PROJECT NO: New

TITLE: Developing methods for an early warning detection system of foliar pathogens of wheat

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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 intends to buy another two. These 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. These Burkard devices were used to validate prototypes of a new integrated unit which combines spore sampling, DNA extraction, nucleic acid detection and alerts via text messaging all into one field deployable unit (Boonham *et al.*, 2016). This device will be made available in two to three years at a likely cost of $25k.

However, despite such advanced spore sampling and testing equipment, several fundamental questions remain about the usefulness of this technology, including the replication required, sampling frequency, influence of environmental conditions, representativeness of each particular spore sampler (field, farm or even county) and the thresholds required for disease development in PNW conditions.

This projects seek to answer these questions and allow the region to be prepared to be an early adopter of such advanced integrated spore sampling units when they become available. The project will also 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. Develop and validate multiplex assays for the key foliar pathogens of wheat
2. Establish an in-depth spore detection study at Parma to determine sampling strategy plus the effect of environment and variety on disease development.
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.
4. Data analysis - correlation of spore populations with diseases observed in the sentinel plots and local weather data.

PROCEDURES:

1. Create multiplex assays for the key foliar pathogens (*Puccinia striiformis* f.sp. *tritici*, *F. graminearum* and *Blumeria graminis* f. sp. *tritici*) to allow cost effective real-time PCR detection. With newly acquired PCR and DNA extraction equipment at Parma and Aberdeen, we can create multiplex assays to test for multiple pathogens simultaneously. This will be coupled with automated, rapid DNA extraction methods to facilitate cost effective testing and rapid dissemination of results through a dedicated internet site and/or twitter feed. Multiplexing these assays requires laboratory validation to ensure the multiplexing does not reduce sensitivity or specificity. Limits of detection, repeatability, assay efficiency and optimum DNA extraction methodology will all be determined.
2. 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 wheat will be evaluated with differing susceptibilities to foliar pathogens (SY Ovation and Brundage). Three spore samplers 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.
3. Establish sentinel plots at Aberdeen and a site yet to be confirmed in central or eastern Idaho with one spore sampler in each. 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.
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 estimated that the project will require funding for two years and this initial year's costs are covering set up and validation. Once the procedures are established, future costs will be less. Long term it is envisaged this technology will enable a pro-active disease alert system which could be funded by industry either directly or through sponsorship.

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

Allocated by	Idaho Wheat Commission	during FY 2016	$	-
Allocated by	Idaho Wheat Commission	during FY 2017	$	-

REQUESTED FY2018 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 9,000	$ 3,677	$ 800	$ 6,900	$ -	$ 20,377

TOTAL BUDGET REQUEST FOR FY 2018: $ 20,377

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Woodhall	*Marshall*	*(PI name)*	*(PI name)*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ 6,000	$ 3,000	$ -	$ -
Fringe Benefits	$ 2,450	$ 1,227	$ -	$ -
Travel	$ 500	$ 300	$ -	$ -
Operating Expenses	$ 4,600	$ 2,300	$ -	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 13,550	$ 6,827	$ -	$ -
			Total Sub-budgets	$ 20,377

Explanatory Comments: (see FY2018 Guidelines for definition)

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