Testing for Aphanomyces and Other Root Rot Pathogens

Adapted from article by Bruce Barker, P.Ag.

Surveys across Western Canada have found two main pathogens responsible for root rots in pulses. Fusarium species are distributed widely, with *F. avenaceum* and *F. solani* the most virulent types of root rot that can cause yield loss. *Aphanomyces euteiches*, first reported in Saskatchewan in 2012, is a more recent concern.

Aphanomyces root rot is most common under good soil moisture conditions. The pathogen is a water mould that depends on moisture for the zoospores to move in the soil and infect plant roots. Infection can happen anytime during the growing season, and spores can persist for many years in the soil. Root rots may still show up in drier growing conditions, and may or not be Aphanomyces root rot.

The seedling stage seems to be the most susceptible for Fusarium root rot, but symptoms do not typically become visible until late flower. In drier years, root rot symptoms showing up later in the season may be caused by one of many different pathogens.

Aphanomyces root rot is very difficult to identify and isolate with conventional methods, and requires a DNA test for confirmation. For fields with a history of root rot, recommendations are for growers to test their fields for the presence of *A. euteiches* so that they can implement practices to manage the disease if is present.

Several labs in Western Canada can test for the Aphanomyces root rot pathogen. These labs may analyze either root tissue or a soil sample. While each lab has its own sampling and submission protocol, the following are general recommendations for soil and plant sampling.

### Soil

- Sample field in a W shaped pattern across entire field or focus on taking samples from low spots, water runs, compacted areas, or other suspect areas where yellowing and poor growth in previous pea or lentil crop was noted.
- Collect soil from four to eight inches (10 to 20 centimetres) deep in the surface A-horizon layer, or less as depth allows, without taking any of the B-horizon subsoil. Do not include thatch or surface residue layers in the sample.
- Submit a minimum two cup sample of soil.

### Plant Tissue

- Infected plants are concentrated sources of pathogen, and represent a significant escalation in the amount of inoculum present in a field.
- Scout for suspect plants showing signs of root rots such as wilting and yellowing of the plant, and discoloured roots. Submit root tissue only (fresh, dried, or frozen) for testing.

Some labs provide their own sample kit. Contact the lab for their specific sampling and submission requirements.

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What The Results Mean

The results from a lab test in the fall can mean several things depending on the reporting lab. The first is positive or negative. This simply means that the pathogen is present in the sample, but does not put a number on the amount of spores per gram of soil, or level of infection of the root tissue.

Some labs take the positive/negative test one step further in their reporting, where a positive result means that the pathogen is present at levels that would be capable of causing disease. A negative result cannot guarantee that the pathogen is not present in the field, but just not present in the soil sample.

Finally, some labs provide a quantitative result reporting on the number of spores per gram of soil or levels of risk for root rots. Research by Agriculture and Agri-Food Canada has suggested higher risk for *A. euteiches* in fields when levels are above 100 oospores per gram of soil in the Dark Brown soils, and 750 oospores per gram in the Brown and Black soils. However, the presence of *Fusarium* spp., particularly in the Brown soils, increases disease severity. Because *Fusarium* spp. are so widespread on the Prairies, the risk threshold for *A. euteiches* has been adjusted. The number of *A. euteiches* oospores where the pathogen may start to cause an impact on the crop has been set at 100 oospores per gram of soil for all soil types.

If *A. euteiches* is confirmed in a field, growers should follow recommended management practices that include rotating to non-susceptible crops or more tolerant pulse crops such as faba beans, chickpeas, or soybeans, and maintaining a minimum of six to eight years between susceptible hosts such as peas, lentils, dry beans, and alfalfa.

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Figure 1. Healthy seedlings (left) vs root rot infected seedlings (right).
Pea Root Rot Test on plant specimens through plating for Fusarium species, Pythium species, and Rhizoctonia solani, in addition to DNA extraction for Aphanomyces euteiches. This test can be done on other pulse crops besides just peas. Requires at least five plants and provides the percentage of infection by each pathogen. Soil test results are based on extraction of oospores from soil and DNA analysis for Aphanomyces only. Results are reported as positive or negative. A positive result means the pathogen is present at levels capable of causing the disease.

A & L Canada Labs

Plant specimens and soil are subjected to DNA extraction and PCR analysis to detect various pathogens including the root rot pathogens Aphanomyces eutiches, Fusarium oxysporum, F. solani, Pythium, and Rhizoctonia solani. Results presented in semi-quantitative analysis (low, moderate, high).

AFL Agriculture & Food Laboratory

Plant specimen and soil are subjected to DNA extraction and PCR analysis to detect various pathogens including root rot pathogens Aphanomyces eutiches and Rhizoctonia solani as well as various Fusarium and Pythium species. Results presented quantitatively and identified as low, moderate, and high levels.

BDS Laboratories

Plant specimen and soil samples are plated and the cultures produced are identified. Pathogens able to detect including root rot pathogens Aphanomyces eutiches, Fusarium, Pythium, and Rhizoctonia. Results presented as detect or not detected.
Plant specimens are analyzed for Aphanomyces through DNA extraction and reported as detected or not detected. Fusarium is also screened for as requested.

Soil tests use both DNA extraction directly from the soil as well as from roots grown in the Bait test. Bait testing involves growing germinated pea seedlings in submitted soil for seven days and then extracting the DNA from the roots to screen for pathogens. The combination of the tests are used to confirm *Aphanomyces eutiches* presence. Fusarium can also be identified in the roots from the Bait test but is not reported to the species level. Results are reported as detected or not detected and the lab will assist with interpretation or further risk analysis based on individual results. Discovery Seed Labs has the ability to quantify the number of oospores per gram of soil and have done some quantitative reports for agronomists/farmers to establish a baseline for a field to allow monitoring over time.

Q Protect kits are available from select retailers for submitting and detecting presence of organisms in soil or plant tissues. Detection or quantification is offered for Aphanomyces, *Fusarium avenaceum*, and Rhizoctonia as well as for root rot complex. The test uses DNA extraction and PCR techniques for detection and quantification and can provide the number of oospores per gram of soil.

Plant specimen and soil are subjected to DNA extraction and PCR analysis to detect various pathogens including root rot pathogens *Aphanomyces eutiches*, Fusarium, Pythium and Rhizoctonia. For soil, results are expressed as detected if all replicates have detectable PCR product greater than 100 oospores/gram of soil, otherwise the result will be reported as not detected. For root tissue, results are expressed as detected or not detected.