PROJECT NO: BJKU82, BJKU83

TITLE: Developing Methods for an Early Warning Detection System of Foliar Pathogens of Wheat

PERSONNEL: James Woodhall and Juliet Marshall

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JUSTIFICATION: Foliar pathogens of wheat can cause severe yield losses if not managed correctly and if the conditions are optimum for disease. Presently such foliar pathogens are often treated with little or no knowledge of the local optimum conditions for disease development or if the spores are already present in the area at the required threshold for disease development. The widespread and increasingly frequent occurrence of stripe rust has forced growers to preventively apply fungicides on susceptible varieties, even without data on presence of the pathogen. The wide spread occurrence of Fusarium Head Blight (FHB) in wheat in southern and eastern Idaho likely results from airborne spores of *Fusarium graminearum* derived from localized corn residue throughout the area. Powdery mildew occurs at damaging levels in wheat crops when planted at exceedingly high rates. Recent advances in molecular detection technology, such as real-time Polymerase Chain Reaction (PCR), can allow the rapid detection and quantification of spores in samples to species and sometimes even to strain level (Woodhall & Wharton, 2016). This detection technology coupled with the latest air sampling devices could provide a powerful tool to provide early warning of when spore populations are at critical thresholds in each vicinity and inform growers of when to take preventative measures, thereby reducing the need for unnecessary treatments.

There have been some recent advances in spore sampling technology. The University of Idaho has already invested in four state-of-the-art Burkard Automatic Multi-Vial Cyclone samplers and with support from other commodity groups and the University of Idaho, another 12 spore samplers will be place for April 2018. These spore samplers possess a degree of automation in that they can automatically sample at eight different time points and produce a sample, which will be immediately suitable for rapid DNA extraction and subsequent PCR in the laboratory. In 2017, this project developed much of the technology required to operate these spore samplers to detect wheat diseases. Firstly, a robust high-throughput DNA extraction was developed for spore samples enabling a rapid turnaround of samples to results (within 24 hours). Also, robust qPCR (TaqMan) assays and standards were developed for key wheat pathogens including *Puccinia striiformis* f.sp. *tritici*, *F. graminearum* and *Blumeria graminis* f. sp. *tritici*. The project operated four spore samplers from May to September at three R&E Centers in Idaho in 2017 and planted 'sentinel' plots consisting of treated and untreated plots adjacent to the spore sampler at Parma and Aberdeen. At Parma, plant samples were tested for *Puccinia striiformis* f.sp. *tritici* and showed many of the plants were latently infected with the pathogen, with little or no spores of *Puccinia* detected between May to September suggesting infection occurs much earlier in the season for winter wheat – this is concordant with recent findings from the UK (F . Ritchie Per. Comm). The other diseases were not observed at Parma. At Aberdeen, *Fusarium* was observed but the analysis for this is still underway. A low percentage of stripe rust was observed in UI Pettit (19% of leaf area affected where no fungicide was applied versus no stripe rust in fungicide treated plots) and in IDO851(5% of leaf area affected where no fungicide was

applied versus no stripe rust in fungicide treated plots). Despite disease conditions not being ideal in 2017 to show the predictive power for wheat diseases, promising results were obtained for sugar beet powdery mildew using the same spore sampler at Parma – where a definite spike in mildew spore levels was detected two to three weeks prior to disease observations.

In this year of the project we aim to continue sampling for plant diseases using the Burkard sampler. Initially we will use spore samplers at four R&E Centers (Parma, Aberdeen, Kimberley and Tetonia) but have the potential to use additional sites from the wider network of 12 samplers should we see disease present in those areas. We will also seek to integrate better weather data into the project using more sophisticated weather stations than currently available. Finally, we will test a greater array of plant material from the sentinel plots at more intervals to gain an understanding on the role of latent infection for stripe rust. The project will ultimately produce data on long term monitoring of airborne spore populations linked to the local environmental conditions which can provide us vital insights into the epidemiology of the foliar pathogens.

HYPOTHESIS & OBJECTIVES: Using an automated spore sampling in conjunction with real-time PCR detection permits early warning of foliar diseases allowing early and / or preventative treatment. Specific goals are:

1. Operate spore samplers at four R&E Centers in Idaho (Parma, Aberdeen, Kimberley and Tetonia).
2. Establish sentinel treated and untreated plots at Parma and Aberdeen.
3. Data analysis – correlate spore populations with disease incidences and weather data
4. Investigate importance of latent infection for stripe rust

PROCEDURES:

