Finding Pathogens in Your Soil

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Introduction

- Many soilborne diseases affecting potato, including common scab (*Streptomyces* spp.), powdery scab (*Spongospora subterranea*), black dot (*Colletotrichum coccodes*), Verticillium wilt (*Verticillium dahliae*), black scurf (*Rhizoctonia solani*), and white mold (*Sclerotinia sclerotiorum*) can cause persistent and recurring economic losses. *S. subterranea* also vectors Potato mop-top virus (PMTV), which causes internal necrosis of potato tubers. Detection and quantification of these pathogens in soil plays a pivotal role in disease management.

Objective:

To develop and validate sensitive and efficient molecular assays to quantify soil-borne potato pathogens *S. subterranea*, *C. coccodes*, *V. dahliae*, and PMTV from soil.

Methods

- Powdery scab (*S. subterranea*) and black dot (*C. coccodes*): The powdery scab and black dot pathogens were quantified using specific primers/probe and probe (1,2) along with external control pUC primer-probe to normalize the data calculations (3). The qPCR protocol and program used in quantifying these two pathogens were similar, allowing for the potential to quantify these pathogens in one assay (4).
- Verticillium (*V. dahliae*): The Verticillium wilt pathogen was quantified using previously published PCR primers adapted to the digital droplet system (5).
- PMTV: A one-step reverse-transcription droplet digital PCR (ddPCR) assay was performed using previously published primers and a probe adapted to the digital droplet system (6).

Results

- The real-time qPCR and ddPCR protocols developed and validated in this and previous studies (1-6) were specific for each pathogen. They did not amplify other closely related soil-borne potato pathogens.
- The qPCR reliably detected less than ten *S. subterranea* (powdery scab) sporosori and *C. coccodes* (black dot) sclerotia per gram of soil, and two *V. dahliae* (Verticillium wilt) gene copies per µl of DNA extracted from soil. The reverse transcriptase ddPCR detected less than one PMTV copy per µl of RNA extracted from soil.
- The *S. subterranea* (powdery scab) and PMTV assays were used to quantity of both pathogens from field samples taken in a grid pattern and data were used to develop a heat map (Figure 1).
- The *C. coccodes* assay was used to quantify the pathogen from soils sampled from 9 fields from several potato growing regions (Figure 2). Pathogen quantity was classified as low, moderate, and high. No samples were determined to be in the high (>1,000 sclerotia / g soil) category.
- *V. dahliae* quantification from 144 soil samples using the ddPCR assay compared to quantification by Pest Pros (Figure 3a). A significant statistical correlation was observed between the quantification results obtained from Pest Pros and ddPCR (Figure 3b).

Conclusions

- A single DNA extraction from a soil sample can be utilized for quantification of *S. subterranea* (powdery scab), *C. coccodes* (black dot), and *V. dahliae* (Verticillium wilt).
- An RNA extraction from that same sample can be used to quantify PMTV.
- In the future, molecular assays for additional pathogens and pests could potentially be added, allowing growers to submit one soil sample to test for multiple soil-borne pathogen/pests.
- Assays could potentially be combined to quantify more than one pathogen/pest simultaneously, reducing the time and cost of determining the pathogen/pest density in soil.
- Efficient quantification of multiple soil-borne pathogens/pests will enhance management to minimize the loss of potato yield to soil-borne diseases.

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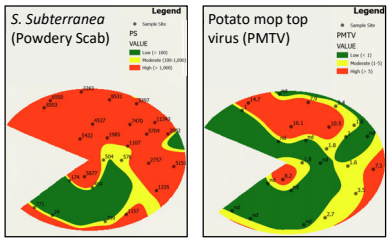


Figure 1. Field map illustrating PCR quantification of *S. subterranea* (left) and PMTV (right) in soil.

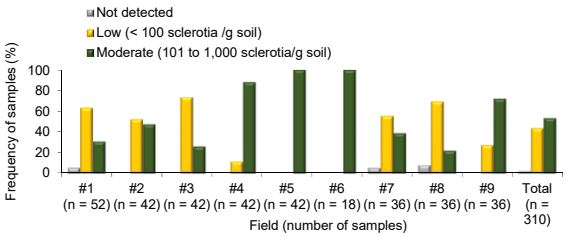


Figure 2. Quantification of *C. coccodes* using qPCR in soil samples collected from multiple potato growing fields across the US. No samples were determined to be in the high (>1,000 sclerotia / g soil) category.

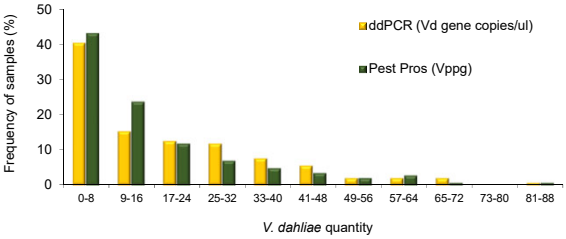
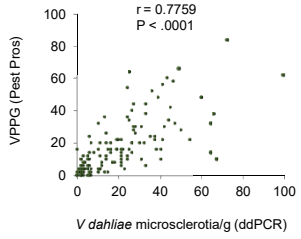


Figure 3a. Comparison of *V. dahliae* quantification in 144 soil samples as determined by ddPCR and Pest Pros.

Figure 3b. Pearson correlation between *V. dahliae* quantification comparing ddPCR and Pest Pros.



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