

SURVEY OF POTATO EARLY DYING DISEASE COMPLEX IN ALBERTA'S COMMERCIAL POTATO FIELDS 2021-2022**CROP:** Potato**LOCATION:** Alberta**NAMES AND AGENCIES:**A.U. RAHMAN¹, M. MUNAWAR¹, M. KONSCHUH¹, M. TENUTA², M.W. HARDING³ & D.P. YEVTUSHENKO¹¹Department of Biological Sciences, University of Lethbridge, Lethbridge, AB T1K 3M4**Telephone:** (403) 317-2879; **Facsimile:** (403) 329-5159; **E-mail:** dmytro.yevtushenko@uleth.ca²Department of Soil Science, University of Manitoba, Winnipeg, MB R3T 2N2³Alberta Agriculture and Irrigation, Crop Diversification Centre South, Brooks, AB T1R 1E6

ABSTRACT: Potato fields were evaluated for incidence and severity of early dying disease complex in the growing seasons of 2021 and 2022. Disease pressure varied among the fields in both years. Fields containing higher inoculum levels of *Verticillium dahliae* in the fall soil samples as determined by qPCR of soil samples typically showed more potato early dying (PED) symptom development in the subsequent growing season. The incidence of PED was higher in 2022 than in 2021. Results demonstrated the potential of qPCR evaluation of representative soil samples as an aid for risk prediction activities in potato production systems dealing with PED.

INTRODUCTION AND METHODS: Over the last decade, Alberta has had the highest provincial potato yield per hectare in Canada, averaging 45.28 metric tons per hectare (Agriculture and Agri-Food Canada 2021). Despite having the highest yield per hectare, plant diseases continue to constrain potato production. Among them is the potato early dying (PED) disease complex. The disease affects the plant's xylem tissue, hindering water transport and resulting in premature death of the plant. Initial symptoms of the disease include chlorosis of the basal leaves, followed by necrosis, internal stem brown discoloration and flagging. Infected plants usually die 4–6 weeks before reaching maturity (Rowe et al. 1987). PED complex is caused either by *Verticillium* spp. or by a combination of *V. dahliae* and other soil-borne pathogens, including *Colletotrichum coccodes*, *Fusarium* spp., and the root lesion nematode *Pratylenchus penetrans* (Cole et al. 2020; Johnson and Dung 2010; Powelson and Rowe 1993; Rowe et al. 1987; Rowe and Powelson 2002; Tsrer and Hazanovsky 2001; Wheeler et al. 1992). Therefore, disease surveys, early diagnosis and detection of PED are essential to further increase or maintain the average yield of potatoes grown in Alberta.

A survey of 30 commercial potato fields was conducted in 2020 and 2021 to determine the number of *V. dahliae* and *V. albo-atrum* cells in fields scheduled for potato production the following year. The fields were located in southern Alberta and were irrigated. For each field, 40 soil cores up to 30 cm depth were collected using a 6-cm diameter Dutch auger along a zigzag W-pattern. Soil from all 40 cores sampled in each field was mixed and stored at 4 °C before further analysis. Soil samples were air-dried, passed through a 2-mm-pore sieve, and used to identify and quantify *V. dahliae* and *V. albo-atrum* at Agricultural Certification Services Inc. (Fredericton, New Brunswick) according to their standard method (Singh et al. unpublished). Briefly, DNA was extracted from about 250mg of soil using EZNA® Soil DNA extraction kit (Omega Bio-tek). Real-time PCR amplifications were performed using PerfeCTa® Multiplex qPCR ToughMix (Quantabio). The quantity of *V. dahliae* and *V. albo-atrum* in soil samples was determined using the standard curve (absolute quantification) method. The standard curve was generated from a dilution series (1:10 dilutions covering 1 pg to 0.001 fg of DNA) of g-block DNA. The amount of DNA corresponding to one *V. dahliae* cell was estimated at 36.5 fg/genome, considering a genome size of 33.8 Mb (Klosterman et al. 2011). Based on the qPCR results for the presence of *V. dahliae*, four fields were designated as high-risk and four as low-risk. Five areas (two rows x 10 m) in each field were selected and geo-tagged by a Global Positioning System (GPS) device. Each area was assessed for disease severity starting ten weeks after planting, then every two weeks until harvest. Fifteen consecutive plants in the middle of each area were rated individually for PED symptoms, using a scale proposed by MacGuidwin and Rouse (1990): 0 = no symptoms; 1 = 1–33% of the foliage showing wilting, necrosis, or chlorosis; 2 = 34–66%; 3 = 67–99%, and 4 = dead plant.