1. An in-depth spore detection study will be established at Parma to determine required replication and sampling frequency. This will involve establishing a field trial with multiple plots with three treatments: conventionally treated with fungicides, untreated, and untreated with elevated humidity through misting. In addition, two varieties of winter wheat will be evaluated with differing susceptibilities to foliar pathogens (SY Ovation and Brundage) and one variety of spring wheat. The spore sampler will be placed ahead of the field plots relative to the prevailing wind. This will run throughout the season. Plots will be monitored for disease and correlated with detection in the spore sampler. qPCR will be used on plant material such as petals or anthers to verify visual disease and determine the prevalence and timing of asymptomatic infection.
2. The Parma experiment will be used to investigate latent infection with these foliar pathogens. Plant material will be collected each month and tested for the various foliar diseases to determine relative disease levels in asymptomatic tissue over time.
3. Establish sentinel plots at Aberdeen. Collect spores at regular intervals and determine relative levels of spores for key pathogens. Plots will be scouted for disease regularly in the sentinel plots and correlated with spore detection. At the other R&E centers where spore samplers are situated as well as the 12 sites in the wider network, wheat fields near to the spore sampler will be periodically scouted and tested for wheat diseases retrospectively should a key disease be present.

4. Correlation of spore populations with disease symptoms observed in the sentinel plots and local weather data. At the end of the season the data will be analyzed to determine relationships between spore populations, disease observations and weather conditions.

DURATION: It is expected that this will be the second and final year of the project. If the technology demonstrates usefulness in early disease warning, it may be appropriate to investigate subsequent projects delivering spore sample data to industry.

COOPERATION: The project leader will be James Woodhall based at Parma where the main field experiment exploring sampling frequency and replication will be based along with molecular assay development. Juliet Marshall will provide input into in field disease assessments, data analysis and interpretation of results along with managing a sentinel plot in Aberdeen. Funding is being sought from other commodity groups (potatoes, barley and sugar beet) for studying spore populations in those crops. Therefore, considerable efficiency savings in spore collection and DNA extraction will be envisaged if all are funded. Additional U of I plant pathologists (Phill Wharton and Kasia Kinzer) are involved in this wider spore detection initiative across all crops.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER: This project will position the region for rapid deployment of advanced spore sampling equipment when it becomes commercially available. The industry will benefit from early warnings on the presence of these foliar pathogens prior to symptoms development. Insights into the epidemiology of foliar pathogens in the region will also be gained. Such an early warning system will provide growers with additional resources enabling them to make rapid management decisions to prevent spread of the disease. It is hoped a website displaying current detection events and risk levels will be developed. This will build upon the PNW Pest Alert system but will be focused on risk prediction rather than reporting disease when symptoms first occur.

LITERATURE REVIEW: Aerial dispersal of spores is one of many mechanisms by which plant pathogens can spread to reach new hosts either within the same field or even in a completely different continent (Aylor, 2003, West & Kimber, 2015). Although Gregory (1973) predicted that most spores of plant pathogens do not disperse beyond the field in which they were produced, longer distance dispersal is also of great significance in allowing spores to reach new locations. It is possible for pathogens to travel to new continents, surviving environmental extremes and still remain viable to cause disease (Morris *et al.*, 2013). Rust spores in particular have been documented surviving intercontinental dispersal (Schneider, *et al.*, 2005). For wheat, airborne pathogens of *Puccinia striiformis* f.sp. *tritici* and *Fusarium graminearum* are capable of long-distance dispersal (Schmale, *et al,* 2012, Hovmøller, *et al*, 2002). The wide spread occurrence of FHB in malt barley in eastern Idaho likely resulted from airborne spores of *Fusarium graminearum* derived from localized corn residue in western Jefferson county. With the increasing acreage of corn throughout southern Idaho, localized dispersal of *Fusarium* spores occurs in the same areas where the highest concentration of wheat acres are produced, putting wheat at increasing vulnerability.

Airborne particulates can be sampled in several ways, from impaction of particles onto adhesive surfaces, such as a petroleum-jelly coated microscope slide or thin glass rod, to automated samplers which use miniature cyclone technology. Several advantages exist with the

cyclone based samplers including limited need for downstream processing prior to PCR (West and Kimber, 2015) and superior sensitivity when combined with PCR detection methods. For example, Pashley *et al.* (2012) used a cyclone to sample air that and found that over 86% of genera detected were not routinely identifiable by microscopy.

The use of cyclone sampling technology has already been use with immunological assays for the detection of Brassica disease in the Brassica Alert network of spore traps that provide a direct, inoculum-based warning of airborne spores (Syngenta Brassica Alert in the UK). They have also been used to provide warnings of *Peronospora destructor* (onion downy mildew) (Kennedy & Wakeham, 2008). The use of molecular technology combined with innovations in automation will enable more applications of disease forecasting through airborne detection with much greater adoption by growers or industry support workers to aid in crop protection decisions. Presently a prototype device integrating cyclone spore sampling, DNA extraction, molecular detection and subsequent SMS alerts has been developed with efforts now underway to incorporate molecular tests for fungicide resistance within the device (Boonham et al., 2016). In the UK, this device has could detect the presence of stripe rust four weeks prior to symptoms development (Allison, 2016), whilst other spore samples could detect it 15 days prior to symptom development (Dedeurwaerder *et al.*, 2011). Such a device when combined with robust knowledge on pathogen epidemiology and sampling protocols will allow for ever greater precision in crop disease management.

