Grant Code: AN5477

Title: A survey of central and eastern Idaho wireworm species and evaluating ecological and chemical approaches to maximize cereal production

Personnel: A. Rashed, X. Liang, I. Popova, J. M. Marshall

Address: University of Idaho, 875 Perimeter Dr., Moscow, ID 83844; 208-885-5972; arashed@uidaho.edu

Justification/Rationale: Wireworms (Coleoptera: Elateridae) are significant pests of wheat in the Pacific Northwest region. The long-awaited Broflanilide is a new insecticide class introduced to the market in the spring of 2021. However, not much is known about the efficacy of this class of insecticide against other pests such as Hessian flies; this chemistry is not effective against aphids. Such information would be critical as we try to keep input costs low to maximize economic return, especially in rainfed production, where the return is generally lower than irrigated production.

To date, our state-wide survey of wireworms in approximately 215 fields across northern and southern Idaho resulted in the identification of 14 pest species (larvae and adults). We have also collected relevant soil physical, chemical, and biological data to evaluate their impact on wireworm activity. Our extensive data is being analyzed, and we continuously provide updates on detected patterns through reports and growers' meetings. The sugar beet wireworm, *Limonius californicus,* and the western field wireworm, *L. infuscatus,* are prevalent in southern and northern Idaho. We have focused our research efforts on these two most damaging species. We have established collaborations with IBEST, WSU, USDA-ARS, and MustGrow Biologics Corp. to understand genetic variations both within and among species and evaluate the efficacy of biological and cultural control approaches in managing wireworms. We have also continued with researchers in AgriFood Canada, USDA, the University of California to develop pheromones effective in collecting adult beetles. We have received exemplary support from Idaho growers over the years; more than 53 cereal growers have participated in our monitoring program. Numerous articles, book chapters, and conference presentations have been produced or are currently in production.

There were several bacterial species associated with *L. californicus*. These bacteria varied across eastern, central, and western Idaho; the presence and density of certain microbes can affect insect susceptibility to insecticides. Thus, our program has not only supported efforts in evaluating the newly developed insecticide but also continued biological (entomopathogens) and cultural control (crop rotation) fronts to establish effective location-specific pest management practices to minimize damage. We are continuing our research on short-term management solutions by evaluating the efficacy of upcoming insecticides against wireworms, as well as cultivar tolerance to wireworm damage.

The continuous support from and the involvement of Idaho wheat growers have been precursors to the success of this solid collaborative program, which has also attracted support from federal sources of funding to match grower dollars. This work has also provided a backbone for other research proposals as it continuously provides wireworms for experiments in other programs.

Hypothesis & Objectives: The degree of wireworm damage is species-dependent and a clear understanding of their interaction with the surrounding biotic and abiotic environment will assist

with developing integrated management practices to minimize their negative impact. Our objectives include (some modified to include new directions):

I) Quantify *L. californicus* susceptibility to neonicotinoids with respect to their associated microbe species and densities,
II) Study the impact of crop rotation options [*new focus* on mustard concentrated seed meal extract] on wireworm pressure and damage,
III) Quantify among-cultivar differences in susceptibility to wireworms in relation to tiller number (*completed*) and root structure in low and high organic matter soil (*new focus*)
IV) *New:* Determine wireworm response to heat and cold stress in different species, and possible relation to soil water content (*L. californicus*, only)
V) *New:* Determine the need for neonicotinoids in broflanilide-treated seeds to protect against Hessian flies (and aphids)

