the condition does not apply, then continue one row at a time until you find the condition that does apply.

Table 1-1 How to Use Decision Tables

If you:	And if the condition applies:	Then:
Read this column cell and row first	Continue in this cell	TAKE the action listed in this cell
Find the previous condition did not apply, then read this column cell	Continue in this cell	TAKE the action listed in this cell

Footnotes

Footnotes comment on or cite a reference to text and are referenced by number. The footnotes used in the guidelines include general text footnotes, figure footnotes, and table footnotes. General text footnotes are located at the bottom of the page.

When space allows, figure and table footnotes are located directly below the associated figure or table. However, for multi-page tables or tables that cover the length of a page, footnote numbers and footnote text cannot be listed on the same page. If a table or figure continues beyond one page, the associated footnotes will appear on the page following the end of the figure or table.

Heading Levels

Within each chapter and section there can be four heading levels; each heading is green and is located within the middle and right side of the page. The first-level heading is indicated by a horizontal line across the page, and the heading follows directly below. The second-, third-, and fourth-level headings each have a font size smaller than the preceding heading level. The fourth-level heading runs in with the text that follows.

Hypertext Links

Figures, headings, and tables are cross-referenced in the body of the guidelines and are highlighted in boldface type. These appear in blue hypertext in the online guidelines.

Italics

The following items are italicized throughout the guidelines:

- Cross-references to headings and titles
- Names of publications
- Scientific names

Numbering Scheme

A two-level numbering scheme is used in the guidelines for pages, tables, and figures. The first number represents the chapter. The second number represented the page, table, or figure. This numbering scheme allows for identifying and updating. Dashes are used in page numbering to differentiate page numbers from decimal points.

Transmittal Number

The transmittal number contains the month, year, and a consecutively-issued number (beginning with -01 for the first edition and increasing consecutively for each update to the edition). The transmittal number is only changed when the specific chapter sections, appendixes, or glossary, tables, or index is updated. If no changes are made, then the transmittal number remains the unchanged. The transmittal number only changes for the entire guidelines when a new edition is issued or changes are made to the entire guidelines.

Acknowledgements

Writers, editors, reviewers, creators of cover images, and other contributors to the guidelines, are acknowledged in the acknowledgements section. Names, affiliations, and Web site addresses of the creators of photographic images, illustrations, and diagrams, are acknowledged in the caption accompanying the figure.

How to Cite the Guidelines

Cite the guidelines as follows: U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2011. *New Pest Response Guidelines: Selected* Candidatus *Phytoplasma spp.'of Apple, Grape and Peach*. Washington, D.C. http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

How to Find More Information

Contact USDA–APHIS–PPQ–EDP-Emergency Management for more information about the guidelines. Refer to *Resources* on page A-1 for contact information.

Chapter

2

Pest Information

Contents

Introduction

Use *Chapter 2 Pest Information* to learn more about the classification, history, host range, and biology of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach: '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'. The systemic plant diseases associated with these phytoplasmas are respectively: apple proliferation, Australian grapevine yellows, and European stone fruit yellows. The three phytoplasma species are discussed here as these are of particular concern due to their impact on economically important food crops and ornamentals.

Classification

Use *Table 2-1* on page 2-2 and *Table 2-2* on page 2-3 as aids to classify the three phytoplasma species: '*Candidatus* Phytoplasma mali' ('*Ca*. P. mali'), '*Candidatus* Phytoplasma australiense' ('*Ca*. P. australiense') and '*Candidatus* Phytoplasma prunorum' ('*Ca*. P. prunorum').

Table 2-1 Classification of Phytoplasmas

Domain	Bacteria[1]
Phylum	Firmicutes
Class	Mollicutes
Order	Acholeplasmatales
Family	Acholeplasmataceae
Genus	'*Candidatus*[2] Phytoplasma'

1 CABI 2011.

2 The provisional taxonomic status '*Candidatus*' was established for incompletely described prokaryotes (Murray and Schleifer 1994). Because this designation does not qualify as a valid name under the Bacteriological code, it should be printed within quotation marks.

Table 2-2 Scientific and Common Names of 'Ca. P. mali', 'Ca. P. australiense' and 'Ca. P. prunorum'

Scientific Name	16S rDNA Group-Subgroup[1]	Synonym	Common Names[2]
'*Candidatus* Phytoplasma mali' Seemüller and Schneider 2004	16SrX-A	"*Candidatus* Phytoplasma mali"; Phytoplasma AP-MLO; *Phytoplasma mali*; Apple proliferation mycoplasma-like organism; Apple proliferation phytoplasma; Phytoplasma mali (Candidatus) Seemüller & Schneider 2004	apple proliferation, witches' broom, 16SrX (apple proliferation group)
'*Candidatus* Phytoplasma australiense' Davis et al. (1997)	16SrXII-B	'*Candidatus* Phytoplasma australiense', *Phytoplasma australiense*, *Phytoplasma australiense* (*Candidatus*) R.E. Davis et al.(1997)	Australian grapevine yellows, Phytoplasma australiense, liquidambar yellows (LaY), papaya dieback (PDB), phormium yellow leaf (PYL), strawberry lethal yellows (SLY), cordyline sudden decline (CSD), coprosma lethal decline (CLD)
'*Candidatus* Phytoplasma prunorum' Seemüller and Schneider (2004)	16SrX-F	'*Candidatus Phytoplasma prunorum*', *Phytoplasma prunorum*, *Phytoplasma prunorum* Seemüller and Schneider, *Phytoplasma prunorum* (*Candidatus*) Seemüller and Schneider (2004), '*Candidatus* Phytoplasma prunorum', 16SrX (apple proliferation group)	apricot chlorotic leaf roll (ACLR), plum leptonecrosis (PLN), European stone fruit yellows mycoplasma-like organism (ESFY-MLO), European stone fruit yellows (ESFY), ESFY 16SrX-B

1 For classification purposes, phytoplasmas are grouped based on their distinct restriction fragment length polymorphism (RFLP) pattern of PCR amplicons, derived from their 16S rDNA conserved sequences, which have been subjected to enzymatic restriction with a number of enzymes (Ahrens and Seemüller (1992), Deng and Hiruki (199) Firrao et al. (2004), Lee et al. (1998), Lee et al. (1993), Zhao et al. (2009)).

2 Refer to *Taxonomic Support for Surveys* on page D-1 for a list of common names disease names and acronyms.

Biology

Phytoplasmas are insect-transmitted, gram-positive wall-less bacteria that have resisted all attempts of isolation and culturing in artificial media. Phytoplasma cells reside in plant phloem sieve elements and in the tissues of phloem-feeding insect vectors, including leafhoppers, plant hoppers and psyllids. Refer to *Known Phytoplasma Vectors* on page G-1 for a list of confirmed phytoplasma vectors. Phytoplasmas, first discovered in 1967 (Doi et al. 1967), were referred as plant-pathogenic mycoplasma-like organisms or MLOs until 1993 (ICSB 1993). They are associated with diseases in over hundreds of plant species worldwide that were often thought to be of viral origin.

Phytoplasmas are wall-less, nonhelical prokaryotes characterized by a minute size (0.3 to 0.5 µm), bounded by a single membrane and are pleomorphic. Phytoplasmas genomes are relatively small (530 to 1,350 kb) and appear to have suffered extreme genome reductions compared with their gram-positive walled relatives (Marcone et al. 1999). Their genomes are composed of a single chromosome (circular or linear) and may include one or more plasmids. It has been suggested that some of these plasmids may also play a role in the specific interaction between insect and phytoplasmas (Ishii et al. 2009; Nishigawa et al. 2002). Their genomes are characteristically AT-rich, with a GC content that ranges from 21.4 percent to 29 mol percent (Kollar and Seemüller 1989). They have similar numbers of tRNA genes and two copies of the rRNA operon. Phytoplasmas cannot be morphologically or ultrastructurally distinguished from one another using electron or light microscopy (McCoy 1979). A distinction between different groups of phytoplasmas has been achieved by sequence comparisons of their respective 16S rRNA genes (Gundersen et al. 1994; Kuske and Kirkpatrick 1992; Lee et al. 1993; Seemüller et al. 1994).

Phytoplasmas induce severe symptoms in several hundred plant species worldwide, such as stunting, phyllody (development of floral parts into leafy structures), virescence (development of green flowers in place of normal color), abnormal proliferation of shoots giving rise to witches' broom growths, yellowing of leaves. They are detrimental to vegetable, flower, field crop and fruit production industries, the natural forest ecosystems and ornamentals.

Historical Information

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

The first reported case of apple proliferation (AP) was recorded in Veneto (northeastern Italy) in 1950 (Rui et al. 1950). The disease is known in Europe where it represents one of the most economically important threats to apple trees. The disease affects the overall tree vigor resulting in significantly smaller fruits with poor taste that cannot be commercialized.

In the late 1990s, damages caused by the AP phytoplasma became significant, especially in orchards in northern Italy and southwest Germany. '*Candidatus* Phytoplasma mali' belongs to the 16SrX-A apple proliferation group-subgroup of phytoplasmas and is phylogenetically and genetically related to the pathogens associated with two other diseases, pear decline (PD) '*Candidatus* Phytoplasma pyri', and European stone fruit yellows ESFY '*Candidatus* Phytoplasma prunorum' (Seemüller and Schneider 2004).

'*Candidatus* Phytoplasma mali' forms, together with '*Ca*. P. pyri' and '*Ca*. P. prunorum' and a few other phytoplasmas, a major subclade in phytoplasma phylogenetic tree (Seemüller et al. 2002). Nucleotide sequence analysis of their 16S rDNA revealed differences that range between 1.0 to 1.5 percent, which is below the 2.5 percent threshold to assign an individual species rank. However, the species distinction between AP, ESFY and PD phytoplasmas was granted based on other molecular markers (16S-23S rRNA spacer region and ribosomal protein), serological comparisons (based on recognition of *imp*: immunodominant membrane protein) as well as vector transmission and host range specificity (Loi et al. 2002; Seemüller and Schneider 2004).

Initially, several closely related subtypes of AP phytoplasma (AT-1; AT-2; AP) were detected with PCR-RFLP of a nonribosomal protein, however, the variation did not appear associated to geographic parameters (Jarausch et al. 2000). Strain discrimination of AP phytoplasma greatly improved utilizing single strand conformation polymorphism (SSCP) and sequence analyses of the *hfl*B gene (Schneider and Seemüller 2009). The higher resolution allowed for the identification of apple trees infected by multiple distinct strains that revealed possible interaction among them and their effect on virulence (Seemüller et al. 2010).

The three phytoplasmas are also characterized by their genomes organized into a linear chromosome, a rare feature in other phytoplasma and among bacteria. In 2007 the complete genome sequence of '*Ca.* P. mali' (strain AT) was determined (Kube et al. 2008; Kube et al. 2007).

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

Australian grapevine yellows (AGY) was first reported in 1976 in Australia and described by Magarey and Wachtel in 1978. The associated pathogen of AGY, '*Candidatus* Phytoplasma australiense', was taxonomically recognized as a distinct phytoplasma, more closely related to the European stolbur phytoplasma, causing disease on Australian 'Chardonnay' grapevines in 1997 (Davis et al. 1997a).

Recent improvements in identification techniques for phytoplasmas revealed that '*Ca*. P. australiense' related strains are associated with *Phormium* yellow leaf (PYL), (Liefting et al. 1998), Papaya dieback (PDB), (Gibb et al. 1996; Liu et al. 1996), Strawberry lethal yellows (SLY), (Andersen et al. 1998b), and other severe and economically important plant diseases (Andersen et al. 2006; White et al. 1998). Sequence analysis of the *tuf* gene by Andersen et al. (2006) revealed that there are three distinct subgroups of '*Ca*. P. australiense': tuf 1, tuf 2 and tuf 3. Subgroup tuf 1 is found in both Australia and New Zealand, tuf 2 isolates are only found in New Zealand and tuf 3 isolates are only found in Australia.

The New Zealand isolates from subgroups tuf 1 and tuf 2 can be further divided into nine *tuf* variant groups (I-IX) (Andersen et al. 2006). In 2007, the complete genome sequence of '*Ca.* P. australiense' (subgroup tuf I), was determined (Tran-Nguyen et al. 2007; Tran-Nguyen et al. 2008).

'Candidatus Phytoplasma prunorum' (European Stone Fruit Yellows)

A severe decline affecting Japanese plum and apricot trees was described at the beginning of the 20th century in orchards present across the southern part of France and in Italy. A decline by apoplexy in apricot trees was first reported by Chabrolin (1924). The diseases infecting several stone fruit species were later referred to as apricot chlorotic leaf roll (ACLR), peach yellows, plum leptonecrosis (PLN) or plum decline and were originally thought to be caused by a virus since they were transmissible by grafting.

The decline of apricot, cherry, peach and Japanese plum are now collectively refered as European stone fruit yellows (ESFY) disease (Lorenz et al. 1994; Marcone et al. 1996b; Seemüller and Foster 1995). The first reported case of PLN was recorded in Italy on Japanese plum (*Prunus salicina*) by Goidanich 1933. In Europe, the disease was found to be associated with the presence of phytoplasmas that were closely related to the apple proliferation (AP) group and distinct from those isolated from stone fruit species from the United States showing western X-disease symptoms.

'*Candidatus* Phytoplasma prunorum' is the aetiological agent of ESFY and it is considered the most important pathogen causing decline and death of cultivated *Prunus* species. Apricot trees are killed 12 to 24 months after first appearance of symptoms. This period may be reduced in duration to weeks if the rootstock source is peach. Spontaneous recovery is rare for apricot, but does occur more often with *Prunus salicina*.

In France, ESFY phytoplasma is probably responsible for 60 to 70 percent of cases of apricot decline. Serious effects begin to arise when trees first start bearing fruit after 5 years; 5 percent of trees may then be killed every successive year. In other countries where the ESFY phytoplasma occurs, *P. salicina* seems to be more important as a host. In southwestern France the disease (known as Moliéres disease), caused thousands of plum and cherry trees to decline and ultimately die. ESFY has increased its prevalence in Europe in recent decades and is now a major economic problem on apricot (*Prunus armeniaca*) and Japanese plum (*P. salicina*) throughout Europe.

'*Candidatus* Phytoplasma prunorum' belongs to the 16SrX-F apple proliferation group-subgroup of phytoplasmas and is phylogenetically and genetically related to the agents that cause pear decline (PD) '*Ca*. P. pyri', and apple proliferation (AP) '*Ca*. P. mali' (Seemüller and Schneider 2004). '*Ca*. P. prunorum' forms, together with '*Ca*. P. pyri' and '*Ca*. P. mali', and a few other phytoplasmas, a major subclade in phytoplasma phylogenetic tree (Seemüller et al. 2002).

Nucleotide sequence analysis of their 16S rDNA revealed differences that range between 1.0 to 1.5 percent, which is below the 2.5 percent threshold to assign an individual species rank. However, the species distinction between ESFY phytoplasma, AP phytoplasma and PD phytoplasma was granted based on other molecular markers (16S-23S rRNA spacer region and ribosomal protein), serological comparisons (based on recognition of *imp*: Immunodominant Membrane Protein) as well as vector transmission and host range specificity (Loi et al. 2002; Seemüller and Schneider 2004). The three phytoplasmas are also characterized by having their genomes organized into a linear chromosome, a rare feature in other phytoplasma and among bacteria.

Damage

The presence of phytoplasmas within the phloem of infected plants affects their normal development causing symptoms that suggest an alteration of the natural balance of plant nutrients and growth regulators. Tree flowering is affected reducing the overall number and quality of fruits produced that can also fall prematurely. The anticipated break of normal bud dormancy can produce a proliferation of weak shoots with shorter internodes that are susceptible to phloem damages by freezing temperatures. Leaf chlorosis and upward curling will also appear later during the growing season as well as early defoliation.

The detrimental effect caused by phytoplasmas can vary in severity depending on plant species as well as time of its occurrence during the plant development. Some plant species may tolerate the infection with mild or no symptoms, while other may suffer a general rapid decline and occasionally death. These observations indicate the possibility that the concentration (titer) of phytoplasma within the plant and perhaps the location and/or distribution of phytoplasmas within the plant may influence symptom expression. Environmental factors such as temperature and its interaction with phytoplasma multiplication and survival may also play a role in the expression of symptoms.

Economic Impact

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

The apple proliferation (AP) phytoplasma is considered among the most economically important threats in pome fruit growing areas of southern and central Europe. This is one of the most important phytoplasma diseases of apple, affecting almost all cultivars, reducing size (by about 50 percent), weight (by 63 to 74 percent) and quality of fruit, as well as reducing tree vigor and increasing susceptibility to powdery mildew (*Podosphaera leucotricha*) (Maszkiewicz et al. 1980) as well as the silver leaf fungus (*Chondrostereum purpureum*) (Németh 1986).

During the first 2 years of infection, AP phytoplasma has been reported to result in fruit losses up to 80 percent. In commercial orchards in Europe, AP phytoplasma can spread as much as 18 percent per year. While infected trees recover, the fruit produced from infected trees are often undersized. Once a tree becomes infected with AP phytoplasma it remains infected throughout its life. In recovered trees, AP phytoplasma disappears from the canopy, but remains active in the roots and can still be transmitted to healthy trees. The tree

decline induced by infection of AP phytoplasma can also lead to premature death of trees (Németh 1986, Seemüller 1990).

To evaluate the potential economic impact to the United States caused by the introduction and establishment of phytoplasma diseases caused by AP phytoplasma, major crops that would be affected were taken into consideration and summarized in *Table 2-3* on page 2-9. The estimated total value of these main crops is valued at $7.2 billion.

Table 2-3 Value of U.S. Production Potentially Affected by Apple Proliferation Phytoplasma[1]

Crop	Value of US Production (1,000 $)	Main Producing States
Grapes	3,171,814	CA (90%), WA, NY
Apples	2,222,759	WA (59%), NY, MI
Peaches	595,103	CA (74%), SC, NJ
Cherries	504,879	WA, CA, OR
Pears	350,615	WA (>50%), CA, OR
Prunes and plumes	261,881	CA (99%)
Hazelnuts	74,730	OR (99 %)
Apricot	44,078	CA (92 %), WA, UT
Total	7,225,859	

1 NASS 2011.

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

In Australia, the pathogen is responsible for several economically important diseases of food crops and ornamentals: Australian grapevine yellows (Padovan et al. 1995; Schneider et al. 1999), papaya dieback (Guthrie et al. 2001), strawberry lethal yellows, strawberry green petal (Padovan et al. 2000), pumpkin yellow leaf curl (Streten et al. 2005a), diseases of red clover and paddy melon in southwest Australia (Saqib et al. 2006), and Australian Lucerne yellows (Getachew et al. 2007).

Researchers have documented vineyard yield losses as high as 13 percent (CABI 2011). Severely affected grape vines can produce up to 54 percent less fruit than healthy grape vines (CABI 2011). Grapevines of the cultivars Riesling and Chardonnay affected by AGY in South Australia and Victoria had an average 10 percent reduction in their annual yield (Padovan et al. 1995). Other reports indicated that 5 percent of all vines in a vineyard exhibited yellows symptoms, with yield reduction ranging between 40 and 50 percent in severely diseased vines (Magarey and Wachtel 1986a).

Reports on Other Hosts

Papaya dieback can completely destroy a plantation (CABI 2011). *Phormium* yellow leaf disease was responsible for the contraction of the fiber industries based on New Zealand flax (*Phormium tenax*) (Andersen et al. 1998a). Australian lucerne yellows costs the pasture seed industry millions of dollars each year (Getachew et al. 2007). Establishment of '*Ca*. P. australiense' in the United States could impact trade, because some countries, like Morocco, classify '*Ca*. P. australiense' as a dangerous quarantine pest (WTO 2004).

To evaluate the potential economic impact to the United States that could be caused by the introduction and establishment of phytoplasmal diseases associated with AGY, the value of major crops that would be affected were taken into consideration and summarized in *Table 2-4* on page 2-10. The estimated total value of these crops is valued at $17.6 billion.

Table 2-4 Value of U.S. Production Potentially Affected by Australian Grapevine Yellows Phytoplasma[1]

Crop	Value of US Production (1,000 $)	Main Producing States
Alfalfa	7,997,221	CA; SD; ID
Potatoes	3,521,219	ID; WA; WI
Grapes	3,171,814	CA (90%); WA; NY
Strawberries	2,123,735	CA (80%); FL; OR
Celery	404,039	CA; MI
Beans	255,650	ND; MI; NE
Pumpkins	102,700	IL; CA; OH
Papaya	14,186	HI
Total	17,590,564	

1 NASS 2011.

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

The European stone fruit yellows (ESFY) phytoplasma is considered an economically important threat for stone fruit growing areas throughout Europe. Significant losses are reported mainly for apricot and the Japanese plum production. Infection rates for susceptible cultivars can reach 50 percent reducing tree vigor and fruit production and rendering orchard unproductive in eight to ten years from planting.

To evaluate the potential economic impact to the United States that could be caused by the introduction and establishment of phytoplasmal diseases associated with ESFY phytoplasma, the value of major crops that would be

affected were taken into consideration and summarized in *Table 2-5* on page 2-11. The estimated total value of these main crops is valued at $4.5 billion.

Table 2-5 Value of U. S. Production Potentially Affected by ESFY Phytoplasma[1]

Crop	Value of US Production (1,000 $)	Main Producing States
Almonds	2,694,450	CA
Cherries	766,982	WA; CA; OR
Peaches	614,619	CA(74%); SC; NJ
Prunes & plumes	237,131	CA (99%)
Nectarines	130,794	CA (96%); WA
Hazelnuts	59,670	OR (99%)
Apricot	47,498	CA (92%); WA; UT
Total	4,551,144	

1 NASS 2011.

Ecological Range

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

The apple proliferation (AP) phytoplasma is not present in the United States. AP phytoplasma has been reported to occur throughout Europe, specifically in Albania, Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Moldova, Norway, Poland, Romania, Serbia and Montenegro, Slovakia, Slovenia, Spain, Switzerland, Ukraine.

Apple proliferation phytoplasma has been found in Turkey and in Denmark and the Netherlands where it is not believed to be established. Additionally AP phytoplasma was found in 1978 and eradicated in 1979 from the United Kingdom.

Records from Austria, Bulgaria, Greece, Norway, Romania, Switzerland, former USSR, former Yugoslavia (Németh 1986), India and South Africa (Seemüller 1990) are based on symptoms and may require further confirmation.

Table 2-6 Apple Proliferation Phytoplasma Reported World Distribution

Region	Country	References
Asia	Turkey	CABI (2011)
Africa	Tunisia	Ben Khalifa and Fakhfakh (2011a)

Table 2-6 Apple Proliferation Phytoplasma Reported World Distribution

Region	Country	References
Europe	Albania	Myrta et al. (2003)
	Austria	Németh (1986)
	Belgium	Németh (1986)
	Bulgaria	Németh (1986)
	Croatia	CABI (2011)
	Czech Republic	Bertaccini et al. (1997)
	France	Jarausch et al. (1994)
	Germany	Lorenz et al. (1995), Seemüller et al. (1998a)
	Greece	Németh (1986)
	Hungary	Del Serrone et al. (1998)
	Italy	Firrao et al. (1993), Osler et al. (2001)
	Moldova	CABI (2011)
	Norway	Németh (1986)
	Poland	CABI (2011)
	Romania	Németh (1986)
	Serbia and Montenegro	Németh (1986)
	Slovakia	CABI (2011)
	Slovenia	Osler et al. (2001)
	Spain	Avinent and Llácer (1995)
	Switzerland	Németh (1986)
	Ukraine	CABI (2011)

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

The Australian grapevine yellows (AGY) phytoplasma is not present in the United States. AGY-affected grapevines are found in most viticultural regions of Australia (Bonfiglioli et al. 1996; Magarey and Wachtel 1986a). A particularly high incidence occurs in the warmer inland districts of Sunraysia in New South Wales and Victoria, Riverina in New South Wales, and the Riverland in South Australia. ‘Chardonnay’ and ‘Riesling’ appear to be most often affected (Magarey and Wachtel 1986a), but phytoplasmas have been detected in other white and red varieties (Bonfiglioli et al. 1996).

Australian grapevine yellows is widespread in Australia, specifically in New South Wales, Queensland, South Australia, Victoria and Western Australia (Davis et al. 1997a; Liefting et al. 1998), and New Zealand (Andersen et al. 2001), where it is also reported affecting potato plants (Liefting et al. 2009b).

Two incorrect reports of '*Ca*. P. australiense' occur in the literature: Nivum Haamir dieback of papaya in Israel (Gera et al. 2005) and yellow leaf roll of

peach in Bolivia (Jones et al. 2005). In both cases, the 16S rRNA gene sequence did not fulfill the criteria to be classified as '*Ca*. P. australiense'.

Table 2-7 Australian Grapevine Yellows Phytoplasma Reported World Distribution

Region	Country	References
Oceania	Australia	Magarey and Wachtel (1986a), Bonfiglioli et al. (1996)
	New Zealand	Andersen et al. (2001), Boyce and Newhook (1953)

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

The European stone fruit yellows (ESFY) phytoplasma is not present in the United States. ESFY phytoplasma has been reported to occur mostly on susceptible apricot (*Prunus armeniaca*), Japanese plum (*P. salicina*), European plum (*P. domestica*) and peach (*P. persica*) throughout southern Europe (France, Italy, Spain, Bosnia and Herzegovina, Croatia). The report of ESFY present in South Africa by Németh (1986), remains isolated and unconfirmed.

Table 2-8 European Stone Fruit Yellows Phytoplasma Reported World Distribution

Region	Country	References
Africa	Tunisia	Ben Khalifa and Fakhfakh 2011b
Asia	Azerbaijan	Danet et al. 2008
	Turkey	Gazel et al. 2009
Europe	Albania	CABI 2011
	Austria	Laimer Da Câmara Machado et al. 2001
	Belgium	CABI 2011
	Bosnia and Herzegov-ina	Delic et al. 2005
	Croatia	Krizanac et al. 2010
	Czech Republic	Navratil et al. 2001
	Engalnd	Davies and Adams 2000
	France	Desvignes and Cornaggia 1983
	Germany	Lederer and Seemüller 1992
	Greece	Rumbos and Bosabalidis 1985
	Hungary	Seemüller and Foster 1995
	Italy	Giunchedi et al. 1978
	Poland	Cieslinska and Morgas 2011

Table 2-8 European Stone Fruit Yellows Phytoplasma Reported World Distribution

Region	Country	References
	Romania	Ionica 1985
	Serbia and Montenegro	CABI 2011
	Slovenia	Brzin et al. 2001
	Spain	Sánchez-Capuchino and Forner 1975; Torres et al. 2004
	Switzerland	CABI 2011

Potential Distribution

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

A map based on density of susceptible hosts indicates several counties in the North East area of the United States (New York, Pennsylvania, Vermont, and Massachusetts) to be at intermediate risk within the continental United States (*Figure 2-1* on page 2-15). The leafhopper *Fieberiella florii*, one of the known AP phytoplasma vectors, is already established in North America and would likely spread AP phytoplasma if it was introduced into the United States. For this reason, '*Candidatus* Phytoplasma mali' should be considered an imminent threat that could be introduced into the United States with imported plants intended for planting and viable plant parts infected by this pathogen.