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IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: James Woodhall

Allocated by	Idaho Wheat Commission	during FY 2017	$ -
Allocated by	Idaho Wheat Commission	during FY 2018	$ 20,377

REQUESTED FY2019 SUPPORT:

	Salary (*staff, post-docs, etc.*)	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 5,000	$ 385	$ 600	$ 3,600	$ -	$ 9,585

TOTAL BUDGET REQUEST FOR FY 2019: $ 9,585

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Woodhall	*Marshall*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ 3,000	$ 2,000	$ -	$ -
Fringe Benefits	$ 231	$ 154	$ -	$ -
Travel	$ 300	$ 300	$ -	$ -
Operating Expenses	$ 2,800	$ 800	$ -	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 6,331	$ 3,254	$ -	$ -

Total Sub-budgets $ 9,585

Explanatory Comments: (see FY2019 RFP for definition)

Fall 2017 Version

ANNUAL REPORT

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ACCOMPLISHMENTS:

Progress for each objective is as follows:

1. Develop and validate multiplex assays for the key foliar pathogens of wheat

A robust, automated high throughput DNA extraction method for spore material was developed based from adapting a Wizard Food DNA extraction method (Promega) for use with a Kingfisher ML magnetic particle processor. A new DNA extraction buffer was developed to mitigate for the presence of inhibitors in spore samples.

TaqMan PCR assays with specificity to *Puccinia striiformis* f.sp. *tritici*, *F. graminearum* and *Blumeria graminis* f. sp. *tritici* were developed and designed to be suitable for use in a multiplex qPCR. These assays were validated in the lab at Parma to ensure good specificity and sensitivity.

2. Establish an in-depth spore detection study at Parma to determine sampling strategy plus the effect of environment and variety on disease development.

A spore sampler was run from May to September at Parma adjacent to a field trial where three varieties of wheat were planted. Stripe rust was observed in this field from June. No other diseases were observed. Regular disease assessments were undertaken as well as plant sampling and testing of leaves for stipe rust to investigate latent infection. An experiment with replicate spore samplers to inform sampling strategy is planned for early Spring 2018. The spore samplers will be deployed in late January again to investigate stripe rust spores in the air in winter and spring. In addition, plant samples will be taken and tested for the presence of stripe rust to investigate the timing of latent infection.

3. Establish sentinel plots at Parma, Aberdeen and an additional site yet to be confirmed in Idaho. Collect spores at regular intervals and determine relative levels for key pathogens.

Spore samplers were set up at Parma, Kimberly and Aberdeen. Analysis is still underway but little to no stripe rust or Fusarium was detected in the Parma spore sampler between late May and September. However, qPCR on plant material showed many plants were latently infected with *Puccinia striiformis* f.sp. *tritici*. This suggests that much of the stripe rust infection is early season and similar findings have been found in the UK (F . Ritchie Per. Comm).

At Aberdeen, *Fusarium* was observed but the spore sample PCR analysis for this is still underway. A low percentage of stripe rust was observed in UI Pettit (19% of leaf area affected where no fungicide was applied versus no stripe rust in fungicide treated plots) and in IDO851(5% of leaf area affected where no fungicide was applied versus no stripe rust in fungicide treated plots). Despite disease conditions not being ideal in 2017 to show the predictive power for wheat diseases, promising results were obtained for sugar beet powdery mildew using the same spore sampler at Parma – where a definite spike in mildew spore levels was detected two to three weeks prior to disease observations.

4. Data analysis - correlation of spore populations with diseases observed in the sentinel plots and local weather data.

This analysis is dependent upon spore sample qPCR data and is consequently still underway.

PROJECTIONS:

In 2018, we aim to continue sampling for plant diseases using the Burkard sampler. Initially we will use spore samplers at four R&E Centers (Parma, Aberdeen, Kimberley and Tetonia) but have the potential to use additional sites from a wider network of 12 more samplers should we see disease present in those areas. We will also seek to integrate better weather data into the project using more sophisticated weather stations than currently available. Finally, we will test a greater array of plant material from the sentinel plots at more intervals to gain an understanding on the role of latent infection for stripe rust. The project will ultimately produce data on long term monitoring of airborne spore populations linked to the local environmental conditions which can provide us vital insights into the epidemiology of the foliar pathogens.

PUBLICATIONS: None as yet.