PROJECT NO: BJKT74, BJKT75

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
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JUSTIFICATION:
Idaho, one of the largest cereal producers in the United States, is facing a significant outbreak of CCN that can reduce wheat yield. Widespread damage was seen in spring cereal production in 2015 and 2016 from Ashton to St. Anthony and Rexburg areas. Due to weather conditions (a very warm February through April), early planting of cereal crops, and a very wet May in 2015, large numbers of nematodes found homes on cereal roots, causing extensive damage and building residual soil populations. Integrated pest and disease management and control strategies for this pathogen require accurate and extensive knowledge on genetics of the pathogen. Currently, we have no knowledge of the present biotypes, including the genetic diversity, and population density. Research has been published on variety resistance and susceptibility (Smiley and Marshall, 2015, Marshall and Smiley, 2015) of currently grown wheat and barley varieties and interaction of host varieties with nematode pathotypes. The continuation of the screening project will establish a study platform for further experiments on controlling the CCN in southeastern Idaho and be a key pilot research project in Aberdeen.

Pooria Ensafi, a PhD student studying with Dr. Juliet Marshall, 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 four 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 12 species reported on cereals. PCR-RFLP molecular technique and advanced qPCR and High Resolution Melt Analysis (HRM) will be used for detection, quantification and distinguishing different haplotypes.

b) CCN Pathotype screening
Cyst nematodes have relatively limited host range, 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 tests 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. Due to environmental concerns, effective chemistries for nematode control have been removed from the market. The remaining available registered insecticides for wireworm control in cereals (i.e., neonicotinoids) have provided very limited and ineffective protection. Investigation into biocontrol methods to reduce wireworm pressure may be the only sustainable long-term solution. Parasitic nematode species will be used to begin efforts for non-chemical alternatives to wireworms.

HYPOTHESIS & OBJECTIVES:

a) Investigating CCN Genetic diversity. The main purpose of this study is to identify different haplotypes of 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) Haplotype identification and analysis of the "Host races" for regional population of *Heterodera* (which has never been done in southeastern Idaho). We are collecting nematode samples from various locations in southern and southeastern Idaho to extract DNA for haplotype screening.
2) Comparative analysis: 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 avenae group. This main objective can be translate 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 will be studied to evaluate the impact in cereal yield. The objectives of this study are:

1) Conduct a nematode population analysis of the soil in the field following biocontrol treatments.
2) Evaluate the quantitative effect of treatments on the CCN population (both cyst and eggs) to determine the correlation between the yield loss and cyst population.

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

In order to have an extensive overview of current Entomopathogenic Nematodes (EPNs) in the region, soil samples from the currently running wireworm project, plus new soil samples from the area close to the cereal fields are tested for naturally occurring EPNs. Focusing on genetic diversity of the EPNs, we will identify the genera and species of those nematodes with pathogenicity on wireworm.

In this study we will target three consecutive goals:

1) Identification of naturally occurring EPN species using molecular and morphological techniques.
2) Conducting laboratory bioassay analysis of nematode pathogenicity on wireworm of different developmental stages (more specifically on young larvae).
3) Testing the efficacy nematode juveniles of EPNs in the field situation.
4)

PROCEDURES:

The research (all 4 chapters) have been started so far. The study area for all chapters is southeastern Idaho. CCN haplotype/pathotype screening started last year by collecting samples from St. Anthony, Rexburg and Ashton, and will be continued this year to cover additional portions of the geographic area.

1) CCN genetic diversity:
   Cysts from collected soils are sampled, and the DNA has been extracted and prepared for purification and PCR-RFLP analysis. The morphological analysis of cysts so far indicates the presence of a single species, *H. avenae*. Haplotype identification will be conducted using more advance techniques including HRM on nematodes collected in more soil samples from the wider geographical area that represents the southern Idaho cereal production.
2) Seven different cultivars of spring wheat were planted last year for assessing the tolerance and resistance of these cultivars against the local CCN pathotype in the field and green house. Preliminary results show different responses of the cultivars that represent differing geographical development of the cultivars. The experiment will be repeated this year in a different field in the same area.
3) The Biological control of CCN using the predatory nematode species of higher colonizer-persister value (c-p value) will be continued this year. The initial stage of extracting CCN juvenile from the cyst on the lab was successful. The predatory nematode will be tested against CCN juvenile population in the greenhouse test this year. We expect challenge in colonization efficacy of the predatory nematode in the soil. We are going to eliminate the disturbance and possible limitation by advanced introduction of predatory nematodes to the soil.
4) Wireworm control study consists of three stages:

i. Extraction of EPNs from samples:
The extraction of possible EPNs is based upon the standard technique of using the Greater Wax Worm (*Galleria mellonella*). One hundred grams of soil samples from each sampling location were placed in separate plastic containers, and 5 Galleria larvae (fishing bait available in the market) were added to the container. The infected larvae are moved to extraction petri plates and will be monitored until having the EPN Juveniles emerged from galleria larva and swam into the water in petri dish. 30 samples of ten locations are in the process so far.

ii. Identification:
The identification of EPN species will be based upon the morphological and molecular analysis of collected nematodes

iii. Test of pathogenicity against wireworm:
Occurrence of any EPNs, the Juveniles (stage one) especially the new local species will be used to conduct a laboratory bioassay against the wireworms' species. Since the biggest challenge in this experiment is the penetration of J1 into wireworm body, different techniques and timing will be used aiming the assessment of possibility and practicality of the application in both laboratory and field situation.

