Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports

(Begin 1 page limit)

Project #:3061-7667

Progress Report Year: *_3___of _3___(maximum of 3 year funding cycle)*

Title: **Management of Nematode Diseases with Genetic Resistance**

Scot Hulbert, Kimberly Garland Campbell and Timothy Paulitz

Executive summary:

- To determine the distribution of cereal cyst nematode (CCN) in eastern Washington and the Palouse, we surveyed over 300 fields from 2013-2017. Cysts were identified to species level with DNA techniques developed in previous grants. *H. filipjevi* was only found in southern Whitman County, and *H. avenae* primarily in eastern Whitman County. We also identified fields with high inoculum levels for use in greenhouse testing. A paper has been submitted for publication
- We developed a high throughput greenhouse screening method to identify CCN resistance in wheat. This method assesses roots of young plants grown in cone-tainers containing soil collected from highly infested fields in fall and vernalized at 4 C prior to planting.
- We completed resistance testing of 1209 wheat lines from the programs of Carter, Pumphrey, Campbell, and Morris, regional nurseries, and a Campbell mapping population, all in the greenhouse using above method.
- From above screening, we identified resistance in 10 to 21% of the advanced winter wheat lines, but less than 2% of spring wheat lines.
- We established greenhouse pot cultures of *H. avenae* and *H. filipjevi.* These cultures will be grown in the greenhouse to increase nematode populations and then used for screening
- We developed KASP markers for QTLs for resistance to *H. filipjevi* that were identified in a CIMMYT study and assayed the breeding lines that we evaluated above.
- We tested SSR markers linked to *Cre1, Cre3, Cre5, Cre8, CreX*, and *CreY* genes. These markers may facilitate the understanding of the resistance background of our material.
- We imported differential lines for identification of CCN pathotypes from Turkey, increased the seeds in the greenhouse and did initial experiments, but our pathotypes do not exactly match known ones. .

Impact:

- Using the high throughput greenhouse screening system, we can now screen material at an earlier stage and report results to breeders. We identified many good sources of resistance to cereal cyst nematode *H. filipjevi* and *avenae* in the adapted winter wheat and a few in spring wheat breeding lines and varieties. Planting these varieties will reduce the inoculum levels in infested fields.
- We collected all the data needed to identify markers associated with specific *Cre* genes, which should speed up selection and possibly identify new sources of resistance.

- **What measurable impact(s) has your project had in the most recent funding cycle?**

    - Because of the greenhouse techniques that we developed, breeders can now screen more material and are incorporating *Heterodera* resistance into their selections.

Nematode Grant 2016-2018

3061-7667

Management of Nematode Diseases with Genetic Resistance

S. Hulbert, K. Garland-Campbell, and T. Paulitz

3 year summary and final report.

Over the last three years, we have made significant progress on genetic solutions to the management of cereal cyst nematode (CCN, *Heterodera* spp.) for Washington wheat growers. Until 2010, it was considered primarily a problem in NE Oregon, and Dr. Richard Smiley had been investigating it since the mid 1980s. His work documented the losses caused by this nematode, which causes whiteheads, stunting and malformations of the root system, interfering with the uptake of water and nutrients. However, discoveries in 2010 showed that it has spread to the Palouse area of eastern Washington. Given that the first thing in management is defining the problem (where is the pathogen, how much is in a field?), we initiated a series of surveys to find out where the nematode had spread. Over the life of this grant, we surveyed over 300 locations, and have submitted a paper for publication. However, in 2014, we discovered another species in eastern Washington- *H. filipjevi.* Until this discovery, we had assumed that *H. avenae* was the only species. This necessitated developing new techniques that could distinguish the two species, which is almost impossible to do morphologically since the two species look very similar. We developed a DNA technique by sequencing the ITS region of the ribosomal gene, and could extract DNA from a single cyst isolated from the soil and determine its identity. This was then used in subsequent surveys, which found *H. avenae* in 15-25% of the fields in the Palouse region. At the same time, *H. filipjevi* and *H. avenae* have been found in Montana and Idaho.