Infected propagative material may be asymptomatic and may thus move undetected in trade. If infected plants or vectors were to enter the United States, they could transmit the pathogen to U.S. apple crops and cause significant losses to the U.S. apple industry.

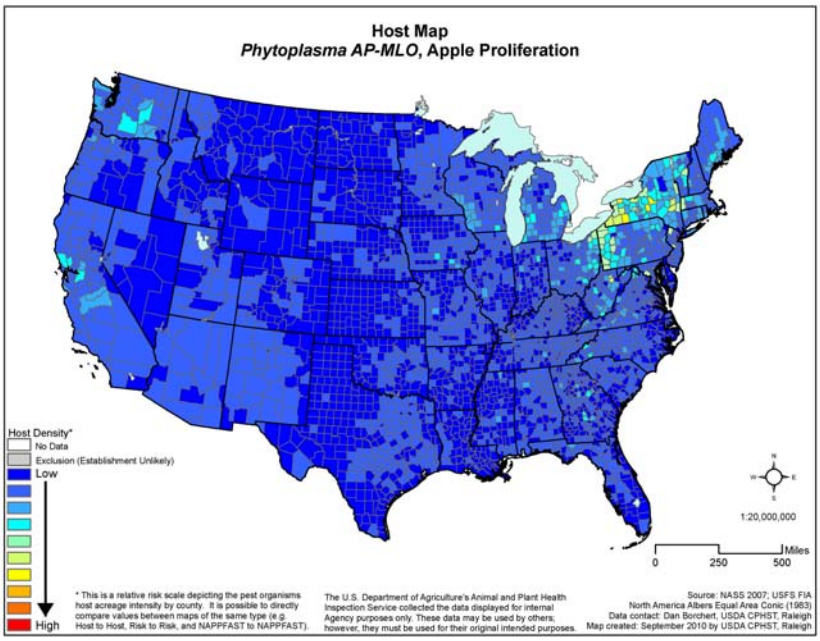


Figure 2-1 Risk Map for Establishment Potential of '*Ca*. P. mali' Within the Continental United States

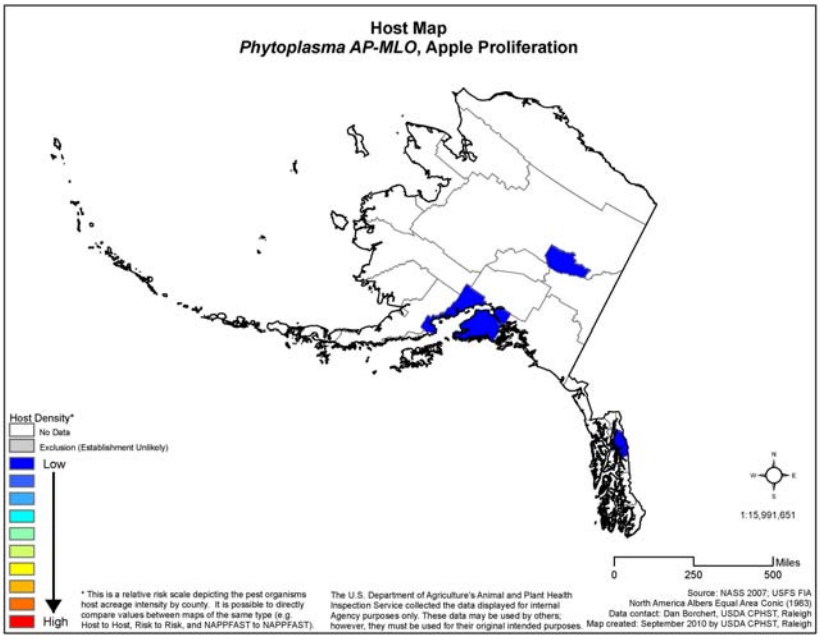


Figure 2-2 Risk Map for Establishment Potential of '*Ca*. P. mali' Within Alaska

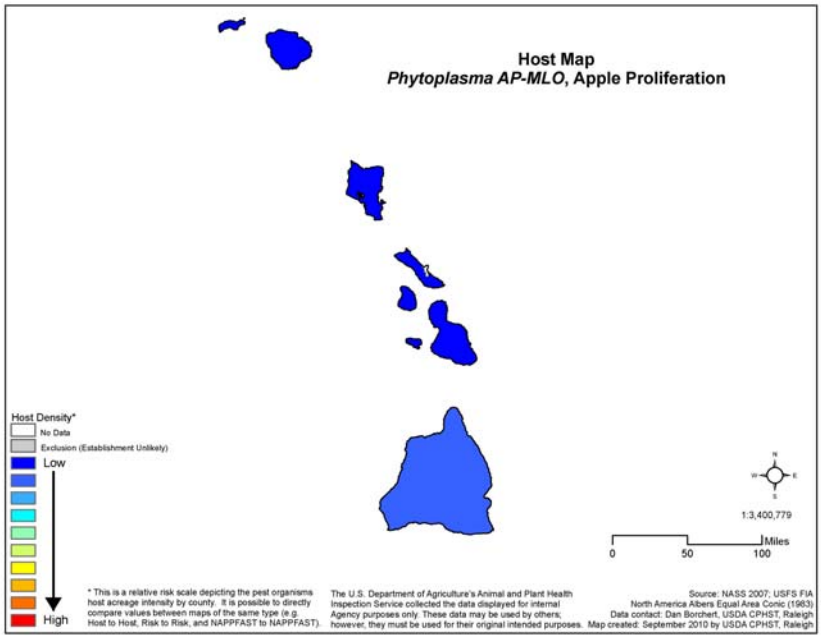


Figure 2-3 Risk Map for Establishment Potential of '*Ca*. P. mali' Within Hawaii

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

'*Candidatus* Phytoplasma australiense' is an imminent threat that could be introduced into the United States with imported plants intended for planting and viable plant parts infected by this pathogen. The Pareto risk map summarizes the overall risk based on combined climate, host and pathways data (*Figure 2-2* on page 2-15). This map shows that portions of the southeastern United States would be favorable for disease development associated with this phytoplasma species. The central and northern areas of the United States have a low to moderate risk of '*Candidatus* Phytoplasma australiense' establishment.

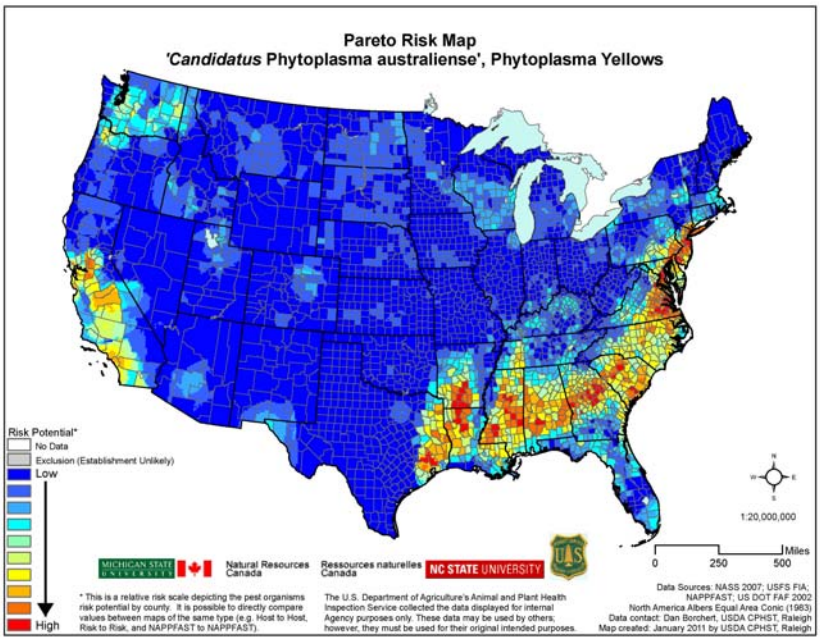


Figure 2-4 Risk Map for Establishment Potential of '*Ca.* P. australiense' Within the Continental United States

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

'*Candidatus* Phytoplasma prunorum' could potentially establish in all areas of the United States where *Prunus* spp. are grown. For this reason, this phytoplasma should be considered an imminent threat that could be introduced into the United States with imported plants intended for planting and viable plant parts infected by this pathogen. A United States map based on density of susceptible hosts indicates the counties in the central part of California to be at moderate risk within the continental United States (*Figure 2-3* on page 2-16).

The only known vector of this phytoplasma, the psyllid *Cacopsylla pruni*, is not present in North America. It is possible that other insects already present in these regions could become vectors once the European stone fruit yellows phytoplasma is accidentally introduced in the United States. The rate of spread for disease associated with phytoplasma is slow and strictly dependent on the flight behavior of the infectious vectors. Infected propagative material may be asymptomatic and may thus move undetected in trade. If infected plants or vectors were to enter the United States, they could transmit the pathogen to U.S. apricot crops and causes significant losses to the U.S. stone fruit industry.

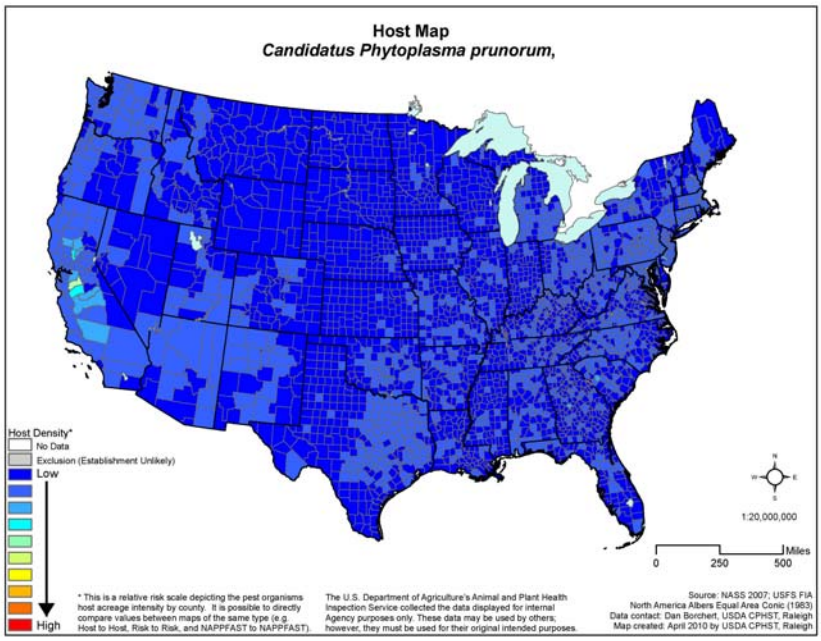


Figure 2-5 Risk Map for Establishment Potential of '*Ca*. P. prunorum' Within the Continental United States

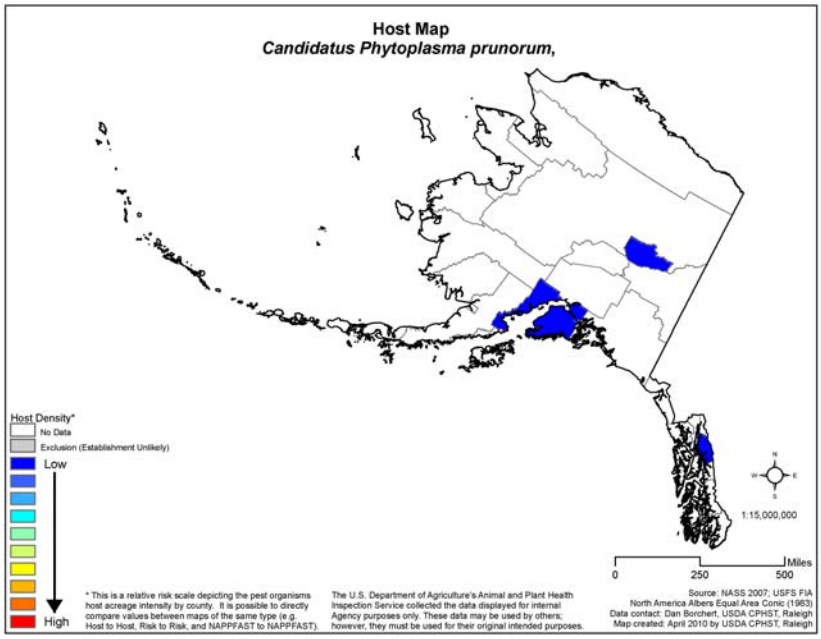


Figure 2-6 Risk Map for Establishment Potential of '*Ca*. P. prunorum' Within Alaska

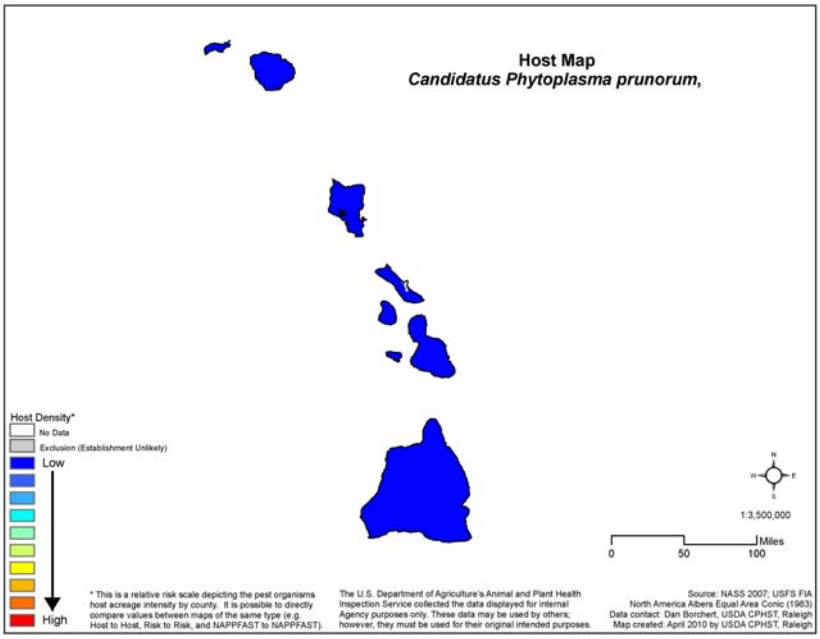


Figure 2-7 Risk Map for Establishment Potential of '*Ca.* P. prunorum' Within Hawaii

Hosts

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

The primary host of apple proliferation (AP) phytoplasma is apple (Rosaceae: *Malus* spp.). CABI lists additional hosts in the plant families Rosaceae (*Pyrus communis* (European pear)), Apocynaceae (*Catharanthus roseus* (pink periwinkle)), Betulaceae (*Corylus avellana* (hazel)), Vitaceae (*Vitis vinifera* (grapevine)), Convolvulaceae (*Convolvulus arvensis* (bindweed)), and Poaceae (*Cynodon dactylon* (Bermuda grass)) (CABI 2011). *Table 2-9* on page 2-20 includes a more comprehensive list of hosts, their geographical location, and their original reference.

Apples are the main host, and most cultivars are susceptible (CABI 2011). Susceptible apple cultivars known to be affected by AP phytoplasma include Belle de Booskop, Gravestein, Golden Delicious and Winter Banana. Highly susceptible apple cultivars known to be affected by AP phytoplasma include Florina, Prima and Priscilla (Loi et al. 1995a). Apple cultivars of medium susceptibility known to be affected by AP phytoplasma include of medium susceptibility, Idared, McIntosh, Starking and Starkrimson (Németh 1986). Tolerant cultivars include Roja de Benejama, Antonokova, Cortland, Spartan, Yellow transparent, Wealthy (Németh 1986). In northern Italy serious

epidemics have been reported to occur on the cultivars Golden Delicious, Florina, Canadian Renette and Granny Smith, grafted on different rootstocks (Osler et al. 2001).

The cultivars Prima, Florina and Priscilla, which are known to be resistant to scab (*Venturia inaequalis*), were derived from cultivars susceptible to apple proliferation such as Golden Delicious, Starking Delicious, McIntosh, Jonathan, Rome Beauty and Malus floribunda 821 (Kartte and Seemüller 1988). The phytoplasma can also be artificially inoculated to *Malus baccata*, *M. coronaria*, *M. domestica*, *M. floribunda*, *M. fusca*, *M. gloriosa*, *M. ionensis*, *M.* × *platycarpa*, *M. purpurea*, *M.* × *robusta* (Németh 1986).

Magnolia species and cultivars have been found to be hosts for phytoplasmas of both the apple proliferation and aster yellows phytoplasma groups, although the specific phytoplasma within the AP group/clade was not identified.

Table 2-9 List of Reported Plant Host of Apple Proliferation Phytoplasma

Latin Name	Common Name	Origin	References
Malus domestica Borkh.	apple	Europe	Kirkpatrick et al. 1994, Seemüller et al. 1994, Lorenz et al. 1995; Rui et al. 1950
Malus prunifolia Desf. ex Steud.[1]	plumleaf crabapple	No data	CABI 2011
Malus pumila Mill.[1]	apple	No data	CABI 2011
Pyrus communis L.	European pear	Hungary	Del Serrone et al. 1998
Catharanthus roseus (L.) G. Don	pink periwinkle	Polland	Davis and Dally 2000
Convolvulus arvensis L.[1]	bindweed	Germany	Schneider et al. 1997
Corylus avellana L.	hazel	Italy	Marcone et al. 1996a
Prunus domestica L.	plum	Tunisia	Ben Khalifa and Fakhfakh 2011a
Prunus salicina Lindl.[1]	Japanese plum	No data	CABI 2011
Prunus persica var. *nucipersica* (Suckow) C.K.Schneid.	nectarine	Polland	Cieslinska and Morgas 2011
Ribes rubrum L.	Ribes	Czech Republic	Navratil et al. 2005
Vitis vinifera L.	grapevine	Chile	Matus et al. 2008
Crataegus monogyna Jacq.	hawthorn	Italy	Tedeschi et al. 2009
Cynodon dactylon (L.) Pers.[1]	Bermuda grass	No data	CABI 2011

Table 2-9 List of Reported Plant Host of Apple Proliferation Phytoplasma

Latin Name	Common Name	Origin	References
Dahlia × *cultorum*	dahlia	Polland	Kamińska and Śliwa 2008b
Lilium spp. L.	lily (cultivar Siberia)	Polland	Kamińska and Śliwa 2008a
Nicotiana occidentalis	native tobacco	No data	Berg et al. 1999
Nicotiana tabacum L.	cultivated tobacco	No data	Berg et al. 1999
Prunus avium (L.) L.	cherry	Slovenia	Mehle et al. 2007
Prunus armeniaca L.	apricot	Slovenia	Mehle et al. 2007
Prunus domestica L.	plum	Slovenia	Mehle et al. 2007

1 Host is not confirmed.

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

Since the 16S rRNA gene sequence of Australian grapevine yellows (AGY) phytoplasma is distinct from that of most other known phytoplasmas, AGY phytoplasma has been provisionally named '*Ca*. P. australiense' (Davis et al. 1997a), and the described strain represents now the reference strain for this species. AGY phytoplasma is classified in 16S rDNA RFLP subgroup 16SrXII-B. Papaya dieback (PDB) phytoplasma from Australia, the *Phormium* yellow leaf (PYL) phytoplasma from New Zealand, and all other phytoplasma strains related to AGY phytoplasma have 16S rRNA gene sequences with minimal nucleotide differences in respect to the AGY phytoplasma reference sequence. Thus, based on the current taxonomic system, all these phytoplasmas are considered strains of '*Candidatus* Phytoplasma australiense' or at least related to it (Liefting et al. 1998).

The 16S rRNA gene sequence of the AGY phytoplasma has a 99.5 percent homology with the PYL phytoplasma 16S rRNA gene sequence (Padovan et al. 1996) and a 99.7 percent homology with the 16S rRNA gene sequence of the PDB phytoplasma (White et al. 1998). Significant sequence variation is likely to occur elsewhere within the genomes of these phytoplasma strains and may reflect differences, among the strains, in biological properties such as host range. However, this circumstance does not affect taxonomic classification, since their 16S rRNA genes share greater than 97.5 percent nucleotide sequence identity with the 16S rRNA gene sequence (GenBank no. L76865) of the reference strain of '*Ca*. Phytoplasma australiense'.

Other than grapevine (*Vitis vinifera*), papaya (*Carica papaya*), strawberry (*Fragaria × ananassa)*, pumpkin (*Cucurbita maxima* and *C. moschata*), mountain flax (*Phormium cookianum*), cabbage tree (*Cordyline australis*), common bean (*Phaseolus vulgaris*), New Zealand flax (*Phormium tenax*) and loganberry (*Rubus loganobaccus*) are the reported alternative host of AGY phytoplasma (CABI 2011). Sweet gum (*Liquidambar styraciflua*) is also affected by a close but distinct (related) strain of AGY phytoplasma (Habili et al. 2007). *Table 2-10* on page 2-22 includes a more comprehensive list of hosts, their geographical location, and their original reference.

Table 2-10 List of Reported Plant Host of Australian Grapevine Yellows Phytoplasma

Scientific Name	Common Name	Origin	References
Apium graveolens L.	celery	New Zealand	Liefting et al. 2011
Carica papaya L.	papaya (papaw)	Australia	Gibb et al. 1996
Catharanthus roseus (L.) G. Don	periwinkle	Australia	Davis et al. 2003
Cicer arietinum L.[1]	chickpea	Australia	Saqib et al. 2006; Saqib et al. 2005
Coprosma robusta Raoul	coprosma	New Zealand	Beever et al. 2004
Coprosma macrocarpa Cheeseman	coprosma	New Zealand	Beever et al. 2004
Cordyline australis (G. Forst.) Endl.	cabbage tree	New Zealand	Andersen et al. 2001
Cordyline banksii Hook. f.	cabbage tree	New Zealand	Andersen et al. 2001
Cucumis myriocarpus Naudin	paddy, melon	Australia	Saqib et al. 2006
Cucurbita maxima Duchesne	pumpkin, great	Australia	Streten et al. 2005a
Cucurbita moschata Duchesne	pumpkin	Australia	Streten et al. 2005a
Exocarpus cupressiformis Labill.	native cherry, cherry ballart	Australia	Streten et al. 2005b
Fragaria spp. L.	strawberry	Australia	Padovan et al. 1998
Fragaria virginiana Duchesne	strawberry	Australia	Padovan et al. 1998
Fragaria × ananassa Duchesne ex Rozier	strawberry	Australia-New Zealand	Padovan et al. 2000; Andersen et al. 1998b
Gomphocarpus fruticosus (L.) W. T. Aiton	swan plant	New Zealand	Liefting et al. 2011

Table 2-10 List of Reported Plant Host of Australian Grapevine Yellows Phytoplasma

Scientific Name	Common Name	Origin	References
Asclepias physocarpa (E. Mey.) Schlechter; synonym: *Gomphocarpus physocarpus* E. Mey	cottonbush balloonplant balloon cotton-bush swan plant	Australia	Streten et al. 2005b
Melilotus indicus (L.) All	hexham scent	Australia	Streten et al. 2005b
Jacksonia scoparia Sm.	dogwood	Australia	Streten et al. 2005b
Liquidambar styraciflua L.	sweetgum	Australia	Habili et al. 2007
Medicago sativa L.	alfalfa	Australia	Getachew et al. 2007
Medicago polymorpha L.	toothed medick	Australia	Streten et al. 2005b
Paulownia fortunei (Seem.) Hemsl.	dragon tree	Australia	Bayliss et al. 2005
Paulownia spp. Siebold & Zucc.	paulownia	Australia	Bayliss et al. 2005
Phaseolus vulgaris L.	bean	Australia	Schneider et al. 1999
Phormium cookianum Le Jol.	mountain fla	New Zealand	Boyce and Newhook 1953
Phormium spp. J.R. & G. Forst.	phormium	New Zealand	Boyce and Newhook 1953
Phormium tenax J.R. and G. Forst.	new zealand flax	New Zealand	Andersen et al. 1998a
Rubus loganobaccus L. H. Bail.[1]	loganberry	Australia	CABI 2011
Rubus ursinus Cham. and Schlecht.	California blackberry	New Zealand	Wood et al. 1999; Liefting et al. 2011
Solanum pseudocapsicum L.	Jerusalem-cherry	New Zealand	Liefting et al. 2011
Solanum tuberosum L.	potato	New Zealand	Liefting et al. 2009b
Trifolium pratense L.	clover, red	Australia	Saqib et al. 2006
Vigna radiata (L.) R. Wilczek [1]	mung bean	Australia	Davis et al. 1997b
Vitis spp. L.	grape	Australia	Padovan et al. 1995
Vitis vinifera L.	grape	Australia	Magarey and Wachtel 1986a
Vitis vinifera L.	grapevine	Australia	Padovan et al. 1995

Table 2-10 List of Reported Plant Host of Australian Grapevine Yellows Phytoplasma

Scientific Name	Common Name	Origin	References
Maireana brevifolia (R. Br.) Paul G. Wilson	small-leaf bluebush	Australia	Magarey et al. 2005
Enchylaena tomentosa R. Br.	ruby saltbush	Australia	Magarey et al. 2005
Euphorbia terracina L.	false caper	Australia	Magarey et al. 2005
Einadia nutans subsp. *linifolia* (R.Br.) Paul G.Wilson	Synonyms: *Rhagodia linifolia* R.Br.	Australia	Magarey et al. 2005

1 Host is not confirmed.

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

'*Candidatus* Phytoplasma prunorom' is generally identified with *Prunus* spp. plants. The primary hosts of European stone fruit yellows (ESFY) phytoplasma are the stone fruit trees *P. armeniaca* (apricot), *P. domestica* (plum) and *P. persica* (peach). CABI (2011) lists additional hosts in the plant families Rosaceae: *P. avium* (sweet cherry), *P. dulcis* (almond), *P. persica* (peach) and *P. salicina* (Japanese plum).

Apocynaceae: *Catharanthus roseus* (pink periwinkle), Betulaceae: *Corylus avellana* (hazel), Convolvulaceae: *Convolvulus arvensis* (bindweed), and Poaceae: *Cynodon dactylon* (Bermuda grass) (CABI 2011).

Table 2-11 on page 2-24 includes a more comprehensive list of hosts, their geographical location, and their original reference.

