

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

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**Project #:** 3061-4949

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

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

**Investigators: S. Hulbert, K. Garland-Campbell, and T. Paulitz**

**Cooperators: R. Smiley**

**Executive summary:**

- Over the last three years (2013, 2014, and 2015) we have screened over 300 lines from the Western Spring Regional Nursery in a field infested with cereal cyst nematode (*H. filipjevi*) near Colton, WA. **In 2013**, we identified UC 1711, AUBR3059W, SY Steelhead and WA 8163 with resistance. UC 1711 and SY Steelhead also showed resistance in our 2012 trial. **In 2014**, SY Steelhead and AUS28451 (an Iranian landrace) showed resistance. Glee (WA 8074), Glee 0W and SY605 CL showed moderate resistance. A newly tested line, Svevo, showed resistance. Ouyen, a resistant check to *H. avenae*, was susceptible, while Chara, another resistant check to *H. avenae*, showed moderate resistance to *H. filipjevi*. **In 2015**, we reconfirmed the resistance in WA 8163, UC 1711, UC 1741, SY605 CL, SY-B041418, AUS28451 and Svevo. Ouyen continues to be susceptible, while Chara and Sonmez continue to be resistant. We also identified three new lines that were completely resistant- Pretty Wheat, Soft Alzada, and SY3051-9. S346 also showed resistance. Svevo, Soft Alzada and 'Pretty wheat' are all durum.
- **In Spring, 2014**, *Heterodera filipjevi* was discovered in Washington for the first time, near Colton. This discovery required us to work with APHIS to provide information for possible regulatory action, as well as numerous talks and publications to growers. But we needed to answer the question of how far the infestation extended. We have extended our survey to cover all of Whitman County in Fall, 2014 and 2015. We developed a molecular method for species identification from a single cyst, based on ITS and 28S primers. We went back to all the soil samples we took in 2013, 2014, and 2015 to extract single cysts.
- **In 2013**, 19% of the 53 sampled fields were infected with cysts, and all were identified as *H. avenae*, except for the Colton site. **In 2014**, 10% of the 76 fields were infested with cysts, and all were *H. avenae* except for the Colton site. **In 2015**, 31% of the 81 sampled fields were infested, and all were *H. avenae* except for the Colton site and a site south of Uniontown, which had *H. filipjevi*. Surveys by Dr. Smiley in Spring 2014 also found *H. filipjevi* only in the Colton-Uniontown area, with one mixed infection north of Pullman.
- **In 2014, for the first time, we were successful in conducting greenhouse screening using infested soil collected in the spring.** This nematode is difficult to work with in the greenhouse, because it requires a cold treatment or vernalization period to break dormancy of the nematodes and allow them to hatch. We screened 112 varieties using infested soil collected in the spring 2014. SY Steelhead and AUS28451 were resistant. The resistant check for *H. avenae* Ouyen was moderately resistant to *H. filipjevi*, while

another resistant check Chara showed resistance. In addition, we discovered two additional resistant durum wheat lines, Svevo and Soft Svevo.