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:
Cereal cyst nematode has been found at damaging levels in southern and southeastern Idaho cereal production for many years (Smiley, 2009, Harry Kreeft, *personal communication*). Research has documented yield losses in wheat due to the cereal cyst nematode, *Heterodera avenae* Woll., in localized regions of Washington, Oregon and Idaho (Smiley et al 2005a, 2005b, 2011b, 2013). An initial density of more than 3,000 *H. avenae* eggs plus juveniles is generally capable of reducing yields of wheat, barley, oats and rye (Andersson 1982), and there are many areas in southeastern Idaho where populations exceed that density. Spring cereals are far more vulnerable than winter cereals due to the life cycle of the nematode, specifically egg hatch that is coordinated with the most vulnerable plant growth stage (seedling) of spring wheat (Smiley et al 1994). Crops produced under dry land systems are more vulnerable than under irrigation due to increased potential for drought stress that exacerbates nematode damage (Smiley and Machado,

2009).

Rotation of cereal crops that host *H. avenae* with two years of non-host broadleaf crop species or fallow is strongly recommended to reduce the density of this nematode on highly-infested fields (Andersson, 1992), but is difficult to practice as it is seldom profitable or practical in the high elevation areas of southeast Idaho. There are no chemical or biological nematicides are currently available to manage *H. avenae* (Smiley et al. 2011, 2012). Host plant resistance is characterized by cultivars that greatly suppress or prevent reproduction of nematodes (Cook and Evans, 1987). Many of the adapted varieties that significantly reduce nematode reproduction are not currently available (Smiley and Marshall, 2016). Efforts need to continue to identify resistance in currently available varieties, to characterize resistance in new cultivars, and to understand pathogen population dynamics in southeastern Idaho.

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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 2016	$	-
Allocated by	Idaho Wheat Commission	during FY 2017	$	-

REQUESTED FY2018 SUPPORT:

	Salary	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	$ -	$ 9,461	$ 3,870	$ 6,300	$ 6,000	$ -	$ 25,631

TOTAL BUDGET REQUEST FOR FY 2018: $ 25,631

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	Marshall	*Rashed*	*(PI name)*	*(PI name)*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ 7,620	$ 1,841	$ -	$ -
Fringe Benefits	$ 3,117	$ 753	$ -	$ -
Travel	$ 4,300	$ 2,000	$ -	$ -
Operating Expenses	$ 3,200	$ 2,800	$ -	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 18,237	$ 7,394	$ -	$ -

Total Sub-budgets $ 25,631

Explanatory Comments: (see FY2018 Guidelines for definition)

11.21.2016 - Version

ANNUAL REPORT

PROJECT NO: BJKT38, BJKT75

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

ACCOMPLISHMENTS:

The research has been started in southeastern Idaho as the study area for all chapters. More specific, CCN haplotype/pathotype screening started last year by collecting samples from St. Anthony, Rexburg and Shelley, which will be continued this year to cover the most geographic area including Ashton.

1) CCN genetic diversity:
   Cysts from soil samples are collected, the DNA has been extracted and ready for purification and PCR-RFLP analysis. The morphological analysis of cyst indicates the presence of a single species *Heterodera avenae* (so far). Haplotype identification will be conducted using high resolution melt (HRM) analysis in more soil samples from wider geographical area, to be a good representative of southeastern Idaho.
2) Seven different cultivars of spring wheat were planted last year for assessing the tolerance and resistance against the local CCN pathotype in the field and green house. Preliminary results represent different regional response of introduced cultivars. The experiment will be repeated this year in a different field for the test repeatability.
3) Biological control of CCN using the predatory nematode species with high colonizer-persister value (c-p value) was successful in extracting second stage juveniles from the cysts in the lab. The naturally occurring predatory nematode from a local field successfully collected from the soil and will be tested against CCN juvenile population in the greenhouse this year. We expect challenges in colonization efficacy of the predatory nematode in the soil. We are going to eliminate the disturbance and possible limitations by advanced introduction of predatory nematodes to the soil.
4) Wireworm control study consists of three stages:
   a. Extraction of entomological pathogenic nematodes (EPNs) from samples:
      The extraction of possible EPNs is based upon the standard technique of using the

Greater Wax Worm (*Galleria mellonella*). One hundred grams of soil samples from each sampling location were placed in separate plastic containers, and 5 Galleria larvae (fishing bait available in the market) were added to the container. The infected larvae are transferred to extraction petri plates and will be monitored until having the EPN Juveniles emerged from galleria larva and swam into the water in petri dish. 30 samples of ten locations are in the process so far.

b. Identification:
The identification of EPN species will be based on morphological and molecular analysis of collected nematodes that will be conducted this year.

c. Test of pathogenicity against wireworm:

d. Occurrence of any EPNs, the Juveniles (stage one) specially of the new local species will be used to conduct a laboratory bioassay against the wireworm species. Since the biggest challenge in this experiment is the penetration of J1 into wireworm body, different techniques and timing will be used aiming the assessment of possibility and practicality of the application in both laboratory and field situation.

PROJECTIONS:

Control methodologies for CCN is closely related to identification of occurring species, because the resistance response of most cereal cultivars can be extensively varied in different geographical and environmental areas. Besides, introducing new resistant or tolerant lines to the farmers should be evaluated against local pathotypes in the region. Considering the population density and resistance level of most cultivated cereals, results from the ongoing project would provide us a good understanding of the pathogenicity of CCN that could facilitate configuring a threshold level for the pathogen. Possible yield reduction for different rotations in cereal production and designing a plausible control methodology requires testing new treatments compliant with strict regulations and environmental concerns. Direct molecular analysis of soil in our experiments for identification of different pathogens concurrently will provide a quick and accurate technique that will reduce the time and expenses in soil analysis which will be a breakthrough in the field of nematology.

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