Table 2-11 List of Reported Plant Host of ESFY Phytoplasma

Latin Name	Common Name	Origin	References
Catharanthus roseus (L.) G. Don	pink periwinkle	Italy	Loi et al. 1995b
Celtis australis L.	hackberry	France	Jarausch et al. 2001b
Convolvulus arvensis L.[1]	bindweed	Spain	Sánchez-Capuchino et al. 1983
Corylus avellana L.	hazel	Italy	Lederer and Seemüller 1992; Marcone et al. 1996a
Cuscuta campestris Yuncker	dodder	Italy	Loi et al. 1995b
Cynodon dactylon (L.) Pers.[1]	Bermuda grass	Spain	Sánchez-Capuchino et al. 1983

Table 2-11 List of Reported Plant Host of ESFY Phytoplasma

Latin Name	Common Name	Origin	References
Fraxinus excelsior L.	ash, European	France	Jarausch et al. 2001b
Prunus amygdalus Batsch	almond	France	Jarausch et al. 2001b
Prunus armeniaca L.	apricot	Spain	Yvon et al. 2009
Prunus avium (L.) L.	cherry sweet	Italy	Carraro et al. 2004
Prunus cerasifera Ehrh.	plum, cherry Myrobolan	Azerbaijan	Carraro et al. 2004
Prunus domestica L.	plum	Germany	Lorenz et al. 1994
Prunus dulcis (Mill.) D. A. Webb	almond	Germany	Lorenz et al. 1994
Prunus laurocerasus L.	cherry-laurel	Italy	Carraro et al. 2004
Prunus mahaleb L.	cherry, Mahaleb	Italy	Carraro et al. 2004
Prunus padus L.	cherry, bird	Italy	Carraro et al. 2004
Prunus persica (L.) Batsch	peach	Germany	Kirkpatrick et al. 1994
Prunus persica var. *nucipersica* (Suckow) C. K. Schneid	nectarine	Germany	Kirkpatrick et al. 1994
Prunus salicina Lindl.	Japanese plum	Spain	Lorenz et al. 1994
Prunus spinosa L.	blackthorn	France	Jarausch et al. 2001b
Prunus serotina Ehrh.[1]	cherry, black		
Prunus serrulata Lindl.	cherry, Japanese flowering	Germany	Lorenz et al. 1994; Yvon et al. 2009
Prunus tomentosa Thunb.	cherry, Nanking	Italy	Carraro et al. 2004
Rosa canina L.	dog rose	France	Jarausch et al. 2001b

1 Host is not confirmed.

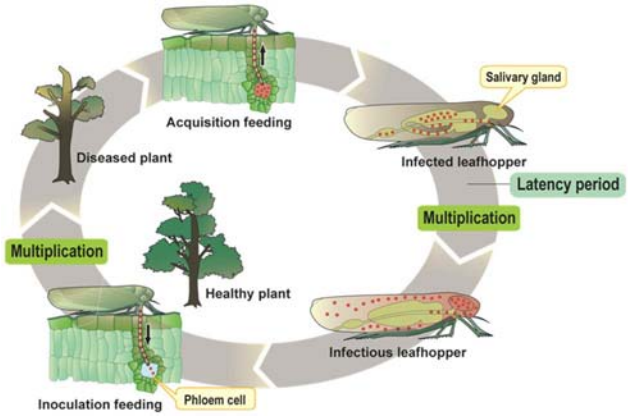
Life Cycle

The biology and reproductive strategies of phytoplasmas are not completely understood. Phytoplasmas are obligate intracellular parasites that occur in the phloem sieve tubes of infected plants and the hemolymph, tissues and salivary glands of insect vectors (*Figure 2-8* on page 2-27). Phytoplasmas are mainly spread by vegetative propagation or grafting of infected plant material and phloem feeding insects. In some hosts, natural transmission by root fusion may sometimes occur, for example, apple proliferation phytoplasma in apple trees. Experimentally, phytoplasmas can be transmitted by the parasitic plant, dodder (*Cuscuta* spp.).

Adult and nymph of the insect vectors acquire the pathogen when they feed on sap from infected trees. Once phytoplasmas have been acquired, the phytoplasma cells move first into the insect midgut, multiply in the hemolymph, and subsequently colonize the salivary glands where they can be expelled during feeding (Tedeschi and Alma 2004). Some phytoplasma vectors retain their infectivity throughout their lives and while overwintering on alternative hosts. Several studies suggest that the overwintering adult population most likely plays the largest role in ESFY phytoplasma transmission (Tedeschi et al. 2002; Thébaud et al. 2009).

Phytoplasma ransmission through seed has been suggested for alfalfa (Khan et al. 2002), coconut fruit (Cordova et al. 2003), lime, oilseed rape and tomato (Botti and Bertaccini 2006) but, in many cases, remains unconfirmed (Nipah et al. 2007a; Nipah et al. 2007b). In the northern hemisphere, symptom development is less likely to occur beyond January/February and the percentage of samples that test positive for phytoplasma using PCR techniques can decline in autumn (Gibb et al. 1999). Distribution of phytoplasmas in the tree is not constant over the year. In winter the content of phytoplasmas declines in the tree due to sieve tube degeneration. They also concentrate more in the roots but, during April to May, reinvade the stem from the roots and reach a peak in late summer or early autumn (Seemüller et al. 1984). The distribution pattern of the phytoplasmas in the tree is also dependent on temperature. In France, phytoplasmas could be found throughout the trees at temperatures of 21 to 25°C, causing symptoms; at 29 to 32°C symptoms were inhibited and phytoplasmas were found only in the roots, but reinvaded the stems when plantlets were stored at the lower temperature (Ducrocquet et al. 1986). Infected trees are particularly sensitive to powdery mildew (*Podosphaera leucotricha*). There appears to be an interaction between apple

rubbery wood disease and apple proliferation, the former promoting transmission of the latter (Bovey 1963 1972; Seidl and Komarkova 1974).



Oshima et al. 2011

Figure 2-8 Phytoplasma Life Cycle

Insect Vectors

Phytoplasmas are spread by insects belonging to order Hemiptera and specifically the families Fulgoridae (planthoppers), Cicadellidae (leafhoppers), and Psyllidae (psyllids).

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

'*Candidatus* Phytoplasma mali' is transmitted in a circulative, propagative manner by two species of psyllids present in Europe: *Cacopsylla melanoneura* (Foerster) and *C. picta* (Foerster) (Frisinghelli et al. 2000; Jarausch et al. 2003; Tedeschi et al. 2002). These psyllids have similar life cycles with one generation per year but different efficiencies as phytoplasmas vectors.

In Europe, *C. picta* (syn. *C. costalis*) is monophagous on *Malus* spp. and is considered the main vector in northeastern Italy (Carraro et al. 2001). *Cacopsylla melanoneura* is oligophagous on Rosaceae (*Malus*, *Pyrus* and *Crataegus* spp.) with a Palaearctic distribution including Germany and northwestern Italy (Tedeschi et al. 2002). A third reported vector of AP phytoplasma is the leafhopper *Fieberiella florii* (Stål), (Krczal et al. 1989; Tedeschi and Alma 2006) (*Table 2-12* on page 2-29). These species spend only a few months during the winter and spring feeding and reproducing on wild or cultivated rosaceous before newly emerged adults migrate to alternative hosts at the end of spring (Carraro et al. 2001; Tedeschi and Alma 2004).

The leafhopper *Fieberiella florii* occurs both in Europe and North America. In North America, *F. florii* is a vector of X-disease of stone fruit (Tedeschi and

Alma 2006). *Fieberiella florii* completes one generation per year, overwintering on woody plants in the egg stage. In Europe, *F. florii* is a vector of AP phytoplasma, occupying apple trees in the late spring through summer (during the time when *Cacopsylla melanoneura* and *C. costalis* are absent) (Tedeschi and Alma 2006) (*Figure 2-9* on page 2-29). *Fieberiella florii* is therefore present during times when the AP phytoplasma titer within the tree is high. In general, however, *F. florii* is believed to be an inefficient vector of AP phytoplasma, and typically appears in apple orchards in low densities (Tedeschi and Alma 2006).

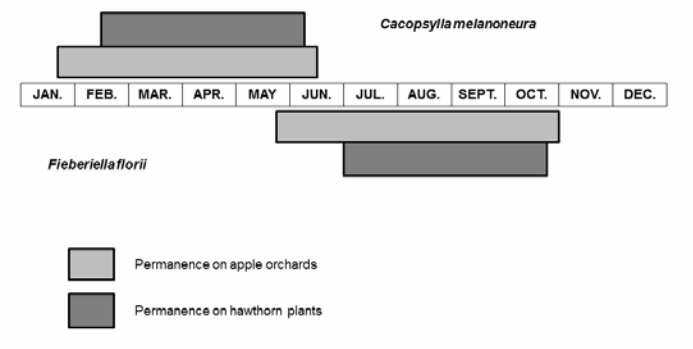
While *F. florii* may not transmit AP phytoplasma as frequently or as well as *C. melanoneura* and *C. costalis*, its presence during months when trees have higher concentration of AP phytoplasma within the foliage, make this a potentially significant vector of AP phytoplasma (Tedeschi and Alma 2007). Moreover, *F. florii* is a highly polyphagous species, making this leafhopper a potentially serious vector of AP phytoplasma.

A possible role as phytoplasma vector was suggested for other two psyllid species that feed on *Crataegus monogyna* (Hawthorn), *Cacopsylla peregrina* (Foerster) and *C. affinis* (Löw) (Tedeschi et al. 2009). Detection of '*Candidatus* Phytoplasma mali' through nested PCR was confirmed for *C. peregrina* but is not yet established for *C. affinis*. There is also one isolated record for '*Ca*. P. mali' detected in *Empoasca* sp. in Cuba (Arocha et al. 2004). *Figure 2-10* on page 2-29 through *Figure 2-22* on page 2-33 represent nymph and adult images of these known and potential vectors of '*Candidatus* Phytoplasma mali'.

Cacopsylla melanoneura is a difficult species to confirm photographically, requiring examination of the male genitalia to separate from *C. affinis*.

Table 2-12 Vectors of Apple Proliferation Phytoplasma

Phylum	Arthropoda	Arthropoda	Arthropoda
Class	Insecta	Insecta	Insecta
Order	Hemiptera	Hemiptera	Hemiptera
Superfamily	Psylloidea	Psylloidea	Cicadelloidea
Family	Psyllidae	Psyllidae	Cicadellidae
Genus	Cacopsylla	Cacopsylla	Fieberiella
Scientific Name	*Cacopsylla melanoneura* (Foerster)	*Cacopsylla picta* (Foerster)	*Fieberiella florii* (Stål)



Tedeschi and Alma 2007

Figure 2-9 Presence Period of *Cacopsylla melanoneura* and *Fieberiella florii* in Apple Orchards and on Hawthorn Bushes

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-10 *Cacopsylla affinis* Mating Pair

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-11 *Cacopsylla melanoneura* Adult Female

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-12 *Cacopsylla melanoneura* Adult Female

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-13 *Cacopsylla melanoneura* 5th instar nymph

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-14 *Cacopsylla melanoneura* Adult Male

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-15 *Cacopsylla melanoneura* Adult Female

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-16 *Cacopsylla peregrina* Nymph

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-17 *Cacophylla peregrina* Adult Female

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-18 *Cacopsylla peregrina* Adult Male

Julius Kühn Institute,
Federal Research Centre for Cultivated Plants

Figure 2-19 *Cacopsylla picta* (Foerster)

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-20 *Fieberiella florii* Adult

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-21 *Fieberiella florii* Adult

Joe Botting
Tristan Bantock http://www.britishbugs.org.uk

Figure 2-22 *Fieberiella florii* Nymph

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

The only confirmed insect vectors of '*Candidatus* Phytoplasma australiense' are the planthoppers *Zeoliarus atkinsoni* (Myers 1924; *Oliarus*) and *Zeoliarus oppositus* (Walker, 1851; *Oliarus*) (comb. Larivière & Fletcher (2008)) (Beever et al. 2008; Cumber 1953; Liefting et al. 1997) (*Figure 2-23* on page 2-35 through *Figure 2-25* on page 2-36). Both species of planthopper are endemic to New Zealand where they are widespread. *Z. atkinsoni* is uniquely associated with *Phormium*. In contrast, *Z. oppositus* is polyphagous and has been reported from many different plants.

The insect vector of '*Candidatus* Phytoplasma australiense' in grapevines and other host in Australia is still undetermined. AGY phytoplasma has been detected in the common brown leafhopper, *Orosius orientalis* (Matsumura) (also *Orosius argentatus* (Evans)), using PCR techniques (Beanland et al. 1999), however transmission studies need to be carried out to establish its role as a vector. *O. orientalis* is a vector of phytoplasmal diseases in Australia other than AGY. It has a very wide host range and is common throughout southern Australia, is the only opsiine to be found in New Zealand, and its range extends through Indonesia, Norfolk Island, Fiji, Polynesia, Java, Melanesia, Africa, New Britain to Korea, Taiwan and Japan. Refer to *Resources* on page A-1 for the Web site address for the key to leafhopper and treehopper genera occurring in New Zealand.

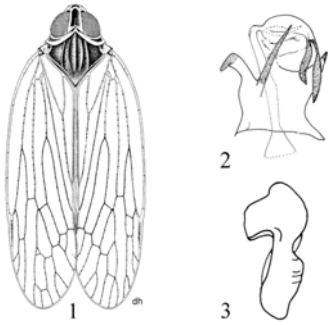
Orosius orientalis was the most abundant of all phloem-feeding insects captured in Australian vineyards during several seasons, although higher numbers were located on the vineyard floor amongst weeds and cover crops rather than within the grapevine canopy (Beanland et al. 1999). There may be other planthoppers and leafhoppers that are vectors of '*Ca*. P. australiense' in Australia and New Zealand.

Zeoliarus oppositus (Walker, 1851), a species closely related to *Z. atkinsoni*, is more polyphagous and was found on both *Coprosoma robusta* and *Phormium tenax* (Beever et al. 2004) (*Figure 2-24* on page 2-35). Its ability to vector '*Ca*. Phytoplasma australiense' needs to be confirmed.

Orosius orientalis (Matsumura 1914), the common brown leafhopper (synonym = O. argentatus (Evans)), is a vector of several phytoplasma diseases including legume little leaf, tomato big bud, lucerne witches broom, potato purple top wilt and pawpaw yellow crinkle (*Figure 2-25* on page 2-36). It has also been implicated as a possible vector of grapevine yellows and pawpaw yellows.

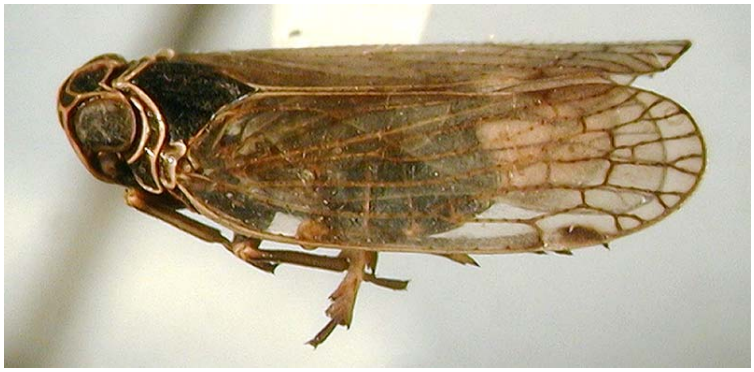
Table 2-13 Vectors of Australian Grapevine Yellows Phytoplasma

Phylum	Arthropoda	Arthropoda
Class	Insecta	Insecta
Order	Hemiptera	Hemiptera
Superfamily	Fulgoroidea	Fulgoroidea
Family	Cixiidae	Cixiidae
Genus	Zeoliarus	Zeoliarus
Scientific Name	*Zeoliarus atkinsoni* (Myers 1924; *Oliarus*) (comb. Larivière & Fletcher (2008))	*Zeoliarus oppositus* (Walker, 1851; *Oliarus*) (comb. Larivière & Fletcher (2008))



Lariviere and Fletcher 2008

Figure 2-23 *Zeoliarus atkinsoni* (Myers, 1924) 1: Habitus, Dorsal Aspect (Body Length: 8mm); 2: Male Aedeagus, Ventral Aspect; 3: Male Genital Style, Ventrolateral Aspect



http://www1.dpi.nsw.gov.au/keys/fulgor/nz/species/oopposit.htm

Figure 2-24 *Zeoliarus oppositus* (Walker, 1851)

http://commons.wikimedia.org/wiki/File:Orosius_orientalis.jpg

Figure 2-25 *Orosius orientalis* (Matsumura, 1914) Common Brown Leafhopper

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

'*Candidatus* Phytoplasma prunorum' is transmitted in a persistent manner by the psyllid *Cacopsylla pruni* (Carraro et al. 2001; Carraro et al. 1998) in various Europen countries. *Cacopsylla pruni* is narrowly oligophagus on *Prunus* spp. and wild *Prunus* plants, mainly blackthorn (*P. spinosa*), are the preferred host. This psillid is monovoltine and hibernates as adults on conifers. The period of egg deposition occurs on *Prunus* spp. soon after the winter. Five larval instars can be observed up to early summer. Later, immature adults abandon fruit trees and can be observed on conifers where they overwinter. A study from Thébaud (2009) indicats that *C. pruni* increases their transmission efficiency to 60 percent after a latency period of eight months and only after it has moved to conifers. A Web link address for a pictorial key of central European *Cacopsylla* species associated with *Rosaceae* is provided in the References.

A second vector of ESFY phytoplasma has been reported, the leafhopper *Empoasca decedens* (*Asymmetrasca decedens*) (original genus: Empoasca) and other *Empoasca* spp. (*Figure 2-27* on page 2-38 through *Figure 2-28* on page 2-38). In apricot-plum experimental orchard in central Italy, *Empoasca* spp. were tested and confirmed vectors of 16SrX-B phytoplasma subgroup (Nicotina and Ragozzino 1991; Pastore et al. 2004; Pastore et al. 2010). The relevance of *Empoasca* spp. in the spread of ESFY remains to be evaluated.

Other cicadellids, species in the subfamilies Agalliinae (*Austroagallia sinuata*) or Deltocephalinae (*Euscelis lineolata*, *Neoaliturus fenestratus*, *N. haematoceps* and *Psammotettix striatus*) were considered to be the most likely vectors of ESFY in some apricot orchards from Valencia, Spain (Llacer et al.

1986). A positive identification of ESFY phytoplasma was reported in a single individual of the deltocephalid *Synophropsis lauri* captured in an experimental orchard with apricot trees in southern France (Jarausch et al. 2001a).

The possible transovarial transmission of two phytoplasmas, '*Candidatus* Phytoplasma prunorum' (associated with European stone fruit yellows) and '*Candidatus* Phytoplasma mali' (Apple proliferation) by their respective psyllid vectors *Cacopsylla pruni* and *C. melanoneura*, was investigated in Italy (Tedeschi et al. 2006). Results showed that *C. pruni* could transmit '*Ca*. P. prunorum' transovarially, as it could be detected in the progeny of infected females (i.e. eggs, nymphs and newly emerged adults). It was also shown that psyllids which had acquired the phytoplasma transovarially could then transmit it by feeding on a healthy plum seedling. The fact that the insect is not only a vector but also a reservoir for the phytoplasma has implications for disease management.

Table 2-14 Vectors of European Stone Fruit Yellows Phytoplasma

Phylum	Arthropoda	Arthropoda
Class	Insecta	Insecta
Order	Hemiptera	Hemiptera
Superfamily	Psylloidea	Cicadelloidea
Family	Psyllidae	Cicadellidae
Genus	Cacopsylla	Asymmetrasca
Scientific Name	*Cacopsylla pruni* (Scopoli, 1763)	*Asymmetrasca decedens* (Paoli 1932)

Alberto Loschi, http://www.fitoplasmi.it

Wolfgang Jarausch

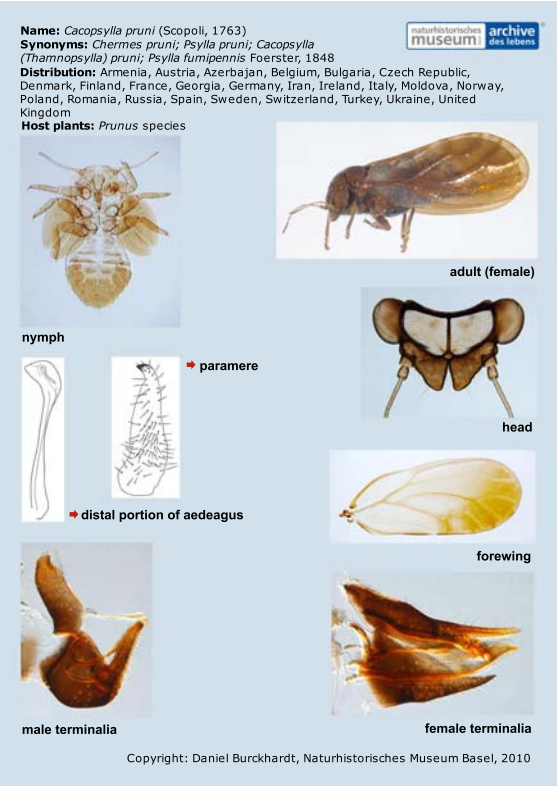
Figure 2-26 *Cacopsylla pruni*, Vector of '*Candidatus* Phytoplasma prunorum'

Joaquín Torres (IVIA-Valencia)

Figure 2-27 *Asymmetrasca decedens* Adult

Joaquín Torres (IVIA-Valencia)

Figure 2-28 *Asymmetrasca decedens* Nymph



Burckhardt, Naturhistorisches Museum Basel http://www.psyllidkey.com

Figure 2-29 *Cacopsylla pruni* Fact Sheets

Environmental Impact

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

Introduction of this pathogen could have some negative impacts on the environment. Plant hosts of the apple proliferation (AP) phytoplasma may include *Lilium occidentale, L. pardalinum* subsp. *pitkinense* and *Prunus geniculata* which are listed as federally threatened or endangered (USFWS 2011). Chemical control programs may be initiated in the event of an introduction of the AP phytoplasma in the United States, which may negatively impact nontarget pests and the environment.

'*Candidatus* Phytoplasma australiense' *(*Australian Grapevine Yellows)

Introduction of this pathogen into the United States could have negative impacts on the natural environment. Plant hosts of Australian grapevine yellows (AGY) phytoplasma include *Cucurbita okeechobeensis* ssp. *okeechobeensis* and *Euphorbia* spp.*, Coprosma* spp.*,* and *Asclepias* spp., some of which are listed as federally threatened or endangered in the United States (USFWS 2011). Chemical control programs for the insect vector(s) that could be initiated in the event of an introduction of the AGY phytoplasma into the United States, may negatively impact nontarget pests and the environment.

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

Introduction of this pathogen into the United States could have negative impacts on the environment. Plant hosts of the European stone fruit yellows ESFY phytoplasma may include *Prunus geniculata*, which is listed as federally threatened or endangered, as well as *Prunus alleghaniensis*, *Prunus alleghaniensis* var. *davisii*, *Rosa minutifolia* and *Rosa stellata* subsp. *abyssa* listed as species of concern (USFWS 2011). Chemical control programs may be initiated in the event of an introduction of the ESFY phytoplasma in the United States may negatively impact nontarget pests and the environment.

Chapter

3

Identification

Contents

Introduction

Use *Chapter 3 Identification* as a guide to recognizing any of the selected '*Candidatus* Phytoplasma' species of apple, grape and peach: '*Candidatus* Phytoplasma mali' (apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows). Accurate identification of these pathogens can only be achieved with molecular techniques. Recognition of plant characteristic symptoms associated with phytoplasma diseases is the initial but essential step towards this process.

Authorities

Qualified State, County, or cooperating university personnel may perform preliminary identification and screening of suspect '*Candidatus* Phytoplasma spp.' Before survey and control activities are initiated in the United States, an authority recognized by USDA–APHIS–PPQ-National Identification Services must confirm the identity of such pests. Submit specimens to the USDA National Identification Services (NIS). For further information refer to *How to Submit Plant Samples* on page C-1 and *Taxonomic Support for Surveys* on page D-1.

Reporting

Forward reports of positive identifications by national specialists to PPQ-National Identification Service (NIS) in Riverdale, Maryland, according to Agency protocol. NIS will report the identification status of these tentative and confirmed records to PPQ-Emergency and Domestic Programs (EDP). EDP will report the results to all other appropriate parties. For further information refer to *Taxonomic Support for Surveys* on page D-1.

Symptoms

This section describes the plant symptoms that are characteristic of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach.

Before the introduction of more discriminating serological and molecular detection techniques, phytoplasmas were identified and grouped based on the induced plant symptoms, their reported host range and known vectors. In the past, detection methods relied on direct examination of phloem tissues by electron microscopy or by fluorescent staining. Unfortunately this detection method is complicated by phytoplasmas variable titer levels and uneven distribution, especially in nonherbaceous plants. For a list of characteristic symptoms of plants infected with phytoplasmas refer to *Detection Survey* on page 4-4.

Diagnostic Test

Biological Assay

Positive identification requires transmission to a woody indicator species.

Biological Assay Available for Apple Proliferation

The apple species *Malus* × *dawsoniana* Rehder is considered a very sensitive indicator, when grafted in June on the scion, develops symptoms the following autumn (Morvan and Castelain 1975). Using the double budding technique, the reaction appears after budbreak. Alternatively, *M. pumila* 'Golden Delicious' can be utilized for field testing with root grafting in five replicates and for two consecutive years.

DAPI Staining

Thin sections of young tissues (petioles of young leaves, or phloem tissues of shoots, branches and roots), are stained with 1 µg/mL DAPI solution (4'6 diamidino-2-phenylindole). Sections are observed under a fluorescence microscope (*Figure 3-1* on page 3-5). A bluish fluorescence (at a wavelength of 460 nm) in the sieve tubes indicates the likely presence of phytoplasmas (Seemüller 1976). This method requires good experience of observing slides and is not always sufficiently sensitive. The advantages of this method include rapidity and low cost, but it is not specific for any particular phytoplasma. As for all other diagnostic test, for each sample, sections of young tissues should be taken from different parts of the plant, because of the uneven distribution of the phytoplasma.

Serological Assay

For detection, especially for testing a large number of samples, ELISA tests have been used when phytoplasma-specific polyclonal and/or monoclonal antibodies were available (Berg et al. 1999; Seemüller and Schneider 2004).

Serological Assay Available for Apple Proliferation

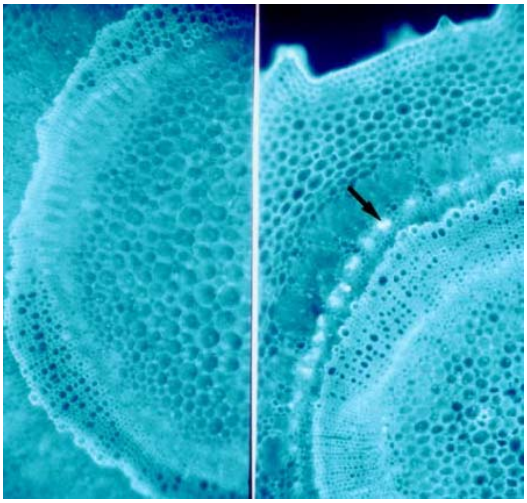
'*Candidatus* Phytoplasma mali' was detected in infected apple trees using monoclonal antibodies (mAbs) to AP phytoplasma obtained from infected *Catharanthus roseus* as source of antigen (Loi et al. 2002). Alternatively, specific polyclonal antibodies (pABs) were prepared to the expression product, in *E. coli*, of an individual immunodominant membrane protein (IMP) isolated from AP phytoplasma (Berg et al. 1999).

