

X-disease phytoplasma (*Candidatus Phytoplasma pruni*)

Sampling Recommendations for Nurseries of Stone Fruit

Adapted by BC Ministry of Agriculture and Food from Washington State University
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Nursery Mother Trees

Where to sample

- **Trees with fruit symptoms:** Sample from symptomatic limbs.
- **Trees with no symptoms or no fruit:** Sample from each leader or compass point (N,E,S,W) of 1/4"-1/2" (pencil diameter wood) with leaves. Sample from the lower limbs of the tree as close to the trunk as possible.

Materials to sample:

- Collect four 5" cuttings of 1/4"-1/2" (pencil diameter) diameter limb which include wood from the previous season or older, flower buds, fruit stems, or leaves depending on time of year (Table 1).
- Trim off excess tissue (Figure 1). Do not include dead or dying tissue.

Table 1. The recommended tissue for testing cherry or other stone fruit tissue for *Candidatus Phytoplasma Pruni* (*Ca. P. pruni*) varies depending on the time of year or season, the growth state of the plant, and how advanced the infection is. Sequential testing (Wright et al. 2022) of heavily infected trees over the course of three years suggests the following tissues are most optimal:

Time of Year	Growth Stage	Best Tissue	Secondary Tissues
April	Green tissue	Second-year from symptomatic limbs if possible.	
May	Shuckfall		
May-June	Preharvest		Fruit Stem (Pedicel)
June-September	Harvest		Fruit Stem (Pedicel)
September	Postharvest		
Early October	Pre-dormancy		
Late October-March	Dormancy	Not Recommended	Not Recommended



Figure 1. Collection of woody stem tissue at harvest. Both the trimmed cutting and pedicel (fruit stem) will be submitted together for testing. Leaves are suboptimal at this time and are discarded. The fruit itself should not be submitted, as it is difficult to work with, bulky for storage, and prone to fungal growth that can contaminate the sample.

When to sample

- The week before harvest to mid-August is standard for commercial orchard trees.
- **Target sampling low on the tree in mid-to-late June for mother trees.** Early in the season the concentration of the pathogen in the upper parts of the tree is low and may provide false negatives. June timing will allow for results before August budding. Do not sample after mid-August when phytoplasma concentration in the tree begins to drop.
- Sample when temperatures are below 32 C (90 F). Elevated temperatures can suppress virus and phytoplasma concentration, leading to false negative results. After collection and bagging, samples should be directly enclosed in a cooler with an ice pack.

If the tree is dormant, sampling is not recommended as phytoplasma replication and concentration drops with lower temperature, and there is little to no active movement of solutes or the phytoplasma in the phloem. It may be detectable in large lower limbs and/or the trunk, however the probability of false negative test results is high. The phytoplasma can be detected in primary and secondary root tissues of infected trees, often prior to the phytoplasma becoming detectable in the limbs of the tree. However, sampling of roots of planted, actively growing orchard or mother trees is difficult and not recommended for routine testing. Check with your diagnostic lab whether they can extract DNA from roots successfully before testing this tissue. A longer description of the seasonal concentration and distribution of *Ca*.

P. pruni at different stages of infection can be found in: Wright et al. (2022) Titer and Distribution of *Candidatus Phytoplasma pruni* in *Prunus avium*. Phytopathology, <https://doi.org/10.1094/PHYTO-11-21-0468-R>.

Sampling sanitation

- Clean tools with 10% bleach between sampling each tree to reduce risk of contamination. Sodium hypochlorite (bleach) denatures both viral coat proteins and the phytoplasma cell membranes.
- 70% or greater ethanol or isopropanol can also be used but are less effective than bleach against viruses.
- The chance of mechanically transmitting LChV-2 or *Ca. P. pruni* is minimal, but there is always a risk of transmitting other pathogens such as Prune dwarf virus or Prunus necrotic ringspot virus which are common in the Pacific Northwest.

Packaging and sending samples

- Keep tissue moist and cool (with ice packs).
- Old or dried tissue or tissue which has gotten hot is likely to have false negatives.
- Send to labs with current proficiency test results. The BC Ministry of Agriculture and Food (BCMAF) Plant Health Laboratory in Abbotsford can test for LChV1, LChV2 and *Ca. P. pruni*.
- Shipping on a Monday or Tuesday to get to labs on time is recommended.

