

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

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Project #:3061-7667

Progress Report Year: 1 of 3 (maximum of 3 year funding cycle)

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

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Executive summary and Final Report for Year 1 of grant.

This grant was not funded for the 2nd year of this cycle. A previous 3-year summary was submitted for the previous cycle (Jan. 2019) and Year 1 of the grant (Jan. 2020). Here, we report on our progress from Jan 1, 2020 to June 30, 2020, which covers the last half of Year 1 grant. Because of COVID, activity was severely curtailed in the lab, but we did accomplish the following

-collected infested soil in Fall, 2019 to use in greenhouse screening and testing in 2020.

-continue to cross resistant/susceptible lines for RIL and NIL populations, as described in the previous report.

- we used a known *Cre* sequence to blast against the Chinese Spring genome, identified similar sequences, found SNPs near the region (this was recorded in last year's report) and developed markers for these SNPs. However, at present, without reliable *Cre* gene differential lines with single *Cre* genes, it was difficult to draw conclusions from these markers.

-started screening a winter wheat population, as part of an USDA pre-doctoral scholarship for Nikayla Strauss, a PhD student with Drs. Campbell and Paulitz. The synthetic wheat population used in this project is the D-genome Nested Association Mapping (DNAM) population. The DNAM population is the result of 8 different hybridizations of *Ae. tauschii* with a hexaploid hard white Kansas breeding line. The *Ae. tauschii* accessions were chosen for their diversity compared to the D-genome in modern wheat varieties. All Recombinant Inbred Lines (RILs) in this population have the same genetic material for the A and B genomes, varying only in genes in the D-genome. During preliminary greenhouse CCN screenings, it was discovered that lines within DNAM family U6713 have resistance to both species of CCN found in the US, *Heterodera avenae* and *Heterodera filipjevi*, especially TA1642 (Figure 1). KS05HW14 exhibited resistance to *H. avenae* but not *H. filipjevi*. Thus, this resistance would be extremely useful if incorporated into elite adapted varieties because the germplasm we are starting from is relatively well adapted.

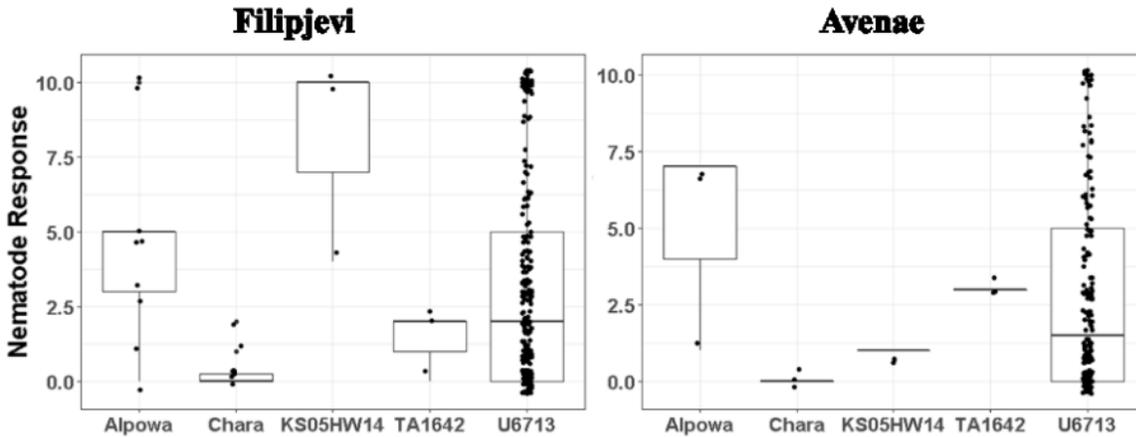


Figure 1. Results of multiple greenhouse screenings of family U6713. For these screenings, the checks are as follows: Alpowa (S), Chara (R), KS05HW14 (recurrent parent), TA1642 (*Ae. tauschii* parent of U6713 family). KS05HW14 exhibits different responses to *H. filipjevi* and *H. avenae*. The U6713 population varies in score but is skewed towards resistance.

Report from Year 1, submitted Jan. 2020.

- Over the last 2 funding cycles, we have developed a greenhouse method to screen PNW varieties and germplasm for resistance to cereal cyst nematode (CCN) which has become a problem in eastern Washington, first recognized in 2010. We have screened over 1000 lines, but are limited by finding field inoculum with high levels of the nematode. The populations in some of our field sites has declined, so we have had to locate new sources of inoculum. This resulted in less lines screened this year. Working in the greenhouse, we are constrained by the number of lines that can be screened.
- The ideal solution to replace phenotyping in the greenhouse is to develop DNA markers linked to CCN resistance genes or loci. To that end, we have developed two series of markers. The first was from mining the Chinese Spring genome and looking for sequences close to known *Cre* genes. These were converted to KASP markers which can be run in a high throughput fashion. We have identified four potential markers that show a correct reaction on a set of differentials with known *Cre* genes and resistance. The second set of SSR markers for Cre 3, 5, 8, and X were designed from the literature. But only *Cre* 3 and 8 worked, and it appears that lines in the differential set may have more than one *Cre* gene in them. Both sets of markers will now be tested on a wider range of PNW material.
- To identify new resistance genes in PNW material, we crossed known resistant varieties such as Chara and ARS Crescent and susceptible lines Alpowa, Seahawk, Bruehl, Ouyen and Louise. We are now backcrossing them to develop a mapping population which will be phenotyped, so we can locate new QTL or resistance genes.
- We imported differential lines for identification of CCN pathotypes from Turkey. We have done preliminary pathotype screening with this set of differentials, and our isolates of *H. filipjevi* do not match known races. We also made a new collection of cysts from a number of locations in Aug. 2019 and a colleague in Turkey extracted cysts to take back to Turkey to also run the pathotype test. In addition, we developed a collection of fungi from the cysts that may be potential biocontrol agents.