The most reliable results can be obtained when leaf midribs or stems collected from late spring to end of summer (June to end of September) are tested. Leaf samples should be collected randomly all around the plant, because of the uneven distribution of phytoplasma cells in the foliage. In cooler climates and in case of latent infections, in Northern and Western Europe, ELISA may not be sensitive enough to detect the relatively low concentrations, so testing may be unreliable. This method has been replaced in practice by PCR, which is versatile, more specific and highly sensitive.

PCR Analysis

Molecular techniques may constitute the only method of specific identification for this pathogen. Polymerase chain reaction (PCR) based assays have been developed to selectively amplify phytoplasma 16S rRNA gene because of its conservation throughout the prokaryotes (Ahrens and Seemüller 1992; Deng and Hiruki 1991; Lee et al. 1993; Smart et al. 1996). The taxonomic classification of phytoplasmas is based on direct comparisons of the nucleotide sequence of 16S ribosomal ribonucleic acid (rRNA) genes. 16S rRNA genes are present in all prokaryotic organisms and contain both variable and highly conserved regions, which make them suitable for phylogenetic classifications of many bacteria including the mollicutes (*Figure F-1* on page F-3).

Phytoplasmas have two 16S rRNA genes and each gene is ca 1500 base pairs (Schneider and Seemüller 1994). Based on the sequence analysis of the 16S rRNA gene it has been shown that phytoplasmas form a distinct cluster within the class Mollicutes (Gundersen et al. 1994; Kuske and Kirkpatrick 1992; Lee et al. 1993; Lim and Sears 1989; Seemüller et al. 1994). An alternative method of studying phytoplasmas' genetic diversity utilizes single strand conformation polymorphism (SSCP). The electrophoretic separation of heat-denatured PCR products can be used to discriminate between similar sequences derived from closely related strains. In particular, the membrane associated ATP-dependent Zn proteases *hflB* gene has proven to be an appropriate target to exploit its highly variable sequence for SSCP based analysis (Schneider and Seemüller 2009). A more exhaustive description of PCR procedures for the identification of phytoplasmas can be found in *Disease and Pathogen Common Names and Acronyms* on page E-1 together with a partial list of Phytoplasma semi-universal primers (*Table E-1* on page E-2).



Musetti and Favali 2004

Figure 3-1 *Catharanthus roseus* L. Stems, Healthy (left) and Infected With Phytoplasma (right)[1]

1 Arrow indicates fluorescent bright spots, visible within the phloem, diagnostic for the presence of phytoplasmas.

Similar Species

The identification of specific phytoplasmas relies solely on PCR-based analysis of their 16S rRNA gene (Lee et al. 1993, Seemüller et al. 1998b) (*Disease and Pathogen Common Names and Acronyms* on page E-1). The GenBank deposited reference sequence associated with the three selected '*Candidatus* Phytoplasma spp.' are reported in *Disease and Pathogen Common Names and Acronyms* on page E-1 with their respective Accession number AJ542541.

The IRPCM Phytoplasma/Spiroplasma Working Team-Phytoplasma Taxonomy Group (Firrao et al. 2004) concluded that “a '*Candidatus* Phytoplasma' species description refers to a single, unique 16S rRNA gene sequence (>1200 bp)”, and that “a strain can be recognized as a novel species if its 16S rRNA gene sequence has <97.5 percent similarity to that of any previously described '*Ca*. Phytoplasma' species”. A series of phytoplasma generic (universal) primers are available for detection of a wide array of phytoplasmas (*Table E-1* on page E-2).

Many of the plant host, where infection of '*Ca*. P. mali', '*Ca*. P. australiense' and '*Ca*. P. prunorum' has been documented, can be co-infected by several strains at

the same time (Lee et al. 1995). The observation of characteristic phytoplasma infected plant symptoms is consequently a fundamental but initial step towards targeted phytoplasma identification.

Similar Diseases

Similar to Apple Proliferation

Similar symptoms are caused by phylogenetically related but distinct phytoplasmas referred as European stone fruit yellows phytoplasma (ESFY phytoplasma), '*Candidatus* Phytoplasma prunorum' and pear decline phytoplasma (PD phytoplasma), '*Candidatus* Phytoplasma pyri'. Based on their 16S rRNA gene sequence, these strains are 98.6 percent and 99.0 percent similar. Plant hosts reported for ESFY phytoplasma shared with '*Candidatus* Phytoplasma mali' include: *Convolvulus arvensis* (bindweed); *Cynodon dactylon* (Bermuda grass); *Prunus armeniaca* (apricot); *P. avium* (sweet cherry); *P. domestica* (plum); *P. salicina* (Japanese plum). While plant hosts reported for PD phytoplasma in common with AP phytoplasma include: *Catharanthus roseus* (Pink periwinkle); *Corylus avellana* (hazel); *Malus domestica* (apple); *Prunus salicina* (Japanese plum); *Pyrus communis* (European pear). A distantly related phytoplasma, '*Candidatus* Phytoplasma pruni' belonging to the 16SrIII group, is the aetiological agent of Western X-disease also affecting peach and cherry in North America with symilar symptoms.

Similar to Australian Grapevine Yellows

Symptoms similar to those associated with '*Candidatus* Phytoplasma australiense' are caused by phylogenetically distinct phytoplasmas referred to as grapevine yellows (GY) diseases. In Europe: Flavescence dorée, bois noir (black wood), Jaunisse de la Vigne, and German grapevine yellows or Vergilbungskrankheit (VK). North America: Virginia grapevine yellows I, Virginia grapevine yellows III, and New York grapevine yellows. Some other grapevine yellows are also reported in South Africa and Chile. Symptoms of general stunting and yellowing of leaves can also be observed in cultivated strawberry infected with StrawY phytoplasma. Comparison of a 1.3 kb region of the StrawY 16S rRNA gene with the corresponding region from '*Ca.* P. australiense' revealed a 97.4 percent similarity and allowed recognition of StrawY phytoplasma as representative of a new taxon: '*Candidatus* Phytoplasma fragariae' (Valiunas et al. 2006).

Similar to European Stone Fruit Yellows

Symptoms similar to those associated with '*Candidatus* Phytoplasma prunorum' on *Prunus* spp. are caused by AP phytoplasma, '*Candidatus* Phytoplasma mali' and PD phytoplasma, '*Candidatus* Phytoplasma pyri' Several strains associated with peach yellow leaf roll (PYLR) reported on *Prunus* spp. in parts of California are closely related to the pear decline agent (Kison et al. 1997). The two phytoplasmas share 99.6 percent 16S rDNA sequence similarity (Seemüller and Schneider 2004). A distantly related phytoplasma, '*Candidatus* Phytoplasma pruni' belonging to the 16SrIII group, is the aetiological agent of Western X-disease also affecting peach and cherry in North America with symilar symptoms. General yellows symptoms are observed on peach trees infected with '*Ca*. P. phoenicium', '*Ca*. P. asteris' and '*Ca*. P. australiense'. A *Prunus persica* tree exhibiting chlorosis and leaf malformation was observed, in 2007, during a survey of fruit tree orchards in Azerbaijan (Balakishiyeva et al. 2010). Molecular analysis indicated the presence of '*Candidatus* Phytoplasma brasiliense', a phytoplasma belonging to the 16SrXV group. This was the first report of this phytoplasma associated with peach. '*Ca*. P. brasiliense' is also associated with *Hibiscus* spp. and was previously reported in Brazil. It is not known to occur in the United States.

Plant hosts reported for ESFY phytoplasma include: *Catharanthus roseus* (Pink periwinkle); *Convolvulus arvensis* (bindweed); *Cynodon dactylon* (Bermuda grass); *Prunus armeniaca* (apricot); *P. avium* (sweet cherry); *P. domestica* (plum); *P. salicina* (Japanese plum). Shared plant hosts reported for PD phytoplasma include: *Catharanthus roseus* (Pink periwinkle); *Corylus avellana* (hazel) and *P. salicina* (Japanese plum). Shared plant hosts reported for AP phytoplasma: *Catharanthus roseus*; *Convolvulus arvensis*; *Corylus avellana; Cynodon dactylon*; *P. salicina; P. persica*; *P. avium*; *P. armeniaca* and *P. domestica*.

Chapter

4

Survey Procedures

Contents

Introduction

Use *Chapter 4 Survey Procedures* as a guide when conducting a survey for any of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach: '*Candidatus* Phytoplasma mali' (Apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows).

Survey Types

Plant regulatory officials will conduct detection, delimiting, and monitoring surveys for any of the selected '*Candidatus* Phytoplasma spp.'. Conduct a detection survey to ascertain the presence or absence of '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum' in an area where it is **not** known to occur. After a new U.S. detection, conduct a delimiting survey to define the extent of an infestation. Conduct a monitoring survey to determine the success of control or mitigation activities conducted against the pest.

Preparation, Sanitization, and Clean-Up

This section provides information that will help personnel prepare to conduct a survey; procedures to follow during a survey; and instructions for proper cleaning and sanitizing of supplies and equipment after the survey is finished.

1. Before starting a survey, determine if there have been recent pesticide applications that would make it unsafe to inspect the vineyards, rootstock nursery, or landscape planting. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or greenhouses.
2. Conduct the survey at the proper time. Studies have shown that the phytoplasma is easier to be detected during warmer months, in areas that experience seasonal weather. Based upon the pests reported global distribution, scientists believe '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum' could establish throughout the apple, grape and peach producing area in the United States. For surveys directed at their vectors, survey and trapping efforts should focus on months when host plants are actively growing.

3. Obtain permission from the landowner before entering a property.
4. Determine if quarantines for other pests, or other crops, are in effect for the area being surveyed. Comply with any and all quarantine requirements.
5. When visiting the area to conduct surveys or to take samples, everyone must take strict measures to prevent contamination by '*Ca*. P. mali', '*Ca*. P. australiense' and '*Ca*. P. prunorum' or other pests between properties during inspections.
6. Before entering a new property, make certain that clothing and footwear are clean and free of pests and soil to avoid moving soilborne pests and arthropods from one property to another.

   Wash hands with an approved antimicrobial soap. If not using an antimicrobial soap, wash hands with regular soap and warm water to remove soil and debris. Then use an alcohol-based antimicrobial lotion, with an equivalent of 63 percent ethyl alcohol. If hands are free of soil or dirt, the lotion can be applied without washing. Unlike some antimicrobial soaps, antimicrobial lotions are less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.
7. Gather together all supplies. Confirm the equipment and tools are clean. When taking plant samples, disinfest tools with bleach to avoid spreading diseases or other pests. A brief spray or immersion of the cutting portion of the tool in a 5 percent solution of sodium hypochlorite (bleach) is an effective way to inactivate bacterial and other diseases and prevent their spread.
8. Mark the plant, tree or sampled location with flagging whenever possible, and draw a map of the immediate area and indicate reference points so that the areas can be found in the future if necessary. Do not rely totally on the flagging or other markers to re-locate a site as they may be removed. Record the GPS coordinates for each trap or infested tree location so that the area or plant may be re-sampled if necessary.
9. Survey task forces should consist of an experienced survey specialist or entomologist familiar with '*Ca*. P. mali', '*Ca*. P. australiense', '*Ca*. P. prunorum' and the symptoms of their damage.

Detection Survey

The purpose of a detection survey is to determine if a pest is present in a defined area. This can be broad in scope, as when assessing the presence of the pest over large areas or it may be restricted to determining if a specific pest is present in a focused area.

Statistically, a detection survey is not a valid tool to claim that a pest does not exist in an area, even if results are negative. Negative results can be used to provide clues about mode of dispersal, temporal occurrence, or industry practices. Negative results are also important when compared with results from sites that are topographically, spatially, or geographically similar.

Procedure

Follow this procedure when conducting a detection survey for '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'.

1. Use visual inspection to examine the cultivated host plants apple, grape and peach for yellowing symptoms. Refer to *Visual Inspection for Detection Survey* on page 4-21 for further information on inspection procedures.

Important Detection surveys for plant infected by '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'or other cultivated hosts in fields should be conducted by State inspectors in conjunction with Federal PPQ inspectors.

2. To confirm disease, collect plants showing typical symptoms. Place samples in plastic bags. Keep samples cool. Double bag the samples and deliver promptly to a diagnostic laboratory.

The CAPS-approved survey method for all of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach is based on collecting symptomatic plant tissue identified by visual survey. The best period to collect aboveground tissue sample is between late summer to early fall. At least five samples per plant should be collected due to the low titer and erratic distribution of the pathogen in the plant phloem. Phytoplasmas are present in the roots of infected plant year round.

Symptoms begin to appear in late spring and increase in incidence until January or February. Beyond this time, symptoms begin to disappear as symptomatic leaves and shoots fall from the tree. New symptoms are less likely to develop after February. Plant diseases caused by phytoplasmas can

produce both external and internal symptoms, *Table 4-1* on page 4-5 list the most common ones and shown below is a series of images of typical symptoms associated with infections by the three phytoplasma species ('*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum') on their main hosts (*Figure 4-1* on page 4-8 through *Figure 4-15* on page 4-17).

Based on the CAPS survey manuals, described in the remainder of this section are the symptoms present in association with the three selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach.

Table 4-1 External and Internal Symptoms of Plants Infected by Phytoplasma[1]

External	Internal
Generalized growth reduction (stunting), reduced foliar size	Excess growth of phloem tissue
Generalized decline	Swollen veins
Proliferations of axillary buds and little leaf (witches'broom)	Phloem necrosis
Flower sterility, premature fruit drop	Root decay
Virescence (development of green flowers)	Formation of bunchy fibrous secondary roots
Rosettes (shortened internodes with many leaves)	
Bolting (growth of elongated stalks)	
Enlarged stipules	
Phyllody (production of leaf-like petals and sepals)	
Discoloration of leaves and shoots	
Rolling of leaves	
Unseasonal yellowing or reddening of leaves and stems	
Reduced quality and quantity of fruit	

1 Plants infected by phytoplasmas may exhibit one or more of the following symptoms.

Symptoms of Apple Proliferation Associated with '*Candidatus* Phytoplasma mali'

Trees infected by apple proliferation often occur in clusters, and these clusters grow year by year (Bliefernicht and Krczal 1995). Symptoms are unevenly distributed on the plants. Additionally, there is considerable variability in virulence in '*Candidatus* Phytoplasma mali'. Based on symptomatology, the phytoplasma strains can be defined as avirulent to mildly, moderately, or highly virulent; and the trees can be simultaneously affected by more than on strain of the apple proliferation phytoplasma (Seemüller et al. 2010; Seemüller and Schneider 2007).

Apple

Trees affected by the apple proliferation phytoplasma in 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. Diseased trees may die, but often recover if adequately fertilized.

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' broom) (*Figure 4-1* on page 4-8). 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. The angle between these secondary shoots and the main shoots is abnormally narrow on infected trees (Bovey 1963). The witches’ brooms do not develop repeatedly on the same branch. They may appear successively on various parts of the tree, or all at once over the whole tree, but usually develop only during the first two or three years following infection.

Leaves will 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 (*Figure 4-2* on page 4-8). 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 (*Figure 4-3* on page 4-8). 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).

Cherry

Symptoms of apple proliferation in cherry include wilting, dying, and floral and phloem necrosis (Mehle et al. 2007).

Apricot

Symptoms of apple proliferation in apricot include stem necrosis and leaf wilting (Mehle et al. 2007).

Plum

The primary symptom of apple proliferation in plum is late blooming (Mehle et al. 2007).

Dahlia

Symptoms of apple proliferation in dahlia include bushy growth accompanied by shoot proliferation, narrowed leaves, and flower bud deficiency (Kamińska and Śliwa 2008b). Note: plants in this study were co-infected with apple proliferation and aster yellows phytoplasmas.

Rose

Symptoms of apple proliferation in rose include dieback, witches' broom, bud proliferation, stunted growth, leaf and flower malformation, and shoot and flower proliferation (Kaminska and Sliwa 2004). Plants in this study were co-infected with apple proliferation and aster yellows phytoplasmas.

Lily

Symptoms of apple proliferation in lily include leaf scorch/leaf burn, leaf malformation, necrosis, and flower bud abscission (Kamińska and Śliwa 2008a).

Alberto Loschi http://www.fitoplasmi.it/index1.htm

Figure 4-1 Shoots Displaying Witches'Broom Caused by Apple Proliferation

Biologische Bundesanstalt für Land- und Forstwirtschaft Archive, Biologische Bundesanstalt für Land- und Forstwirtschaft, http://www.bugwood.org

Figure 4-2 Healthy Apple Leaf (top) and Leaf Infected with Apple Proliferation (bottom)

Alberto Loschi http://www.fitoplasmi.it/index1.htm

Figure 4-3 Elongated Pedicel of Flowers Displaying Apple Proliferation

Alberto Loschi http://www.fitoplasmi.it/index1.htm

Figure 4-4 Misshaped Apple Fruits Displaying AP Symptoms

Alberto Loschi http://www.fitoplasmi.it/index1.htm

Figure 4-5 Rosette of Leaves Displaying AP symptoms

Symptoms of Australian Grapevine Yellows Associated with '*Candidatus* Phytoplasma australiense'

Symptoms in grape include yellow and downward curled leaves that fall prematurely; reddening may be seen in red varieties (*Figure 4-6* on page 4-11 to *Figure 4-9* on page 4-13). 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 (Constable et al. 2004).

Any time from flowering, bunches may shrivel and fall (Magarey and Wachtel 1986b). Stems of affected shoots often take on a bluish hue (Constable et al. 2004). Only a few shoots on grapevine are usually affected, and inflorescence and fruit are only affected on symptomatic shoots. Later in the season, affected shoots tend to be green and rubbery. The symptoms associated with '*Candidatus* Phytoplasma australiense' can be influenced by the environment. Infected grapevines are less likely to show symptoms in summer than winter.

Although the infected vines are likely to show symptoms year after year, the disease can go into remission and not express symptoms.

Papaya
Symptoms include dieback, bending of the growing tip, bunching and chlorosis of the crown leaves, followed by necrosis of the young leaves and stem. Laticifer discoloration, particularly in the vicinity of the vascular tissue is evident. Plant death is observed within 2 to 3 weeks of first visible symptoms.

Pumpkin, Strawberry, and Peach
Plant growth is stunted and leaves turn yellow and curl (roll) (Streten et al. 2005a).

New Zealand Flax
Abnormal yellowing of the leaves, stunted growth, increased root death, phloem necrosis, and xylem gummosis of the rhizome vascular system are observed.

Coprosma
Lethal decline causes leaf reddening and bronzing, heavy leaf loss, dieback, and plant death (Beever et al. 2004).

Cordyline
Symptoms start as leaf yellowing and leaf desiccation ultimately causing rapid death of mature cabbage trees (Lucas 2005).

Liquidambar
Patchy chlorosis of the crown, chlorotic shoots with comparatively few leaves, dieback of apical and lateral branches, smaller leaves showing tip necrosis and vein clearing early senescing compared to healthy trees, and reduced fruit production (Habili et al. 2007).

Sweetgum
The crown may have patchy chlorosis, chlorotic shoots with comparatively few leaves, dieback of apical and lateral branches, small leaves showing tip necrosis, and reduced fruit production (Habili et al. 2007).

Paulownia
Yellows stunts plant growth, causes leaves to yellow, and reduces internode length and leaf size (Bayliss et al. 2005).

Red Clover
Diminished leaf size, pallor, rugosity, leaf deformation shoot proliferation, and severe stunting were observed (Saqib et al. 2006).

Alfalfa

Symptoms range from a yellow to red discoloration of the leaves, a yellowish-brown root discoloration under the periderm to plant death (Pilkington et al. 2003).

Potato

Upward rolling and purpling of the leaves. The symptoms appeared similar to those of zebra chip associated with '*Candidatus* Liberibacter solanacearum' in New Zealand and the United States (Liefting et al. 2009a).

Other Hosts

While phytoplasma infections are usually detrimental to plant growth, some plants exhibit minor symptoms or are symptomless.

Two other phytoplasmas distinct from '*Ca*. P. australiense' have been detected in grapevine displaying Australian grapevine yellows symptoms in Australia (Gibb et al. 1999). One of these is the tomato big bud (TBB) phytoplasma from Australia, which is a member of the peanut witches' broom phytoplasma group, 16SrII (Lee et al. 1998). Additionally, the TBB phytoplasma is considered to be a strain of the provisional taxon '*Candidatus* Phytoplasma australasiae' (White et al. 1998, not to be confused with '*Candidatus* Phytoplasma australiense'). A second phytoplasma found in Australian grapes is an as yet uncharacterized phytoplasma found only in the Buckland Valley of Victoria, Australia (Gibb et al. 1999); this phytoplasma has been suggested to represent a distinct '*Candidatus* Phytoplasma' species that has not been named (Wei et al. 2007; Zhao et al. 2009a).

Fiona Constable (CABI 2011)

Figure 4-6 Leaves of Shiraz' Grapevine Displaying Irregular Reddening of Leaves, Backward Curling of Leaves, and Overlapping Leaves Caused by Australian Grapevine Yellows

Fiona Constable (CABI 2011)

Figure 4-7 Shoot of Chardonnay Grape Displaying Mild, Irregular Chlorosis of Leaves, Backward Curling of Leaves, Overlapping Leaves, and Tip Death Caused by Early Australian Grapevine Yellows

Fiona Constable (CABI 2011)

Figure 4-8 Shoot of Chardonnay Grape Displaying Chlorosis Along Veins and Backward Curling Leaves Caused by Early Australian Grapevine Yellows

Fiona Constable (CABI 2011)

Figure 4-9 Shoot of Cabernet Sauvignon Grape Displaying Irregular Reddening, Unseasonal Yellowing, and Reddening of Leaves Associated with Phytoplasma Infection

Symptoms of European Stone Fruit Yellows Associated With '*Candidatus* Phytoplasma Prunorum'

Symptoms of European stone fruit yellows (ESFY) are influenced by species, cultivar, root stock, and environmental factors. There are many tolerant hosts that do not show any symptoms of disease but can harbor infections.

Symptoms first appeared in late summer in Italy with latent bud production occurring in September (Poggi Pollini et al. 2001). ESFY affects tree flowers and shoots in winter, which leads to lack of fruit production and chlorosis of the leaves later in the growing season. The early break in dormancy increases the susceptibility of affected trees to frost, which can cause damage to the phloem (*Figure 4-12* on page 4-15). Disease often starts with only a few branches affected but the whole tree may become affected as the disease progresses. Infected shoots are typically shorter and bear smaller, deformed leaves. Leaves can drop prematurely. Shoots may die back. Yield is reduced. Fruit on affected branches develops poorly and may fall prematurely.

Peaches exhibit early leaf reddening, severe upward longitudinal rolling of leaves, abnormal thickening and suberization of the midribs and primary veins (*Figure 4-15* on page 4-17), autumnal growth of latent buds which produce tiny chlorotic leaves and sometimes flowers, and early phylloptosis (leaf fall) (Poggi Pollini et al. 2001). The leaves also tend to be more brittle than normal.

Apricot and Japanese plum trees show typical yellows symptoms accompanied by leaf roll (*Figure 4-10* on page 4-14 through *Figure 4-13* on page 4-16) followed by leaf reddening (*Figure 4-14* on page 4-16), reduction, or suppression of dormancy with the consequent risk of frost damage, severe and

progressive necrosis, decline, and eventual death of the tree. Symptoms are mainly present on shoots and leaves and they are generally not uniform in appearance and distribution. Some plants varieties are tolerant and can be symptomless carriers of the disease. Environmental condition and plant seasonal changes can complicate the recognition of phytoplasma induced symptoms. Mechanical disruption to the phloem of tree shoots can cause symptoms similar to those associated with phytoplasma infection. It is important to inspect symptomatic shoots for damage to vascular tissue due to breakage, restrictions of the vascular tissue due to tendrils or string wrapping tightly around shoots, and damage to vascular tissue by boring insects.

Symptoms of ESFY disease on *Prunus* spp. are offseason production of new growth especially evident during winter dormancy. Symptoms are more apparent before flowering and at the end of the summer with leaf yellowing or reddening. During spring, affected trees bear leaves before the break of flower buds. If temperatures during winter are lower than -5°C, infected trees with poor lignification of young shoots show extensive phloem necrosis. Leafroll symptoms develop through the summer, becoming most clear towards the end of September. Rolling or curling of leaves is observed along the midrib giving the leaf a cone or a polygonal shape. Other symptoms include swollen midribs resulting from corky deposition and a yellow or red coloration of the enlarged lateral veins. Vigour and productivity of infected tree are reduced, scaffold branches exhibit dieback and trees decline within a few years or die (Seemüller and Foster 1995).

Bernd Schneider

Figure 4-10 Chlorosis and Rolling of Plum Leaves Affected by ESFY (right) Compared to Unaffected Leaf (left)

Bernd Schneider

Figure 4-11 Chlorosis and Rolling of Apricot Leaves on Shoot Affected by ESFY (Right) Compared to Unaffected Apricot (left)

Bernd Schneider

Figure 4-12 Necrosis of Vascular Tissue of *Prunus* Affected by ESFY

Bernd Schneider

Figure 4-13 Chlorosis and Rolling of Apricot Leaves Affected by ESFY (left) Compared to Unaffected Leaf (right)

Bernd Schneider

Figure 4-14 Reddening of Plum Leaves Affected by ESFY (right) Compared to Unaffected Leaf (left)

Bernd Schneider

Figure 4-15 Chlorosis and Rolling of Peach Leaves on Shoot Affected by ESFY (right) Compared to Unaffected Peach (left)

Bernd Schneider

Figure 4-16 Development of Corky Tissue along Lateral Vein of Peach Leaf Affected by ESFY

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Figure 4-17 Early Bud-Break and Foliation on ESFY-Affected Plum (left) and Peach (right)

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Figure 4-18 Longitudinally Rolled Leaves of Peach Affected by ESFY (right) and Unseasonal Reddened Leaves (left)

Bernd Schneider

Figure 4-19 Early Defoliation and Decline of *Prunus* Affected by ESFY (left) Compared to Unaffected Tree (right)

Delimiting Survey after Initial U.S. Detection

If any of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach is detected in the United States, surveys will be conducted in the area to determine the distribution of the pathogen. In large areas, locating the actual source of a phytoplasma infestation could be difficult depending on season, age of infected plants and time elapsed from the initial infection.

Procedure

Follow the same procedure used for detection surveys on page *Detection Survey* on page 4-4. Once any of the three phytoplasma species ('*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum') has been confirmed surveys should be most intensive around the known positive detections and any discovered through traceback and trace-forward investigations.

Traceback and Trace-Forward Investigations

Traceback and trace-forward investigations help determine priorities for delimiting survey activities after an initial U.S. detection. Traceback investigations attempt to determine the source of infection. Trace-forward investigations attempt to define further potential dissemination through means of natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

Due to the risk of '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum' spreading through infected plants, USDA–APHIS–PPQ has prohibited the importation of plants for planting of the listed host genera, with the exception of seed, until a pest risk analysis has been completed and appropriate effective mitigation measures have been established. However, the three phytoplasma species may enter through the illegal importation of nursery stock.

Homeowner Properties

For positive detections on homeowner properties, ask the owner of the infected material to determine where it originated (nursery, neighbors, etc.) and where it might have been further distributed.