How can we manage this disease? Like many soilborne pathogens, we have no registered or economical chemical control methods, unlike with foliar pathogens. In higher value crops, like potatoes, some nematicides are registered, but not with wheat. Seed treatments have also been tested by R. Smiley and chemical companies, but nothing has been effective. Crop rotation can provide a limit on the buildup of inoculum, since the CCN only attacks cereals, but not broadleaf crops like peas or chickpeas. However, because the cysts can survive in the soil for many years, they can survive between cereal crops so the pathogen is not eliminated. This leaves genetic resistance as the only economical management technique. Luckily, a number of major resistance genes have been identified to control this disease, called *Cre* genes. These were deployed in Australia over 30 years ago, when this nematode was a major limit on cereal production. Presently, CCN is now a minor problem in Australia. This is what we hope for the PNW.

How to incorporate resistance to CCN into PNW varieties? This can be done by extensive breeding over a long period. But we hypothesized that there may already be resistance in existing adapted varieties, brought in from their pedigrees. This would be the fastest way to

proceed. But there were many challenges to screen varieties for resistance to nematodes. Unlike fungi, which can be grown in culture in the laboratory, nematodes require living plants to infect and reproduce. Instead, we looked for fields that already had high populations of the nematodes, that could be planted with lines and then assess the reproduction of the females on the root. We identified a site in Colton, WA and used it for several years in the previous grant cycles. However, we were limited to screen only about 100 lines. Because of the natural variability in the field, we needed replicated small plots, and in each 4 row plot, paired the unknown with 2 rows of a susceptible variety for comparison. We also discovered that we could bring in soil in April, as the nematodes were hatching, and plant in containers in the greenhouse to screen lines. But this was a limited time window. However, we discovered that we could collect soil in the fall, vernalize in the cold room at 4 C for a few months, and then warm up the soil to get the nematodes to hatch and infect plants. With this breakthrough, we were able to increase our capacity to screen lines- not only adapted lines from the winter and spring nurseries and variety testing, but earlier material in the 4 breeding programs at WSU- club, winter, spring and durum. This also expanded our ability to look at both *H. filipjevi* and *H. avenae*. Having two species has further complicated breeding efforts, since resistance to one species may not be effective against the other. But for the first time, breeders could select earlier material.

Because we cannot always depend on field sources of inoculum, we are also developing pot cultures in the greenhouse, for both *H. avenae* and *H. filipjevi.* This involves growing wheat in large containers, harvesting the soil, and replanting for multiple cycles to increase the nematode numbers.

Can we identify the resistance genes we are finding in PNW material, to develop genetic markers to eliminate the costly need to phenotype plants in the greenhouse? This was the next logical step that we have started in this funding cycle. If we could identify DNA markers to the *Cre* genes (or possibly new genes in our PNW material), we could use these to quickly screen material. However, little has been published on *H. filipjevi.* But a recent paper was published on *H. filipjevi* based on Turkish (CIMMY) material, and we developed KASP markers to identify these QTLs in our material. We also tested SSR markers for known *Cre* genes. Finally, to look at the pathogen races (pathotypes) of *H. filipjevi,* we imported differential lines from Turkey and did initial screens with our cyst populations. We also completed a QTL association mapping analysis of a large population, and discovered six QTLs that can be further investigated. This was from the PhD thesis of Yvonne Thompson, who was funded by this research.

In the following few pages, we will address our objective separately and give more details of our results. These were the objectives from last year's proposal.

Objective 1. Screen adapted PNW and US varieties and advanced material in WA breeding programs for resistance to *Heterodera* in infested soil in the greenhouse, identify the *Cre* genes involved, and use markers to incorporate this resistance into breeding programs

We developed a high throughput greenhouse screening method to identify CCN resistance in wheat. This method assesses roots of young plants grown in cone-tainers containing soil collected from highly infested fields in fall and vernalized at 4 C prior to planting. In addition, this soil can be stored in the cold room after vernalization, and be used for up to a year.

We completed resistance testing of 1209 wheat lines from the programs of Carter, Pumphrey, Campbell, and Morris, regional nurseries, and a Campbell mapping population (NEMAX), all in the greenhouse using above method.

From above screening, we identified resistance in 10 to 21% of the advanced winter wheat lines, but less than 2% of spring wheat lines. Named and advanced lines resistant to *filipjevi* in one or more trials include ARS Crescent and Selbu, Cara, Otto, Masami, Madsen, Foote, ORCF-102, Prichett, SY605CL and Steelhead; WA 8235, 8206, 8163, 8194; Svevo and Soft Svevo. Preliminary resistance to *H. avenae* was found in Norwest 553, Jasper, and WA 8227. Chara and WA 8235 showed resistance to both *H. filipjevi* and *H. avenae*. In addition, three HRW and 12 SWW showed resistance.