Impact:

- By developing molecular markers for CCN resistance genes, we can greatly enlarge the amount of germplasm and variety testing, since we will not be reliant on lengthy greenhouse testing and

dependent on field inoculum which may not be stable.

- We will continue to identify resistance sources that can be incorporated into PNW adapted varieties.

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

- We have a set of markers that may be useful in detecting multiple *Cre* genes, and will be tested with a wider range of PNW varieties to determine their utility.
- Identified resistance in a new mapping population of D-genotype (D-genome Nested Association Mapping (DNAM) population). These RILs developed from this population will enable us to identify the genes and move them into elite lines.

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. 2019. Distribution of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in Eastern Washington State. *Plant Disease* 103:2171-2178.

Thompson-Manning, Y., Wen, N., Garland-Campbell, K. and Paulitz, T. C. 2021. Genome-wide association mapping for cereal cyst nematode resistance in Pacific Northwest wheat germplasm. In preparation

Abstracts

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. 7th International Cereal Cyst Workshop, Nov. 2019. New Delhi, India

Presentations

Paulitz, T. C. 2019. What's New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 6, 2019

Paulitz, T. C. 2019. Nematode Diseases of Cereals. Far West Agribusiness Association, Pasco, WA. Dec 9, 2019.

Paulitz, T. C. 2020. What's New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 5, 2020

Paulitz, T. C. 2020. Nematode diseases of cereal crops. Presented to Agricultural Research Council of the Ukraine, Sept. 11, 2020.

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: 7/1/2019

This report covers the last half of Project Year 1, Jan. 2020- June 30, 2020. Section in **Bold** indicates new work started not reported in previous report

Project year 1: 2019-2020

Objective	Deliverable	Progress	Timeline	Communication
<p>1. Continue to identify CCN (specifically <i>H. filipjevi</i> and <i>H. avenae</i>) resistance in wheat varieties adapted to PNW.</p>	<p>List of resistant US and PNW varieties and lines, knowledge of what <i>Cre</i> genes we have in our backgrounds</p>	<p>To date, we have screened over 1000 lines in our greenhouse assay, using infested field soil. However, our source of soil for <i>H. filipjevi</i> declined in population, but we located another hot location in Aug. 2019. We have collected soil for vernalization, to use in 2020, along with <i>H. avenae</i> soil.</p>	<p>Will continue greenhouse testing next year using vernalized, infested soil in the greenhouse.</p>	<p>Paulitz, T. C. 2019. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 26 2019. (presentation). Paulitz, T. C. 2019. Nematode Diseases of Cereals. Far West Agribusiness Association, Pasco, WA. Dec 9, 2019 (presentation) Paulitz, T. C. 2020. What's New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 5, 2020 (presentation) Paulitz, T. C. 2020. Nematode diseases of cereal crops. Presented to Agricultural Research Council of the Ukraine, Sept. 11, 2020 (Presentation)</p>

<p>2. Develop more knowledge about specific resistance genes and develop molecular markers</p>	<p>Usable markers that can be incorporated in the breeding programs.</p>	<p>We have made considerable progress this year. Using the <i>Cre</i> 1 sequence from the database, we mined the genome of Chinese Spring and found a number of <i>Cre</i> suspects. We developed KASP markers and tested them on the Australian differentials with know <i>Cre</i> gene resistance. A number of markers look good, and seem to identify both <i>Cre</i> 1 and <i>Cre</i> 3. The best is IWA3381 which will now be tested on PNW lines with know resistance. SSR markers were tested for <i>Cre</i> 3, 5, 8, and X based on published literature. The markers for <i>Cre</i> 3 and 8 worked, but in the differential collection, many lines with supposedly a single <i>Cre</i> gene also were detected with <i>Cre</i> 3 and 8. This indicates that these lines may have multiple <i>Cre</i> genes. Despite the fact that these markers seem to pick up multiple <i>Cre</i> genes, they will be useful for germplasm screening.</p> <p>We started screening a winter wheat population, as part of an NSF pre-doctoral scholarship for Nikayla Strauss, a PhD student with Drs. Campbell and Paulitz. The synthetic wheat population used in this project is the D-genome Nested Association Mapping (DNAM) population. The DNAM population is the result of 8 different hybridizations of Ae. tauschii with a hexaploid hard white Kansas breeding line. The Ae. tauschii accessions were chosen for their diversity compared to the D-genome in modern wheat varieties. All Recombinant Inbred Lines (RILs) in this population have the same genetic material for the A</p>	<p>Markers will be tested in 2020 on PNW material with known resistance and susceptibility Delayed because of COVID</p>	
<p>3. Identify unknown resistance genes in adapted PNW material.</p>	<p>New resistance genes for cereal cyst nematode</p>	<p>Lines (NILs) will be developed by crossing CCN resistant lines Chara and ARS-Crescent with CCN susceptible lines Alpowa, Seahawk, Bruehl, Ouyen and Louise, then backcrossing. We have made all the initial crosses, and will continue to develop these mapping populations</p>	<p>Backcrosses will be made in 2020. Continuing despite COVID</p>	
<p>4. Determine pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i>, using differential lines being imported from Turkey.</p>	<p>knowledge of pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i></p>	<p>Collections of CNN populations were made in Aug. 2019, with a Borlaug Fellow from Turkey. We will pathotype these on our differential varieties imported from Turkey, but he also took back a collection to pathotype there. In addition, we isolated a collection of fungi from cysts to look for potential biocontrol agents</p>	<p>Pathotyping will be done in WA and Turkey in 2020 Delayed because of COVID</p>	