Nursery Properties

For nursery hosts, a list of facilities associated with infected nursery stock from those testing positive for '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum' will be compiled. These lists will be distributed by the State to the field offices, and are not to be shared with individuals outside USDA–APHIS–PPQ regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited.

Each State is only authorized to see locations within their State and sharing of confidential business information may be restricted between State and Federal entities. Check the privacy laws with the State Plant Health Director for the State.

When notifying growers on the list, be sure to identify yourself as a USDA or State regulatory official conducting an investigation of facilities that may have received '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'-infested material. Speak to the growers or farm managers and obtain proper permission before entering private property.

Several actions need to occur immediately upon confirmation that a nursery sample is positive for any of the tree phytoplasma species:

- Check nursery records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from infested nursery during the previous 6 months).
- Evaluate the disease situation, including identification and inspection of the budwood source(s) of the diseased tree(s), the location within the nursery, and the disease severity.

Refer to *Control Procedures* on page 6-1 and *Regulatory Procedures* on page 5-1 for further information.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. If any of the tree phytoplasma species ('*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum') is detected in the United States, CPHST personnel will assemble a technical working group to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments on the pathogen population. Refer to *Control Procedures* on page 6-1 for further information on control options.

Procedure

Once any of the tree phytoplasma species ('*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum') has been confirmed from a particular field, and infected and potentially infected plants have been destroyed, additional monitoring will be necessary. Use the following tools:

- Visual inspection in the field

- Collection of samples from potential weed hosts for several years and multiple times per season

Refer to *Visual Inspection for Detection Survey* on page 4-21 and *Visual Inspection for Delimiting Survey* on page 4-22 for further information concerning the inspection of host plants.

Visual Inspection for Detection Survey

Use visual inspection as a tool when surveying for any of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach in field crops. This section includes also instructions for inspecting plants for damage and determining where to deploy traps for preliminary survey of potential vectors.

Any of the selected phytoplasma diseases of apple, grape and peach have common symptoms, including generalized stunting, leaf yellowing, downward rolling of leaves, and reduced quality and quantity of fruit. Symptoms are not uniform, and may appear on some or all shoots of infected vines or branches. A few rootstock varieties are tolerant to phytoplasma infections, and can be symptomless carriers of the disease. Field surveys are conducted visually by looking for plants with typical phytoplasma yellows symptoms. The distinction between AP, AGY and ESFY from other similar disease should be solely based on the combined presence of plant symptoms and positive RFLP analysis of PCR-amplified 16S ribosomal DNA in plant tissue samples. Serological test may be conducted as preliminary test followed by more discriminating molecular techniques.

Use the following tools singly or in any combination to detect potential vectors of '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum':

- Focus on the areas in the vicinity of diseased plants associated with high risk pathways
- Establish regular sites to inspect along your normal surveying route
- Monitor all season long
- Check plants for disease symptoms associated with phytoplasmas
- Employ sticky traps in and near crops
- Use sweep netting and vacuum sampling in field crops and for live insects
- Shake branches over collection trays
- Use unidirectional or bidirectional malaise trap

Servicing Traps

The number of insects captured should be recorded each week.

Trap Placement

Hang the traps from trees, or place them partially buried next to host trees.

Visual Inspection for Delimiting Survey

Construct delimiting surveys in an area based on known positive testing, associated positive testing, or potentially infested areas to define the geographic location of the pathogen population. However, it may be necessary to do random samples in a growing area to detect new infestations not discovered through investigations.

The delimiting survey in a growing area can include random sampling of wild and cultivated host species throughout a geographical area, with more intensive sampling near known infestations. As the distance away from the epicenter of a known infestation increases, decrease the rate of random sampling. Based on the epidemiology and grower practices, an evaluation of risk and resources available will help determine the extent of these random sampling surveys.

Sentinel Sites

Sentinel sites are locations that are regularly inspected along the surveyor's normal route. The sites can be established using a known host plant. The plant used as a sentinel site should be inspected for visual signs of damage; if available, test the host plant. Use GPS to record the location of the host plant, and draw a map of the immediate area that includes reference points so that the area can be found by others if necessary. Once the sentinel site is established the surveyor should re-inspect the site on a regular basis (bimonthly or monthly) as permitted by the persons regular survey schedule. GIS can be use to map the sentinel site locations to help visualize an even coverage, particularly high risk areas.

Targeted Surveys

Conduct targeted surveys at nurseries associated with high risk pathways. Areas with regular traffic from countries with known infestations, that may carry hitchhiker insect vectors, should also be targeted for regular surveys.

Procedure

A defined method is unavailable.

Survey Records

Records should be kept for each survey site. Negative survey data must be recorded even in the absence of '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'. Record also the absence of samples at surveyed sites. Survey records and data recording formats should be consistent, to allow for standardized collection of information.

If automated field collection devices are used, such as the Integrated Survey Information System (ISIS), ensure that all surveyors are trained in the technology before beginning the survey. Use the appropriate ISIS templates for this pathogen. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders before working in the field. At the end of the survey, all survey data should be entered into a designated State or national pest database.

Data Collection

Surveyors visiting sites to place holds or take samples should collect the following information:

- Date of collection or observations
- Collector’s name
- Grower’s field identification numbers
- GPS coordinates
- Variety of host plants grown
- Methods of irrigation
- History of farm machinery usage
- Observations of yellowing symptoms
- Other relevant information

In the absence of inspection officials, take the following actions immediately if yellowing symptoms are noticed:

1. Mark the location
2. Remove the plants and flag the location in the field

3. Notify the State or PPQ inspector
4. Place the whole plant inside two resealable plastic bags
5. Label the sealed bags with the following information:
   A. Date
   B. Name of person responsible
   C. Location of sample collection
6. Keep bagged plants cool or refrigerated until the inspector arrives
7. Do not freeze the sample

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize outbreaks that could be associated with the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach.

Chapter

5

Regulatory Procedures

Contents

Introduction

Use *Chapter 5 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against '*Candidatus* Phytoplasma mali' (apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows).

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to people interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in line with labeling restrictions. During all field visits, ensure that proper sanitation procedures are followed as outlined in *Preparation, Sanitization, and Clean-Up* on page 4-2.

Regulatory Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification may be issued to hold articles or facilities, pending positive identification by a USDA–APHIS–PPQ-recognized authority and/or further instruction from the PPQ Deputy Administrator. If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register*.

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides the authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under State authority.

State departments of agriculture normally work in conjunction with Federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that the States measures are inadequate, USDA can take intrastate regulatory action provided that the governor of the State has been consulted and a notice has been published in the Federal Register. If intrastate action cannot or will not be taken by a State, PPQ may find it necessary to quarantine an entire State.

PPQ works in conjunction with State departments of agriculture to conduct surveys, enforce regulations, and take control actions. PPQ employees must have permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property without owner permission. PPQ prefers to work with the State to facilitate access when permission is denied, however each State government has varying authorities regarding entering private property.

A General Memorandum of Understanding (MOU) exists between PPQ and each State that specifies various areas where PPQ and the State department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected State. Refer to *Resources* on page A-1 for information on identifying SPHD's and SPRO's.

Tribal Governments

USDA–APHIS–PPQ also works with federally-recognized Indian Tribes to conduct surveys, enforce regulations and take control actions. Each Tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to State governments. Permission is required to enter and access Tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Indian Tribal governments about actions that may have substantial direct effects on Tribes. Whether an action is substantial and direct is determined by the Tribes. Effects are not limited to Tribal land boundaries (reservations) and may include effects on off-reservation land or resources which Tribes customarily use or even effects on historic or sacred sites in States where Tribes no longer exist.

Consultation is a specialized form of communication and coordination between the Federal and Tribal governments. Consultation must be conducted early in the development of a regulatory action to ensure that Tribes have opportunity to identify resources which may be affected by the action and to recommend the best ways to take actions on Tribal lands or affecting Tribal resources. Communication with Tribal leadership follows special communication protocols. For more information, contact PPQ's Tribal Liaison. Refer to *Table A-1* on page A-2 for information on identifying PPQ's Tribal Liaison.

To determine if there are federally-recognized Tribes in a State, contact the State Plant Health Director (SPHD). To determine if there are sacred or historic sites in an area, contact the State Historic Preservation Officer (SHPO). For clarification, check with your SPHD or State Plant Regulatory Official (SPRO) in the affected State. Refer to *Resources* on page A-1 for contact information.

Overview of Regulatory Program After Detection

Once an initial U.S. detection is confirmed, holds will be placed on the property by the issuance of an Emergency Action Notification. Immediately put a hold on the property to prevent the removal of any host plants of the pest.

Traceback and trace-forward investigations from the property will determine the need for subsequent holds for testing and/or further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures.

Record-Keeping

Record-keeping and documentation are important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by the owners, researchers or manager for information on destination of shipped plant material, movement of plant material within the facility, and any management (cultural or sanitation) practices employed.

Keep a detailed account of the numbers and types of plants held, destroyed, and/or requiring treatments in control actions. Consult a master list of properties, distributed with the lists of suspect nurseries based on traceback and trace-forward investigations, or nurseries within a quarantine area. Draw maps of the facility layout to located suspect plants, and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout, and document plant propagation methods, labeling, and any other information that may be useful for further investigations and analysis.

Keep all written records filed with the Emergency Action Notification copies, including copies of sample submission forms, documentation of control activities, and related State issued documents if available.

Issuing an Emergency Action Notification

Issue an Emergency Action Notification to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested, or testing determines there is no risk, the material may be released and the release documented on the EAN.

Regulated Area Requirements Under Regulatory Control

Depending upon decisions made by Federal and State regulatory officials in consultation with a Technical Working Group, quarantine areas may have certain other requirements for commercial or research fields in that area, such as plant removal and destruction, cultural control measures, or plant waste material disposal.

Any regulatory treatments used to control this pest or herbicides used to treat plants will be labeled for that use or exemptions will be in place to allow the use of other materials.

Establishing a Federal Regulatory Area or Action

Regulatory actions undertaken using Emergency Action Notifications continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfestation orders or longer term requirements for growers that include prohibiting the planting of host crops for a period of time. Over the long term, producers, shippers, and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing, and risk assessment will determine the area to be designated for a Federal and parallel State regulatory action. Risk factors will take into account positive testing, positive associated, and potentially infested exposed plants. Boundaries drawn may include a buffer area determined based on risk factors and epidemiology.

Regulatory Records

Maintain standardized regulatory records and databases in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

Use of Chemicals

The PPQ *Treatment Manual* and the guidelines identify the authorized chemicals, and describe the methods and rates of application, and any special instructions. For further information refer to *Control Procedures* on page 6-1. Agreement by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for this pest.

Chapter

6

Control Procedures

Contents

Introduction

Use *Chapter 6 Control Procedures* as a guide to controlling the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach. Consider the treatment options described within this chapter when taking action to eradicate, contain, or suppress any of the three selected '*Candidatus* Phytoplasma' species: '*Candidatus* Phytoplasma mali' (Apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows).

Because of phytoplasmas cryptic nature, phytoplasma-associated diseases are difficult to manage. The control of these pathogens is obtained implementing a combination of preventive strategies including control of their vectors,

eradication of infected material and alternative plant hosts, and planting of certified pest-free material.

Overview of Emergency Programs

APHIS–PPQ develops and makes control measures available to involved States. United States Environmental Protection Agency-approved treatments will be recommended when available. If the selected treatments are not labeled for use against the pest or in a particular environment, PPQ's FIFRA Coordinator is available to explore the appropriateness in developing an Emergency Exemption under Section 18, or a State Special Local Need under section 24(c) of FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act), as amended.

The PPQ FIFRA Coordinator is also available upon request to work with EPA to rush the approval of a product that may not be registered in the United States, or to get labeling for a new use. The PPQ FIFRA Coordinator is available for guidance pertaining to pesticide use and registration. Refer to *Resources* on page A-1 for information on contacting the Coordinator.

Treatment Options

Consider the treatment options described within this chapter when taking action to eradicate or control any of the three selected '*Candidatus* Phytoplasma' species: '*Candidatus* Phytoplasma mali' (Apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows). Treatments may include the following:

- *Resistant Plants* on page 6-4
- *Clean Propagation Material* on page 6-4
- *Roguing and Weed Control* on page 6-4
- *Mulching* on page 6-5
- *Barriers* on page 6-5
- *Barrier Sprays and Insecticides* on page 6-5
- *Biological Control* on page 6-6
- *Antibiotic Therapy* on page 6-6
- *Genetic Engineering* on page 6-6

Environmental Documentation and Monitoring

Obtain all required environmental documentation before beginning. For further information, refer to *Environmental Compliance* on page 7-1. Contact Environmental Services staff for the most recent documentation. Refer to *Resources* on page A-1 for contact information.

Efficacy of Treatment

Eradication measures should be continued for several years to ensure that populations of exotic '*Candidatus* Phytoplasma spp.' have been eliminated. Once the pathogen has been eradicated, monitoring of the site should be continued for 1 to 2 years. For further information, refer to *Monitoring Survey* on page 4-20.

Site Assessment

When visiting a site keep a log of observations, flag the infested areas, and record the coordinates. Record also the name of the property owner. Some of this information may have been recorded during the survey. Communicate frequently with the person responsible for the site.

Classification

Information on the type of property needs to be recorded to help develop a control plan. Site access, security, containment, and ownership type may dictate a particular direction in control options. Prepare a concise overview of the infested area. Record information about the infested property, including the following:

- Location
- Type of property ownership (government, private, Tribal, commercial, residential, or agricultural)
- Current and past users of the property
- Distribution of infected plants
- Status of security and containment
- Modes of artificial movement

Resistant Plants

Several projects focus on breeding plants that are less susceptible or resistant to phytoplasmas as well as plants that inhibit or deter vector feeding. This approach may constitute the most economically and effective option to control the disease. Natural resistance and tolerance are being investigated. The use of disease-free certified planting material should be recommended.

The colonization behavior of phytoplasmas within the tree varies annually between the stems in the spring and the roots where the pathogen overwinters (Schaper and Seemüller 1982; Seemüller et al. 1984). Thanks to this seasonal fluctuation scion derived from susceptible cultivars can be successfully grown on resistant rootstocks.

Apple Proliferation

Sources of genetic resistance have been reported in the wild apomictic *Malus sieboldii* and hybrids derived from this *Malus* species (Kartte and Seemüller 1988). Apple rootstocks selections are being conducted among material derived from crosses between *M. domestica* genotypes and several of the *M. sieboldii* derived genotypes. The use of disease-free certified planting material should be recommended.

Clean Propagation Material

A preventive measure should include planting material from reliable approved sources and produced in areas where phytoplasma diseases are not present. The management of irrigation and nutrition to improve or maintain tree health may limit the impact of phytoplasma infections. Also, vectors and pests should be eliminated before transporting potentially contaminated material to new areas. Hot water treatment of dormant wood can be used to eliminate phytoplasmas from infected propagating material (Caudwell et al. 1997; Mannini 2007). This treatment requires cuttings to be immersed in water at 50°C for 45 min. The same treatment was reported to be beneficial against leafhoppers overwintering eggs.

Roguing and Weed Control

Frequent inspection and direct removal of infected plants and or other phytoplasma reservoir hosts may help reducing the spread or combined with insecticide spraying. Ratooning (removing the main stem of diseased plants to promote new growth) is not an effective management practice.

Apple Proliferation and European Stone Fruit Yellows

Crataegus monogyna (common hawthorn) is a shrub or small tree from the Rosaceae family often found growing wild near orchards. A recent study by Tedeschi et al. has shown that hawthorn is a natural host of both the vectors and phytoplasma belonging to the apple proliferation group (Tedeschi et al. 2009). Based on these findings, control of hawthorn shrubs as well as other broadleaf weeds near orchards should be recommended.

Australian Grapevine Yellows

While there is no evidence implicating alternative plant hosts of the Australian grapevine yellows (AGY) strain of '*Candidatus* Phytoplasma australiense' within affected vineyards or surrounding affected vineyards, research on other phytoplasma diseases has shown that likely candidates for alternative hosts are often broadleaf weeds. For example, in Germany, the alternative host for German grapevine yellows phytoplasma, to which '*Candidatus* Phytoplasma australiense' is closely related, is *Convolvulus arvensis* (bindweed) (Maixner et al. 1995). Also, the TBB phytoplasma in Australia has an extensive range of broad leaf plant hosts (Davis et al. 1997b). From this information, control of broadleaf weeds has been recommended.

Mulching

The application of various types of mulches can physically interfere or prevent the movement of vectors or repel them away from the plant.

Barriers

A reliable means of controlling phytoplasma vectors is by covering the crop with insect exclusion screening. It may be recommended for production of clonal or mother plants.

Barrier Sprays and Insecticides

Kaolin is an aluminosilicate mineral that can be applied to directly control the insect or coating the plant and obstructing feeding and oviposition (Tedeschi et al. 2007b). The efficacy of kaolin is greatly hindered by water; it may be effective in dry areas. A list of other tested insecticides to control psillids is provided in *Table 6-1* on page 6-6. Other studies do not find the application of pesticide to be an effective method of controlling the disease spread (Pollini et al. 2007).

Table 6-1 Insecticides Used to Control Psillid Vectors[1]

MOA[2]	Class	Ingredient	Reference
15	Benzoylureas	diflubenzuron	Baldessari et al. 2010
7B	Carbamate	fenoxycarb	Baldessari et al. 2010
6	Avermectins	abamectin	Baldessari et al. 2010
3A	pyrethroid	etofenprox	Baldessari et al. 2010
1B	organophosphate	chlorpyrifos	Baldessari et al. 2010
N/A	kaolin clay	-	Tedeschi et al. 2007a

1 Insecticides are EPA-registered according to the National Pesticide Information Retrieval System database (NPIRS 2009) queried October 4 2010.

2 Mode of action (IRAC 2010).

Biological Control

Leafhoppers and planthoppers are attacked by a range of predators. Spiders are very important predators of both adults and nymphs, especially in grassland ecosystems, while Miridae (Hemiptera) may be significant egg predators. Other potential biological control agents include *Anthocoris nemorum* (L.), *Chrysopa carnea* (Stephens), *Forficula auricularia* L., Coccinellidae, Araneae and parasitoids (Riedl et al. 2007; Sigsgaard,).

Antibiotic Therapy

Treatment of phytoplasma-associated disease may be achieved by application of tetracycline antibiotics, also oxytetracycline HCl (OTC), administered by high-pressure injection or by gravity infusion into the trunk. This treatment is costly, not curative and reduces the disease severity only temporarily.

Genetic Engineering

A paratransgenic is a vector harboring symbiotic bacteria that have been genetically altered to prevent the transmission of pathogens from the vector populations. In many arthropods these transformed bacteria are maternally inherited. This symbiont-based strategy may be a potential tool to reduce the vectoring capacity of the host (Bextine et al. 2005).

Engineering plants to constitutively express specific antibodies (plantibodies) capable of interfering with the phytoplasmas' normal multiplication process

can provide an efficient way to induce resistance. Phytoplasma cells are protected by a single cytoplasmic membrane. For this reason, their growth and reproduction is inhibited by antibodies targeting their membrane epitopes (Le Gall et al. 1998).

Apple Proliferation

Several antibodies were derived from antigens of distinct AP phytoplasma strains and their specificity has already been tested (Berg et al. 1999; Seemüller and Schneider 2004).

Another form of genetic manipulation involves the modification of plant lectins, which affects various physiological functions of vectors, including blocking the absorption of free amino acids and sugars (Saha et al. 2006). In plants, systemic acquired resistance is a resistance response that is activated after initial exposure to a pathogen. The use of certain chemical compounds (for example, benzothiadiazole) can artificially activate this plant response. The application of such chemicals could be used to induce a protective effect against phytoplasma establishment and replication with the host (Bressan and Purcell 2005).

Chapter

7

Environmental Compliance

Contents

Introduction

Use *Chapter 7 Environmental Compliance* as a guide to '*Candidatus* Phytoplasma mali' (apple proliferation), '*Candidatus* Phytoplasma australiense' (Australian grapevine yellows) and '*Candidatus* Phytoplasma prunorum' (European stone fruit yellows).

Overview

Program managers of Federal emergency response or domestic pest control programs must ensure that their programs comply with all Federal Acts and Executive Orders pertaining to the environment, as applicable. Two primary Federal Acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may begin.

Program managers should also seek guidance and advice as needed from Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development (PPD) staff. ERAS is available to give guidance

and advice to program managers and prepare drafts of applicable environmental documentation.

In preparing draft NEPA documentation ERAS may also perform and incorporate assessments that pertain to other acts and executive orders described below, as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), will assist ERAS in the development of documents, and will implement any environmental monitoring.

Leaders of programs are strongly advised to meet with ERAS and/or ECT early in the development of a program in order to conduct a preliminary review of applicable environmental statutes and to ensure timely compliance. Environmental monitoring of APHIS pest control activities may be required as part of compliance with environmental statutes, as requested by program managers, or as suggested to address concerns with controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition, or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from ECT will work with the program manager to develop an environmental monitoring plan, conduct training to carry out the plan, give day-to-day guidance on monitoring, and provide an interpretive report of monitoring activities.

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all Federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decisionmaker before taking action, and to tell the public of the decision. Actions that are excluded from this examination, that normally require an Environmental Assessment, and that normally require Environmental Impact Statements, are codified in APHIS' NEPA Implementing Procedures located in 7 CFR 372.5.

The three types of NEPA documentation are Categorical Exclusions, Environmental Assessments, and Environmental Impact Statements.

Categorical Exclusion

Categorical Exclusions (CE) are classes of actions that do not have a significant effect on the quality of the human environment and for which neither an Environmental Assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have been built into the actions themselves (7 CFR 372.5(c)).

Environmental Assessment

An Environmental Assessment (EA) is a public document that succinctly presents information and analysis for the decisionmaker of the proposed action. An EA can lead to the preparation of an environmental impact statement (EIS), a finding of no significant impact (FONSI), or the abandonment of a proposed action.

Environmental Impact Statement

If a major Federal action may significantly affect the quality of the human environment (adverse or beneficial) or the proposed action may result in public controversy, then prepare an Environmental Impact Statement (EIS).

Endangered Species Act

The Endangered Species Act (ESA) is a statute requiring that programs consider their potential effects on federally-protected species. The ESA requires programs to identify protected species and their habitat in or near program areas, and document how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act

The statute requires that programs avoid harm to over 800 endemic bird species, eggs, and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

Clean Water Act

The statute requires various permits for work in wetlands and for potential discharges of program chemicals into water. This may require coordination with the Environmental Protection Agency, individual States, and the Army Corps of Engineers. Such permits would be needed even if the pesticide label allows for direct application to water.

Tribal Consultation

The Executive Order requires formal government-to-government communication and interaction if a program might have substantial direct effects on any federally-recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed before public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed Federal actions intersect with Tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act

The statute requires programs to consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local State Historic Preservation Offices. Documentation under this act involves preparing an inventory of the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may need public involvement and comment before the start of program activities.

Coastal Zone Management Act

The statute requires coordination with States where programs may impact Coastal Zone Management Plans. Federal activities that may affect coastal resources are evaluated through a process called Federal consistency. This process allows the public, local governments, Tribes, and State agencies an opportunity to review the Federal action. The Federal consistency process is administered individually by states with Coastal Zone Management Plans.

Environmental Justice

The Executive Order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to consider if the actions might impact minority or economically disadvantaged populations and if so, how such impact will be avoided.

Protection of Children

The Executive Order requires Federal agencies to identify, assess, and address environmental health risks and safety risks that may affect children. If such a risk is identified, then measures must be described and carried out to minimize such risks.

Chapter

8

Pathways

Contents

Introduction

Use *Chapter 8 Pathways* as a source of information on the pathways of introduction in the United States for any of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach: '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum'. These phytoplasma species could potentially enter the continental United States through commerce, or the movement of infected planting material and insect vector. Any of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach should be regarded as a moderate threat to United States production.

Natural Movement

Natural spread of the selected '*Candidatus* Phytoplasma spp.' of apple, grape and peach into the continental United States is considered a possibility.

'*Candidatus* Phytoplasma mali' (Apple Proliferation)

Natural spread of AP phytoplasma into the continental United States is considered a possibility. One of the reported vectors for this phytoplasma, the leafhopper *Fieberiella florii*, is present both in Europe and in the United States. In North America *F. florii* is a known vector of a phytoplasma belonging to the X-disease group (16SrIII), which caused major economic losses on peach and cherry trees (Lee et al. 2000). It is also possible that other plant hoppers or psillids already present in the United States may serve as potential vectors for this phytoplasma once established. Spread of phytoplasmas by use of infected cuttings and through mechanical grafting is also considered a possibility. The phytoplasma could also spread over long distances when infected insect vectors are accidentally introduced into new areas. According to the Pest ID database, U.S. agricultural inspectors have not intercepted the other two known vector of '*Ca*. P. mali', the psyllids *Cacopsylla melanoneura* and *C. picta* (queried 17 December 2010), while *F. florii* remains listed as nonreportable/ nonactionable

'*Candidatus* Phytoplasma australiense' (Australian Grapevine Yellows)

Natural spread of Australian grapevine yellows phytoplasma into the continental United States is considered a possibility. Despite the fact that *Zeoliarus atkinsoni* and *Z. oppositus*, the only known vectors of '*Ca.* P. australiense', have not been reported in the United States, other insect species may be potential vectors for this phytoplasma. Spread of phytoplasmas by use of infected grapevine cuttings in Australia has not been reported. However, this means of spread is considered a possibility. The phytoplasma could also spread over long distances when infected insect vectors are accidentally introduced into new areas. According to the Pest ID database, U.S. agricultural inspectors have not intercepted the only two known vectors of '*Ca*. P. australiense', *Z. atkinsoni* and *Z. oppositus* (queried 17 December 2010); however, various sources mention that there are probably at least one unknown vector and probably more (Charles et al. 2002; Saqib et al. 2006).

'*Candidatus* Phytoplasma prunorum' (European Stone Fruit Yellows)

Natural spread of ESFY phytoplasma into the continental United States is considered a possibility. Despite the fact that *Cacopsylla pruni* and *Asymmetrasca decedens*, the only known vectors of '*Ca.* P. prunorum', have not been reported in the United States, other insect species may be potential vectors for this phytoplasma. Spread of phytoplasmas by use of infected cuttings and through mechanical grafting is also considered a possibility. The phytoplasma could also spread over long distances when infected insect vectors are accidentally introduced into new areas. According to the Pest ID database, U.S. agricultural inspectors have not intercepted the only two known vector of '*Ca*. P. prunorum', the psyllids *C. pruni* and leafhopper *A. decedens* (queried 17 December 2010).

Commerce

Plant parts liable to carry the pest in trade/transport are leaves, seedlings, micropropagated plants roots, stems, shoots, trunks, branches and all plant tissues containing sieve tube elements. Other plant parts suspected to harbor the pathogen may include flowers, inflorescences, cones, calyx, and true seeds. While plant parts not known to carry the pathogen are fruits (including pods), growing medium accompanying plants and wood. In some cases (alfalfa (Bertaccini 2007; Khan et al. 2002), coconut fruit (Cordova et al. 2003;), lime, oilseed rape and tomato (Botti and Bertaccini 2006)) seed transmission has been suggested but not yet confirmed (Nipah et al. 2007a; Nipah et al. 2007b).

