

PROJECT NO: AN4567

TITLE: Fungal and oomycete soil-borne diseases of cereals in Idaho: causal agents, relative importance and disease management tools.

PERSONNEL: James Woodhall, Kurtis Schroeder and Juliet Marshall

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JUSTIFICATION: Throughout the PNW, rotations are limited and concentrated in cereal production, especially under dryland and rainfed production conditions. Several soil-borne diseases constrain yield including various strains of *Rhizoctonia*, *Pythium* species, the eyespot species: *Oculimacula yallundae* and *O. acuformis* and the take-all pathogen: *Gaeumannomyces graminis* var. *tritici*. Presently there is little to no resistance germplasm available for *Rhizoctonia* and *Pythium* species and we have no knowledge of relative incidence of the major pathogen groups present in Idaho. *Rhizoctonia* is a particular challenge as it exists as a species complex of approximately 40 strains called anastomosis groups (AGs), which are typically further divided into 30 subgroups. These AGs and possibly subgroups can vary widely in pathogenicity, plant part infected, optimum growth temperature and even fungicide sensitivity. Typically, an AG associated with a particular host like AG3 with potatoes or AG2-2 with sugar beet. Knowledge of the relative importance of the individual pathogen species/strains will be invaluable in developing effective disease management strategies.

The main aim of this project is to determine the soil-borne disease-causing agents in cereal production throughout Idaho. Since cropping practices and climate can radically differ over the state, this approach will enable us to gain a unique understanding of the various factors influencing the relative incidence and impact of the pathogen species present. Work so far in the project has focused on surveying fields throughout Idaho to determine which soil-borne disease are present. This was done by sampling soil pre-planting and re-visiting the field and determine which diseases were present and isolating. Over the 2018-2020 sampling period of the project, 115 fields were visited, and 1,612 fungal isolates collected. Work has focused on characterizing *Rhizoctonia* isolates in the first instance and from 176 isolates, the following AGs were found: 2-1, 2-2, 3, 4 HG-II, 5, 11, C,D, H and K. Within AG11, new subgroups were found during this project which need to be further characterized. Several of these are first reports for the region. Other fungi frequently isolated included *Gaeumannomyces graminis* var. *tritici*, *Microdochium nivale*, *Microdochium bolleyi* and *Pythium ultimum*. Through other sources of funding, we have collected *Rhizoctonia* isolates from potato, onion, sugar beet and dry bean. This has enabled us to build up a large isolate collection over several crops and conduct pathogenicity testing to determine host range of the various strains. This project will gain insights into soil-borne disease across rotations and an understanding of the mechanism of pathogenicity of individual strains between different hosts. We are also investigating the use of molecular tools for determining the DNA levels of the various pathogens in bulk soil samples pre-planting to see if it can be used in decision support systems for determining disease risk. In Australia, this is routinely done in cereal production for take-all, cereal cyst nematode and *Rhizoctonia solani* AG 8 through the service called PredictaB (Ophel-Keller et al., 2008). We currently are developing new PCR assays for *R. solani* AG11 subgroups and *G. graminis* var. *tritici*. Previously published tests for *G. graminis* appear to be poorly designed. These new assays will be used, along with other PCR assays developed in the

project, on the soil samples collected between 2018 to 2020 to evaluate their suitability as part of a predictive soil test for soil-borne disease.

HYPOTHESIS & OBJECTIVES: The hypothesis is that soil-borne diseases are causing a yield constraint on cereal production in Idaho. The project will investigate the casual agents, population dynamics in soil, disease development *in planta*, fungicide efficacy and develop predictive diagnostic methods for determining risk prior to planting. These are broken down into the following objectives for the final year of the project: 1. Gain an understanding on the presence of *G. graminis* var. *tritici* in Idaho cereal fields and develop a predictive risk test for this organism as well as for individual *Rhizoctonia* AGs. 2. Complete characterization of *Rhizoctonia* isolates. 3. Write extension bulletins for *Rhizoctonia* cereal disease and take-all. 4. Preliminary research into understanding the host-pathogen/soil health aspect at the molecular level for *Rhizoctonia*.

PROCEDURES:

1. Develop a *G. graminis* var. *tritici* TaqMan qPCR assay and test previously collected pre-plant soil samples, along with assays for specific *Rhizoctonia* AGs. DNA will be extracted from bulk soil samples (250 g) and we will determine if visual root disease assessment correlates with pre-planting inoculum DNA.
2. Isolates of *Rhizoctonia* will be sequenced and characterized for pathogenicity and fungicide EC₅₀. Pathogenicity testing will be done in the greenhouse. We will also investigate the effect of variety.
3. The latest data from these research project will be incorporated into the extension bulletins which will be aimed at a grower audience.
4. RNAseq experiments will be done on wheat plants challenged with pathogens in the greenhouse. This will determine what genes are expressed when roots are challenged with a particular pathogen. We have secured funding to use this methodology to investigate pink root in onions, so we can gain efficiencies in method development. A literature review will also be conducted on this topic by the graduate student.

