**Ohio Vegetable & Small Fruit Research & Development Program**

**Final Report**

**2018**

**Project Title:** Developing robust diagnostic methods for key soilborne diseases in Ohio high tunnels

**Principle Investigator(s)**: Sally A. Miller

**Phone Number**: 330-263-3678

**Fax**: 330-263-3841

**E-mail**: miller.769@osu.edu

**Cooperating Institution**: The Ohio State University Department of Plant Pathology

**Mailing Address**: 1680 Madison Ave., Wooster, OH 44691

**Other Key Personnel**: Anna Testen and Francesca Rotondo, The Ohio State University

**Why was this project funded?** Soilborne diseases are an important constraint to Ohio high tunnel tomato production and their diagnosis is difficult. Corky root rot and black dot root rot are far more common in Ohio high tunnels than anticipated. The corky root rot pathogen was identified in 33 of 68 (49%) Ohio high tunnels surveyed in 2017, and the black dot root rot pathogen was identified in 61 of 68 high tunnels (90%). The diagnostic techniques developed in this study will provide a method for rapid diagnosis and quantification of these pathogens in high tunnel soils.

**Project outline**. Our objective was to develop quantitative polymerase chain reaction (qPCR) assays for two key fungal pathogens: the corky root rot pathogen (*Pyrenochaeta lycopersici*) and black dot root rot pathogen (*Colletotrichum coccodes*). Diagnosis of corky root rot is further complicated by the presence of two distinct types, Types 1 and 2, that differ genetically and in ability to cause disease. Both corky root rot types are present in individual high tunnels in Ohio. Black dot root rot nearly always occurs with corky root rot and has only one genetic type. Our approach was to sequence specific segments of DNA in these pathogens and design primers and probes for molecular diagnostics (real-time quantitative PCR).

**What was discovered?** We developed realtime PCR (polymerase chain reaction) primers and probes for rapid and sensitive detection of *Pyrenochaeta lycopersici* Types 1 and 2, causal agents of corky root rot of tomato. We developed two sets of primers and probes for each type of *P. lycopersici* and tested them for specificity. Each set of primers and probes for each type were highly specific, and amplified only the correct type of *P. lycopersici.* The primers for Type 1 are Py1TQ3F/Py1TQ3R and the probe is Py1TQ3P, while the primers for Type 2 are Py2TQ5F/Py2TQ5R and the probe is

Py2TQ5P. These are currently being further tested for sensitivity, and specificity against other common soilborne pathogens. We can successfully use both sets of primers and probes (duplexing) in one reaction to detect both types of *P. lycopersici* at the same time. These primers and probes can be used to amplify *Pyrenochaeta* DNA from fungal hyphae, soil, and root tissue.

We sequenced Ohio strains of *C. coccodes* (black dot root rot) and found the target sequences to be identical to the sequences of *C. coccodes* for which primers and probes have already been developed (Cullen et al. 2002), therefore it was not necessary to design additional tests.

**Take-home messages.**

- Isolation of the causal agent of corky root rot, *Pyrenochaeta lycopersici* from plant tissues or soil is slow and difficult by traditional culture methods. Development of these molecular primers and probes speeds up the diagnosis/confirmation of corky root rot in plant samples from weeks to one day, and also allows us to differentiate between the two types.
- The ability to detect both types of the pathogen in soil will allow us to alert growers about the presence or absence of the pathogen in their high tunnels with a rapid soil test. In addition, it may help us to predict the potential future severity of the disease in tomatoes in high tunnels, although additional research to establish threshold values for this pathogen is still needed.
- The *Pyrenochaeta* tests, along with the verified molecular assay for *Colletotrichum coccodes*, will improve the speed and accuracy of soil tests for tomato pathogens, and provide valuable information to growers.