- **In 2015, we successfully screened soft white and hard red winter varieties for the first time in the greenhouse.** From the Winter Wheat Soft White Regional Nursery, a number of ARS lines from the program of Kim Campbell were identified with resistance, including ARS-Crescent, ARS-Selbu, ARS010719-4L, and ARS2006-126-13C. Other lines included IDN-04-1001A 03PN062-21, 03PN071-4, 4J070874-1 and Chara. From the Winter Wheat Hard Red Regional Nursery, ARS070141-18L, 09X199-0-t-4, and HE9817/1.2 were identified with resistance.
- We hypothesized that the source of resistance was *Cre5* because it is linked to the stripe rust resistance gene *Yr17* which is prevalent in PNW wheat. We assayed for the presence of *Cre5*, and discovered that *Cre5* is present in UC1711, WB Rockland, Chara, 4J070874-1, ARS2006-126-13C, ARS070141-18L, and 09X199-0-t-4, but *Cre5* is not present in Somnez, SY Steelhead, Ouyen, ARS-Crescent, ARS-Selbu, ARS010719-4L, IDN-04-1001A 03PN062-21, 03PN071-4, and HE9817/1.2. **The resistance that we have identified is due to other unknown genes.**
- For root lesion nematode (*Pratylenchus thorneii* and *P. neglectus*), we have screened a collection of Iranian landraces and identified thirty two with dual resistance to both species. Six of these accessions also had moderate adult plant resistance to stripe rust in the field. A recombinant inbred line population between the dual resistant accession AUS28451 and Louise was assayed for resistance to Lesion Nematodes, other soil born diseases, and for lignin content. This population was genotyped with the 90K Iselect SNP chip and we identified QTLs associated with higher lignin content and with multiple soil borne disease resistance. We developed multiple segregating populations from a backcrosses of AUS28451 to Louise that perform well in infested field sites in Pendleton. We discovered high populations of lesion nematode at Spillman Farm in Pullman and screened the backcross populations in Pullman in 2015. The identification of screening locations in Pullman facilitates our ability to work with lesion nematodes.

#### **Impact:**

- We have now identified 10 locally-adapted spring wheat varieties and 11 winter wheat varieties with resistance to cereal cyst nematode *H. filipjevi*. These varieties may be available to growers in the next 1-2 years, allowing them to reduce the inoculum levels in their fields.
- The resistance to cereal cyst nematode that is present in these varieties is being used in crossing and breeding additional varieties in the ARS and WSU breeding program.
- *Cre5* may be useful in combination with other genes that have yet to be identified.
- We have shown that *H. filipjevi* has a restricted distribution, based on present sampling. However, more sampling is needed to detect possible mixed populations of both species which may be present.
- Resistant lines from the AUS28451/Louise backcross populations have been used as breeding lines to improve resistance to Lesion nematodes and other soil borne diseases in PNW wheat. The six dual resistance accessions with stripe rust resistance are being crossed to spring wheat breeding lines.
- **What measurable impact(s) has your project had in the most recent funding cycle?**

- **Discovery of new species of cereal cyst nematode (*H. filipjevi*), a coarse understanding of its distribution, and a handful of spring and winter wheat varieties with resistance to this species.**

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WGC project number: 3061-5745  
WGC project title: Management of nematode diseases with genetic resistance  
Project PI(s): S. Hulbert, T. Paulitz, K. Campbell  
Project initiation date: 7/1/2013  
Project year: 2014-2015