Dormant, Bare-Rooted or ‘Production’ Trees

The sampling of bare-rooted trees for sale, either immediately prior to, during, and after dormancy is more difficult than sampling larger, mature trees because sampling is destructive, and these small trees lack branching limbs that can be safely removed without harming the tree. Also, as the young trees enter dormancy, phytoplasma concentration and distribution becomes primarily focused on the roots.

Materials to sample

- One 3-4" cutting from the tip of the tree including buds and one 3-4" cutting from the main lateral roots (at least ¼" thick), or from the taproot (including root core) should be present (Figure 2). Diagnostic laboratories should process these two tissues together.

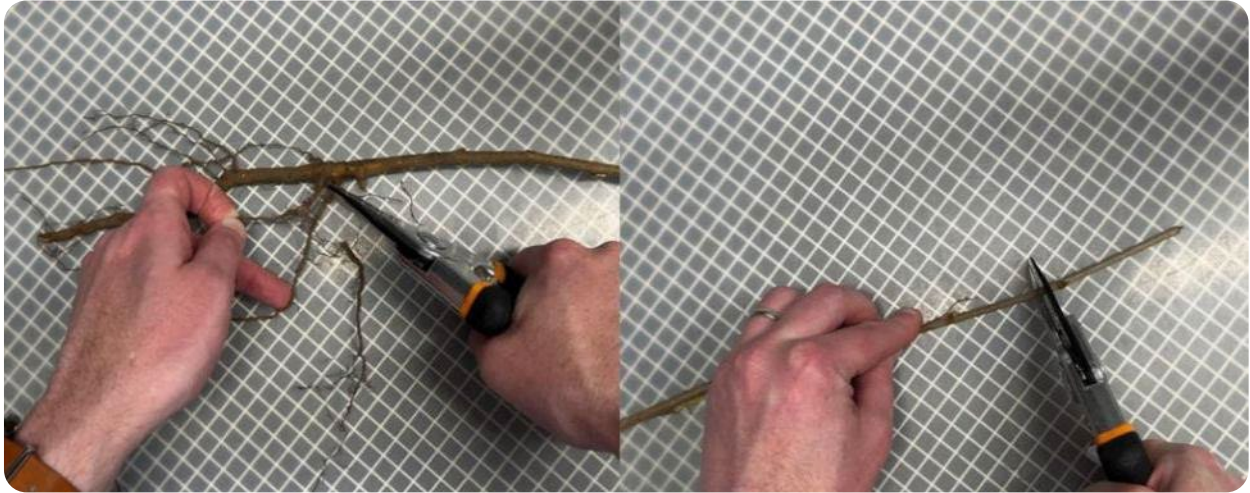


Figure 2. Collection of root and stem tissue from a dormant rootstock. Both the trimmed stem cutting and root will be submitted together for testing. Samples should be as free of dirt as possible to aid the laboratories in sample processing.

When to sample

- Sampling young dormant trees after digging them out of the ground, or once they are in cold storage and easily accessible, is an option but the chance of false negatives is a risk.

Sampling sanitation

- Clean tools with 10% bleach between each tree sampling. Sodium hypochlorite (bleach) denatures both viral coat proteins and the phytoplasma cell membranes.
- 70% or greater ethanol or isopropanol can also be used but are less effective than bleach against viruses.
- The chance of mechanically transmitting LChV-2 or *Ca. P. pruni* is minimal, but there is always a risk of transmitting other pathogens such as Prune dwarf virus or Prunus necrotic ringspot virus which are common in the Pacific Northwest.

Packaging and sending samples

- Keep tissue moist and cool.
- Old or dried tissue or tissue which has gotten hot is likely to have false negatives and is not suitable for testing.
- Send to labs with current proficiency test results. The [BCMAF Plant Health Lab](#) in Abbotsford can test for LChV1, LChV2 and *Ca. P. pruni*.

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Hours of operation: Monday to Friday 8:30 A.M. to 4:30 P.M.

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Disclaimer: Failure to recover or identify a disease or insect in a sample does not imply that a field or commodity represented by the sample is free of the organism. Due to uneven distribution and/or seasonal fluctuations of disease or insect population in plant tissue and limitations of the sampling procedure used, the Plant Health Laboratory does not guarantee, warrant or imply, as a result of negative test results, freedom of infection in the population from which the sample was withdrawn.