BIOAg Project Report

Report Type: Final

Title: A SmartChip for Pathogenic and Beneficial Microbes Underlying Soil Health

Principal Investigator(s) and Cooperator(s):

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Abstract:

High-throughput, low-cost diagnostics for multiple aspects of soil health are a necessary component to maximize the ability of researchers to understand soil health processes and for farmers to effectively adopt soil health management practices. Across many growing regions of Washington, soilborne diseases are a major limitation, and given their patchy nature a large number of samples are required to detect their presence in fields. Additionally, many growers are taking action to improve soil health through the fostering of beneficial microbes. This project will focus on two areas: optimizing a high-throughput low-cost qPCR screen for disease-causing agents in organic vegetable systems in western Washington and in dryland cereal-legume-oilseed cropping systems across Washington State. We will focus on the pathogenic fungi *Phytophthora, Pythium, Rhizoctonia*, *Verticillium,* and *Fusarium* as well as the protist *Plasmodiophora brassicae,* include primers specific for the 18S region of arbuscular mycorrhizal fungi, and consider additional taxa based on stakeholder interest. This work will connect farm management to the presence of key microbial taxa that impact soil health.

Project Description:

The proposed research will determine how farm management practices impact key microbial populations–pathogens and mutualists through two main steps: the development of the HT-qPCR SmartChip and an examination of microbial abundance in soils from both diversified organic vegetable systems in western Washington as well as the Cook Farm LTAR where extensive metadata on management and crop performance/yield have been collected over two decades.

We focus on pathogenic fungi and fungal organisms *Phytophthora, Pythium, Rhizoctonia, Verticillium,* and *Fusarium* as well as the protist *Plasmodiophora brassicae* (causal organism of clubroot, a widespread, soilborne brassica disease in western Washington). We also include primers specific for the 18S region of arbuscular mycorrhizal fungi. Primers will be tested on pure fungal cultures and then soil samples.

Outputs:

Overview of Work Completed and in Progress:

- Pathogen and AMF primer sets obtained.
- DNA extracted from cultures and soils. In addition to Cook Farm, three farms in western Washington were sampled in consultation with each farmer to design a small on-farm experiment investigating pathogens of interest.
- Primers validated against pure fungal cultures.
- Clubroot primers validated against infected plant tissue.
- SmartChip runs designed – have not been able to complete due to machine being out of service, but all materials are on hand.

Table 1. Primer pairs for soil health assessment with HT-qPCR system.

Primer pair	Function	Target
F515/R907	Bacterial community	16S rRNA V4-V5 region
1380F/1510R	Fungal community	18S V9 region
ULT1F/ULT4R	Pathogen detection	*Pythium ultimum*
IIV7F/ABA1R	Pathogen detection	*Pythium irregulare group IV*
ABA1bF/ABA1R	Pathogen detection	*Pythium abappressorium*
PROSF2/PROSR2	Pathogen detection	*Pythium rostratifingens*
FCKY648945F/FCKY648945R	Pathogen detection	*Fusarium culmorum*
FPKY927890F/FPKY927890R	Pathogen detection	*Fusarium pseudograminearum*
RoGr3F/RoGr3R	Pathogen detection	*Rhizoctonia oryzae geno III*
Rs8F/Rs8R	Pathogen detection	*Rhizoctonia solani AG-8*
AG2-1F/AG2-1R	Pathogen detection	*Rhizoctonia solani AG2-1*
PbF/PbR	Pathogen detection	*Plasmodiophora brassicae*
AMG1F/AM1	AMF detection	AMF community

Table 2. Pathogen DNA samples for primer pair validation with HT-qPCR system.

Sample Name	Primer Pair
Pythium ultimum	ULT1F/ULT4R
Pythium irregulare group IV	IIV7F/ABA1R
Pythium abappressorium	ABA1bF/ABA1R
Pythium rostratifingens	PROSF2/PROSR2
Fusarium culmorum	FCKY648945F/FCKY648945R
Fusarium pseudograminearum	FPKY927890F/FPKY927890R
Rhizoctonia oryzae geno III	RoGr3F/RoGr3R
Rhizoctonia solani AG-8	Rs8F/Rs8R
Rhizoctonia solani AG2-1	AG2-1F/AG2-1R
Plasmodiophora brassicae	PbF/PbR

Methods, Results, and Discussion:

- Samples were collected from fields with different disease histories from three farms in western Washington.
- DNA from pure cultures and from soils was extracted using the Kingfisher Powersoil kit.
- PCR was conducted using the primers listed above. A representative gel image showing success of the primers targeting clubroot *Plasmodiophora brassicae* alongside primers targeting multiple fungal pathogens is shown in Figure 1.