A disease severity index (DSI) was calculated as a percentage of the maximum using the following formula: $DSI = [\text{sum}(\text{class frequency} \times \text{score of rating class})] / [(\text{total number of plants}) \times (\text{maximal disease index})] \times 100$ (Safi et al. 2020). Percent disease incidence (DI) was calculated as: $DI = (\text{number of plants with PED symptoms} / \text{total number of plants observed}) \times 100$. During each survey, one plant sample was collected from each area for molecular detection of *V. dahliae* and *C. coccodes*. A total of 200 potato stems were evaluated in 2021 and 240 stems were evaluated in 2022.

RESULTS AND COMMENTS: The qPCR analyses confirmed the presence of *V. dahliae* in 22 (n=30) fields in 2020 and 14 (n=30) fields in 2021. The inoculum level of *V. dahliae* in these fields was highly variable, ranging from 0 to 19139 and 0 to 1887 *V. dahliae* cells per gram of soil in 2020 and 2021, respectively (Tables 1 and 2). The heaviest infestation recorded was 19139 *V. dahliae* cells per gram of soil in 2020. The incidence of *V. albo-atrum* was low in both years, with only one field showing small traces of the pathogen in 2021.

There is currently no risk prediction tool for PED based on qPCR results. In Wisconsin, soil infestation levels of 6 to 10 microsclerotia/g resulted in 100% stem infection, whereas 8, and 18 to 23 microsclerotia/g of soil are considered as economic thresholds in Idaho and Colorado, respectively (Davis and Sorensen 1986; Nicot and Rouse 1987; Nnodu and Harrison 1979). As per the threshold level set by the Ontario Ministry of

Agriculture Food and Rural Affairs (OMAFRA 2009), fields having 0–5 cfu per gram of soil are at low risk, fields with 5–12 cfu/g are at moderated risk, and fields having more than 12 cfu/g are at high risk of PED development. Comparing our qPCR results of *V. dahliae* cells per gram of soil to the inoculum threshold set by OMAFRA, 22 fields in 2020 and 14 fields in 2021 were at high risk of PED disease development. Eight fields in 2020 and 16 in 2021 were at low risk of PED disease development. Four low-risk and four high-risk fields were selected for surveillance in the summers of 2021 and 2022. The incidence (percentage of affected plants per field) and severity (extent of damage on affected plants) of PED were recorded from each field.

The disease severity index (DSI) at the end of the growing season was high for high-risk fields in both years (Figs. 1 and 2). In 2021, the DSI's of the low-risk and high-risk fields were less than 10% until the end of July, except for the AB55 field, which showed a DSI of 12% (Fig.1). The DSI progressed more rapidly in high-risk fields than in low-risk fields. The DSI at the start of September was 54–68% and 5–30% for high-risk and low-risk fields, respectively (Fig.1). In 2022, at the end of July, all the high-risk fields showed a DSI of more than 20% (Fig. 2). AB81 and AB105, designated as low-risk fields, also showed more than a 20% DSI at the end of July (Fig.2). In the beginning of September 2022, the DSI was 59–82% and 12–69% for high- and low-risk fields, respectively (Fig. 2).

Table 1. Number of *V. dahliae* cells per gram of soil in Alberta commercial potato fields in 2021.

Fields	<i>V. dahliae</i> cells/g of soil	Risk designation
AB42	0	Low-risk
AB47	0	Low-risk
AB60	0	Low-risk
AB62	0	Low-risk
AB45	3934	High-risk
AB48	19139	High-risk
AB55	2304	High-risk
AB66	3161	High-risk

Table 2. Number of *V. dahliae* cells per gram of soil in Alberta commercial potato fields in 2022.

