

Raspberry Root Rot Sampling Guidelines for *Phytophthora fragariae rubi* Identification using PCR

Root rot of raspberry is a condition of root damage that can be caused by poor cultural conditions in the root zone or disease organisms such as *Phytophthora fragariae rubi*. Raspberry roots are sensitive to low oxygen levels in the soil. With low soil aeration, perhaps due to flooding, sufficient oxygen will not be available to the roots, allowing for root damage and slow or no root growth. Insufficient oxygen to the roots may consequently cause shoot symptoms such as decreased vigor, wilting, and leaf scorch. Damaged roots are also more susceptible to infection by disease causing organisms such as *Phytophthora*.

When present, *Phytophthora fragariae rubi* (raspberry root rot) is able to survive in the soil for several years. When soil temperature and moisture are favorable, it produces a swimming stage called a zoospore which enables it to infect healthy plant roots. In fields which are infected with this fungus, optimum discharge of zoospores occurs when soils become completely saturated with water. Many fields are free of this disease, and therefore soil-applied fungicides should not be used as a preventative fungicide. Root rot fungicides should not be used unless *Phytophthora* symptoms are present and the disease causing organism has been positively identified through laboratory analysis of root and crown tissue.

The regular and blanket use of fungicides will drive the mechanism that develops resistant pathogen strains. Fungicides with specific modes of action— as many *Phytophthora*-active fungicides are — can be at a higher risk for proliferating *Phytophthora* resistant pathogenic strains. Minimizing fungicide use, in any way, is the first priority to prevent resistant strains from developing.

Since the currently registered fungicide Ridomil® does not eradicate this disease, but does mask symptoms, the fungicide should not be used by propagators.

A new tool is available for use in the identification of *Phytophthora fragariae rubi* as a disease causing root rot in raspberry. The use of PCR (Polymerase Chain Reaction) can be used to detect if a specific gene is present in an organism. In this case, it will be used to detect a gene present only in *Phytophthora fragariae rubi*. With this data, it can be determined which tissue samples, if any, carry the root rot causing disease

organism, *Phytophthora fragariae rubi*. PCR technology has been used in Europe for the past 10 years to identify *Phytophthora* in raspberry.

The following guidelines describe root rot symptoms and methods for collecting samples for the use in PCR identification of *Phytophthora fragariae rubi*. To make results of the PCR testing meaningful, be sure to develop a coding system that allows you to locate infected fields and areas of fields while concealing your identity. Please follow the guidelines closely to avoid contamination of samples and ensure quality samples are submitted for testing.

Record Keeping

Good sample record keeping is critical. Be sure to attach a code for each sample bag. This code should contain at least eight characters (letters and numbers) and the code should be unique and only recognizable by you. Ensure that you can track the origin of the field for each sample bag using your code. For example, your code might be 8025AB10W with the "8025" being the code you have chosen for your farm, the "AB" as the field code, and the "10W" as the location within the field or the code for the nursery stock.

After each field sampled, be sure to fill out the REQUIRED information on the data sheet to be sent with your samples. Include your eight character code, the county where the sample was taken, the raspberry variety and whether it is a pre-plant or in-field sample. Keep a copy for your records and include the physical location for each sample taken so you can back track your samples. Only samples with the required information recorded and the correct payment will be processed.

Avoiding Contamination of Samples:

Phytophthora fragariae rubi has the potential of being spread by propagules, soil, contaminated equipment, tools, boots, or any other mechanism by which infested soil or drainage water may be moved. Growers should take care not to increase the risk of spread through these mechanisms while taking samples. For example; shovels, pruners, or other equipment suspected of coming in contact with infected tissue should be dipped in a 10% bleach solution prior to coming in contact with other tissue.

Pre-plant sampling:

To sample pre-plant raspberry stock, bare roots should be selected straight from the box. While at this point growers will not be able to submit enough samples for a statistically valid sample, the accurate identification of *Phytophthora fragariae rubi* using PCR, if it exists, is a valid objective of this program.

Useful Tools and equipment:

- Cooler and cold packs
- Shovel
- Pruners
- Flagging tape or other field marker
- Sampling Kit (provided):
 - Bags
 - Permanent Markers
 - Coding Tags
 - Record sheets

Randomly select plants straight from boxes and place them directly into sample bags provided. Samples may be grouped in ten plants per bag. All plants in one bag should be from the same lot. A lot can be described as a single complete shipment of a single variety from one propagator or the total number of plants of a single variety about to be planted in a single field.

