A brief report on screening for the common root rot pathogen *Aphanomyces euteiches*

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Root rot caused by soil-borne pathogen *Aphanomyces euteiches* is considered a significant threat to pulse industry globally. Since there is no control available to cure the disease once established, disease avoidance remains the best disease management strategy. Therefore, it is very important to determine soil capabilities, in term of quantifiable amount of disease causing agent, to cause disease before planting a susceptible crop. Soil pH and texture play crucial role is root rot establishment. Soil biological and molecular assays have been developed to accurately determine the quantities of root rot pathogen at any time in any soil.

This report describes the analysis of the soil and root samples for the presence and abundance of root rot pathogen *A. euteiches* using soil physio-chemical parameters, biological, and molecular assays.

A total of nine samples (soil and pea roots) were received at Lethbridge Research and Development Centre on August 31, 2022 (Table 1). The cropping history and incidence of root rot in these soils is unknown.

Table 1: Samples received from Dawson Creek, BC in August 2022.

Field	Sampling date	Latitude	Longitude
Altona	18-Aug	56.85883	-120.877
Baldonnel	17-Aug	56.236	-120.688
Buick	18-Aug	56.79085	-121.094
Clayhurst	16-Aug	56.18724	-120.103
Doe River	16-Aug	55.96237	-120.109
Farmington	16-Aug	55.91211	-120.568
Flatrock	16-Aug	56.2602	-120.424
Pineview	17-Aug	56.34924	-120.789
Tower Lake	18-Aug	56.02297	-120.632

In order to perform molecular assay on the quantitation of root rot pathogen *A. euteiches*, DNA was extracted form soil (as received) using DNeasy PowerSoil Pro kit, Qiagen, Canada following manufacturer protocol. DNA was extracted in triplicates from each soil sample. The received roots were gently washed, chopped into small pieces, and flash dried. Approximately 30 milligram of these roots were used to extract DNA in duplicates using DNeasy Plant mini kit, Qiagen, Canada following the manufacturer protocol. Roots for the field sample Tower Lake got lost during processing hence DNA could not be extracted. DNA extracted from soil and roots was analysed for the presence and abundance of *A. euteiches* using quantitative PCR technique. Moreover, about 500 gram of soil from each field was sent to a commercial soil testing lab in Lethbridge, AB. for the analysis of soil pH and texture (Table 2).

Table 2: Soil physio-chemical analysis.

Field	Sand	Silt	Clay	Soil Texture	pH
Altona	26.2	37.8	36	Clay Loam	5.2
Baldonnel	25.3	50.7	24	Silt Loam	5.6
Buick	24.4	39.6	36	Clay Loam	5.4
Clayhurst	21.1	44.9	34	Clay Loam	5.2
Doe River	20.7	25.3	54	Clay	5.4
Farmington	24.6	49.4	26	Loam	6.7
Flatrock	23.9	54.1	22	Silt Loam	5.5
Pineview	25.7	40.3	34	Clay Loam	4.6
Tower Lake	30.3	51.7	18	Silt Loam	5.5

Greenhouse soil bioassay: Approximately 250 ml of soil from each of the nine fields was used to plant four pea seeds per pot with five replications. All the samples were planted and harvested the same time. Plants were grown in a greenhouse bench for four weeks. Seed germination data was acquired for each pot from day five after planting until at least 90% of emergence was achieved (Fig. 1). After four week of growth, plants were harvested and rated for disease severity of root rot on a 1 – 7 disease severity scale based on root (dis)coloration and root mass reduction as described by Chatterton et al (2019).

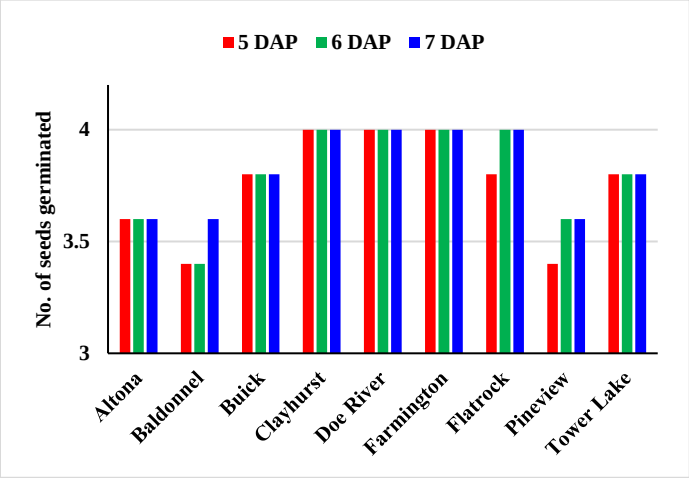

Figure 1: Pea germination data in soils received from Dawson Creek, BC

Roots from each pot was processed individually as previously. Soil from all five replications of the same field was pooled. DNA was extracted from processed root samples and pooled soil samples using same methods and subjected to quantify the abundance of *A. euteiches*. Soil DNA was extracted in three replications from the pooled sample whereas two root DNA extraction was performed from each pot totalling 10 extractions from each field except for the samples Flatrock and Altona where disease pressure was very high and not enough roots tissues were left at harvest time, thereby DNA was extracted in only duplicates. Quantitative PCR were performed using *A. euteiches* species specific probes and primers on each extracted sample, the number of *A. euteiches* was calculated using a standard curve, and presented per gram of soil and root sample. All the technical replications were averaged as shown in the following Tables of Fig 2.