'*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma australiense' and '*Candidatus* Phytoplasma prunorum' are an imminent threat that could be introduced into the United States with imported leaves, stems, and roots of infected plants (CABI 2011). Due to the relatively slow rate of potential disease spread caused by its vectors, containment and eradication should be actively pursued. The success of such strategy would rely on accurate and timely identification of the pathogen matched by quarantine action and targeted eradication implementing all necessary control measures. According to the USDA Nursery Stock Restrictions Manual, the following hosts may enter the United States, as propagative materials, without additional safeguards to prevent the introduction of '*Ca*. P. australiense': *Catharanthus roseus*, *Coprosma robusta*, *Cordyline australis*, *Fragaria ananassa*, *Liquidambar styraciflua*, *Paulownia fortunei*, and *Phormium tenax* (queried 08 March 2007). Moreover, none of the phytoplasmas infecting Australian grapes are considered a quarantine pathogen between Australian grape growing regions.

Once a positive identification has been made confirming the presence of infected plants or vectors by any of the phytoplasma species '*Ca*. P. mali', '*Ca.* P. australiense', and '*Ca.* P. prunorum', investigations should be initiated to determine the probable origin of the initial infections/ introduction and the extent of distribution of potentially infected plants in the U.S. territory.

After investigations are performed and the risk of pathogen establishment is evaluated the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific actions under the Plant Protection Act. The Plant Protection Act of 2000 provides for authority for emergency quarantine action. Program personnel must maintain records and maps noting the location of all detections, the number and type of plants subjected to control actions, and the materials and chemical formulations used in each treated area.

Candidatus Phytoplasma

References

Use *References* to learn more about the publications, Web sites, and other resources that were consulted during the production of the guidelines.

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Candidatus Phytoplasma

Glossary

Use this glossary to find the meaning of specialized words, abbreviations, acronyms, and terms used by PPQ–EDP. To locate where in the manual a given definition, term, or abbreviation is mentioned, refer to the index.

Definitions, Terms, and Abbreviations

amplicon. piece of DNA synthesized using amplification techniques such as PCR

APA. American Phytopathological Society

APHIS. USDA–Animal and Plant Health Inspection Service

approved landfill. State licensed municipal or private landfill managed under state regulation to prevent leaching of potential pollutants into groundwater

bp. base pair

bunchy top. shortening of internodes at and near the tip of a branch, resulting in bunched growth at the end of the branch

CAPS. Cooperative Agricultural Pest Survey Program, a partnership between all 50 States and USDA to detect and monitor exotic pests of economic impact

chlorosis. yellowing of normally green tissue due to chlorophyll destruction in infected plants

CPB. U.S. Department of Homeland Security-Customs and Border Protection

CPHST. PPQ-Center for Plant Health Science and Technology

decontamination. application of approved chemical or other treatment to contaminated implements, material, or buildings for killing or deactivating a pathogen

detection survey. conducted in an environmentally favorable area where the pathogen is not known to occur

DHS. U.S. Department of Homeland Security

dieback. death of branches on woody plants, shrubs, trees; typically young shoots, twigs, and distal portions of branches die progressively toward older plant parts

disposal. Method used to eliminate diseased plant material or material associated with diseased plant material, usually at an approved landfill

EDP. PPQ-Emergency and Domestic Programs

ELISA. enzyme linked immunosorbent assay, is a biochemical technique used to detect the presence of an antibody or an antigen in a sample

EM. PPQ-Emergency Management

fastidious phloem-limited. quality of a pathogen that describes its ability to only survive within the phloem vascular system of a plant

FIFRA. Federal Insecticide, Fungicide, and Rodenticide Act

GC content. percentage of nitrogenous bases on a DNA molecule which are either guanine or cytosine (from a possibility of four different ones, also including adenine and thymine

host. plant which is invaded by a parasite or pathogen and from which it obtains its nutrients
ICS. Incident Command System
infection. establishment of a parasite on or within a host plant
ISIS. Integrated Survey Information System
little leaf. development of abnormally small leaves
MLOs. mycoplasma-like organisms
monitoring survey. conducted at a site where a disease was found and an eradication program is being performed; also known as evaluation survey
necrosis. dead or discolored plant tissue
NEPA. National Environmental Policy Act
NIS. PPQ-National Identification Service
NPAG. PPQ New Pest Advisory Group
NPRG. New Pest Response Guidelines
pathogen. any organism that can incite a disease
PCR. polymerase chain reaction, a laboratory technique that amplifies DNA sequences in order to determine if a host is infected with a known pathogen
PCR primers. short fragments of single stranded DNA (15 to 30 nucleotides in length), complementary to DNA sequences that flank the target region of interest; necessary components for the polymerase chain reaction
PERAL. Plant Epidemiology and Risk Analysis Laboratory
pest. includes insects, weeds, plant disease agents, and microorganisms
phyllody. development of leaf-like growths in place of normal flower parts
plasmid. small circle of DNA that replicates independently of chromosomal DNA
pleomorphic. capable of assuming different shapes
PPQ. APHIS-Plant Protection and Quarantine|
proliferation. abnormal growth of numerous shoots
RFLP. restriction fragment length polymorphism, difference between two or more samples of homologous DNA molecules arising from differing locations of restriction sites, and to a related laboratory technique by which these segments can be distinguished
SEL. USDA–ARS-Systematic Entomology Laboratory
SPHD. State Plant Health Director
SPRO. State Plant Regulatory Official
stunting. overall reduction of plant height due to shortening of internodes
symptom. external and internal reactions or alterations of a plant as the result of a disease
traceback. investigation of the origin of infested plants through intermediate steps in commercial distribution channels to the origin
trace-forward. investigation to determine where infected plants may have been distributed from a known infestation through steps in commercial distribution channels or wholesale or retail procurement
TWG. Technical Working Group
USDA. United States Department of Agriculture

vector. carrier of an infectious agent capable of transmitting infection from one host to another, especially the animal that transfers an infectious agent from one host to another, usually an arthropod
virescence. development of green color in place of normal flower color
witches broom. abnormal, excessive proliferation of axillary shoots resulting in a broom-like growth
yellowing. leaves loose normal green color and become yellow

Appendix

A

Resources

Use *Appendix A Resources* to find the Web site addresses, street addresses, and telephone numbers of resources mentioned in the guidelines. To locate where in the guidelines a topic is mentioned, refer to the index.

Table A-1 Resources for *Candidatus* Phytoplasma spp. of Apple, Grape and Peach

Resource	Contact Information
Center for Plant Health, Science, and Technology (USDA–APHIS–PPQ–CPHST)	http://www.aphis.usda.gov/plant_health/cphst/index.shtml
Emergency and Domestic Programs, Emergency Management (USDA–APHIS–PPQ–EDP–EM)	http://www.aphis.usda.gov/plant_health/plant_pest_info/index.shtml
PPQ *Manual for Agricultural Clearance*	http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml
PPQ *Treatment Manual*	http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml
Host or Risk Maps	http://www.nappfast.org/caps_pests/CAPs_Top_50.htm
Plant, Organism, and Soil Permits (APHIS–PPQ	http://www.aphis.usda.gov/plant_health/permits/index.shtml
National Program Manager for Native American Program Delivery and Tribal Liaison (USDA–APHIS–PPQ)	14082 S. Poston Place Tucson, AZ 85736 Telephone: (520) 822-544
Biological Control Coordinator (USDA–APHIS–CPHST)	http://www.aphis.usda.gov/plant_health/cphst/projects/arthropod-pests.shtml
FIFRA Coordinator (USDA–APHIS–PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: (301) 734-5861
Environmental Compliance Coordinator (USDA–APHIS–PPQ-EDP)	4700 River Road Riverdale, MD 20737 Telephone: (301) 734-7175
PPQ Form 391	http://www.aphis.usda.gov/library/forms/
List of State Plant Health Directors (SPHD)	http://www.aphis.usda.gov/services/report_pest_disease/report_pest_disease.shtml
List of State Plant Regulatory Officials (SPRO)	http://nationalplantboard.org/member/index.html
National Climatic Center, Data Base Administration, Box 34, Federal Building, Asheville, North Carolina 28801	http://www.ncdc.noaa.gov/oa/ncdc.html
CAPS Survey Manuals	http://caps.ceris.purdue.edu/
Leafhopper and treehopper genera in New Zealand	http://www1.dpi.nsw.gov.au/keys/leafhop/deltocephalinae/opsiini.htm
GenBank®	http://www.ncbi.nlm.nih.gov/
iPhyClassifier	http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi

Appendix

B

Forms

Use *Appendix B Forms* to learn how to complete the forms mentioned in the guidelines. To locate where in the guidelines a form is mentioned, refer to the index.

Contents

PPQ Form 391 Specimens For Determination

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)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS*)*
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
F. ☐ Possible Immigrant *(Explain in* REMARKS*)*
G. ☐ Survey *(Explain in* REMARKS*)*
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 ☐ Leaves, Lower Surface ☐ Petiole ☐ Stem
☐ Trunk/Bark ☐ Branches ☐ Growing Tips ☐ Roots
☐ Bulbs, Tubers, Corms ☐ Buds ☐ Flowers ☐ Fruits or Nuts
☐ Seeds

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

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

SIGNATURE

DATE

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

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

Figure B-1 Example of PPQ Form 391 Specimens For Determination, side 1

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.

Figure B-2 Example of PPQ Form 391 Specimens For Determination, side 2

Purpose

Submit PPQ Form 391, Specimens for Determination, along with specimens sent for positive or negative identification.

Instructions

Follow the instructions in *Table B-1* on page B-5. Inspectors must provide all relevant collection information with samples. This information should be shared within a State and with the regional office program contact. If a sample tracking database is available at the time of the detection, please enter collection information in the system as soon as possible.

Distribution

Distribute PPQ Form 391 as follows:

1. Send the original along with the sample to your area identifier
2. Keep and file a copy for your records

Table B-1 Instructions for Completing PPQ Form 391, Specimens for Determination

Block	Description	Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: 2-letter State code–5-digit sample number (Survey Identification Number in Parentheses) Example: PA-1234 (04202010001) 2. CONTINUE consecutive numbering for each subsequent collection 3. ENTER the collection number
2	DATE	ENTER the date of the collection
3	SUBMITTING AGENCY	PLACE an X in the PPQ block
4	NAME OF SENDER	ENTER the sender's or collector's name
5	TYPE OF PROPERTY	ENTER the type of property where the specimen was collected (farm, feed mill, nursery, etc.)
6	ADDRESS OF SENDER	ENTER the sender's or collector's address
7	NAME AND ADDRESS OF PROPERTY OR OWNER	ENTER the name and address of the property where the specimen was collected
8A-8H	REASONS FOR IDENTIFICATION	PLACE an X in the correct block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE GIVE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE blank; ENTER remarks in *Block 22*
10	HOST INFORMATION NAME OF HOST	If known, ENTER the scientific name of the host
11	QUANTITY OF HOST	If applicable, ENTER the number of acres planted with the host
12	PLANT DISTRIBUTION	PLACE an X in the applicable box
13	PLANT PARTS AFFECTED	PLACE an X in the applicable box
14	PEST DISTRIBUTION FEW/COMMON/ ABUNDANT/EXTREME	PLACE an X in the appropriate block
15	INSECTS/NEMATODES/ MOLLUSKS	PLACE an X in the applicable box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage
16	SAMPLING METHOD	ENTER the type of sample
17	TYPE OF TRAP AND LURE	ENTER the type of sample
18	TRAP NUMBER	ENTER the sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check the appropriate box; otherwise LEAVE blank
20	WEED DENSITY	If applicable, check the appropriate box; otherwise LEAVE blank

Table B-1 Instructions for Completing PPQ Form 391, Specimens for Determination (continued)

Block	Description	Instructions
21	WEED GROWTH STAGE	If applicable, check the appropriate box; otherwise LEAVE blank
22	REMARKS	ENTER the name of the office or diagnostic laboratory forwarding the sample; include a contact name, email address, phone number of the contact; also include the date forwarded to the State diagnostic laboratory or USDA–APHIS–NIS
23	TENTATIVE DETERMINATION	ENTER the preliminary diagnosis
24	DETERMINATION AND NOTES (**Not** for Field Use)	LEAVE blank; will be completed by the official identifier

PPQ 523 Emergency Action Notification

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 is 0579-0102. The time required to complete this information collection is estimated to average 1 hour 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.

FORM APPROVED - OMB NO. 0579-0102

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

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION | 2. DATE ISSUED

3. NAME AND QUANTITY OF ARTICLE(S)

4. LOCATION OF ARTICLES

5. DESTINATION OF ARTICLES

6. SHIPPER

7. NAME OF CARRIER

8. SHIPMENT ID NO.(S)

9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. | FAX NO.

SS NO. | TAX ID NO.

10. PORT OF LADING | 11. DATE OF ARRIVAL

12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)

12a. PEST ID NO. | 12b. DATE INTERCEPTED

13. COUNTRY OF ORIGIN | 14. GROWER NO.

15. FOREIGN CERTIFICATE NO.

15a. PLACE ISSUED | 15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT:

☐ RE-EXPORTATION:

☐ DESTRUCTION:

☐ OTHER:

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):

18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE: | DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER: | DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

Figure B-3 Example of PPQ 523 Emergency Action Notification

Purpose

Issue a PPQ 523, Emergency Action Notification (EAN), to hold all host plant material at facilities that have the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines the plant material is not infested, or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision to destroy plants is made, or in the case of submitted samples, once positive confirmation is received, the same EAN which placed plants on hold also is used to document any actions taken, such as destruction and disinfection. More action may be warranted in the case of other fields testing positive for this pest.

Instructions

If plant lots or shipments are held as separate units, issue separate EAN's for each unit of suspected plant material and associated material held. EAN's are issued under the authority of the Plant Protection Act of 2000 (statute 7 USC 7701-7758). States are advised to issue their own hold orders parallel to the EAN to ensure that plant material cannot move intrastate.

When using EAN's to hold articles, it is most important that the EAN language clearly specify actions to be taken. An EAN issued for positive testing and positive-associated plant material must clearly state that the material must be disposed of, or destroyed, and areas disinfected. Include language that these actions will take place at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infested material, then document on the same EAN, any disposal, destruction, and disinfection orders resulting from investigations or testing.

Find more instructions for completing, using, and distributing this form in the PPQ *Manual for Agricultural Clearance*.

Appendix

C

How to Submit Plant Samples

Plant Samples for Plant Pathology Analysis

1. Sampling

   Please submit adequate amounts of suspect leaf material when possible. This helps ensure that there is sufficient material if downstream diagnostic techniques are required. Twelve or more leaves per sample are desired.

2. Storing

   Refrigerate samples while awaiting shipment to the diagnostic laboratory. Place leaves without paper towel in a sealed and labeled ziplock bag.

3. Documentation

   Each sample should be documented on, and accompanied by its own completed PPQ Form 391 ‘Specimens for Determination’. It is good practice to keep a partially filled electronic copy of this form on your computer with your address and other information filled out in the interest of saving time. Please make sure all fields that apply are filled out and the bottom field (block 24: Determination and Notes) is left blank to be completed by the Identifier. Include the phone number and/or e-mail address of the submitter. Other documentation in the form of notes, images, etc. can be sent along with this if it useful to the determination. It is important that there be a way to cross-reference the sample with the accompanying form. For example, write the “Collection Number” both on the Form 391 and on the sample bag.

4. Packing

   To provide extra insurance against accidental release during shipping, specimens should be double-bagged – i.e. first place the specimen in a self-locking plastic bag and then place that bag within a second self-locking plastic bag. **The Form 391 should not be placed in the bag holding the sample! Rather, it should be placed inside the outer bag**

Place double-bagged samples in a sturdy cardboard box or heavy styrofoam container so that the samples are not damaged during shipping and handling. Ideally, samples should be packed with freezer blocks or wet ice to maintain their integrity during the shipping process. Thoroughly seal all seams on the container with shipping tape.

5. Shipping

The Identifier Laboratory should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded, what types of samples they are (e.g. SOD-suspect Camellia leaves), when the samples will be shipped, and the package tracking number. Label the shipping box as 'URGENT' and send via overnight express courier (FedEx, UPS, Airborne, DHL, etc) to the appropriate Identifier.

Appendix

D

Taxonomic Support for Surveys

Contents

Background

The National Identification Services (NIS) coordinates the identification of plant pests in support of USDA's regulatory programs. Accurate and timely identifications are the foundation of quarantine action decisions and are essential in the effort to safeguard the nation's agricultural and natural resources.

NIS employs and collaborates with scientists who specialize in various plant pest groups, including weeds, insects, mites, mollusks and plant diseases. These scientists are stationed at a variety of institutions around the country, including federal research laboratories, plant inspection stations, land-grant universities, and natural history museums. Additionally, the NIS Molecular Diagnostics Laboratory is responsible for providing biochemical testing services in support of the agency's pest monitoring programs.

On June 13, 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02 which established the role of PPQ NIS as the point of contact for all domestically- detected, introduced plant pest confirmations and communications. A Domestic Diagnostics Coordinator (DDS) position was established to administer the policy and coordinate domestic diagnostic needs for NIS. This position was filled in October of 2007 by Joel Floyd (USDA, APHIS, PPQ-PSPI,NIS 4700 River Rd., Unit 52, Riverdale, MD 20737, phone (301) 734-4396, fax (301) 734-5276, e-mail: joel.p.floyd@aphis.usda.gov).

Taxonomic Support and Survey Activity

Taxonomic support for pest surveillance is basic to conducting quality surveys. A misidentification or incorrectly screened target pest can mean a missed opportunity for early detection when control strategies would be more viable and cost effective. The importance of good sorting, screening, and identifications in our domestic survey activity cannot be overemphasized.

Fortunately most states have, or have access to, good taxonomic support within their states. Taxonomic support should be accounted for in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the State often may require supplies, develop training materials, or need to hire technicians to meet the needs of screening and identification. As well, when considering whether to survey for a particular pest a given year, consider the challenges of taxonomic support.

Sorting and Screening

For survey activity, samples that are properly sorted and screened before being examined by an identifier will result in quicker turn around times for identification.

Sorting

Sorting is the first level of activity that assures samples submitted are of the correct target group of pests being surveyed, that is, after removal of debris, ensure that the correct order, or in some cases family, of insects is submitted; or for plant disease survey samples, select those that are symptomatic if appropriate. There should be a minimum level of sorting expected of surveyors depending on the target group, training, experience, or demonstrated ability.

Screening

Screening is a higher level of discrimination of samples such that the suspect target pests are separated from the known non-target, or native species of similar taxa. For example, only the suspect target species or those that appear similar to the target species are forwarded to an identifier for confirmation. There can be first level screening and second level depending on the difficulty and complexity of the group. Again, the degree of screening appropriate is dependent on the target group, training, experience, and demonstrated ability of the screener.

Check individual survey protocols to determine if samples should be sorted, screened or sent entire (raw) before submitting for identification. If not specified in the protocol, assume that samples should be sorted at some level.

Resources for Sorting, Screening, and Identification

Sorting, screening, and identification resources and aids useful to CAPS and PPQ surveys are best developed by taxonomists who are knowledgeable of the taxa that includes the target pests and the established or native organisms in the same group that are likely to be in samples and can be confused with the target. Many times these aids can be regionally based. They can be in the form of dichotomous keys, picture guides, or reference collections. NIS encourages the development of these resources, and when aids are complete, post them in the CAPS Web site so others can benefit. If local screening aids are developed,

please notify Joel Floyd, the Domestic Diagnostics Coordinator, as to their availability. Please see the following for some screening aids available: http://pest.ceris.purdue.edu/caps/screening.php

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a state is not adequate for a particular survey, in some cases other entities may assist including PPQ identifiers, universities and state departments of agriculture in other states, and independent institutions. Check with the PPQ regional CAPS coordinators about the availability of taxonomic assistance.

Universities and State Departments of Agriculture

Depending on the taxonomic group, there are a few cases where these two entities are interested in receiving samples from other states. Arrangements for payment, if required for these taxonomic services, can be made through cooperative agreements. The National Plant Diagnostic Network (NPDN) also has five hubs that can provide service identifications of plant diseases in their respective regions.

Independent Institutions

The Eastern Region PPQ office has set up multi-state arrangements for Carnegie Museum of Natural History to identify insects from trap samples. They prefer to receive unscreened material and work on a fee basis per sample.

PPQ Port Identifiers

There are over 70 identifiers in PPQ that are stationed at ports of entry who primarily identify pests encountered in international commerce including conveyances, imported cargo, passenger baggage, and propagative material. In some cases, these identifiers process survey samples generated in PPQ conducted surveys, and occasionally from CAPS surveys. They can also enter into our Pest ID database the PPQ form 391 for suspect CAPS target or other suspect new pests, prior to being forwarded for confirmation by an NIS recognized authority.

PPQ Domestic Identifiers

PPQ also has a limited number of domestic identifiers (three entomologists and two plant pathologists) normally stationed at universities who are primarily responsible for survey samples. Domestic identifiers can be used to handle unscreened, or partially screened samples, with prior arrangement through the PPQ regional survey coordinator. They can also as an intermediary alternative to sending an unknown suspect to, for example, the ARS Systematic Entomology Lab (SEL), depending on their specialty and area of coverage.

They can also enter into our Pest ID database the PPQ form 391 for suspect CAPS target or other suspect new pests, prior to being forwarded for confirmation by an NIS recognized authority.

PPQ Domestic Identifiers
Bobby Brown
Domestic Entomology Identifier
Specialty: forest pests (coleopteran, hymenoptera)
Area of coverage: primarily Eastern Region

USDA, APHIS, PPQ
901 W. State Street
Smith Hall, Purdue University
Lafayette, IN 47907-2089
Phone: 765-496-9673
Fax: 765-494-0420
e-mail: robert.c.brown@aphis.usda.gov

Julieta Brambila
Domestic Entomology Identifier
Specialty: adult Lepidoptera, Hemiptera
Area of Coverage: primarily Eastern Region
USDA APHIS PPQ
P.O. Box 147100
Gainesville, FL 32614-7100
Office phone: 352- 372-3505 ext. 438, 182
Fax: 352-334-1729
e-mail: julieta.bramila@aphis.usda.gov

Kira Zhaurova
Domestic Entomology Identifier
Specialty: to be determine
Area of Coverage: primarily Western Region
USDA, APHIS, PPQ
Minnie Belle Heep 216D
2475 TAMU
College Station, TX 77843
Phone: 979-450-5492
e-mail: kira.zhaurova@aphis.usda.gov

Grace O'Keefe
Domestic Plant Pathology Identifier
Specialty: Molecular diagnostics (citrus greening, P. ramorum, bacteriology, cyst nematode screening)
Area of Coverage: primarily Eastern Region

USDA, APHIS, PPQ
105 Buckhout Lab
Penn State University
University Park, PA 16802
Lab: 814 - 865 - 9896
Cell: 814 – 450- 7186
Fax: 814 - 863 – 8265
e-mail: grace.okeefe@aphis.usda.gov

Craig A. Webb, Ph.D.
Domestic Plant Pathology Identifier
Specialty: Molecular diagnostics (citrus greening, P. ramorum, cyst nematode screening)
Area of Coverage: primarily Western Region
USDA, APHIS, PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
Manhattan, KS 66506-5502
Cell (785) 633-9117
Office (785) 532-1349
Fax: 785-532-5692
e-mail: craig.a.webb@aphis.usda.gov

Final Confirmations

If identifiers or laboratories at the state, university, or institution level suspect they have detected a CAPS target, a plant pest new to the United States, or a quarantine pest of limited distribution in a new state, the specimens should be forwarded to an NIS recognized taxonomic authority for final confirmation. State cooperator and university taxonomists can go through a PPQ area identifier or the appropriate domestic identifier that covers their area to get the specimen in the PPQ system (for those identifiers, see table G-1-1 in the Agriculture Clearance Manual, Appendix G link below). They will then send it to the NIS recognized authority for that taxonomic group.

State level taxonomists, who are reasonably sure they have a new United States. record, CAPS target, or new federal quarantine pest, can send the specimen directly to the NIS recognized authority, but must notify their State Survey Coordinator (SSC), PPQ Pest Survey Specialist (PSS), State Plant Health Director (SPHD), and State Plant Regulatory Official (SPRO).

Before forwarding these suspect specimens to identifiers or for confirmation by the NIS recognized authority, please complete a PPQ form 391 with the tentative determination. Also fax a copy of the completed PPQ Form 391 to

"Attention: Domestic Diagnostics Coordinator" at 301-734-5276, or send a PDF file in an e-mail to mailto:nis.urgents@aphis.usda.govwith the overnight carrier tracking number.

The addresses of NIS recognized authorities of where suspect specimens are to be sent can be found in The Agriculture Clearance Manual, Appendix G, tables G-1-4 and G-1-5: http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/mac_pdf/g_app_identifiers.pdf

Only use Table G-1-4, the "Urgent" listings, for suspected new United States records, or state record of a significant pest, and Table G-1-5, the "Prompt" listings, for all others.

When the specimen is being forwarded to a specialist for NIS confirmation, use an overnight carrier, insure it is properly and securely packaged, and include the hard copy of the PPQ form 391 marked "Urgent" if it is a suspect new pest, or "Prompt" as above.

Please contact Joel Floyd, the Domestic Diagnostics Coordinator if you have questions about a particular sample routing, at phone number: 301-734-5276, or e-mail: joel.p.floyd@aphis.usda.gov

Digital Images for Confirmation of Domestic Detections

For the above confirmations, do not send digital images for confirmation. Send specimens in these instances. For entry into NAPIS, digital imaging confirmations can be used for new county records for widespread pests by state taxonomists or identifiers if they approve it first. They always have the prerogative to request the specimens be sent.

Communications of Results

If no suspect CAPS target, program pests, or new detections are found, communication of these identification results can be made by domestic identifiers or taxonomists at other institutions directly back to the submitter. They can be in spread sheet form, on hard copy PPQ form 391's, or other informal means with the species found, or "no CAPS target or new suspect pest species found". Good record keeping by the intermediate taxonomists performing these identifications is essential.

All confirmations received from NIS recognized authorities, positive or negative, are communicated by NIS to the PPQ Emergency and Domestic Programs (EDP) staff in PPQ headquarters. EDP then notifies the appropriate PPQ program managers and the SPHD and SPRO simultaneously. One of these contacts should forward the results to the originating laboratory, diagnostician, or identifier.

Data Entry

Cooperative Agricultural Pest Survey (CAPS)

For survey data entered into NAPIS, new country and state records should be confirmed by an NIS recognized authority, while for others that are more widespread, use the identifications from PPQ identifiers or state taxonomists.