Objective	Deliverable	Progress	Timeline	Communication
Obj. 1. Conduct surveys for CCN	Maps of CCN around all of Eastern and Central Washington	From 2013-2015, we surveyed 210 fields in eastern Washington and the Palouse. Initially, the survey was conducted assessing white females. With the discovery of <i>H. filipjevi</i> in 2014, we developed a technique to assess the species identification of an individual cyst, based on ITS and 28S primers. Species cannot be easily distinguished from morphology. We extracted cysts from the 2013 and 2014 samples, along with soil from 2015 sampling. Almost all of the positive samples were identified as <i>H. avenae</i> . <i>H. filipjevi</i> was only found in the Colton-Uniontown area.	The species-specific survey for the Palouse has been completed. However, other areas of eastern Washington and possibly northern Idaho should be surveyed. In addition, we should use methods that have the ability to pick up mixed populations of the two species.	Paulitz, T. C. 2015. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 4, 2015. (presentation). Paulitz, T. C. 2014. A new nematode on the block: Cereal cyst nematode adds a new twist to wheat farming. <i>Wheat Life</i> , Dec. 2014. <b>See attached list for communications and publications during during the 3-years of the grant.</b>
Obj. 2) Screen adapted PNW and US varieties in infested grower fields for resistance to CCN, 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	Conducted 3 years (2013-2015) of resistance testing in a field site in Colton, infested with <i>H. filipjevi</i> . Screened over 300 lines from the Regional Spring Wheat Nursery. Evaluated lines based on number of white females on the root. Each line was paired with the susceptible "Alpowa" in each 4-row head row.	Have completed testing of adapted varieties against <i>H. filipjevi</i> . Will need to verify resistance against <i>H. avenae</i> .	Manning-Thompson, Y., Thompson, A., Smiley, R., Paulitz, T., Garland-Campbell, K., 2016. Cereal Cyst Nematode Screening in Locally Adapted Spring Wheat ( <i>Triticum aestivum</i> L.) Germplasm of the Pacific Northwest, 2015. <i>Plant Dis Manag. Rep.</i> SUBMITTED.
	Germplasm rated for resistance to CCN	Field trial was conducted in summer, 2014 in Colton. This is the field where <i>H. filipjevi</i> was discovered. SY SY Steelhead continued to show resistance. Glee (WA 8074), Glee OW and SY605 CL showed moderate resistance, with some reps showing very few cysts. These should be retested to verify. Ouyen, which is resistant to <i>H. avenae</i> , was susceptible in this field, while Chara showed moderate resistance. AUS28451, used in root lesion resistance work, showed resistance to <i>H. filipjevi</i> . We also identified Svevo with a high level of resistance. For the first time, we have identified locally adapted varieties with resistance. In 2015, we reconfirmed the resistance in WA 8163, UC 1711, UC 1741, SY605 CL, SY-B041418, AUS28451 and Svevo. Ouyen continues to be susceptible, while Chara and Sonmez continue to be resistant. We also identified three new lines that were completely resistant- Pretty Wheat, Soft Alzada, and SY3051-9. S346 also showed resistance.	Testing in field will continue in summer, 2016, but instead we will test crosses and populations made with CCN resistant parents and locally adapted varieties.	Manning, Thompson, Y., Thompson, A., Smiley, R., Garland-Campbell, K., and Paulitz, T. 2015. Screening for Resistance to Cereal Cyst Nematode in Locally Adapted Spring Wheat Cultivars of the Pacific Northwest 2014 Dryland Field Day Abstracts. Dept. of Crop and Soil Sciences Technical Report 15-1. Pg. 68. <b>See attached list for communications and publications during during the 3-years of the grant.</b>
	Greenhouse method of screening was successfully tested	In Spring, 2014, we were successful in greenhouse screening using soil infested with <i>H. filipjevi</i> collected from the field in April. These results confirmed field results. SY Steelhead continued to show a resistant reaction. AUS28451, used in root lesion resistance work, also showed resistance. The resistant check for <i>H. avenae</i> Ouyen was moderately resistant to <i>H. filipjevi</i> , while another resistant check Chara showed resistance. In addition, we discovered two additional resistant lines, Svevo and Soft Svevo. In Spring 2015 tested the Winter Soft White and Hard Red Nursery. We identified a number of soft wheat varieties from the ARS program with resistance, including ARS-Crescent, ARS-Selbu, ARS010719-4L, and ARS2006-126-13C. Other soft white lines with resistance included IDN-04-1001A 03PN062-21, 03PN071-4, 4J070874-1. We also identified three resistant hard red lines- ARS070141-18L, 09X199-0-t-4, and HE9817/1.2	Continue greenhouse testing of varieties in Winter and Spring, 2016. Goal is to test all WSU winter and spring varieties that are in variety testing trials.	
Obj. 3. Complete the identification of and verify QTLs associated with resistance to root-lesion nematodes in AUS28451 and select resistant breeding lines in PNW adapted backcross populations with AUS28451 as a source of resistance.		A major QTL for resistance to both species of lesion nematode was identified on chromosome 5A from AUS 28451. Validation of this QTL is being done using field trials in infested fields, and in the greenhouse. Additional markers are being placed on the linkage map.	7/2013-12/2015	Thompson, AL, Smiley, RW, Paulitz, TC, Garland-Campbell, K. 2015. Identification of dual-resistance to <i>Pratylenchus neglectus</i> and <i>P. thornei</i> in Iranian Landrace accessions of wheat. <i>Crop Sci</i> doi: 10.2135/cropsci2015.07.0438; Date posted: December 01, 2015
		Backcross populations with AUS28451 as a resistance source are being selected. New crosses are being made with other sources of resistance. The selected best backcross lines were grown in nematode infested and drought stress locations at Pendleton and LInd in 2014.	7/2013-12/2015	Thompson, A, Smiley, RW. 2015. Registration of the LouAt (Louise/IWA8608077) Wheat Recombinant Inbred Line Mapping Population. <i>J. Plant Regis.</i> 9:424-429. doi:10.3198/jpr2015.01.0002crmp

