Understanding the Complex Roles of Nematodes and Fungi in Alberta Potato Fields

Project Title: Understanding the role, virulence, and pathogenicity of Pratylenchus neglectus, its interaction with Verticillium dahliae and effect on potato yield.

Overview and Objectives

This project aims to establish a comprehensive experimental setup to study the complex interactions between two key pathogens associated with PED: the root lesion nematode (Pratylenchus neglectus) and the fungal pathogen (Verticillium dahliae). The primary objective during this initial reporting period was to investigate the complex interactions between these pathogens in potato plants, focusing on infection timing, coexistence, and potential synergistic or additive effects.

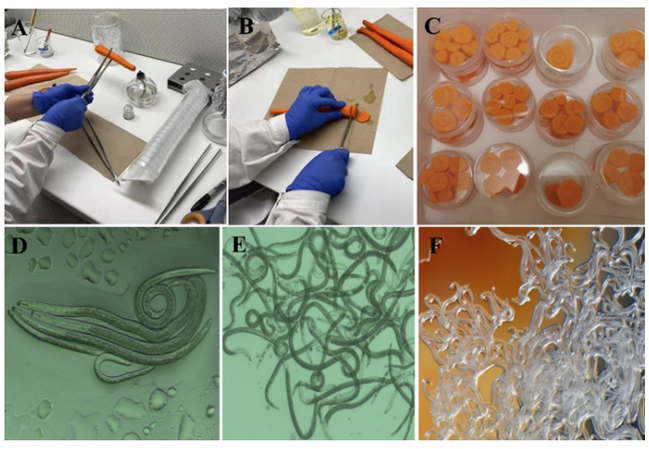
Methodology: Establishing Controlled Pathogen Cultures
To ensure accurate, controlled experiments, the team focused on isolating, confirming the identity of, and culturing pure strains of both pathogens.

Root Lesion Nematode (P. neglectus) Preparation

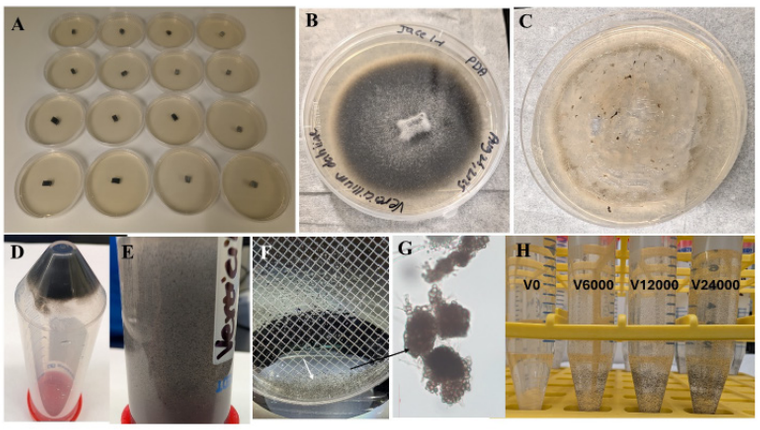
- Nematodes were initially extracted from naturally infested field soil collected in southern Alberta using Cobb's sieving and centrifugal flotation method.

- The species identity was confirmed through both morphological examination and molecular diagnostics. DNA sequencing and BLAST analysis confirmed a 99% similarity to Pratylenchus neglectus.

- For large-scale inoculum production, mature P. neglectus females were surface sterilized and used to establish pure cultures on sterile carrot discs.



Carrot disc culture preparation for nematode multiplication (C: Carrot disc storing; D–F: Root lesion nematode clusters from pure cultures



Verticillium dahliae subculturing and preparation of microsclerotia suspension for inoculation (B: V. dahliae plate with microsclerotia; F–H: Microsclerotia counting in water, and inoculum preparation

Fungal Pathogen (V. dahliae) Preparation

• V. dahliae was isolated from symptomatic potato stems and cultured on potato dextrose agar (PDA) medium.

• Molecular confirmation was achieved using ITS primers, with resulting sequences showing 99% similarity to Verticillium dahliae reference sequences.

