

PROJECT NO: New

TITLE: Buckets of worms – CCN survey and resistance studies - Observational and Experimental study of Cereal Cyst Nematodes (CCN) in southeastern Idaho, USA

PERSONNEL: Dr. Juliet Marshall, Ext. Crop Mgmt. Specialist (SC and E Idaho)
Dr. Arash Rashed, Entomologist, Aberdeen, ID
Pooria Ensafi, PhD candidate, Aberdeen, ID

ADDRESS: Dr. Juliet Marshall, 1776 Science Center Dr, Suite 205, Idaho Falls, ID, 83402; 208-529-8376; jmarshall@uidaho.edu

JUSTIFICATION:

Idaho, one of the largest cereal producers in the United States, is now facing a significant threat from CCN and wireworms, both of which can negatively impact wheat production. Widespread damage of these pests have been reported in spring cereals in 2015 from Ashton to St. Anthony and Rexburg areas. In particular, weather conditions (a very warm February through April), early planting of cereal crops, and a wet May, resulted in large numbers of nematodes finding homes on cereal roots and causing extensive damage and building soil populations. Integrated pest and disease management and control strategies for this soil pests require accurate and extensive knowledge of genetics and ecology. While in the past two years we have significantly increased our knowledge of species distribution and ecology of wireworms in Idaho, currently, we have no knowledge of the present nematode species, their genetic diversity, and population density. Although several species of nematodes are known to be key pests there are many species that may be used as biocontrol agents of insect pests such as wireworm. In addition as nematodes and wireworms share similar habitats, ecological approaches adapted based on clear understanding of both pest types, would assist in a more effective pest management of the crop. To date, research has been published on variety resistance and susceptibility (Smiley and Marshall, Marshall and Smiley) of currently grown wheat and barley varieties. This project is set to establish a study platform for identifying nematode fauna and their population genetics in the region, experimenting ecological approaches for controlling the CCN (also used in wireworm management), and evaluating the effectiveness of nematodes as biocontrol agents in reducing wireworm populations.

Pooria Ensafi, a PhD student studying with Dr. Juliet Marshall, who has extensive experience in nematode systematics and identification. Pooria is currently funded on a departmental assistantship to conduct research on CCN in southern Idaho. Additional funding is requested to support our ability to complete the proposed research, which includes the following three areas:

a) Investigating CCN genetic diversity

Any short or long term control strategy for CCNs requires a precise identification and quantification of existing populations. The genus *Heterodera* (Schmidt, 1871) comprises 80 valid cyst-forming species. Advanced molecular techniques including qPCR and High Resolution Melt Analysis will be used for detection, quantification and distinguishing different haplotypes.

b) CCN pathotype screening

The genus *Heterodera* has been reported from hosts belong to 14 different orders of plants. Cyst nematodes have a relatively limited host range to even within a single plant genus or family, and morphological and molecular studies support the possibility of coevolution of cyst nematodes and their host. But cereal cultivars with specific resistance or tolerance to CCN do not represent similar response to the same pathogen in different geographical areas. The previous studies in southeastern Idaho by Smiley and Marshall did not attempt to categorize the local population in the current pathotype scheme, but focused on screening varieties to heterogenous populations in the field.

c) Nematodes and Biological control agents

Biological control agents can be considered a management strategy for CCN, and are especially effective when eggs are contained inside the cyst. Biological agents used for our study area are fungal and bacterial based treatments. Although the final yield may explain how effective the treatments are economically, the decrease or even increase in CCN population after treatment is really a response to biocontrol agents that should be investigated. Considering nematodes, agricultural soils tend not to be not very diverse. There are, however a number of fungal and bacterial biocontrol agents that may reduce plant parasitic nematodes. This project will be complementary to an ongoing test to study biological control agents on cereal yield in a naturally and heavily infested soil near St. Anthony.

d) Nematode as Biocontrol Agents - Utilization of Nematodes to Reduce Wireworm Populations

Wireworms, the larval stage of click beetles (Col., Elateridae), continue to be one of the top major concerns of wheat producers in Idaho. Our investigative efforts, to date, have been focused on objectives that help to cover gaps in our knowledge of wireworm species, distribution, ecology, and management in Idaho. Ongoing surveys continue to evaluate the impact of cultural practices and environment on the extent of wireworm damage in the region. However, a clear understanding of the presence and ecology of parasitoid nematodes will help in preserving and using these beneficial species as a key step towards achieving sustainable management practices to control wireworms.

