



Diagnostic Sample Collection for Suspect Laurel Wilt (*Raffaelea lauricola*) and its Beetle Vector

Virginia Tech [Plant Disease Clinic](#) and [Insect Identification Lab](#)

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Note that completely dead tissue is not useful for diagnosis, so avoid collecting tissue from trees and branches that are completely dead. A sample from the main trunk is preferable to branch samples.

Sample collection from the trunk of a mature tree:

1. Using a hatchet, hammer and chisel, or knife, remove the bark down to the surface of the sapwood (xylem) and look for discolored streaking in the wood (Fig. 1).
 - a. If discoloration is observed, using a hatchet or chisel, remove 2-3 substantial chips/sections of the discolored wood. Obtaining a relatively thick ($\geq 3/8''$) and substantial ($\geq 2'' \times 2''$) section of the discolored wood is the best way to get an accurate diagnosis.
 - b. If discoloration is not observed, examine another section of sapwood on the trunk. (Symptoms of discoloration of the sapwood may not be uniform on an infected tree, but only discolored wood should be submitted for diagnostic purposes).
 - c. Immediately after removal, place samples in a sturdy ziplock-type bag and seal.

Sample collection from ~2- to 3-inch diameter stems or branches:

1. Examine branches that have dieback and/or wilted leaves, but still have some green leaves as well (Fig. 2). Using a knife, remove bark down to the surface of the sapwood and look for discoloration in the wood. Another option is to cut through a whole branch or main stem of a tree using a pruner or lopper to check for discoloration of the sapwood in the cross-section (Fig. 3). If discoloration is observed, excise a few short branches or stem sections that have discoloration in the sapwood (do not expose all of the discoloration by removing bark—just confirm that it is present).
2. Place samples in a sturdy plastic bag and seal.

Surface-disinfecting tools used in sample collection: *If you are collecting multiple samples, it is advisable to surface-disinfect tools between samples. Since molecular tests may be used for diagnosis of *Raffaelea lauricola*, bleach is recommended as the most effective option for elimination of contaminants that may interfere with a molecular test. However, bleach is corrosive to tools, so following bleach- disinfection by a rinse in 70% ethanol is recommended to neutralize the bleach.*

1. Wipe tools with 10% sodium hypochlorite (household bleach) solution.
2. Then thoroughly wipe the tool with 70% isopropyl alcohol (rubbing alcohol) and allow the alcohol to have a few minutes of contact time to kill any microorganisms that might be present on the tool before using the tool to collect another sample.

Mailing samples to the Virginia Tech Plant Disease Clinic

1. Individual samples should be submitted through your local Virginia Cooperative Extension office (<https://ext.vt.edu/offices.html>) with a completed Plant Disease Diagnostic form (#450-097), which can be obtained from your local office.
2. Samples suspected to have laurel wilt should be shipped double-bagged in sturdy plastic bags.
3. Write “laurel wilt” on the mailing envelope or box with a permanent marker.
4. Ship early in the week to arrive by Friday.

Collecting Suspect Beetle Vector Samples

The redbay ambrosia beetle (*Xyleborus glabratus*) is the primary insect vector that transmits the laurel wilt pathogen; it is extremely small and spends most of its life cycle within the host tree. Check for beetle galleries in the area of stained sapwood. Redbay ambrosia beetle is smaller than a grain of rice; any beetles larger than a grain of rice are not likely suspects. If you find small beetles, collect them into a vial with alcohol. Fill out VCE form #444-113 and submit it with the vial to the Insect Identification Lab via your local VCE office OR simply include the vial with any trunk specimen submitted to the Plant Clinic. This species can only be confirmed via microscope in the lab. It is possible to do preliminary screening via good, clear photographs, but ultimately, we will need to see the actual beetles to confirm the identification.



Fig. 1. Redbay tree (*Persea borbonia*) infected with *Raffaelea lauricola* showing typical dark staining of the sapwood. (R. Scott Cameron, Advanced Forest Protection, Inc., Bugwood.org).



Fig. 2. Symptoms of laurel wilt on camphor tree (*Cinnamomum camphora*). (Chip Bates, Georgia Forestry Commission, Bugwood.org).



Fig. 3. A main stem of pondspice (*Litsea aestivalis*) infected with the laurel wilt fungus (George Johnson, Georgia Forestry Commission, Bugwood.org).