



Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

# **Oak Wilt**

## ***Ceratocystis fagacearum***

Survey Protocol

## 1. Background and Objectives

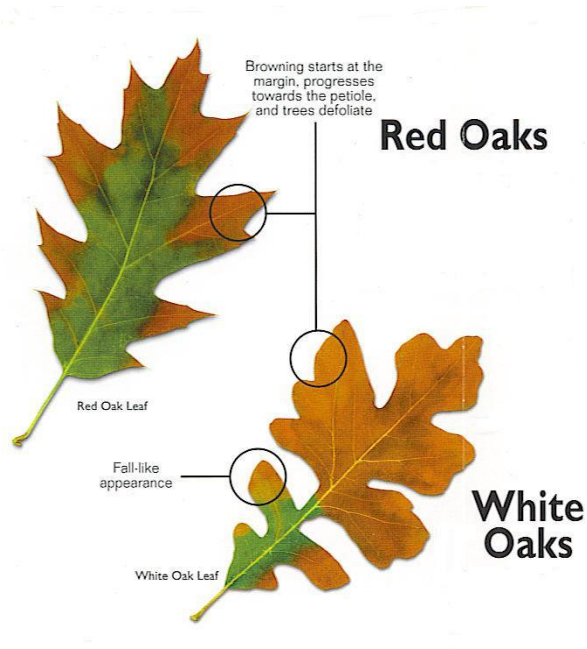
Oak wilt is a vascular wilt disease caused by the fungus *Ceratocystis fagacearum*. The fungus develops on the outer sapwood of the tree, causing its host to develop tyloses and gums which block the movement of water and nutrients within the xylem, resulting in branch wilting and tree death. The disease is capable of killing trees in a single season. Oak wilt was first recognized as an important forest pest in 1944 in Wisconsin and is now known to occur in 23 states within the U.S. (South Dakota to western New York, southward to South Carolina, westward to Texas and northward through Oklahoma to Nebraska). This disease is not known to occur in Canada.

Oak wilt is primarily spread from diseased to healthy trees through root grafting. This disease can also be spread by oak bark beetles, which transmit spores while creating galleries within infected tissue, and by sap beetles, which transmit spores while feeding on fungus mats. Species of red oak are infected more frequently and die more quickly than white oaks. Although thousands of trees have died in the US as a result of oak wilt, mortality rates are higher in the Midwest than in the southeast range of this disease.

This survey is being conducted in support of plant health policy directive D-99-03, *Phytosanitary Measures to Prevent the Entry of Oak Wilt Disease (Ceratocystis fagacearum (Bretz) Hunt) from the Continental United States*. The primary goal of this visual survey is early detection of the pest in areas where it is not known to occur.

## 2. Target Life Stages

Leaves on infected trees will be dull green, bronze, yellow or brown. Colour change will move inward from the leaf margins, starting at the tip of the leaf and progressing towards the midrib and petiole (Figure 1). The border between healthy green tissue and discolouration will be very distinct and sharp (Figure 2). Lightly infected trees may have small clusters of discoloured drooping leaves, which give the appearance of a “flag”. Infected trees may prematurely lose their leaves throughout the summer (June to August), when leaves will fall in various states of discolouration.



**Figure 1.** Leaf colour change pattern.



**Figure 2.** Distinct border on infested leaves (Iowa State University).

Fungus or “compression mats” are composed of dark mycelia and can occur under the bark of infected trees (Figure 3). Sometimes these compression mats can cause the bark to split (Figure 4) and give off an odour similar to “Juicy Fruit” bubble gum.



**Figure 3.** Oak wilt compression mat beneath the bark (Iowa State University).



**Figure 4.** Vertical cracks in bark, indicating presence of spore mats under the bark (John Gibbs, Forestry Commission, Bugwood.org).

### 3. Target Hosts

All oak species are susceptible to oak wilt, but red oaks are more frequently infected and can die quite quickly. Within a target area, prioritize surveys within forests containing red oak, followed by white oaks. However, if there is noticeable crown decline and leaf symptoms on white oaks, these trees must be examined.