Fields	<i>V. dahliae</i> cells/g of soil	Risk designation
AB81	0	Low-risk
AB86	0	Low-risk
AB104	0	Low-risk
AB105	0	Low-risk
AB84	722	High-risk
AB106	504	High-risk
AB107	410	High-risk
AB108	1887	High-risk

PED severity index (%) 2021

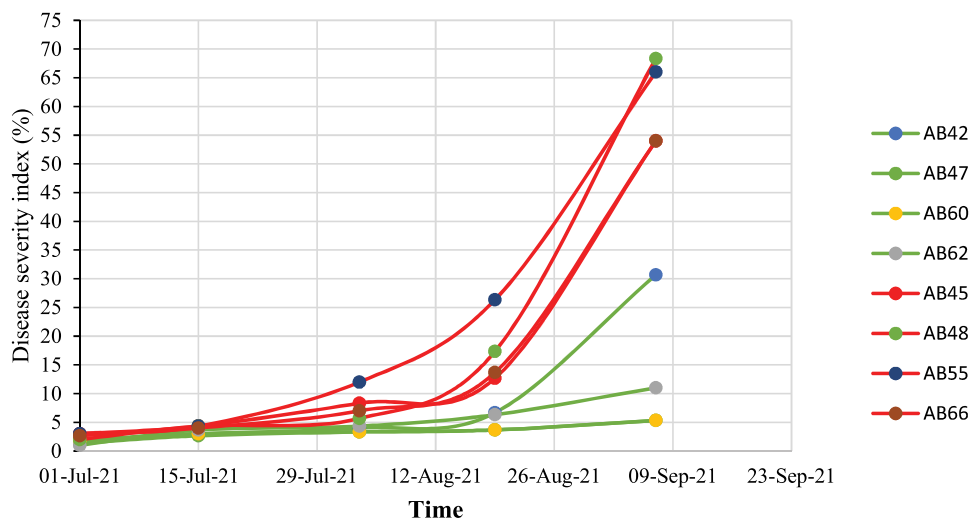


Fig. 1 Disease severity index (DSI) (%) of all the eight fields surveyed in 2021 growing season. The DSI of each field represents the average DSI of five locations. Red lines represent high risk fields whereas green lines represent low risk field.

PED severity index (%) 2022

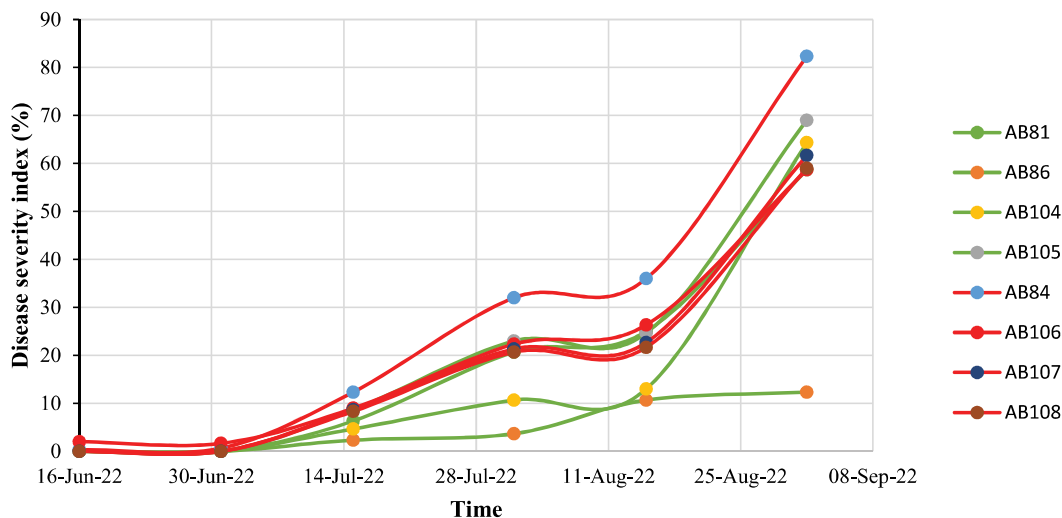


Fig. 2 Disease severity index (DSI) (%) of all the eight fields surveyed in 2022 growing season. The DSI of each field represents the average DSI of five locations. Red lines represent high risk fields whereas green lines represent low risk fields.

The average incidence of PED in all fields surveyed in the beginning of September 2021 was 65%, ranging from 20% to 100% (Table 3). In contrast, the average incidence of PED in all fields surveyed in September 2022 was 93%, ranging from 49-100% (Table 3). This report demonstrates the potential for qPCR evaluation of representative soil samples as an aid for risk

prediction activities in potato production systems with PED risk. While the environment may affect the consistency of risk prediction by qPCR (as was seen in ‘low risk fields’ that developed significant levels of PED by the end of season), this preliminary work also shows some level of correlation between soil inoculum levels of *V. dahliae* and PED risk.