DURATION: 4 years (this is the final year).

COOPERATION: The project leader (James Woodhall) and graduate student (Lara Brown), are based at Parma where the isolate characterization, qPCR testing and glasshouse experiments will take place. Kurtis Schroeder and Juliet Marshall will also be on the student's committee and have assisted with the sampling effort in previous years. They will assist with editing publication outputs in this final year of the project.

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION TRANSFER:

The project is expected to inform us of the key soil-borne pathogens in Idaho cereal production systems. Knowledge of the key pathogens present will enable adequate disease management measures to be developed targeting these pathogens. In addition, qPCR assays and a decision support system for managing soil-borne diseases will be developed.

LITERATURE REVIEW: Soil-borne diseases are capable of causing significant losses. Previous research in eastern Idaho documented the occurrence of several soil-borne pathogens, including *Fusarium* dryland foot rot, cereal cyst nematodes (Smiley, 2009), and *Bipolaris* crown

rot (*B. sorokiniana*) (Strausbaugh and Koehn, 2004). While *Pythium* and *Rhizoctonia* occur, their distribution and frequency of occurrence is not well documented in southern Idaho but have been more thoroughly investigated in the Palouse region (Schroeder et al., 2013;).

For take-all, total crop loss is possible, with losses of 20% can occur even with few visible symptoms. Severe eyespot infection can reduce yields by up to 15% (Ray et al., 2006). In the UK over five seasons, *Rhizoctonia* infection resulted in losses of up to 26% with severe infections (Clarkson and Cook, 1983), and 14% yield losses were observed in New Zealand (Cromey et al., 2002). In China, sharp eyespot is regarded as serious threat to wheat production with losses estimated at over one billion Yuan from 2005 to 2008 (Chen et al., 2008). In Australia, yield losses of 25% or more have been observed (MacNish and Neate, 1996). In Australia, *R. solani* AG-8 is one of the main soil-borne diseases in wheat. This pathogen, as well as take-all and cereal cyst nematode is tested routinely in Australian cereal fields as part of a pre-planting soil testing service based on PCR detection called Predicta B (Ophel-Keller et al., 2008).

The complexity of *Rhizoctonia* presents an additional problem, since it is broken down into multiple strains called Anastomosis Groups (AGs), many of which can have vastly different impacts on the plant. Essentially, *Rhizoctonia* strains are divided into binucleate *Rhizoctonia* (BNR) designated AG-A to AG-U and the multinucleate *Rhizoctonia solani* designated AG1 to AG13. Multiple subgroups can exist within the AGs and typically a subgroup is associated with a particular crop. For cereals, numerous AGs of *Rhizoctonia* have been implicated in disease; the binucleate *Rhizoctonia cerealis* (AG-D) which causes the sharp eyespot symptom on stem bases is arguably one of the most important *Rhizoctonia* species affecting stems, although strains such as AG-5 (Woodhall et al., 2012) and AG-4 have also been found. Interestingly in 2017, a *Rhizoctonia solani* AG-4 isolate was recovered from wheat showing eyespot symptoms in southwest Idaho which resulted in considerable yield losses from early maturity and whiteheads (Parma Diagnostic Lab, 2017). Some *Rhizoctonia* strains such as AG-8 and AG-2-1 can cause root rots. However, there is little data for *Rhizoctonia* in southern Idaho. A previous survey only considered northern Idaho (Ogoshi et al., 1990) where they found AG-8 and AG-4 were present.

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FY2022

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: James Woodhall

If applicable, Allocated by Idaho Wheat Commission during FY 2020 \$ 18,654

If applicable, Allocated by Idaho Wheat Commission during FY 2021 \$ 23,000

REQUESTED FY2022 SUPPORT:

Budget Categories	(10) Salaries (staff, post-docs, etc.)	(12) Temp Help	(11) Fringe	(20) Travel	(30) OE	(70) Graduate Tuition/ Fees	TOTALS
Idaho Wheat Commission	\$ 4,200	\$ -	\$ 88	\$ -	\$ 2,242	\$ 2,470	\$ 9,000

TOTAL BUDGET REQUEST FOR FY 2022:

\$ 9,000

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

Budget Categories	(Insert PI Name)	(Insert CO-PI Name)	(Insert CO-PI Name)	(Insert CO-PI Name)
(10) Salaries	\$ -	\$ -	\$ -	\$ -
(12) Temp Help	\$ -	\$ -	\$ -	\$ -
(11) Fringe Benefits	\$ -	\$ -	\$ -	\$ -
(20) Travel	\$ -	\$ -	\$ -	\$ -
(30) Other Expenses	\$ -	\$ -	\$ -	\$ -
(70) Graduate Student Tuition/Fees	\$ -	\$ -	\$ -	\$ -
TOTALS	\$ -	\$ -	\$ -	\$ -

Total Sub-budgets \$ -

Brief Explanatory Comments: (see FY2022 RFP for guidance)

FY2022 Version

ANNUAL REPORT

Grant Code: AN4567

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Accomplishments

This project has three key objectives: 1. Determine the prevalence of the individual types of soil borne diseases in cereal cropping systems in Idaho, 2. Determine relative importance and inoculum thresholds for individual pathogens and 3. Providing disease management and risk prediction tools for growers. Work in the first three years of the project has focused on the first objective and developing tools to underpin objectives 2 and 3.

Between 2018 and 2020 we have sampled 115 cereal fields throughout the whole of Idaho. Fields were sampled for both soil (pre-planting) and plant material in spring. Each plant collected was assessed for disease severity. DNA was extracted from soil pre-planting and is currently being tested with a range of qPCR assays for various *Rhizoctonia* AGs that have either been developed or validated within the project. This will enable us to determine the relationship between soil inoculum level and disease, which will hopefully underpin a predictive testing and decision support system.

From the plant material collected from these 115 fields, we obtained 1,612 fungal isolates. Based on cultural morphology, 300 isolates were selected for sequencing and 176 isolates were determined to be *Rhizoctonia*. The following AGs of *Rhizoctonia* were found: 2-1, 2-2, 3, 4 HG-II, 5, 11, C,D, H and K. Within AG11, new subgroups were found during this project which need to be further characterized. Many of these findings are first host records for the USA or at least Idaho. The isolation work has initially focused on *Rhizoctonia* in the first instance due to the large amount of isolates taken and due to the complexity of the *Rhizoctonia* fungus – as it exists as multiple anastomosis groups (AGs). These AGs are known to have different pathogenicity, host range, optimum growth temperatures and sensitivities to fungicides. They also have different abilities to produce sclerotia – which is the hardened form of the fungus for longer term survival. AG3 appeared to be the most prolific sclerotia producer. Other species found of importance in the survey were *Gaeumannomyces graminis* var. *tritici*, *Pythium ultimum*, *Microdochium bolleyi* and *M. nivale*.

We have commenced doing fungicide EC₅₀ testing with seven fungicides including Quadris, Proline and Flutolanil. We are also characterising the isolates in terms of pathogenicity. Two green house experiments are under way evaluating the aggressiveness of 60 *Rhizoctonia* isolates to Idaho cereal varieties. Inoculum is currently being prepared for an experiment to investigate the effect of variety on *Rhizoctonia* disease development.

From the soil collected from 115 fields, DNA has been extracted from 80 samples. Preliminary work on soil diagnostic has shown that the published assay for *Gaeumannomyces graminis* var. *tritici* does not appear to produce consistent results and therefore a new assay will be designed in this project. We also aim to design AG 11 subgroup specific qPCR assays.

Projections

Work is presently underway to complete the pathogenicity testing, fungal characterisation and soil testing for *Rhizoctonia* species with presently available TaqMan assays. We aim to publish this work as a paper in Plant Disease and we plan to submit this by May 2021. This work will be a chapter in the graduate student thesis who is on track to complete their MS this academic year with a view to moving to a PhD also at UI.

We also aim to complete glasshouse studies on the effect of variety and also the Fungicide EC₅₀ work by summer 2021.

Soil testing for *Gaeumannomyces graminis* var. *tritici*, *Pythium ultimum*, *Microdochium* spp., and with the new AG 11 subgroup primers will take place in the final year of the project (2021/22). This work will be used to develop a decision support system for predictive risk of soil-borne diseases.

We will also investigate the diversity with AG11 using multi-locus sequence analysis as well as pathogenicity testing to determine if it can influence host range.

The findings of this project will be written up in an extension bulletin aimed at growers and crop consultants. We aim to complete this by May 2022.

Publications

Pizolotto CA, Brown L, Harrington S, Murdock MR, Harrington M, Woodhall JW, Moll M, Marshall JM (2020). First Report of *Rhizoctonia solani* AG4 HG-II infecting barley stems in Idaho. *Plant Disease* 11, 3058.

Brown M, Jayaweera D, Hunt A, Woodhall JW, Ray RV (accepted). Yield losses and control by sedaxane and fludioxonil of soil-borne *Rhizoctonia*, *Microdochium* and *Fusarium* species in winter wheat. *Plant Disease*.