## Publications, Presentations, and Outputs for Nematology Grant 2013-2015

Kandel, S. L., Smiley, R. W., Garland-Campbell, K., Elling, A. A., Abatzoglou, J., Huggins, D., Rupp, R. and **Paulitz, T. C.** 2013. Relationship between climatic factors and distribution of *Pratylenchus* spp. in the dryland wheat production areas of Eastern Washington. *Plant Disease* 97: 1448-1456.

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.* SUBMITTED.

Manning, Thompson. Y., Pumphrey, M., Garland-Campbell, K., and Paulitz, T. 2014. Screening locally adapted spring wheat lines for resistance to cereal cyst nematode. ASA, CSA, and SSSA Annual Meeting, Long Beach, CA. Nov. 2014. (presentation)

Manning, Thompson. Y., Pumphrey, M., Garland-Campbell, K., and Paulitz, T. 2014. Screening locally adapted spring wheat lines for resistance to cereal cyst nematode. 2014 Dryland Field Day Abstracts. Dept. of Crop and Soil Sciences Technical Report 14-1. Pg. 68.

Manning, Thompson. Y., Thompson, A., Smiley, R., Garland-Campbell, K., and Paulitz, T. 2015. Screening for Resistance to Cereal Cyst Nematode in Locally Adapted Spring Wheat Cultivars of the Pacific Northwest 2014 Dryland Field Day Abstracts. Dept. of Crop and Soil Sciences Technical Report 15-1. Pg. 68.

Paulitz, T. C. 2014. A new nematode on the block: Cereal cyst nematode adds a new twist to wheat farming. *Wheat Life*, Dec. 2014.

**Paulitz, T. C.** 2013. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 3, 2013.

Paulitz, T. C. 2014. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 4, 2014. (presentation).

Paulitz, T. C. 2014. Updates in Wheat Disease Research, Garfield Grange, Colfax, Washington, January 25, 2014 (presentation)

Paulitz, T. C. 2015. "Root Disease Research at ARS Pullman-What's New?" Spokane Farm Forum, Ag Expo, Feb. 4, 2015. (presentation).

Smiley, R. et al. Information on *Heterodera filipjevi* presented to APHIS in Fall, 2014.

Smiley, R. W., Marshall, J. M., Gourlie, J. A., **Paulitz, T. C.**, Kandel, S. L., Pumphrey, M. O., Garland-Campbell, K., Yan, G. P., Anderson, M. D. Floers, M. D and Jackson, C.A. 2013. Spring wheat tolerance and resistance to *Heterodera avenae* in the Pacific Northwest. *Plant Disease* 97: 590-600.

Thompson, AL, Smiley, RW, Paulitz, TC, Garland-Campbell, K. 2015. Identification of dual-resistance to *Pratylenchus neglectus* and *P. thornei* in Iranian Landrace accessions of wheat. *Crop Sci* doi: 10.2135/cropsci2015.07.0438; Date posted: December 01, 2015

Thompson, A, Smiley, RW. 2015. Registration of the LouAu (Louise/IWA8608077) Wheat Recombinant Inbred Line Mapping Population. *J. Plant Regis.* 9:424–429. doi:10.3198/jpr2015.01.0002crmp