HYPOTHESIS & OBJECTIVES:

a) Investigating CCN Genetic diversity. The main purpose of this study is to identify different haplotypes of *H. avenae* group that are present in Idaho in order to determine what resistance sources need to be identified in our wheat breeding program. The objectives are:

- 1) Haplotypes identification and analysis of the "Host races" for regional population of *Heterodera* (which has never been done in southeastern Idaho). We will screen to see if there are different haplotypes in the area.
- 2) Comparative analysis with results from the second chapter, we will investigate the possible relationship between haplotypes (virulent populations) of the identified pathotypes in the region.

b) CCN Pathotypes screening

The general objective of the research on CCN pathotypes in southeastern Idaho is to obtain insight into the virulent population diversity of the *H. avenae* group. This main objective can be translated into the following specific objectives:

- 1) To distinguish pathotypes in the study area.
- 2) To assess the specificity of each pathotype population to the local host varieties.

The challenges of these objectives are to fill the gaps in the knowledge of population dynamics and characteristics of CCNs' pathotypes by conducting quantitative molecular analysis of the populations, and to help establish and utilize effective management tactics using host resistance/tolerance strategies.

c) Nematodes and Biological control agents Objectives

New CCN biological control agents are studied to evaluate the impact in cereal yield. The objectives of this study are:

- 1) Conducting a nematode population analysis of the soil in the field following biocontrol treatments.
- 2) The quantitative effect of treatments on the CCN population considering both cyst and eggs and see if there is meaningful correlation between the yield loss and cyst population.
- 3) We will test the null hypothesis that there is no correlation between the changes in CCN population density and treatments.

d) Nematode as Biocontrol Agents - Utilization of Nematodes to Reduce Wireworm Populations

- 1) Potential parasitoid nematode species will be identified from field collected samples
- 2) Their efficiency in infesting sugar beet wireworms will be evaluated in a laboratory setup
- 3) the effectiveness of two selected nematode species, based on laboratory trials, in reducing wireworm fitness will be quantified in greenhouse pot experiments.

PROCEDURES:

Soil samples will be collected from fields. Environmental variables (soil temp, moisture, etc) and data on cultural practices will be recorded at the time of sampling. Using a nematode extraction setup located at the Aberdeen R&E Center, nematodes will be washed from soil and collected. A dissecting microscope will be used to visually identify wireworms to the species level (where possible).

Collected CCN nematodes will be stored in -80 freezer for later genetic analysis. DNeasy blood and tissue extraction kits will be used to extract DNA from nematode samples (Pooria, how many can you extract to get sufficient DNA quantity?). Using Quantifying Polymerase Chain Reaction (qPCR), we will employ High Resolution Melt Curve analysis (HRM) to identify potential haplotype present in eastern Idaho. A distribution map of nematode species, and the predominant haplotypes, in eastern Idaho will be developed. Subsequently, ecological and cultural variables associated with nematode species/haplotypes will be identified.

Potential parasitic species of wireworms will be maintained in pots in the greenhouse to be evaluated as biocontrol agents for wireworms. Wireworm samples collected through wireworm survey project will be used to study interactions between predominantly present sugar beet wireworm, *L. californicus* and parasitic nematodes. Kaplan-Meier survivorship analysis will be used to quantify the nematodes ability in reducing wireworm populations.

DURATION: 2 to 3 years, depending upon success of first two years' of research.

COOPERATION: Dale Daws, a grower supporting research with land use in a very particularly heavily infected area. Other growers have agreed to support us with allowing us to obtain samples from their ground (Alan Baum, Burke Hanks, etc).