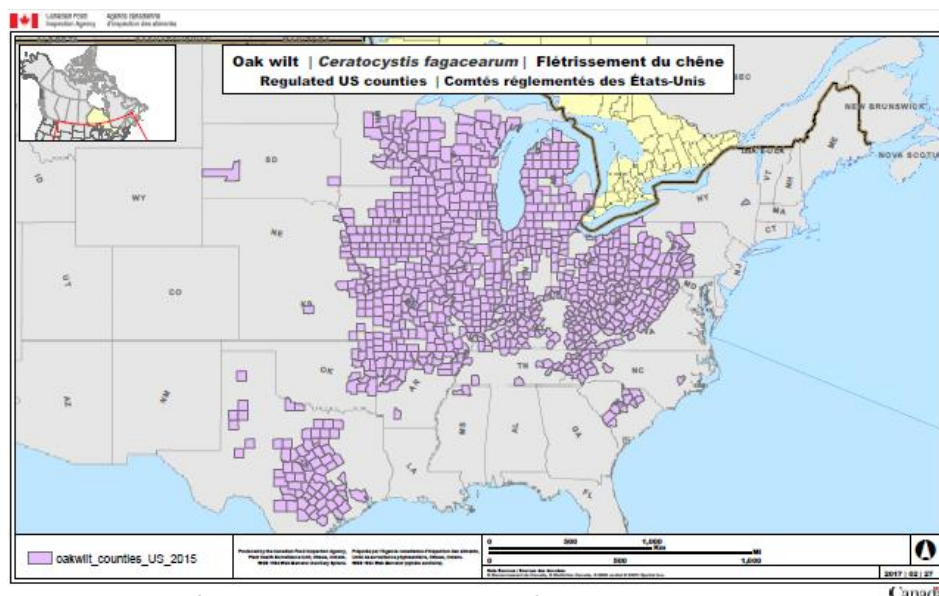
### 4. Timing and Duration

Visual ground surveys will occur between the beginning of July and the 2<sup>nd</sup> week of August.

### 5. High Risk Areas

The primary means for disease transmission is through root grafting from infected to healthy oaks. Currently, oak wilt infected counties in the US are separated from Canada by the Great Lakes or rivers. As the only way that oak wilt can naturally cross roads, rivers and open fields is through transmission by an insect vector, the introduction of oak wilt into Canada will likely occur through the movement of infected commodities such as logs or firewood. Detection surveys should focus on areas where oak wilt could have been introduced through human-assisted movement of infected commodities, focusing on the following target sites:

- Campgrounds (provincial and national parks, private campgrounds) where oak firewood may be transported from infected areas. Prioritize campgrounds hosting visitors from infected US states;
- Mills or other facilities importing oak logs from infected US states;
- Border crossings where firewood may be placed in amnesty bins.
- Areas in Ontario adjacent to US regions where infections are known to occur (Figure 5).



**Figure 5.** Map of Regulated US counties as of 2015.

Note: May not reflect all infested counties but provides an overview of Canadian areas at risk

Surveys should be evenly distributed among each of the 4 site types listed above. Surveys are to occur in forested areas surrounding the target sites, but if oak is absent from the forest, that particular site should not be selected for this survey.

## 6. Survey Methodology

The following survey techniques include excerpts and adaptations from the USDA Forest Service publication *How to Collect Field Samples and Identify the Oak Wilt Fungus in the Laboratory* (Jill Pokorny, 1999). Complete Oak Wilt Visual Survey Site Form (Appendix 1) for all sites surveyed using the CFIA protocol.

### 6.1 Detection Survey

This survey consists of a visual inspection of host trees for signs and symptoms of oak wilt. At each target site, inspection staff will visually examine up to 50 oak trees within the forested areas surrounding the site. If the target site is very large, select 50 oak for inspection over a 500 m distance; this will ensure that a greater proportion of a site will be inspected. If there are no oak within or adjacent to a site, examine the closest forest containing oak within 3 to 5 km of the site.

Binoculars should be used to assist in surveying mature trees which cannot be effectively inspected from the ground. If symptomatic branches occur higher in the canopy, use pole pruners to remove samples. Once oak wilt has become established, it can create an expanding pocket of dead and infested trees. If dead oaks are observed, inspect the tree and ground for signs and symptoms described below.

### 6.2 Signs and Symptoms of Attack

Prior to beginning the survey, it is important to develop the proper search for signs and symptoms typically associated with oak wilt. This is particularly important when conducting detection surveys for new infections or assessing lightly infected stands. Signs and symptoms include:

- Flagging (small cluster of discoloured leaves).
- Oak trees with thin crowns (i.e. loss of leaves).
- Discoloured leaves that have fallen prematurely to the ground
- Bronzing/browning of leaves will have a sharp, distinct border between the healthy green and symptomatic tissue (Figures 1 and 2).
- Dark fungal mats (compression mats) may form under the bark of red oaks that have recently been killed by oak wilt (Figure 3). Occasionally, these mats may rise and cause the bark to split; mycelia may protrude from these cracks (Figure 4). Using the back of an axe head, tap suspect areas, if there is a hollow sound, use the axe to cut a "window" around the hollow area and peel back the bark. Fungal mats will be dark/grey and smells like "Juicy Fruit" chewing gum.