Objective 2. Use markers to identify the *Cre* genes in our lines, and use markers to incorporate this resistance into breeding programs.

We developed KASP markers for QTLs for resistance to *H. filipjevi* that were identified in a CIMMYT study and assayed the breeding lines that we evaluated above. We were not able to identify the same QTLs in our material, except in the durum Svevo and Soft Svevo. These sources of resistance may be specific to CIMMYT derived material.

We tested SSR markers linked to *Cre1, Cre3, Cre5, Cre8, CreX*, and *CreY* genes. These markers may facilitate the understanding of the resistance background of our material.

We selected a subset of the lines that had been screened in the field and greenhouse. Ten varieties showed a strong resistant response in presence of the nematode in both the greenhouse and field. A genome-wide association study was performed using genotype by sequencing (GBS) markers. Although the panel was not large, a marker trait association (MTA) was discovered on genomes 1D, 3A, 5B, and 6BD; and two putative QTL on genomes 1A and 2B with false discovery rate of $P > 0.05$. QTL on 6B and 6D reveal a novel source of resistance to *H. filipjevi*. The introgression of selected MTAs into wheat cultivars will ultimately provide improved resistance to cereal cyst nematode. This work was part of the PhD thesis of Yvonne Thompson, which was completed in Nov. 2018 and will be published.

Objective 3. Conduct surveys for CCN

From 2013-2017, we surveyed 210 fields for Cereal Cyst Nematodes (CCN) infestation in eastern Washington and the Palouse. In 2016, we surveyed 50 locations in Walla Walla, Garfield, Columbia and western Whitman counties. Cysts were identified to species level with DNA techniques developed in previous. *H. filipjevi* was only found in southern Whitman County, and *H. avenae* in eastern Whitman County. No cysts were found in other locations. In 2017, we

concentrated our survey efforts to identify other fields with high levels of *filipjevi* and *avenae* that could be used for greenhouse testing. We identified a field near Colfax for *avenae*, and a field near Colton that may have to be increased for *filipjevi*.

Objective 4. Identify pathotypes of *H. filipjevi*.

A pathotype is like a race of the nematode. Like rusts, CCN has a very specific interaction with the host, which is a gene-for-gene interaction. The nematode produces effectors, which are virulence factors, but can be recognized by the receptors on the plant, leading to a resistance reaction. In order to predict which *Cre* genes are effective, we need to know the pathotype of *H. filipjevi.* This was done with *H. avenae* by R. Smiley in the 1990s, nothing is known about our pathotype of *H. filipjevi*. We imported differential lines for identification of CCN pathotypes from Turkey and increased the seeds in the greenhouse. We conducted initial screens for pathotype identification of our local CCN and results show that our pathotypes are unique, and don't match any of the existing pathotypes.

Deliverables

A growing list of resistant US and PNW varieties and lines, which can be used directly by the growers or incorporated into existing breeding programs.

A greenhouse technique that is optimized for screening more lines for the breeders

Greenhouse pot cultures of *H. avenae* and *H. filipjevi* that can be used for screening of varieties

A beginning knowledge of what *Cre* genes we may have in our backgrounds

A complete understanding of the distribution of *H. avenae* and *H. filipevi* in eastern Washington, including distribution maps.

The first description of the pathotype of *H. filipjevi* in eastern Washington

Refereed papers

Wen, N., Thompson-Manning, Y., Garland-Campbell, K. and Paulitz, T. C. 2018. Distribution of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in Eastern Washington State. Plant Disease: Submitted.

Manning-Thompson, Y, Thompson, A., Smiley, R., Paulitz, T., Garland-Campbell, K., 2016. Cereal cyst nematode screening in locally adapted spring wheat (*Triticum aestivum* L.) germplasm of the Pacific Northwest, 2015. Plant Dis Manag. Rep. 10:N003

Thompson, AL, Mahoney, AK, Smiley, RW, Paulitz, TC, Hulbert, S, Garland-Campbell, K, 2017. Resistance to multiple soil-borne pathogens of the Pacific Northwest is co-located in a

wheat recombinant inbred line population. G3 (Bethesda). 7(4):1109-1116. doi: 10.1534/g3.116.038604.