ANTICIPATED BENEFITS/EXPECTED OUTCOMES/INFORMATION

TRANSFER: Identifying the genetic diversity in the local population will assist us in the development of varietal resistance.

Funding request would include Capital Outlay to support purchase of an appropriate microscope, funding for lab supplies (including molecular support), and travel.

LITERATURE REVIEW:

Smiley, R., and J.M. Marshall. 2015. "Identification of balanced resistance plus tolerance to *Heterodera avenae* in spring wheat." Plant Disease. Manuscript ID is PDIS-09-15-1055-RE. submitted Sept 2015.

Marshall, J.M. and R. Smiley. 2015. Spring Barley Resistance and Tolerance to the Cereal Cyst Nematode *Heterodera avenae*. Plant Disease First Look, 3 Aug 2015. *In press*
<http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-05-15-0498-RE>

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

Smiley, R.W., Gourlie, J.A., Rhinhart, K.E.L., Marshall, J.M., Anderson, M.D. and Yan, G. 2012. Influence of Nematicides and Fungicides on Spring Wheat in Fields Infested with Soilborne Pathogens. Plant Dis. 96:1537-1547.

Smiley, R. W., Marshall, J. M., and Yan, G. P. 2011. Effect of foliarly-applied spirotetramat on reproduction of *Heterodera avenae* on wheat roots. Plant Dis. 95:983-989.

BOOK CHAPTER Smiley, R.W. and J.M. Marshall. 2015. Resistance and tolerance of spring wheat and barley to *Heterodera avenae* in the USA. In. A.A. Dababat and R.W. Smiley (eds.). Nematodes of Small Grain Cereals: Current Status and Research. FAO, Rome, Italy. *In Press*.

Plant Disease Management Reports:

Smiley, R.W., **Marshall, J.M.** "Spring wheat resistance and tolerance to cereal cyst nematode, 2013." Plant Disease Management Reports. March/2014 - Report No. 8:N007

Smiley, R.W., **Marshall, J.M.** "Spring barley resistance and tolerance to cereal cyst nematode, 2013". Plant Disease Management Reports. March/2014 - Plant Disease Management Report No. 8:N008

Smiley, R., and **Marshall, J.** 2014. Spring wheat resistance and tolerance to cereal cyst nematode. Proc. Pacific Northwest Oilseed/Direct-seed Conference, Kennewick, WA. January 20-22. (abstract).

Trade Journal: Smiley, R., and **Marshall, J.** 2014. Resistance and tolerance of Small Grains to Cereal Cyst Nematode. Idaho Grain Producers Magazine. Spring 2014. p. 17-18.

Dailey, P., Smiley, R., **Marshall, J.M.** Are Nematodes Reducing Your Wheat Yield? Idaho Grain Producers Magazine. Fall 2011. Page 21.

IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by	Idaho Wheat Commission	during FY 2015	\$	-
Allocated by	Idaho Wheat Commission	during FY 2016	\$	-

REQUESTED FY 2016 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	\$ -	\$ 9,461	\$ 3,709	\$ 6,300	\$ 6,000	\$ -	25,470

OTHER RESOURCES (not considered cost sharing or match):

TOTAL OTHER RESOURCES \$ 45,000

TOTAL PROJECT ESTIMATE FOR FY 2017:	\$ 25,470 (Requested)	\$ 45,000 (Other)	\$ 70,470 (Total)
--	--------------------------	----------------------	----------------------

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	<i>Marshall</i>	<i>Rashed</i>	<i>(PI name)</i>	<i>(PI name)</i>
Salary	\$ -	\$ -	\$ -	\$ -
Temporary Help	\$ 7,961	\$ 1,500	\$ -	\$ -
Fringe Benefits	\$ 3,121	\$ 588	\$ -	\$ -
Travel	\$ 4,300	\$ 2,000	\$ -	\$ -
Operating Expenses	\$ 3,200	\$ 2,800	\$ -	\$ -
Graduate Student Tuition/Fees	\$ -	\$ -	\$ -	\$ -
TOTALS	\$ 18,582	\$ 6,888	\$ -	\$ -
Total Sub-budgets \$				25,470