## 7. Sampling Procedures

In the event that oak wilt signs or symptoms are encountered during the survey, samples should be collected and prepared for submission to the CFIA lab in collaboration with CFIA inspection staff. Digital photographs of signs and symptoms should be taken and emailed to your local CFIA office <http://www.inspection.gc.ca/about-the-cfia/offices/eng/1313255382836/1313256130232> or Area Survey Biologist [Surveillance@inspection.gc.ca](mailto:Surveillance@inspection.gc.ca). Record the GPS coordinates in Latitude and Longitude in decimal degrees (NAD 83 datum) for the tree sampled, your name, comments about the site (estimated diameter of tree, health, etc.), the date, and other location information on a piece of paper and place this into the bag. A piece of flagging tape should be placed on the tree sampled as you will likely need to revisit the tree if the sample is positive.

Samples must be kept cool at all times and must not be exposed to direct sunlight while in the field; bring a cooler and ice packs to the field (do not use ice cubes or dry ice). Ship samples promptly to the laboratory in coolers via overnight courier. As outlined in the following sections, sample collection methods will vary depending on the type of tissue being sampled.

### 7.1 Symptomatic Branches and Leaves

Although leaf symptoms are important indicators of oak wilt, they are not the diagnostic focus for this survey given that the leaf material is unlikely to contain the pathogen or DNA for this pathogen. Furthermore, competing pathogens may interfere with the qPCR analysis. Symptomatic branches will provide the highest chance of detecting oak wilt if present and should be sampled as follows:

- Select and remove three (3) branches containing discoloured leaves
- Branch samples should be a **minimum of 2.5 cm in diameter** (Figure 6)
- Use a small hand saw to cut a cross section of the branch near the location of the discoloured leaves.
- Look for black staining in the outer sapwood
- In some cases, particularly with red oak, discoloration is not visible in the sawn cross sections and is only observed when the bark is removed longitudinally. If sapwood discoloration is not present in one branch, check other partially wilted branches. Be careful not to remove all of the bark on the branch samples you submit for testing.

- It is critical that the sapwood is moist to the touch, and the inner bark is still alive and green. In the field, check for this by removing the outer bark on a small section of the branch sample. Avoid collecting samples from the extreme tips of branches: this tissue wilts first and is often too dry for successful isolation.
- Cut the entire symptomatic branch into 12-18 cm lengths and placed in re-sealable bags.
- Leaves with discolouration progressing from the leaf tip and margin towards the midrib and petiole may be included on branch samples or in separate plastic bags if fallen leaves are collected from the ground.



**Figure 6.** Fresh, moist branch samples with a minimum diameter of 2.5 cm are the diagnostic focus for this survey.



**Figure 7.** Black staining – cross section (USDA Forest Service).



**Figure 8.** Longitudinal black staining – longitudinal section (USDA Forest Service).

## 7.2 Compression Mats

If there is an area containing a suspect compression mat, use an axe to create a bark window surrounding the mat. Strips of discoloured sapwood tissue can then be cut from the outer growth rings with an axe or chisel. If discolouration is not present in the exposed sapwood, check other locations on the bole. Avoid including bark in the sample bag because the oak wilt fungus competes poorly with fungal contaminants found on the outer and inner bark. Place discoloured sapwood strips in separate plastic bags.

## 8. Biosecurity Precautions

Where sites have biosecurity procedures in place, inspectors should become aware of and follow them.

All equipment (e.g. knives, axes, etc.) used to cut and remove wood samples should be cleaned between samples with an alcohol based sanitizer to prevent inadvertent spread of disease.

Branch samples should not be obtained during the high risk period (April to July) when sap beetles are active.

## 9. Collaborative Data Management

Survey activities conducted for a regulated pest in accordance with the established CFIA survey protocol should be captured so that all collaborative efforts can be captured.

An Excel spreadsheet containing latitude and longitude coordinates and address for the site surveyed, percent oak, organization details and coordinates for any suspect trees can be submitted to the CFIA at [Surveillance@inspection.gc.ca](mailto:Surveillance@inspection.gc.ca) by no later than September 1st each year so that all efforts can be mapped and reported nationally. Possible suspects should always be reported in real time.

