

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

(Begin 1 page limit)

**Project #:3061-7667**

**Progress Report Year:**     *\_1\_ 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:**

- 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 phenotyping in the greenhouse is to develop DNA markers linked to CCN resistance. 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.
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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.

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

Project year 1: 2019-2020

Objective	Deliverable	Progress	Timeline	Communication
<b>1. Continue to identify CCN (specifically <i>H. filipjevi</i> and <i>H. avenae</i>) resistance in wheat varieties adapted to PNW.</b>	List of resistant US and PNW varieties and lines, knowledge of what <i>Cre</i> genes we have in our backgrounds	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.	Will continue greenhouse testing next year using vernalized, infested soil in the greenhouse.	Paulitz, T. C. 2019. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 26 2019. (presentation).
<b>2. Develop more knowledge about specific resistance genes and develop molecular markers</b>	Usable markers that can be incorporated in the breeding programs.	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 developed 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.	Markers will be tested in 2020 on PNW material with known resistance and susceptibility	
<b>3. Identify unknown resistance genes in adapted PNW material.</b>	New resistance genes for cereal cyst nematode	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	Backcrosses will be made in 2020	
<b>4. Determine pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i>, using differential lines being imported from Turkey.</b>	knowledge of pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i>	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	Pathotyping will be done in WA and Turkey in 2020	