Theses

Yvonne Manning. 2018. Identification of Quantitative Trait Loci (QTL) for Resistance to Soil-Borne Pathogens *Fusarium culmorum* and *Heterodera filipjevi* in Wheat (*Triticum aestivum* L). PhD Thesis, Washington State University, Pullman, WA

Abstracts

Paulitz, T. C., Manning-Thompson, Y., Wen, N., Schallter, D., Borneman, J., and Garland-Campbell, K. 2017. Research on Cereal Cyst Nematode in Eastern Washington. 6th International Cereal Nematode Symposium, Agadir, Morocco Sept. 11-15, 2017

Wen, N., Thompson-Manning, Y., Garland-Campbell, K. and Paulitz, T. C. 2019. Distribution of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in Eastern Washington State. International Plant & Animal Genome XXVII, San Diego, CA, USA Jan 12-16, 2019

Popular Publications

Presentations

Paulitz, T. C. 2016. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 3, 2016. (presentation).

Paulitz, T. C. 2017. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 2, 2017. (presentation).

Paulitz, T. C. 2018. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 7, 2018. (presentation).

WGC project number: 3061-7667
WGC project title:Management of nematode diseases with genetic resistance
Project PI(s): S. Hulbert, T. Paulitz, K. Campbell
Project initiation date:7/1/2016
Project year 3: 2018-2019

Objective	Deliverable	Progress	Timeline	Communication
Obj. 1. screen adapted PNW and US varieties and advanced material in WA breeding programs for resistance to *Heterodera* in infested soil in the greenhouse identify the *Cre* genes involved, and use markers to incorporate this resistance into breeding programs	List of resistant US and PNW varieties and lines, knowledge of what *Cre* genes we have in our backgrounds	To date have screened over 1000 lines, see 3-year report of project for detailed results	Will continue greenhouse testing next year using vernalized, infested soil in the greenhouse.	See publication list in full report
	Greenhouse pot cultures of *H. filipjevi* and *H. avenae*	Ideally, instead of relying on naturally infested soil collected in the field, we will produce inoculum in the greenhouse. Because the nematode can only reproduce on living plants, this involves infecting plants in large pots, harvesting the soil after two months, and vernalizing it to induce the nematodes to hatch. We have established pot cultures of both species.	Pot cultures will continue to be replanted and cycled to increase the inoculum density	
Objective 2. Use markers to identify the *Cre* genes in our lines, and use markers to incorporate this resistance into breeding programs	Usable markers that can be incorporated in the breeding programs.	See 3-year report. We are currently testing SSR markers linked to Cre1, Cre3, Cre5, Cre8, CreX, and CreY genes. These markers may facilitate the understanding of the resistance background of our material. We also identfied 6 QTLs and will develop markers. We used an NCBI registered Cre sequence to blast against Chinese Spring, and have identified a serial of Cre suspects.We will develop SNP markers linked to these Cre suspects for potential genetic sources of CCN resistance.	Continue to develop and test markers for other identified *Cre* genes	See publication list in full report
Obj. 3. Conduct surveys for CCN	Maps of CCN around **all** of Eastern and Central Washington	From 2013-2015, we surveyed 210 fields in eastern Washton and the Palouse. In 2016, we surveyed 50 locations in Walla Walla, Garfield, Columbia and western Whitman counties. Cysts were identified to species level with DNA techniques developed in previous. *H. filipjevi* was only found in southern Whitman County, and *H. avenae* in eastern Whitman county. No cysts were found in other locations. In 2017, we concentrated our survey efforts to identify other fields with high levels of *filipjevi* and *avenae* that could be used for greenhouse testing. We identified a field near Colfax for *avenae,* and a field near Colton that may have to be increased for *filipjevi.*	The species-specific survey for the Palouse has been completed. A paper has been submitted to Plant Disease	See publication list in full report
Obj. 4. Identify pathotypes of *H. filipjevi*	Knowledge of pathogen diversity in relation to other world populations, to aid in selecting resistance *Cre* genes	Differential lines were imported from Turkey and seed was increased in the greehouse. Initial screening experiments were done, and tentative pathotypes did not match existing ones.	Pathotype testing will continue in the greenhouse in 2019-2020.	see publication list in full report