Table 3. Mean incidence (range) and PED severity in fields with a high or low *V. dahliae* cells per gram of soil recorded in the start of September 2021 and September 2022

Mean incidence (%) in fields (2021)		Mean incidence (%) in fields (2022)		Mean DSI (%) in fields (2021)		Mean DSI (%) in fields (2022)	
High	Low	High	Low	High	Low	High	Low
96 (92-100)	33 (20-57)	99 (98-100)	87 (49-100)	60 (54-68)	13 (5-30)	65 (59-82)	51 (12-69)

ACKNOWLEDGMENTS: We thank the Potato Growers of Alberta (PGA), the Fruit and Vegetable Growers of Canada (FVGC), and the University of Lethbridge for funding this research project. We also thank Drs. Mathuresh Singh and Tyler MacKenzie from the Agricultural Certification Services Inc. (Fredericton, NB) for qPCR analysis of soil samples. Many thanks to the producers for allowing access to their fields.

REFERENCES

- Agriculture and Agri-Food Canada. 2021. Potato market information review 2020-2021. [accessed 2022 Dec 12]. <https://agriculture.canada.ca/en/canadas-agriculture-sectors/horticulture/horticulture-sector-reports>
- Cole E, Pu J, Chung H, Quintanilla M. 2020. Impacts of manures and manure-based composts on root lesion nematodes and *Verticillium dahliae* in Michigan potatoes. *Phytopathology* 110(6):1226–1234.
- Davis J, Sorensen L. 1986. Influence of soil solarization at moderate temperatures on potato genotypes with differing resistance to *Verticillium dahliae*. *Phytopathology* 76(10):1021–1026.
- Johnson DA and Dung JK. 2010. Verticillium wilt of potato—the pathogen, disease and management. *Can J Plant Pathol.* 32(1):58–67.
- Klosterman SJ, Subbarao KV, Kang S, Veronese P, Gold SE, Thomma BP, Chen Z, Henrissat B, Lee YH, Park J, et al. 2011. Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. *PLoS Pathog.* 7(7): e1002137.
- MacGuidwin A, Rouse D. 1990. Effect of *Meloidogyne hapla*, alone and in combination with subthreshold populations of *Verticillium dahliae*, on disease symptomatology and yield of potato. *Phytopathology* 80(5):482–486.
- Nicot PC, Rouse D. 1987. Relationship between soil inoculum density of *Verticillium dahliae* and systemic colonization of potato stems in commercial fields over time. *Phytopathology* 77(9):1346–1355.
- Nnodu E, Harrison M. 1979. The relationship between *Verticillium albo-atrum* inoculum density and potato yield. *Am J Potato Res.* 56(1):11–25.
- [OMAFRA] Ontario Ministry of Agriculture, Food and Rural Affairs. 2009. Verticillium wilt. Ontario CropIPM. [accessed 2022 Dec 12]. <http://www.omafra.gov.on.ca/IPM/english/potatoes/diseases-and-disorders/verticillium.html>
- Powelson ML, Rowe RC. 1993. Biology and management of early dying of potatoes. *Annu Rev Phytopathol.* 31(1):111–126.
- Rowe RC, Davis JR, Powelson ML, Rouse DI. 1987. Potato early dying: causal agents and management strategies. *Plant Dis.* 71(6):482–489.
- Rowe RC, Powelson ML. 2002. Potato early dying: management challenges in a changing production environment. *Plant Dis.* 86(11):1184–1193.
- Safi H, Hussain S, Shahid M, Nazi M. 2020. Incidence and severity of early blight of tomato in Peshawar, Mardan and Malakand Divisions and variability amongst the isolates of *Alternaria solani* Jones and Mart. *Int J Agric Environ Biotechnol.* 13(2):175–183.
- Tsrer L, Hazanovsky M. 2001. Effect of coinoculation by *Verticillium dahliae* and *Colletotrichum coccodes* on disease symptoms and fungal colonization in four potato cultivars. *Plant Pathol.* 50(4):483–488.
- Wheeler T, Madden L, Rowe R, Riedel R. 1992. Modeling of yield loss in potato early dying caused by *Pratylenchus penetrans* and *Verticillium dahliae*. *J Nematol.* 24(1):99.