• A three-week-old culture was used to prepare the microsclerotia suspension—the infectious structure used for inoculation—by scraping and blending the culture.

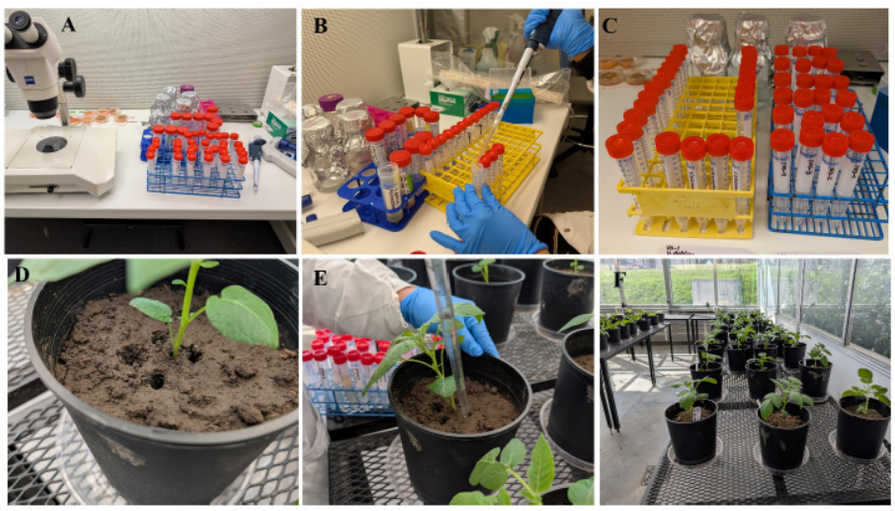
Greenhouse Experimental Setup

To replicate field conditions as closely as possible, the trial was established in a controlled greenhouse environment, excluding the field component due to time constraints.

• Plant Material: Tissue culture plantlets of the potato cultivar Russet Burbank were sourced from the Alberta Seed Repository. Four-week-old plantlets were transplanted into 2-litre pots.

• Soil: Field soil from the Lethbridge Polytechnic Research Farm was used instead of commercial potting mix. The soil was autoclaved twice to ensure the elimination of native soil organisms (especially nematodes) and tested for sterility.

• Design: The 40-pot experiment was arranged in a randomized complete block design (RCBD) with four replications per treatment to control for environmental variation. Plants were inoculated after two weeks of acclimatization



Inoculum preparation and inoculating plantlets (A–C: Counting nematodes and microsclerotia under the microscope; D: Preparing inoculation holes along the stems text

Inoculum Levels Tested

Pathogens were applied at varying concentrations to determine virulence levels and economic thresholds:

• V. dahliae Treatments: Three inoculum levels were used (6,000, 12,000, 24,000 CFU) plus a control). The levels align with literature suggesting that a threshold above 12 CFU (colony forming units) per gram of soil poses a high risk for PED

• P. neglectus Treatments: A range of six levels were tested (0, 1, 10, 100, 1,000, and 5,000 nematodes per pot) to cover a spectrum from sublethal to potentially lethal effects

Preliminary Observations

• Verticillium dahliae: Symptoms appeared rapidly, starting as early as one week post-inoculation with mild wilting. By the second week, symptoms progressed to noticeable leaf yellowing and wilting, severely affecting 5–6 lower leaves per plant.

• P. neglectus: Above-ground symptoms were less visible in nematode-inoculated plants compared to controls. However, these plants were observed to be slightly shorter, suggesting stress at the root level.

The team plans to continue monitoring plants and collecting data until December or until the plants die. Continued monitoring over the coming months is anticipated to reveal more about the disease progression and help the researchers establish threshold levels for infection severity.

• Next Steps: Upcoming activities include harvesting the plants, root staining, and the recovery of nematodes and V. dahliae from soil or plant material between December and March Future research with other nematode species and continued investigation of interaction are suggested.

This is a summary prepared by the PGA from a provided interim report from Dr. Dmytro Yevtushenko and Dr. Maria Munawar's work at the University of Lethbridge. Final project results will be posted once completed.