- SmartChip materials were purchased and we are working with the WSU Genomics Core to get the SmartChip machine serviced in order to complete our planned runs.
- Once we have completed our planned SmartChip runs, we will share the results with each farmer that we collected samples from.

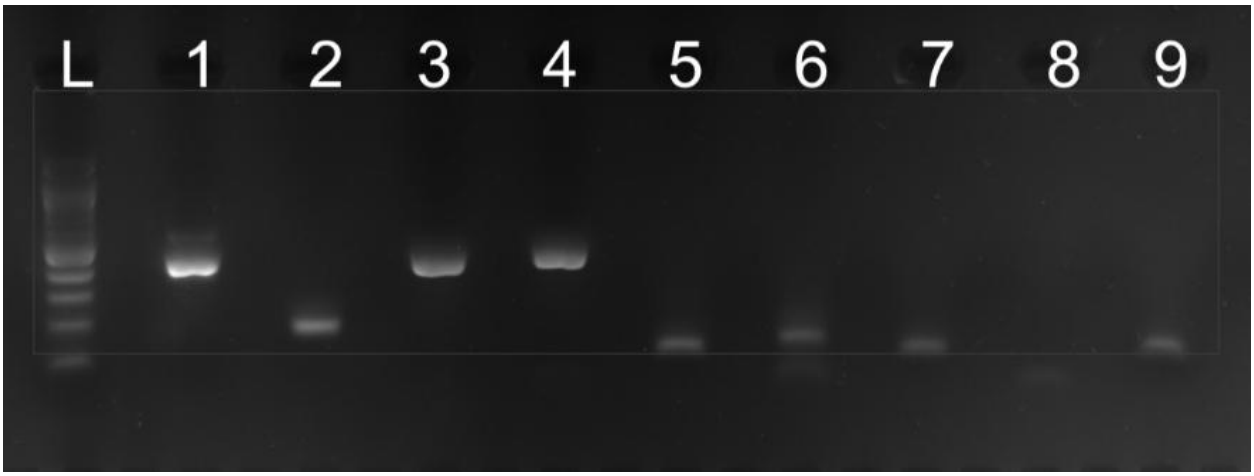

Figure 1. PCR testing of primers. 1: General Bacterial, F515/R907; 2: General Fungal, 1380F/1510R; 3: *Pythium ultimum*, ULT1F/ULT4R; 4: *R. solani* AG2-1, AG2-1F/AG2-1R; 5: *P. rostratifingens,* PROSF2/PROSR2; 6: *Fusarium culmorum*, FCKY648945 F/FCKY648945 R; 7: *P. brassicae*, tissue, PbF/PbR; 8: *P. brassicae*, soil, PbF/PbR; 9: *P. brassicae*, tissue, PbF/PbR.

Publications, Handouts, Other Text & Web Products:
Nothing to report.

Outreach & Education Activities:
Poster titled " A SmartChip for Soil Health" at Regenerative Ag Symposium, Sept 26, 2024, WSU Pullman

Impacts:

- **Short-term:** The proposed research will provide information on optimized high-throughput low-cost molecular protocols for quantifying the abundance of multiple soilborne pathogens and a common mutualist (AMF).
- **Intermediate-term:** The overarching goal of the work is to provide producers with quantitative and easily incorporated information on how management practices impact beneficial and pathogenic microbes. Cost-effective measurements of soilborne pathogens and mutualists will become routine for producers and guide the adoption of management approaches that enhance soil health. In particular, we anticipate that rotational or cover crops will influence populations of key microbes, as well as tillage and chemical inputs; our new tool will enable rapid on-farm research into which management changes impact key microbes so that farmers can make informed decisions.
- **Long-term:** The development of the SmartChip HT-qPCR method focused on two important types of farming in Washington will be conducted with the long-term goal of deploying this technology statewide and ultimately nationally. Adoption of more microbe-friendly farming practices would create a positive environmental impact by stimulating management that is supportive of soil health.

Additional funding applied for/secured:

WOC project to screen for soilborne pathogens and AMF in dryland peaola cropping systems (2025-2027)

Graduate students funded:

N/A

Recommendations for future research:

The SmartChip could be a powerful tool for high-throughput pathogen screening to aid in both research and production. In order to be most beneficial for Washington farmers, region- and crop-specific panels of pathogen primers can be developed. Future research focusing on management and epidemiology of specific pathogens can use this technology as a quantitative tool. Given the flexible nature of this platform, any organism of interest for which there is genetic information could be targeted for molecular quantification.