Log your sample immediately on your data sheet. One copy of the data sheet should be sent in with the sample and one kept by the grower.

In-field sampling

Scouting for plants to sample: Phytophthora root rot can persist in both well-drained and poorly drained soils. Areas of the field that have had standing water are more susceptible to the spread of Phytophthora root rot. Identify areas of fields that have had standing water during spring rains and consider plants in these areas for sampling.

Above ground symptoms of this disease are most evident just prior to and during harvest. Look for reduced number of primocanes, wilted primocanes, and fruiting canes with yellow or scorched lateral shoots. These symptoms are more likely to be found in low areas where saturated soil conditions persist, which favor infection and spread of the disease.

Scout for plants with above ground symptoms. Carefully dig up and remove the soil from around the base of suspect plants. Examine the crown region of suspect plants by scraping away the epidermis. Diseased plants often have red or brown rotted tissue with a clear demarcation between the diseased and adjacent healthy white tissue. A lack of fine feeder roots is also associated with *Phytophthora* root rot.

Sampling techniques for in-field plants: If a plant shows typical below ground symptoms of root rot, remove the entire crown. The entire cane is not needed for a tissue sample however; leave four to six inches of healthy tissue on the sample. Healthy tissue included in the sample is required for accurate identification of *Phytophthora*. Knock off excess soil and place plant sample into the bag. Samples may be grouped in ten plants per bag, but should all be from the same area in a field. Be sure to code the bag so you can easily retrace your steps back to the field and sample area. Leave behind coded flagging tape or otherwise mark the area of the field that you sampled with your corresponding sample code in the field.

Examples of raspberry plants showing root rot symptoms of wilted and yellowing shoots. Root rot may be due to poor cultural conditions or to disease causing organisms such as *Phytophthora fragariae rubi*.



Symptom: wilted and yellowing shoots



Symptom: row sections with plant death



Symptom: scorched drying leaf tips



Symptoms on root and crown. Note the sharp line of demarcation between the healthy (white) and infected (brown) tissue as well as the lack of fine feeder roots.

(Image courtesy of Michael A. Ellis, Department of Plant Pathology, The Ohio State University)

Sample storage, packing and shipping:

All samples should be kept cool and processed as quickly as possible.

Store samples in a cooler or otherwise cool place to avoid further decaying of the tissue. Samples should remain chilled until they are ready to ship.

Place samples in shipping box with a cold gel pack.

The grower cost for each sample (bag submitted) is \$50. Payment should be made upon shipping. Make check payable to Washington Red Raspberry Commission. Samples without payment will NOT be processed.

The Washington Red Raspberry Commission is subsidizing half of the cost. Supplies are limited and growers may submit numbers of samples based on the chart below. If you have questions, contact Henry Bierlink at the WRRC.

Annual Average Raspberry Production	Maximum Samples
More than 5,000,000 pounds	50
4,000,000 to 5,000,000 pounds	40
3,000,000 to 4,000,000 pounds	30
2,000,000 to 3,000,000 pounds	20
1,000,000 to 2,000,000 pounds	10
500,000 to 1,000,000 pounds	5
Less than 500,000 pounds	2

Send or directly deliver the boxes overnight to:

Shipping samples from King County north:

The Washington Red Raspberry Commission
1796 Front Street
Lynden, WA 98264

Shipping samples from Pierce County south:

Peerbolt Crop Management
5261 N. Princeton
Portland, OR 97203

These two receiving locations will transmit the samples to the laboratory for analysis. Growers should not send samples under this program directly to the laboratory.

Results

Results of the survey and more information will be compiled and posted on WSU Extension – Whatcom County's website at http://whatcom.wsu.edu/ag/comhort/com_berries/phytophthora.htm

Results posted will include grower-generated codes, county, raspberry variety, field age and whether sample origin is from pre-plant root stock or field stock.

Funding for this project provided by:

Washington Red Raspberry Commission
Washington State Commission for Pesticide Registration
Whatcom County

For more information, contact Craig MacConnell at (360) 676-6736 or cbmac@wsu.edu, or Henry Bierlink at (360) 354-8767 or Henry@red-raspberry.org.