Altona

A. euteiches per g of soil	
As received	**Bioassay**
33	681
21	437
32	140

A. euteiches per g of root	
As received	**Bioassay**
196060	2527079
315200	14247557

Baldonnel

A. euteiches per g of soil	
As received	**Bioassay**
2	0
2	0
7	1

A. euteiches per g of root	
As received	**Bioassay**
116	8202
108	1856
	1268
	311
	475

Buick

A. euteiches per g of soil	
As received	**Bioassay**
9	327
5	452
5	921

A. euteiches per g of root	
As received	**Bioassay**
328268	3082761
661937	3992135
	1267473
	1550169
	1003160

Clayhurst

A. euteiches per g of soil	
As received	**Bioassay**
30	126
124	15
117	43

A. euteiches per g of root	
As received	**Bioassay**
136	10305
923	3596
	898
	839
	601

Doe River

A. euteiches per g of soil	
As received	**Bioassay**
63	20
303	27
39	20

A. euteiches per g of root	
As received	**Bioassay**
23697	667
53177	35
	49
	70
	272

Farmington

A. euteiches per g of soil	
As received	**Bioassay**
64	2476
49	1726
23	2148

A. euteiches per g of root	
As received	**Bioassay**
274557	1544
846099	13396000
	32599480
	30709152
	37509485

Flatrock

A. euteiches per g of soil	
As received	**Bioassay**
38	430
25	343
18	799

A. euteiches per g of root	
As received	**Bioassay**
53115	3185909
122463	5460797

Pineview

A. euteiches per g of soil	
As received	**Bioassay**
47	4573
64	3987
62	4336

A. euteiches per g of root	
As received	**Bioassay**
42027	58176137
75216	45331425
	14978442
	42232365
	43541865

Tower Lake

A. euteiches per g of soil	
As received	**Bioassay**
237	567
580	476
130	1356

A. euteiches per g of root	
As received	**Bioassay**
Sample lost	39931061
	358
	4333490
	30
	4851

Figure 2: Biological and molecular assay results of soils. Plants were harvested after four weeks of growth. Each value in the tables is an average of two technical replications.

A. euteiches predominantly stays in dormant form (oospore) in soil and only becomes active (germinated mycelium and/or zoospores) under favourable conditions. Under these conditions, dormant oospores germinate, exponentially produce zoospores which infect the plant, utilize host resources up to a nutrition exhaust point leading to plant death. This initiates sexual reproduction in the pathogen to produce oospores completing the disease cycle. Although all the soils were planted and harvested the same time, the disease incidence and severity was found different for each soil, Fig. 2 indicating that each soil has its own intrinsic chemical and biological profile. Table 3 present average root rot rating and quantifiable amounts of *A. euteices* present in soil and root samples. When this data was analysed with soil physio-chemical data (Table 2), several interesting although weak, correlation matrices were found (Table. 4).

Table 3: Root rot rating and abundance of *A. euteiches* in root and soil samples.

Field	Root rot rating^	Root*		Soil*	
		Received	Bioassay	Received	Bioassay
Altona	7	255630	8387318	28	419
Baldonnel	1	112	2423	4	0
Buick	5.6	495103	2179140	6	567
Clayhurst	1.4	529	3248	90	61
Doe River	1	38437	218	51	22
Farmington	3	560328	22843132	45	2117
Flatrock	7	87789	4323353	27	524
Pineview	5.2	58622	40852047	58	4299
Tower Lake	2		8853958	52	799

^ Average root rot rating of five replications

* Average number of *Aphanomyces euteiches* quantified in one gram of soil or root

The most interesting correlation was found between pH of soil and the severity of root rot, -0.24%, which means the higher the soil pH is, the lower the chances of having disease established in that soil. *A. euteiches*, being a fungus loves acidic (lower) pH and the neutral or slightly basic range of pH negatively impacted the growth of this fungus in the soil. Soil texture and disease incidence also showed a weak correlation matrix. There is a trend found in the data that sandy soils tend to have higher root rot incidence under favourable environmental conditions. Similarly, soils that are more clay texture indicated less severity to disease establishment. This may be due to their tightly bound texture which also prevents free flow of water that found essential for *A. euteiches* zoospores to swim towards the host roots. For instance, soils from Doe River and Tower Lake had shown similar amount of *A. euteiches* dormant oospores as received (Table 3), but disease was not successfully developed in Doe River soil within four weeks of plant growth compared to Tower Lake where three out of five pots shown varied disease symptoms and an average root rot rating of 2, which also indicated that the pathogen distribution in the soil was not uniform (Fig. 2).