Appendix

E

Disease and Pathogen Common Names and Acronyms

Table E-1 Disease Names Associated with *Candidatus* Phytoplasma mali

Name of Disease
apple proliferation (AP)
apple witches' broom
AP
AP disease
AP-MLO
AT
almaseprusodes
apfeltriebsucht
apple proliferation phytoplasma
apple proliferation, witches' broom
apple witches' broom
apple witches' broom phytoplasma
besenwuchs
brooming
heksekost
hexenbesen bei apfel
hexenbesenwuchs des apfels
maladie des proliférations du pommier
maladie du proliferation du pommiers
proliferacaija
proliferace janoble
proliferaciones del manzano
proliferatie
proliferation
proliferation of apple
rozet (rosette)
scopazzi del melo
skupa milias
triebsucht des apfels
witches' broom
witches' broom of apple

Table E-2 Diseases Names Associated with *Candidatus* Phytoplasma australiense[1]

Name of Disease
phytoplasma yellows
yellow leaf disease
Australian grapevine yellows (AGY or AusGY)
papaya dieback (PDB)
mung bean witches' broom (MBWB)
Phormium yellow leaf (PYL)
strawberry lethal yellows (SLY, SLY1, SLY2)
witches' broom of garden bean (WBGB)
strawberry green petal (SGP)
Coprosma lethal decline (CLD)
sudden decline of cabbage tree (*Cordyline australis*) (CSD)
periwinkle phyllody
paulownia yellows (PY)
pumpkin yellow leaf curl (PYLC)
Gomphocarpus yellowing (CBRYL, cottonbush reduced yellow leaves)
Gomphocarpus physocarpus witches' broom (CBWB)
liquidambar yellows (LaY)
Australian lucerne yellows (ALuY)
AGY
AUSGY
Australian grapevine yellows
Australian grapevine yellows phytoplasma
Australian yellows of grapevine
Ca. P. australiense
grapevine Australian yellows phytoplasma
jaunisse de la vigne
liquidambar yellows
LaY
PD
PDB
PYL
papaya die-back

Table E-2 Diseases Names Associated with *Candidatus* Phytoplasma australiense[1]

Name of Disease
papaya dieback
pawpaw dieback phytoplasma
phormium yellow leaf
phormium yellow leaf disease
phormium yellow leaf phytoplasma
Phytoplasma australiense (*Candidatus*) (Davis et al. 1997)
SLY
strawberry green petal
strawberry green petal disease
strawberry lethal yellows
strawberry lethal yellows disease
strawberry lethal yellows phytoplasma
sudden decline of cabbage tree
yellow leaf disease

1 Australian grapevine yellows (AGY), which is associated with 'Ca. P. australiense' (IRCPM 2004), is listed on the APHIS (2000) Regulated Plant Pest List (RPPL) (queried 05 March 2007). However, none of the other diseases associated with 'Ca. P. australiense' are listed on the RPPL.

Table E-3 Disease Names Associated with *Candidatus* Phytoplasma prunorum

Name of Disease
almond decline
apricot chlorotic leaf roll
apricot dieback
cherry Molière's disease
decline of European plum
decline of Japanese plum
European plum yellows
flowering cherry decline
leptonecrosis of Japanese plum

Table E-3 Disease Names Associated with *Candidatus* Phytoplasma prunorum

Name of Disease
nectarine chlorotic leaf roll
peach chlorotic leaf roll
peach decline
peach yellows
peach rosette
peach vein clearing
peach vein enlargment
plum leptonecrosis
accartocciamento clorotico dell'albicocco
apricot chlorotic leaf roll
apricot chlorotic leaf roll mycoplasm
apricot chlorotic leaf roll phytoplasma
apricot chlorotic leaf roll virus
apricot chlorotic leafroll
apricot chlorotic leafroll phytoplasma
apricot dieback
cherry Molières disease
cherry molières disease phytoplasma
chlorotic leafroll of apricot
chlorotic leafroll of nectarine
chlorotic leafroll of peach
chlorotische blattrollkrankheit der aprikose
chlorotisches blattrollen
chlorotisches blattrollen
decline of Japanese plum
decline of peach
desarreglos vegetativos del albaricoquero
dieback of apricot
dépérissement de Molières
ESFY
ESFYP
ESFY Phytoplasma
ESFY-P
enrollamiento clorótico
enrollamiento clorótico del albaricoquero

Table E-3 Disease Names Associated with *Candidatus* Phytoplasma prunorum

Name of Disease
enroulement chlorotique de l'abricotier
European peach yellows
European stone fruit yellows mycoplasma-like organism
European stone fruit yellows phytoplasma
European yellows of peach
giallume Europeo delle drupacee
Italian peach rosette
Italian rosette of peach
leptonecrosis of Plum
maladie de Molières
Molieres disease
Molières disease of cherry
moria del Pero
nectarine chlorotic leafroll
peach chlorotic leaf roll
peach chlorotic leaf roll virus
peach decline
peach rosette
peach vein clearing
peach vein enlargement
peach yellows
plum leptonecrosis[1]
vein clearing of peach
vein enlargement of peach

1 First described as Steinobst Chlorotisches Blattrollen.

Appendix

F

Molecular Detection

Contents

DNA Extraction From Plant Tissue

Midrib and main vein tissue (1 g) should be used from leaves collected avoiding any large necrotic areas. Use the following procedure (Ahrens and Seemüller 1992) to obtain DNA for PCR based detection:

1. Incubate the tissue in 6 ml of grinding buffer on ice for 10 min
2. Grind the tissue, then add 8 ml of fresh buffer and grind once more
3. Centrifuge at 1100 g and 4°C for 10 min
4. Decant the supernatant then centrifuge at 14,600 g and 4°C for 25 min
5. Resuspend the pellet in 1.5 ml of warm (60°C) extraction buffer
6. Incubate at 60°C for 30 min
7. Add to the lysate an equal volume of chloroform/isoamylalcohol (24:1, v/v)
8. Precipitate the aqueous layer with two-third volume of -20°C isopropanol
9. Centrifuge at 15,000 g and 4°C

10. Wash the pellet with 70 percent ethanol
11. Dry under vacuum
12. Add 50 µg/ml RNAse at 37°C for 30 min
13. Extract with chloroform/isoamylalcohol (24:1, v/v), precipitate with ethanol, wash and dry pellet as described

Grinding Buffer

potassium phosphate 125 mM
ascorbic acid 30 mM
sucrose 10 percent
bovine serum albumin (BSA fraction V) 0.15 percent
polyvinylpyrrolidone (PVP 15) 2 percent
pH 7.6

Extraction Buffer

Hexadecyltrimethyl-ammonium bromide (CTAB) 2 percent
NaCl 1.4 M
2-mercaptoethanol 0.2 percent
Ethylendiaminetetraacetic (EDTA) 20 mM
Tris- hydroxymethylaminomethane hydrochloride (Tris-HCl) 100 mM
pH 8.0

Polymerase Chain Reaction

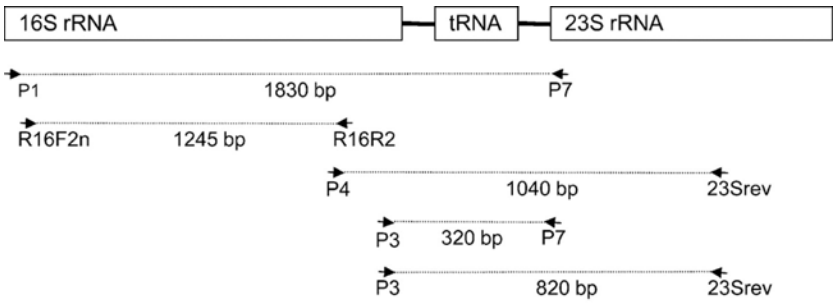
Required elements for positive molecular diagnosis:

1. Water instead of template DNA must be added to reaction mixture for use as negative controls in PCR experiments
2. In case of negative result with phytoplasma specific primer sets, the use of universal rRNA primers is recommended. Suggested universal primers: fU5/RU3 (Lorenz et al. 1995); F2n/R2 (Lee et al. 1995); and P1/Tint (Smart et al. 1996)
3. Then use PCR, with other group- or species-specific primers, and RFLP analysis for differentiation (Lorenz et al. 1995; Smart et al. 1996)
4. Use DNA of reference strains, belonging to the same and to other groups, in all molecular diagnostic methods

For general phytoplasma detection, the most common protocol begins with the amplification of the 16S-23S rDNA sequences using primers P1/ P7 (*Table F-1* on page F-3).

Table F-1 General Phytoplasma Detection Primers for 16S-23S rDNA

Name	Oligonucleotide Sequence	Annealing Temperature	Expected Product Size	Reference
P1	5'-AAGAGTTT-GATCCTGGCTCAG-GATT-3'	55°C	1800 bp	Deng and Hiruki 1991
P7	CGTCCTTCATCG-GCTCTT	55°C	1800 bp	Schneider et al. 1995



Hodgetts et al. 2008

Figure F-1 Diagrammatic Representation of 16S–23S rRNA Gene

The 16S–23S rRNA gene organization in phytoplasmas is represented in *Figure F-1* on page F-3, showing the relative positions of some of the universal primers.

Alternatively, for samples with low titers or inhibitors, a nested-PCR assay using an initial universal primer pair, P1/ P7 or R16mF2/R16mR1, followed by a second amplification with R16F2n/m23sr or R16F2n/ R16R2 (*Table F-2* on page F-4) reportedly can increase detection sensitivity over 100 folds, and readily detect phytoplasmas in woody hosts and insects (Gundersen and Lee 1996). A nested PCR that utilizes a universal primer set followed by a group-specific set of primers as the advantage to allow the detection of phytoplasmas in a sample containing more than one phytoplasma strain.

Three pairs of oligonucleotides were designed to selectively amplify DNA from '*Ca.* P. australiense': AUSGYF1/AUSGYR2 (Davis et al. 1997), fStol/ AGY 2 (Gibb et al. 1998), and FP/RY (Getachew et al. 2007). These sets of primers can be used in a direct (non-nested) PCR or in a nested PCR in

combination with a set of universal primer such as P1/P7 or R16F2n/R16R2 (*Table F-2* on page F-4).

Table F-2 Primers for Phytoplasma Group or Specific Phytoplasma Identitification

Name	Oligonucleotide Sequence	Annealing Temperature	Expected Product Size	Reference
R16F2n	5’-GAAACGACTGCTA-AGACTGG-3’	50°C	1244 bp	Lee et al. 1993
R16R2	5’-TGACGGGCGGT-GTGTACAAACCCCG-3’	50°C	1244 bp	Lee et al. 1993
AUSGY F1	5’-ATCTTTA-AAAGACCTCGCAAG-3’	55°C	644 bp	Davis et al. 1997[1]
AUSGY R2	5’-AGTTTTACCCAAT-GTTTAGTACTC-3’	55°C	644 bp	Davis et al. 1997[1]
fStol	5-GCCATCATTAAGTT-GGGGA-3’	50°C	600 bp	Maixner et al. 1995
AGY 2	5’-GATGTGACCTATTT-TATTTG-3’	50°C	600 bp	Gibb et al. 1998
FP	5'-GCATGTCGCGGT-GAATAC-3'	50°C	267 bp	Getachew et al. 2007
RY	5'-TGAGCTATAG-GCCCTTAATC-3'	50°C	267 bp	Getachew et al. 2007

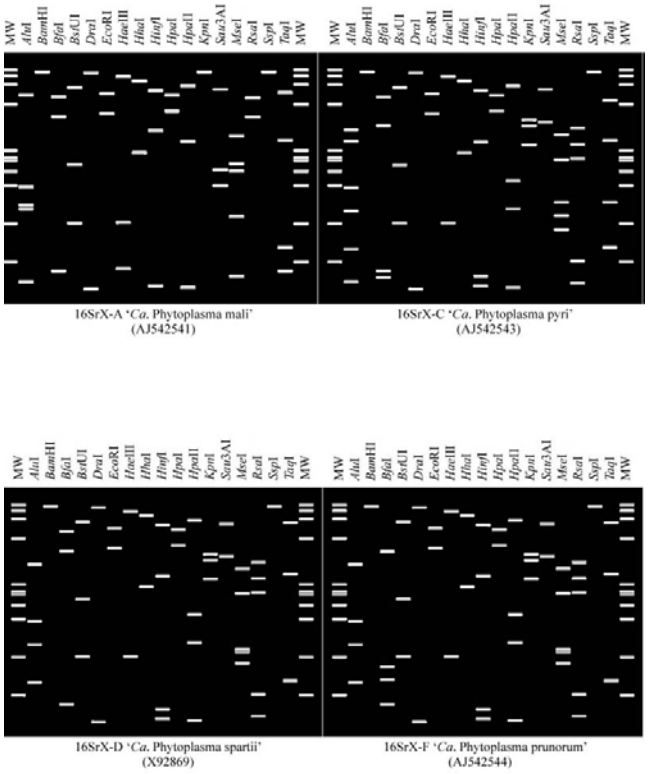
1 An exception was reported by Habili et al. 2007 using this set of primers on a related strain infecting *Liquidambar styraciflua* (sweet gum).

After PCR amplification, the DNA product should be cloned into a vector, purified and sent to appropriate labs for sequencing. The sequence result should be compared for identification with known phytoplasma reference sequences available from GenBank. The 16S rRNA gene sequences associated with '*Ca*. P. mali', '*Ca*. P. australiense' and '*Ca*. P. prunorum' strains are deposited with accession number: AJ542541; L76865; AJ542544 (see below).

The obtained amplified DNA product (amplicon) can be subjected to enzymatic digestion to perform a restriction fragment length polymorphism (RFLP) assay. Digestion with a selected number of endonucleases enzymes (*Alu*I, *Taq*I, *Mse*I, *Rsa*I, *Hha*I) will cut the amplified product at specific sites. The resulting restriction fragments are separated according to their lengths by

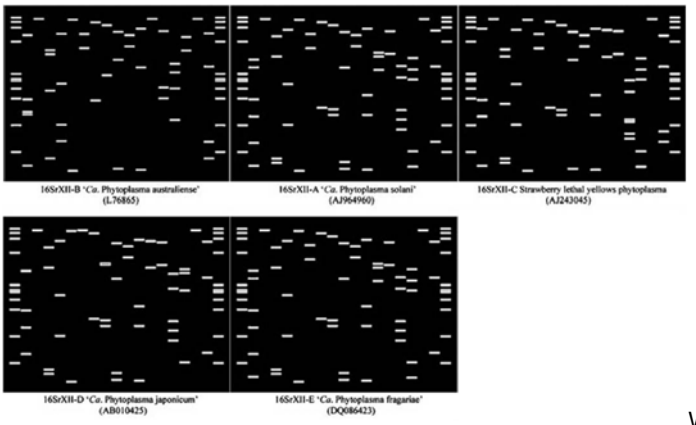
gel electrophoresis generating a unique pattern characteristic of each phytoplasma group. Phytoplasma classification according to RFLP profile is available from Lee et al. 1998.

For identification purposes, an interactive online tool, *iPhyClassifier* has been developed by Zhao et al. (2009), to perform sequence similarity analysis, simulate restriction enzyme digestions and generate virtual RFLP profiles (*Figure F-2* on page F-5 and *Figure F-3* on page F-6). Based on calculated RFLP pattern similarity coefficients and overall sequence identity scores, *iPhyClassifier* makes suggestions on tentative phytoplasma 16Sr group/ subgroup classification status and '*Candidatus* Phytoplasma' species assignment.



Wei et al. 2007

Figure F-2 16SrX Virtual RFLP Patterns; Virtual RFLP patterns of 16S rDNA of Four Members of the 16SrX: Apple Proliferation Group of Phytoplasmas From Use of the iPhyClassifier Program



Wei et al. 2007

Figure F-3 16SrXII Virtual RFLP Patterns; Virtual RFLP Patterns of 16S rDNA of Five Members of the 16SrXII Group of Phytoplasmas from use of the iPhyClassifier Program

The analysis of *rp, secY, tuf* and *secA* genes as well as the 23S rRNA gene and the 16S-23S rRNA intergenic spacer region have been introduced as additional tools to allow better resolution of particular lineages within each 16Sr group. According to Anderson et al. (2006)

> The 16S-23S rDNA spacer region is more variable and can be useful in distinguishing groups of phytoplasmas. However, the two rRNA operons (in a given phytoplasma chromosome) can differ from one another in nucleotide sequence, making it difficult to distinguish variation between paralogous operons within a strain from orthologous genes between strains. Alternatives include protein coding genes such as ribosomal protein genes and the *tuf* gene, which encodes the elongation factor Tu (EF-Tu). In Mollicutes, including phytoplasmas, the *tuf* gene is present as a single copy and can be sequenced readily after amplification using a PCR approach.

The primer pair fTuf AY and rTuf AY is specific to members of the Aster yellows phytoplasma group (*Table F-3* on page F-6). The resulting amplification product can be subjected to RFLP analysis and provide insight into the genetic relatedness of amplified phytoplasma strains.

Table F-3 Primers for Tuf Gene in Phytoplasmas

Name	Oligonucleotide Sequence	Annealing Temperature	Expected Product Size	Reference
fTuf AY	5'-GCTAAAAG-TAGAGCTTATGA-3'	53°C	850 bp.	Schneider and Seemüller 1996
rTuf AY	5'-CGTTGTCACCTG-GCATTACC-3'	53°C	850 bp.	Schneider and Seemüller 1996

16SrX: Apple Proliferation Group PCR Based Detection

Several primers intended for diagnostic procedures for the diagnosis of phytoplasma diseases have been identified, among them are those specific to the apple proliferation group (*Table F-4* on page F-7) (Firrao et al. 1994; Jarausch et al. 1994; Lee et al. 1995; Lee et al. 1993; Lorenz et al. 1995; Smart et al. 1996).

Table F-4 Molecular Diagnosis of Apple Proliferation Phytoplasma by Direct PCR

Name	Oligonucleotide Sequence	Expected Product Size	Phytoplasma Isolates	Reference
f01	5'-CGGAAACTTT-TAGTTTCAGT-3'	1.1 kb	AP group (AP, PD, ESFY, PYLR)	Lorenz et al. 1995
r01	5'-AAGTGCCCAACTA-AATGAT-3'	1.1 kb	AP group (AP, PD, ESFY, PYLR)	Lorenz et al. 1995
fPD	5'-GACCCGTAAGG-TATGCTG-3'	1.0 kb	pome fruit sub-group (AP, PD)	Lorenz et al. 1995
r01	5'-AAGTGCCCAACTA-AATGAT-3'	1.0 kb	pome fruit sub-group (AP, PD)	Lorenz et al. 1995
fAT	5'-CATCATTTAGTT-GGGCACTT-3'	0.5 kb	pome fruit sub-group (AP, PD)	Smart et al. 1996
rAS	5'-GGCCCCGGAC-CATTATTTATT-3'	0.5 kb	pome fruit sub-group (AP, PD)	Smart et al. 1996
AP5	5'-TCTTTTA-ATCTTCAACCATGG C-3'	0.48 kb	Specific for AP	Jarausch et al. 1994
AP4	5'-CCAATGTGT-GAAATCTGTAG-3'	0.48 kb	Specific for AP	Jarausch et al. 1994
AP3	5'-CTAAAACTCAC-GCTTCAGCTACTC-3'	0.67 kb	Specific for AP	Firrao et al. 1994
AP5	5'-TGAGATTTGCTA-AAACTCACG CTT-3'	0.67 kb	Specific for AP	Firrao et al. 1994

The pair fO1/rO1, AP3-AP5 and fPD/rO1 prime in the 16S rDNA sequence and are specific to the first AP group, the second AP, and the third pome fruit subgroup, respectively. The pair fAT/rAS primes in the 16S/23S rDNA spacer region and is specific for pome fruit subgroup phytoplasmas.

The pair AP5-AP4 was designed from a randomly cloned nucleotide sequence (Gene Bank accession number L22217) of the German isolate AT (1812 bp) of

the previously cloned *Hind* III fragment IH196 (3,7 Kb) sequenced from both ends. This pair is specific for AP phytoplasma.

Table F-5 Molecular Diagnosis of Apple Proliferation Phytoplasma by Nested PCR

Name	Oligonucleotide Sequence	Expected Product Size	Phytoplasma Isolates	Reference
R16F2	5'-ACGACTGCTA-AGGACTGG-3'	1.2 kb	All	Lee et al. 1993
R16R2	5'-TGAGGGGCGGT-GTGTACAAACCCCG-3'	1.2 kb	All	Lee et al. 1993
R16(X) F1	5'-GACCCGCAAGTAT-GCTGAGAGATG-3'	1.1 kb	AP group (AP, PD, ESFY)	Lee et al. 1995
R16(X) R1	5'-CAATCCGAACT-GAGACTGT-3'	1.1 kb	AP group (AP, PD, ESFY)	Lee et al. 1995

PCR products initially amplified by using the universal primer pair R16F2/R2 are diluted (1/40) with sterile deionized water and used as template DNA for a subsequent PCR with the pair R16(X)F1/R1 (*Table F-5* on page F-8). This pair

primes on the 16S rDNA sequence (Lee et al. 1995). All these primer pairs work at different reaction mixtures and conditions (*Table F-6* on page F-9).

Table F-6 Reaction Mixtures and Conditions for PCR with Different Primer Pairs for Diagnosis of AP Group and AP Phytoplasma

Primer Pairs	Final Volume	Tem-plate	Primers	Buffer (MgCl2 1.5 mM)	dNTPS	Enzyme (Unit/ reac-tion)	Condi-tions
fO1/rO1; fPD/rO1	40 µl	100-200 ng	0.5 µM	1x	100 µM	0.20-1 Unit	35 cycles: 95°C for 30 s denatur-ation, 55°C for 75 s anneal-ing, 72°C for 90 s exten-sion,
fAT/rAS	30 µl	50 ng	0.5 µM	1x	150 µM	1 Unit	30 cycles: 94°C for 60 s denatur-ation, 55°C for 60 s anneal-ing, 72°C for 120 s exten-sion,

Table F-6 Reaction Mixtures and Conditions for PCR with Different Primer Pairs for Diagnosis of AP Group and AP Phytoplasma

Primer Pairs	Final Volume	Tem-plate	Primers	Buffer (MgCl2 1.5 mM)	dNTPS	Enzyme (Unit/ reac-tion)	Condi-tions
AP5/ AP4	40 µl	10-100 ng	0.5 µM	1x	125 µM	0.5 Unit	95°C for 60 s pre-denatur-ation step, 40 cycles: 95°C for 10 s denatur-ation, 58°C for 15 s anneal-ing, 72°C for 45 s exten-sion, 72°C for 240 s elonga-tion,
fAT/rAS	30 µl	50 ng	0.5 µM	1x	150 µM	1 Unit	30 cycles: 94°C for 60 s denatur-ation, 55°C for 60 s anneal-ing, 72°C for 120 s exten-sion,

Table F-6 Reaction Mixtures and Conditions for PCR with Different Primer Pairs for Diagnosis of AP Group and AP Phytoplasma

Primer Pairs	Final Volume	Tem-plate	Primers	Buffer (MgCl2 1.5 mM)	dNTPS	Enzyme (Unit/ reac-tion)	Condi-tions
AP5/ AP4	40 µl	10-100 ng	0.5 µM	1x	125 µM	0.5 Unit	95°C for 60 s pre-denatur-ation step, 40 cycles: 95°C for 10 s denatur-ation, 58°C for 15 s anneal-ing, 72°C for 45 s exten-sion, 72°C for 240 s elonga-tion,

Table F-6 Reaction Mixtures and Conditions for PCR with Different Primer Pairs for Diagnosis of AP Group and AP Phytoplasma

Primer Pairs	Final Volume	Tem-plate	Primers	Buffer (MgCl2 1.5 mM)	dNTPS	Enzyme (Unit/ reac-tion)	Condi-tions
R16F2/ R2; R16(X)F 2/R2	50 µl	20 ng	0.4-1 µM	1x	200 µM	1 Unit	94°C for 120 sprede-natur-ation step, 35 cycles: 94°C for 60 s denatur-ation, 50°C for 120 s anneal-ing, 70 °C for 180 s exten-sion, 72°C for 600s elonga-tion,
AP3-AP5	50 µl	2 µl	300 ng	1x	100 mM	1 Unit	25 cycles: 94°C for 30 s denatur-ation, 63°C for 30 s anneal-ing, 72°C for 30 s exten-sion, 72°C for 600 s elonga-tion,

General PCR Protocol

For amplification of general phytoplasma genetic material, there are some standard methods and primers available. Specific instructions or other details from this protocol may apply and the original sources should be consulted if satisfactory results are not achieved. Following steps in *DNA Extraction From Plant Tissue* on page F-1, the general protocol is as follows. Create a mixture in a final volume of 50 µl containing 20 to 50 ng of total nucleic acid extracted from plant tissue. Primers (*Table F-7* on page F-13) are used at 0.4 µM/each, 1×DNA polymerase buffer (1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl), 1.25 U of *Taq* DNA polymerase, 0.2 mM dNTP. The PCR machine settings should start with: Initial denaturation 2 min at 94°C; denaturation, 30 sec at 94°C; annealing, 30 sec at 50°C (temperature will vary based on the primer used); extension, 3 min at 72°C (35 cycles). Final extension for 10 min at 72°C. A 5-µl aliquot of the PCR reaction is to be analyzed by electrophoresis in a 1 percent agarose gel, stained with 0.5 µg of ethidium bromide (or similar) per ml and visualized with a UV transilluminator. In nested PCRs, the product from a direct PCR may be diluted (1:50 or 1:100) with sterile deionized distilled water and 1 µl used as the template for the second (nested) PCR. Other protocols do not dilute the first stage PCR product for nested reaction.

Table F-7 Partial List of Phytoplasma Universal Primers

Name	Sequence (5'-3')	PCR Product (kbp)	Reference
R16F0	CTGGCTCAGGAT-TAACGCTGGC-GGC	1.485	Lee et al. 1993
R16R0	GGATACCTTGT-TACGACTTA-ACCCC		
16R758F	GTCTTTACTGAC-GCTGAGGC	0.5	Gibb et al. 1995
16R1232R	CTTCAGC-TACCCTTTGTAAC		
R16F1	AAGACGAGGATA-ACAGTTGG	1.4	Davis and Lee 1993
R16R0	GGATACCTTGT-TACGACTTA-ACCCC		
Gd1	ACGGAGAGTTT-GATCCTG	1.5[1]	Andersen et al. 1998
Berg54	AAAGGAGGT-GATCCAGCCG-CACCTTC		
R16mF2	CATGCAAGTC-GAACGGA	1.4	Gundersen and Lee 1996

Table F-7 Partial List of Phytoplasma Universal Primers

Name	Sequence (5'-3')	PCR Product (kbp)	Reference
R16mR1	CTTAACCCCAAT-CATCGAC		
P3	GGATGGAT-CACCTCCTT	0.32	Schneider et al. 1995
P6	TGG-TAGGGATACCTT-GTTACGACTTA		
rpL2F3	WCCTTGGGG-YAAAAAAGCTC [2]	1.6	Martini et al. 2007
rp(I)R1A	GTTCTTTTTG-GCATTAACAT		
rpF1C	ATGGTDGGDCAY-AARTTAGG [2]	1.212-1.386[3]	Martini et al. 2007
rp(I)R1A	GTTCTTTTTG-GCATTAACAT		

1 General prokaryotic 16S rRNA gene primers.

2 Product size is group-dependent.

3 (W=A+T; Y=C+T; D=A+G+T; and R=A+Gz).

