Canadian Agri-Science Cluster for Horticulture 2

Progress Report December 2014

Activity 15, Potato 14

Development of a Rapid and Sensitive Triplex Nested Real-time PCR Method for Quantification of Verticillium in Soil

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Activity Objectives

- Develop a fast and accurate method for the quantification of V. dahliae (a pathogen of potato and other Canadian crops such as tomato, alfalfa and sunflower) and V. longisporum (a pathogen of canola and other mustard family crops) in soil for Canadian potato growers.
- Adapt the method to a commercial laboratory setting to provide fast enough turn-around of analyses that producers can decide on control options prior to planting their crop.

Research Progress to Date

- Establish a culture collection of the dominant plant pathogenic *Verticillium* species from across Canada to be used in the following objectives - Completed
  Culture collection of microsclerotia and non-microsclerotial soil Verticillium species (V. dahliae, V. albo-atrum, V. longisporum, V. tricorpus, Gibellulopsis nigrescens (formerly V. nigrescens), V. isaacii, and V. klebahnii) has been obtained from the labs of; Soil Ecology (U Manitoba), plant pathology (U Manitoba), CFIA Ottawa, AAFC (National Collection and at AAFC London) and plant pathology (UC Davis)
  Each was purified and made free of contaminants by mono-sporic isolation
  Each was verified for species identity by morphology and sequencing
- Determine effectiveness of published PCR primers for the identification of *V. dahliae* and *V. longisporum* isolates - Completed
  We realized we needed to go further than selection of primers for only two species, we verified or generated new primer sets and now have reliable sets for V. dahliae, V. albo-atrum, V. longisporum, V. tricorpus, Gibellulopsis nigrescens (formerly V. nigrescens), V. isaacii, and V. klebahnii
- Obtain a bank of potato soils from across Canada and characterize them by conventional methods for *Verticillium* propagule densities and to use in the following objectives - Completed for

Manitoba in which at this phase we are using for the method development
Summer of 2015 we will obtain soils from Alberta, Ontario and Quebec
Summer of 2016 we will obtain soils from the Maritimes

- Develop an internal reference of a single-copy gene that would reflect variable efficiencies of PCR amplification of DNA from soils - Not completed
  We are evaluating published methods and determining the requirement for an internal reference. Many publications do not use an internal standard
- Develop a triplex real-time PCR method with internal reference for quantification of *V. dahliae* and *V. longisporum* from soil - We have completed quantitative PCR method development for individual primer set reactions
  Once the species present in soil is confirmed for various regions of Canada, we will decide which primer sets to combine for multispecies determination
- Determine if copy number of rDNA varies within isolates of *V. dahliae* and how it may influence real-time PCR quantification from soil - Not completed
- Determine degree of inhibition of the triplex real-time PCR method for soils by spiking reaction mixtures with known concentrations of the internal reference - Not completed
- Relate the triplex nested real-time PCR quantification to conventional *Verticillium* testing of soil by plate counting - We have related quantitative PCR determinations to standard plate counting for Manitoba
  This objective continues as we progress and needs become clearer based on what species are in Canadian soils
- Determine if soil properties determine the relation in quantification of *Verticillium* between the triplex nested real-time PCR and conventional testing by plate counting - We have optimized the DNA extraction from Manitoba soils
  Once the method is developed we will then calibrate it to plate counts of for a variety of soil types
- Determine if *V. longisporum* is present in appreciable amounts in soil
  We have examined this in Manitoba for 12 potato fields but wish to get more soils to examine in 2015 - Soil from other regions will be examined for V. longisporum but also all the Verticillium species we have in culture and primer sets
- Adapt the triplex nested real-time PCR method for use in a commercial agricultural testing
  There has been great interest from the Manitoba potato industry, Province of Manitoba, and Genome Canada to partner with the Pest Surveillance Initiative (PSI) in Manitoba to provide our methods for commercialization and availability to growers - We believe this is the best way to move forward as the lab has resources and is keen on providing services to growers.

Early Outcomes (if any) or Challenges

One-third of Manitoba soils contain V. tripcorpus. These soils have extremely high plate counts of Verticillium with the high counts due to this species and not V. dahliae. **We have a very low frequency of V. klebhanii and V. isaacii and no V. longisporum**. However, because of the importance of the non V. dahliae species to over estimation of potato disease causing Verticillium by plate counting, we will examine more soil. **Developed primer sets seem to be reliable and able to quantify Verticillium species** to at least to microsclerotia per gram soil.

Key Message(s)

In Manitoba, very high counts of Verticillium are related to the presence on V. tricorpus which is not thought to be a potato pathogen. For grower's interests, we will need to find resources to examine if this is the case. Thus our suspicion that high counts of Verticillium in Manitoba do not warrant intervention such as fumigation seems to be holding true.

We are excited about progress with no major hurdles having been encountered so far to the development of a multi-reaction quantification method.