Table 4: Correlation between soil physio-chemical parameters, root rot severity, and abundance of *A. euteiches* in soil and root tissues.

Multivariate

Correlations

	Root rot rating	Root-A. euteiches	Soil-harvest-A. euteiches	Sand	Silt	Clay	pH
Root rot rating	1.0000	0.2785	0.2717	0.1961	0.0678	-0.1094	-0.2408
Root-A. euteiches	0.2785	1.0000	0.9875	0.3119	0.0440	-0.1200	-0.1349
Soil-harvest-A. euteiches	0.2717	0.9875	1.0000	0.2629	0.0361	-0.1003	-0.1839
Sand	0.1961	0.3119	0.2629	1.0000	0.4773	-0.6691	0.0076
Silt	0.0678	0.0440	0.0361	0.4773	1.0000	-0.9724	0.3566
Clay	-0.1094	-0.1200	-0.1003	-0.6691	-0.9724	1.0000	-0.3036
pH	-0.2408	-0.1349	-0.1839	0.0076	0.3566	-0.3036	1.0000

The correlations are estimated by Row-wise method.

Soil from Baldonnel field exhibited the lowest level of disease incidence, essentially none. The quantifiable amount of *A. euteiches* were the lowest among all other soils in both, roots and soil samples. However, roots at harvest looked a bit coloured and indicated that the plants might suffer with other commonly found pathogen(s) than the *A. euteiches*.

Soils from Altona and Flatrock had the highest disease pressure, both rated 7 and almost all the plants died by week four, which also made DNA extraction from roots very hard resulting only two replications possible from these samples.

Quantification data of *A. euteiches* at different root rot rating levels resulted in a bell shape graph (Fig 3) with essentially similar starting and ending quantities of *A. euteiches* in the soil indicating that the highest amount of quantifiable *A. euteiches* is achieved when disease is on its midway and not necessary showing symptoms in the plant foliar and mild discoloration in associated root systems. This makes the control of

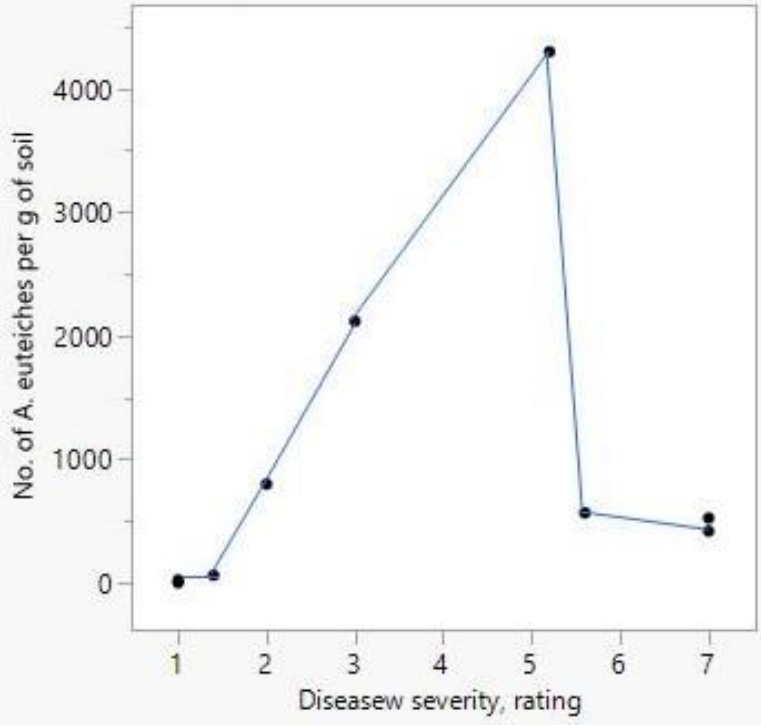

Figure 3: Correlation between root rot severity and the quantifiable amounts of *A. euteiches* in soils.

disease the hardest when the symptom are witnessed in a field of susceptible crop. It is always a good idea to periodically determine the quantifiable levels of *A. euteiches* in soils.

Different soils differ in the amount of *A. euteiches* at various disease rating levels, we hypothesize that this variability among soils is dependent on soil physio-chemical parameters, *A. euteiches* isolate, and initial concentration of dormant oospores. More soils are being tested to understand how these dynamics can be linked to the risk associated with growing a susceptible crop in any given soil.