Restriction Fragment Length Polymorphism Analysis

The use of PCR amplification of the 16S rDNA to differentiate phytoplasmas belonging to the same group is difficult due to the high similarity of their sequences so that it is necessary to digest the amplification products by restriction endonucleases (*Table F-8* on page F-14).

Table F-8 Molecular Characterization of AP Phytoplasma with RFLP Analysis of Amplified Products Obtained with Different Primers

Primer Pair	Endonuclease	Phytoplasma	Reference
f01/r01; fPD/r01	Ssp I, Sfe I	AP	Lorenz et al. 1995
R16F2/R2 + R16(X)F2/R2	Rsa I	AP	Lee et al. 1995
AP5-AP4	Ssp I, Spe I, Hinf I	AP	Jarausch et al. 1994

Loop-Mediated Isothermal Amplification

For field-detection of phytoplasmas in infected plant material, a rapid DNA extraction and loop-mediated isothermal amplification (LAMP) procedure (Tomlinson et al. 2010) could be implemented in association with a colorimetric assay. Information regarding recognized insect vectors and plant hosts are necessary for a clear identification.

Dot-Blot Hybridization

Differential diagnosis of apple proliferation and European stone fruit yellow phytoplasmas can be made by oligonucleotide hybridization in the presence of tetramethylammonium chloride (Malisano et al. 1996). This diagnostic approach may be more practical than PCR plus RFLP in differentiating the two phytoplasmas that cause apple proliferation and plum leptonecrosis, which are economically significant diseases in the main European fruit tree-growing areas. It is also suitable for automatic evaluation of a large number of samples.

DNA Amplification and Hybridization

The primer pair P1-P4 should be used, which primes on 16S rDNA sequence and amplifies a segment of 0.86 Kb (*Table F-9* on page F-16) The amplification product can be used as follows:

1. Add 2 x SSC to 5-45 µl of amplification product (1 x SSC: 150 mM NaCl and 15 mM Na citrate, pH 7.0).
2. vacuum-filter on nylon membranes with a 96-well dot-blot manifold apparatus
3. place dried membrane sequentially on 3 mm paper sheets saturated with: 1.5 M NaCl and 0.5 NaOH for 5 min; 0.5 M Tris pH 7.4; 0.5 M Tris pH 7.4 and 1.5 M NaCl for 5 min
4. dry and bake for 30 min at 120°C
5. pre-hybridize in 5 x SSC; -0.1 percent laurosylsarcosine; 0.02 percent SDS; 1 percent blocking reagent; 42°C for 2 h
6. hybridize in solution as above containing 20 pmol oligonucleotide probe at 42°C for 6 h
7. washings :2 x 30 min in 5x SSC at 4°C; 2 x 20 min at 57°C in tetramethylammonium chloride wash solution (wash solution: 3 M TMACl, 50 mM Tris-HCl; 2 mM EDTA; 0.1 percent sodium dodecyl sulfate (SDS), pH 8)

8. digoxigenin labelling and detection can be performed according to manufacturer's instructions

Table F-9 Dot Blot Hybridization Primer and Probe Sequences

Name	Oligonucleotide Sequence	Reference
Primers[1]		
P1	5'-CAGCAGGYCCGCGTA-ATACATA-3'	Firrao et al. 1993
P4	5'-RMCCCGAGAAGC-TATTCACCG-3'	Firrao et al. 1993
Probes		
LN 11	5'-GTGCGTAGGCGGT-TAAA-3'	Malisano et al. 1996
AP 11	5'-GTGTGTAGGCGGT-TAAA-3'	Malisano et al. 1996

1 Y = C or T; R = A or G; m = A or C.

Real-Time PCR Assay with TaqMan® MGB Probe

This method utilizes a single set of primers for the amplification of a 146-147 bp amplicon in combination with three distinct TaqMan minor groove binder (MGB) probes specifically designed for the detection of AP, PD and ESFY phytoplasmas 16S-23S rRNA intergenic spacer region (IGS) (*Table F-10* on page F-17) (Nikolic et al. 2010). This assay combines speed, sensitivity and specificity for potential high-throughput applications such as phytoplasma free certificiation programs or field test surveys. Additionally a control reaction that uses UniRNA primer set for the amplification of a 73 bp amplicon and a single probe can be added for general phytoplasma detection (Hren et al. 2007)

Table F-10 Real Time PCR Assay Primer/Probe Sequences and Reaction Mixture Conditions

Name	Sequence
Forward primer	5'-TGGTTAGAGCACACGCCTGAT-3'
Reverse primer	5'-TCCACTGTGCGCCCTTAATT-3'
AP-Probe[1]	5' FAM–CAAAGTATTTATCTTAAGAAA ACAAGCT-3' NFQ
PD- Probe[1]	5' FAM–AATATTTATTTTAAAAAAAAGCTCTTT G-3' NFQ
ESFY- Probe[1]	5' FAM–CAAAATATTTATTTTAAAAAAACAAGCTC-3' NFQ
UniRNA Forward Primer	5?-AAATATAGTGGAGGTTATCAGGGATACAG -3?
UniRNA Reverse Primer	5?-AACCTAACATCTCACGACACGAACT-3?
UniRNA Probe	5? FAM-ACGACAACCATGCACCA-3?NFQ

Final Volume	Template	Primers	Probes	Buffer	Reaction Conditions
10 µl	2 µl <100 ng	900 nM	90 nM	1x qPCR Master Mix	50°C for 120 s 95°C for 600 s 45 cycles 95°C for 15 s 60°C for 60 s

1 Probes labeled with 6-carboxyfluorescein (FAM) at the 5' end and a non-fluorescent quencher (NFQ) with MGB at the 3' end.

AP Phytoplasma Reference Sequence: Accession AJ542541

Candidatus Phytoplasma mali 16S rRNA gene, tRNA-Ile gene and 23S rRNA gene (partial), strain AP15 GenBank: AJ542541.1

AGY Phytoplasma Reference Sequence: Accession L76865

Australian grapevine yellows phytoplasma 16S ribosomal RNA (16S rRNA) gene, partial sequence, 16S-23S ribosomal RNA spacer region and tRNA-Ile gene, complete sequence GenBank: L76865.1

ESFY Phytoplasma Reference Sequence: Accession AJ542544

Candidatus Phytoplasma prunorum 16S rRNA gene, tRNA-Ile gene and 23S rRNA gene (partial), strain ESFY-G1 GenBank: AJ542544.1

Appendix G

Known Phytoplasma Vectors

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
Cicadellidae				
Subfamily Agalliinae				
Anaceratagallia torrida (Evans)	rugose leaf curl	clovers	Australia	Grylls et al. 1974
A. ribauti Ossiannilsson	Stolbur (Bois Noir, Tuf type II)	grapevine	Austria	Riedle-Bauer et al. 2008
Subfamily Aphrodinae				
Aphrodes bicincta[3] (Schrank)	clover phyllody16sriv	clovers	Europe	Brcak 1979
	stolbur/16srxii-a	various	Europe	Brcak 1979
	strawberry green petal/ 16sri-c	strawberry, clover	United Kingdom	Posnette & Ellen-berger 1963
A. albifrons (Linnaeus)	phyllody		Europe	Maramorosch 1963
Subfamily Cicadellinae				
Grapohocephala confluens (Uhler)	Western X-disease/ 16SrIII-A	stonefruit	North America	Anthon & Wolfe 1951
Subfamily Coelidiinae				
Coelidia indica[4]	sandal spike	sandal wood	India	Rangaswami & Griffith 1941
Subfamily Deltocephali-nae				
Acinopterus angulatus Lawson	aster yellows	various	North America	Severin 1947a
	western x-disease/16sriii-a	stonefruit	North America	Purcell 1979
Athysanus argentarius Metcalf	aster yellows 16sri		North America	Chiykowski 1979
Cechenotettix quadrino-tatus Mulsant & Rey (= martini Lethierry)	yellow decline/16srxii	*Lavandula* hybrids	France	Boudon-Padieu & Cousins 1999

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
Circulifer haematoceps (Mulsant & Rey)	sesame phyllody	sesame	Middle East	Kersting & Baspinar 1997
	16srv-16srix	*Limonium* spp.	Israel	Weintraub et al. 2004
C. tenellus (Baker)	beet leafhopper-transmitted virescence disease	vegetables	North America	Oldfield et al. 1977
	tomato big bud/16srvi-a	vegetables	North America	Shaw et al. 1993
	16SrV-16SrIX	*Limonium* spp.	Israel	Weintraub et al. 2004
	Columbia baisn potato purple top	potatoes	North America	Munyaneza et al. 2007
Colladonus clitellarius (Say)	Eastern X-disease/ 16SrIII-A	stonefruit trees	North America	Gilmer et al. 1966
Co. geminatus (van Duzee)	aster yellows	weeds, vegetables	North America	Frazier & Severin 1945
	Western X-disease/ 16SrIII-A	stonefruit	North America	Wolfe et al. 1950
Co. montanus (van Duzee)	Western X-disease/ 16SrIII-A	stonefruit	North America	Wolfe 1955
	aster yellows	various	North America	Nielson 1979
Dalbulus elimatus (Ball)	maize bushy stunt/16sri-b	corn	North America/ Mexico	Niederhauser & Cervantes 1950
D. maidis (DeLong and Wolcott)	maize bushy stunt/16sri-b	corn	North America/ Mexico	Kunkel 1946
Deltocephalus flavicosta (Stal.)	phyllody	palms	Jamaica	Dabek 1982
Deltocephalus vulgaris Dash & Viraktamath	grassy shoot disease	sugarcane	India	Singh et al. 2002
Euscelidius variegatus Kirschbaum	clover phyllody/16sri-c	clover	Europe	Giannotti 1969
	aster yellows[5]	vegetables & weeds	North America	Severin 1947b
	Western x-disease[d]/ 16sriii-a	stonefruit	North America	Jensen 1969
	flavescens doree/16srv-c	grape	France	Boudon-Padieu et al. 1989
	chrysathemum yellows/ 16sr-ib	flowers	Europe	Palermo et al. 2001
Euscelis incisus (Kirschbaum) (= *plebeja* Fallen	phyllody	*Rubus* spp.	Europe	Brcak 1979
	Stolbur/16SrXII-A	Solanaceae	Europe	Brcak 1979
	clover witches'-broom/ 16srvi	white clover	England	Posnette & Ellenberger 1963

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
	chrysanthemum yellows 16sri-b		Italy	Alma et al. 2001
E. lineolatus Brulle	clover phyllody/16sri-c	clovers	Europe	Savio & Conti 1983
	green petal disease/ 16sri-c	strawberry	England	Frazier & Posnette 1956
Euscelis obsoletus (Kirschbaum)	bois noir (16srxii-a)	grape	Europe	Lavina et al. 2006
Exitianus capicola Stal	16SrV-16SrIX	*Limonium* spp.	Israel	Weintraub et al. 2004
Endria inimica (Say)	aster yellows/16sri	celery	North America	Chiykowski 1963
Fieberiella florii (Stal)	apple proliferation/16srx-a	apple, stonefruits	Europe	Krczal et al. 1988
	aster yellows/16sri	various families	North America	Severin 1947a
	Western x-disease/ 16sriii-a	stonefruit trees	North America	Anthon & Wolfe 1951
	Eastern x-disease/16sriii-a	stonefruit trees	North America	Gilmer et al. 1966
Graminella nigrifrons (Forbes)	maize bushy stunt	Corn	North America	Nault 1980
Hishimonoides chinensi Anufriev	jujube witches'-broom/ 16srv-b	Chinese jujube tree	China	Tsai et al. 1988
H. sellatiformis Ishihara	mulberry dwarf/16sri-b	mulberry	China	Ishijima & Ishiie 1981
H. phycitis (Distant)	eggplant little leaf	eggplant	East Asia	Maramorosch et al. 1970
H. sellatus Uhler	mulberry dwarf/sr16i-b	mulberry	East Asia	Ishijima & Ishiie 1981
	Rhus yellows 16SrI	*Rhus javanica*, clover, periwinkle	East Asia	Kusunoki et al. 2002
	jujube witches' broom/ 16SrV-B	Ziziphus jujuba	East Asia	Kusunoki et al. 2002
	Cryptotaenia joponica witches'-broom	*Cryptotaeia joponica*	East Asia	Nishimura et al. 1998
Loepotettix dilutior Kirschbaum	Stolbur	brambles, clover	England	Ponnamma & Solomon 1998
Macrosteles cirstata (Ribaut)	clover phyllody/16SrI-C	various families	Europe	Brcak 1979
	clover dwarf/16sriii-b	vegetables	Europe	Brcak 1979
M. laevis (Ribaut)	european aster yellows/ 16sri-b	various	Europe	Brcak 1979
	clover phyllody/16sri-c	various	Europe	Valenta 1960
	clover dwarf/16sriii-b	various	Europe	Brcak 1979
	Stolbur/16SrXII-A	tomato, potato	Turkey	Guclu & Ozbek 1991

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
M. quadrilineatus Forbes (=*fascifrons*)	American aster yellows/ 16SrI-A	vegetables	North America	Giulmer 1954
	European aster yellows/ 16SrI-B	vegetables	Europe	Esau et al. 1976
	Stolbur	vegetables	Europe	Batlle et al. 2008
Macrosteles quadri-punctulatus (Kirsch-baum)	Kok-saghyz yellows		Europe, Russia	Brcak 1979
	chrysanthemum yellows/ 16sr-ib	chrysanthemum	Europe	Palermo et al. 2001
M. severini Hamilton	aster yellows	various families	North America	Hamilton 1983
Macrosteles nr *severini* Hamilton	aster yellows	watercress	Hawaii	Heu et al. 2003
M. sexnotatus (Fallen)	lissers	hyacinths, gladiolus	Europe	van Slogteren & Muler 1972
	aster yellows	flowers	Europe	Savio & Conti 1983
M. striifrons Anufriev (=*orientalis*)	anemone witches' broom	13 plant families	Japan	Kato et al. 1989
	chrysanthemum yellows/ 16sr-ib	chrysanthemum	Europe	Palermo et al. 2001
	eggplant dwarf	solanaceae	Japan	Okuda et al. 1997
	garland chrysanthemum witches'-broom/16sri	chrysanthemum	Japan	Shiomi & Sugiura 1984
	Marguerite yellows	chrysanthemum	Japan	Shiomi & Sugiura 1984
	Mitsuba witches'-broom/ 16SrI	Japanese honewort	Japan	Shiomi & Sugiura 1984
	onion yellows	chrysanthemum	Japan	Shiomi et al. 2001
	tomato yellows	Solanaceae	Japan	Kato et al. 1988
M. viridigriseus (Edwards)	clover phyllody/16sri-c	clover, plantain	England	Frazier & Posnette 1956
	clover witches'-broom	clover, plantain	England	Frazier & Posnette 1956
Matsumuratettix hiro-glyphicus (Matsumoto)	white leaf phytoplasma/ 16srxi-b	sugarcane	Asia	Matsumoto et al. 1968
Neoaliturus fenestratus (Herrich-Schaffer)	phyllody	Compositae	Israel	Klein 1970
	lettuce phyllody	lettuce, periwinkle, sowthistle	Iran	Salehi et al. 2006
Nephotettix cincticeps Uhler	rice yellow dwarf/16srxi-a	rice	Asia	Chancellor & Cook 1995
N. virescens Distant (=*impicticeps* Ishihara)	rice yellow dwarf/16srxi-a	rice	Asia	Chancellor & Cook 1995

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
Nesophrosyne orientalis (Matsumura)	witches'-broom	beans, peanuts	Asia	Lo 1966
Norvellina seminuda (Say)	Eastern X disease/ 16SrIII-A	stonefruit	North America	Gilmer 1954
Ollarianus balli (van Duzee)	Rhynchosia little leaf disease	pea, weeds	Caribbean	Dabek 1982
Orosius argentatus (Evans)	tomato big bud/16srvi-a	solanaceous	Australia	Grylls 1979
	lucerne witches' broom	alfalfa	Australia	Grylls 1979
	potato purple top wilt16sriii-b	solanaceous	Australia	Grylls 1979
	legume little leaf	legumes	Australia	Grylls 1979
	tobacco yellow dwarf	tobacco	Australia	Helson 1950
O. cellulosus Lindberg	cotton phyllody	cotton	Africa	Laboucheix et al. 1972
O. ishidae (Matsumura)	Western X (16SrIII-A)	celery	North America	Rosenberger & Jones 1978
O. lotophagorum (Kirkaldy)	little leaf disease	bellvine	Australia	Behncken 1984
	witches'-broom of sweet potato	sweet potato	Japan	Shinkai 1964
O. orientalis[6] (Matsumura) (=*albicinctus*)	Sesamum phyllody	several families	Middle-Far East	Kersting & Baspinar 1997
	lucerne witches' broom	alfalfa	Iran	Salehi et al. 1995
	purple top	solanaceous	India	Nagaich et al. 1974
	16srv-16srix	*Limonium* spp.	Israel	Weintraub et al. 2004
	garden beet witches'-broom	beets	Iran	Mirzaie et al. 2007
Osbornellus borealis DeLong & Bohr	Western X-disease/ 16SrIII-A	stonefruit	North America	Jensen 1957
Paraphlepsius irroratus (Say)	Eastern X-disease/ 16SrIII-A	stonefruit	North America	Gilmer et al. 1966
	clover phyllody 16SrI-C	clover	North America	Chiykowski 1991
Recilia banda Kramer	Napier stunt	Napier grass - *Pennisetum purpureum*	East Africa	Obura et al. 2009
R. dorsalis Motschulsky	rice orange leaf	rice	Southeast Asia	Cook & Perfect 1989
R. mica Kramer	blast disease	palms	West Africa	de Chenon 1979
Scaphoideus luteolus van Duzee	American elm yellows/ 16SrV-A	*Ulmus* spp.	North America	Baker 1948
S. titanus Ball (=*littoralis* Ball)	Flavescence doree/ 16SrV-C	grape	Europe	Schvester et al. 1963

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
	Flavescence doree/ 16SrV-D	grape	Europe	Mori et al. 2002
	chrysanthemum yellows/ 16sri-b	chrysanthemum	Europe	Mori et al. 2002
Scaphytopius acutus (Say)	soybean bud proliferation	soybean	North America	Derrick & Newson 1984
	Western X-disease/ 16SrIII-A	stonefruit	North America	Nielson 1979
	Eastern X-disease/ 16SrIII-A	stonefruit	North America	Gilmer et al. 1966
S. delongi Young	Western X-disease/ 16SrIII-A	stonefruit trees, celery	North America	Severin 1947b
S. diutius (DeLong and Mohr)	Western X-disease/ 16SrIII-A	stonefruit trees	North America	Purcell 1987
S. fuliginosus Osborn	machismo disease	legume	So. America, Mexico	Granada 1979
S. irroratus (Van Duzee)	Western Aster yellows	celery	North America	Severin 1947b
S. magdalensis Provancher	blueberry stunt/16sri-e	*rhus* spp.	North America	Howard & Thomas 1980
S. nitridus DeLong	western x-disease/16sriii-a	stonefruit, celery	North America	Purcell 1979
Scleroracus flavopictus Ishihara	potato purple top/16sriii-b	solanaceous	Japan	Shiomi & Sugiura 1984
	gentian witches' broom/ 16sriii-b	*Gentiana* spp	Japan	Shiomi & Sugiura 1984
	Tsuwabuki witches' broom	Farfugium japonicum	Japan	Shiomi & Sugiura 1984
Yamatotettis flavovittaus (Matsumura)	sugarcane white leaf disease	sugarcane	Thailand	Hanboonson et al. 2006
Subfamily Iassinae				
Batrachomorphus punctatus (Osborn)	tomato big bud/16srvi-a	Solanaceae	Australia	Grylls 1979
Subfamily Idiocernae				
Rhytidodus decimusquartus (Shrank)	witches'-broom/16sri-a	*Populus* spp.	Europe	Cousin et al. 1999
Tremulicerus vitreus L.	witches'-broom/16sri-a	*Populus* spp.	Europe	Cousin et al. 1999
Subfamily Macropsinae				
Macropsis fuscula (Zetterstedt)	stunt	*Rubus* spp.	Europe	de Fluiter & van der Meer 1958
M. mendax (Fieber)	elm yellows/16sr v	elm	Europe	Carraro et al. 2004

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
M. scotti Edwards	stunt	*Rubus* spp.	Europe	de Fluiter & van der Meer 1958
M. trimaculata (Fitch)	peach yellows	*Prunus* spp.	North America	Seliskar & Wilson 1981
Oncopsis alni (Schrank)	alder yellows	Alnus glutinosa	Europe	Maixner & Reinert 1999
Subfamily Scarinae (=Gyponinae)				
Gyponana lamina DeLong	Eastern X-disease/ 16SrIII-A	stonefruit	North America	Gilmer et al. 1966
Subfamily Typhlocybinae				
Alebroides nigroscutellatus (Distant)	potato purple top roll/ 16sriii-b	potatoes	Southeast Asia	Shatrughna et la. 1983
Amrasca devastans (Distant)	eggplant little leaf	solanaceous	Southeast Asia	Maramorosch et al. 1970
Empoasca papayae Oman	bunchy top disease of papaya	papaya	Caribbean region	Sein & Adsuar 1947
Fulgoridea (=Fulgoromorpha)				
Cixiidae				
Cixius wagneri (China)	Stolbur/16SrXII-A	strawberry	Europe	Foissac et al. 2001
Hyalesthes obsoletus Signoret	Stolbur/16SrXII-A	Solanaceae	Europe – Turkey	Brcak 1979
	Vergilbungskrankheit/ 16SrXII-A	grapevine	Europe	Maixner 1994
	bois noir/16srxii-a	grapevine	Europe	Carraro et al. 1994
Myndus crudus Van Duzee	lethal yellowing 16sriv	palm	Subtropical America	Howard & Thomas 1980
Oliarius atkinsoni Meyers	Phormium yellow leaf/ 16SrXII-B	flax	New Zealand	Liefting et al. 1997
Pentastiridius beieri Wagner	syndrome des basses richesses	sugar beet	Europe	Gatineau et al. 2001
P. leporinus (Linnaeus)	southern stolbur/16srxii-a	various families	Russia	Bogoutdinov 2003
	syndrome des basses richesses	sugar beet-wheat	Europe	Bressan 2009
Reptalus panzeri (Low)	maize redness	corn	Serbia	Jovic et al. 2007
Delphacidae				

Table G-1 Confirmed Phytoplasma Vectors[1,2]

Vector Species	Disease Association/ Phytoplasma	Host Plants	Distribution	Reference
Eumetopina flavipes Muir	ramu stunt disease/ 16sriv	sugarcane	Papua New Guinea	Cronje et al. 19999
Javesella discolor (Boheman)	pseudoclassic stolbur	various families	Europe	Brcak 1979
Nilaparvata lugens Stal	unnamed phytoplasma	rice	Asia	Cook & Perfect 1989
Saccharosydne saccharivora (Westwood)	sugarcane yellow leaf	sugarcane	Cuba	Arocha et al. 2005
Derbidae				
Proutista moesta (Westwood)	coconut root wilt/16sriv	palm	Southeast Asia	Ponnamma & Solomon 1998
	grassy shoot disease	sugarcane	Southeast Asia	Rishi & Chen 1989
Dictyopharidae				
Dictyophara europaea (L.)	flavescence doree (16srv-c)	clematis-grape	Europe	Flippin et al. 2009
Flatidae				
Metcalfa pruinosa (Say)	16SrI-B and –G	various families	Europe	Danielli et al. 1996
Psyllidae				
Bactericera trigonica Hodkinson	16SrXII-A	carrots	Canary Islands	Font et al. 1999
Cacopsylla melanoneura (Forster)	apple proliferation/16srx-a (91)	apple	Europe	Tedeschi et al. 2002
Cacopsylla (=*costalis*) *picta* (Forster)	apple proliferation/16srx-a	apple	Europe	Frisinghelli et al. 2000
Cacopsylla pruni Scopoli	European stone fruit yellows/16SrX-B	stonefruit	Europe	Carraro et al. 2001
Cacopsylla pyri (Linnaeus)	apple proliferation/16srx-a	apple	Europe	Lemoine 1991
	pear decline/16srx-c	pear	Europe	Carraro et al. 1998
Cacopsylla pyricola (Forster)	pear decline/16srx-c	*Pyrus* spp.	North America	Jensen et al. 1964
Cacopsylla pyrisuga (Forster)	pear decline/16srx-c	pear	Russia	Grbic 1974

1 Table was reproduced with permission from COST Action FA 0807 Integrated Management of Phytoplasma Epidemics in Different Crop Systems (http://www.costphytoplasma.eu/WG2/Vector%20table.htm).

2 Every attempt was made to include all species that have been confirmed as phytoplasma vectors. Instances where there was a solitary report with no follow-up or of questionable techniques were not included. Suspected or presumed species were also not included.

3 *Aphrodes bicincta* (Schrank) is the name associated with phytoplasma diseases in Europe but the species if very similar to *A. markarovi* Zachvatkin. So records of these two species may be confused (personal communication, Michael Wilson).

4 Originally incorrectly identified as *Jassus indicus* (Walker).

5 Originally incorrectly identified as *Euscelis maculipennis* DeLong & Davidson.

6 Both species are the same (personal communication, Michael Wilson , Murray Fletcher).

Appendix

H

Research Needs

1. Research is needed on phytoplasma-plant, phytoplasma-vector and phytoplasma- phytoplasma (cross protection) interactions to exploit any existing means of resistance.
2. Develop and maintain collections of phytoplasma strains.
3. Establish tools to identify vector species. Based on morphology, in some cases, only adult males can be identified to species.
4. Define and establish present levels of specific phytoplasma resistance/ tolerance among commercially grown cultivars and rootstocks adapted for the U.S.
5. Collection of worldwide data on known phytoplasma susceptibility/ resistance of different species and cultivars.
6. Determine the potential existence of alternative plant hosts for any of the selected 'Candidatus Phytoplasma spp.' of apple, grape and peach in the U.S.
7. Establish effects of biotic and abiotic environmental factors on disease and symptom development.
8. Establish and validate diagnostic protocols for detection and identification of any the selected 'Candidatus Phytoplasma spp.' of apple, grape and peach.
9. Recommend appropriate changes to current cultural practices that could reduce disease severity. Plant spacing, use of rootstocks etc.
10. Evaluate efficacy of currently available pesticide treatments and other chemical applications that could be used to protect against insect vectors.
11. Initiate cooperation with research institutions for the establishment of survey programs and evaluation of available germplasm as potential breeding sources of natural genetic resistance to any of the selected 'Candidatus Phytoplasma spp.' of apple, grape and peach.
12. Establish the importance of different means of disease spread by vector movement, seed/planting material trade and transmission by root bridges.

13. Provide data about the infectivity of vector species towards the establishment of a risk assessment system
14. Monitor the presence of phytoplasma diseases and their putative vectors in defined regions

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