## 10. Supplies

- Knife, axe
- Small hand saw (e.g. 8-10 inch blade)
- Hand sanitizer, ethanol or rubbing alcohol for sterilizing sampling equipment
- Binoculars
- Paper towels
- HB pencils
- Cooler
- Re-useable ice packs
- Field notebook (with waterproof paper)
- GPS unit
- Insect repellent
- First aid kit
- Permanent markers



- Flagging tape
- Forceps
- Blank LSTS Report For Submission forms
- Disposable latex gloves
- Road map
- Re-sealable plastic bags
- Pole Pruners
- Oak Wilt Pest Cards and fact sheets
- Oak Wilt Survey Site Form
- Tick removal kit (e.g., <https://canlyme.com/product/tick-removal-kit/>)
- Pruning paint

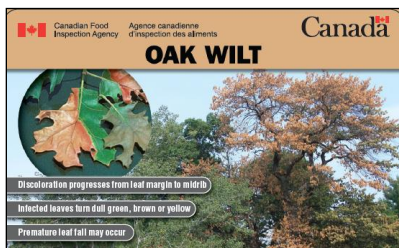
**Note:** Inspectors should have at least a 10% surplus of supplies (sampling bags, disinfectant, etc.) on hand.

## 11. Supporting Documents and Additional Information

*How to Collect Field Samples and Identify the Oak Wilt Fungus in the Laboratory* (Jill Pokorny, 1999).

[http://www.na.fs.fed.us/spfo/pubs/howtos/ht\\_oaklab/toc.htm](http://www.na.fs.fed.us/spfo/pubs/howtos/ht_oaklab/toc.htm)

Oak Wilt Pest Cards



Forest Invasives Oak Wilt Species Profile

<http://forestinvasives.ca/Meet-the-Species/Pathogens/Oak-Wilt>

Oak Wilt Fact Sheets

<http://www.inspection.gc.ca/plants/plant-pests-invasive-species/diseases/oak-wilt/fact-sheet/eng/1325629194844/1325632464641>

[http://forestinvasives.ca/Portals/0/oak\\_wilt\\_factsheet\\_updated\\_genus.pdf?ver=2018-01-08-140226-923](http://forestinvasives.ca/Portals/0/oak_wilt_factsheet_updated_genus.pdf?ver=2018-01-08-140226-923)

Pressure Pad Hunting with the Oak Wilt Guy

<http://forestinvasives.ca/Meet-the-Species/Pathogens/Oak-Wilt#70341-manage>

**Appendix 1: Oak wilt Visual Survey Site**

<b>OAK WILT VISUAL SURVEY SITE FORM</b>														
Date of Survey		Y	Y	Y	Y	M	M	D	D	Surveyors:				
Datum					N	A	D	8	3	Site Type: <input type="checkbox"/> Campground <input type="checkbox"/> Mill/Importer <input type="checkbox"/> Border Crossing <input type="checkbox"/> Adjacent to US Infestation				
Latitude				.										
Longitude		-			.									
Site Name														
Site Address: _____ <small>Number                                  Street Name                                  City                                  Province                                  Postal Code</small>														
Oak Species surveyed: <input type="checkbox"/> Red oak <input type="checkbox"/> Pin oak <input type="checkbox"/> White oak <input type="checkbox"/> Other (red oak group) <input type="checkbox"/> Other (white oak group)							Contact name: _____ Contact number: _____ E-mail (if applicable): _____							
Notes:          														
Suspect tree(s) found? <input type="checkbox"/> Yes <input type="checkbox"/> No							Pictures Taken? <input type="checkbox"/> Yes <input type="checkbox"/> No							
<b>SUSPECT TREE DETAILS (If applicable)</b>														
Datum					N	A	D	8	3	Signs & Symptoms Present: <input type="checkbox"/> Fungal mats <input type="checkbox"/> Thinning crowns <input type="checkbox"/> Flagging <input type="checkbox"/> Vertical bark cracks <input type="checkbox"/> Bronzing/discoloration of leaves				
Latitude				.										
Longitude		-			.									
Samples collected? <input type="checkbox"/> Yes <input type="checkbox"/> No														
Sample Type(s): <input type="checkbox"/> Leaf <input type="checkbox"/> Branch <input type="checkbox"/> Fungal mat							LSTS System ID #: _____							
Notes:          														
<b>LSTS DATA ENTRY</b>														
Entered by: _____														
Signature: _____														
Date entered (e.g. 23-07-2015): _____														
<b>Updated: 01-02-2017</b>														