Procedures/Plan of work: Similar to 2021, we will focus our monitoring (no longer an independent Objective) on a few heavily infested fields since pheromone assays will also be conducted in surrounding areas of the same fields (5 solar bait traps and 18 pheromone traps/field). Wireworm and adult pheromone traps will be replaced every 2 (pheromone) to 4 weeks (bait traps) between April 1st and October 1st in 10 selected fields across Idaho. Subsets of wireworms collected (from 5 geographical locations) will be extracted and analyzed for the presence of endosymbiont DNAs (bacteria), using universal primers (**Objective I**). Although we faced a methodological obstacle last year (low DNA yield), we resolved the issue and successfully extracted sufficient microbial DNA; samples are now submitted for DNA sequencing. We were notified that we would receive the results with a delay. Upon receiving the results, we will set up neonicotinoid susceptibility assays. To address **Objective II**, we have completed efficacy trials of the concentrated extracts and brown mustard plants in the greenhouse and presented our results in last year report. We also incorporated brown and yellow mustard plants (planted late August) and applied concentrated mustard products (brown and yellow mustard seed meal and highly concentrated dehydrated products) in 10x20-ft plot areas late October 2021. These plots are to be planted with spring wheat in April 2022. To address **Objective III**, we continued quantifying wireworm directional response to wheat and legume root volatiles (GC-MS and HPLC) in olfactometers. We will obtain a selection of varieties with different root structures from Dr. Chen (UI) for preference bioassays to identify less preferred wheat genotypes. Through **Objective IV**, in a series of laboratory assays we will continue evaluating thermal thresholds (high and low) for the three predominant Idaho species *L. californicus*. Preliminary assays were completed for *L. infuscatus* during 2021. This assessment will also be conducted in relation to soil organic matter content. We believe that soil organic content can have great direct and indirect influence on wireworm biology and damage rate. While broflanilide, the new chemistry from BASF, has been very effective in reducing wireworms, it is not expected to control aphids and it is not known whether it can protect crops against Hessian flies **Objective V** is set to examine the need for additional neonicotinoid treatment in broflanilide-treated seed through a series of greenhouse bioassays.

Duration: Spring 2020 through Spring 2023; this is a continuing proposal.

Cooperation/Collaboration: This is a cooperative project between Drs. Rashed, Liang, Popova, and Marshall. We established collaborations with UI-IBEST, University of Illinois, University of California, USDA-ARS, WSU, OSU, Agriculture and AgriFood Canada, and MustGrow Biologics Corp (Canada).

Anticipated Benefits, Expected Outcomes and Impacts, and Transfer of Information: This proposal is taking new directions (several not listed here) based upon our 2014-2021 findings. Our objectives are directly relevant to predicting wireworm damage and developing integrated practices to minimize losses. Our results are published in high-impact journals, putting Idaho in the forefront of wireworm research. We routinely communicate our progress with producers.

Literature Review: Historically, wireworms were controlled by using environmentally persistent chemicals that are now banned due to environmental and health concerns (Vernon et al 2009). Although substitute chemicals provided some level of protection, wireworm susceptibility to the new generation of chemicals varied (Van Herk et al. 2007).

Recently, more emphasis has been placed on alternative control measures to achieve more successful and sustainable damage control. The effectiveness of any IPM program in controlling wireworms depends on a clear knowledge of the species and their ecology (Furlan 2005). Unfortunately, knowledge of wireworm ecology and control is generic without taking among-species variations into account. Wireworm species may vary in the range of plant species they feed on (Pazmand and Traugott 2005) and the soil characteristics (such as pH, texture, organic matter, and moisture) where they reside (Barsics et al. 2013). It has been also shown that soil type (Rashed et al. 2016), CO_2 (Barsics et al 2013; Gfeller et al 2013), and volatile organic compounds released from roots (Gfeller et al. 2013) can affect wireworm movement and survival. We now know that the sugar beet wireworm the most damaging species to cereals in Idaho (Rashed et al. 2015), and we have placed our research focus on identifying most effective management practices to control this species in different agroecosystems across Idaho.

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FY2023

COMMODITY COMMISSION BUDGET

Principal Investigator: ***(Rashed)***

Allocated by	*Idaho Wheat Commission*	during FY2021	$ 30,857
Allocated by	*Idaho Wheat Commission*	during FY2022	$ 30,522

REQUESTED SUPPORT: Budget Categories	*Awarded for FY2022*	*Requested for FY2023*
(10) Salary *(staff, post-docs, et NOTE: Faculty salary/fringe not allowed*	$ 10,024	$ 10,024
(12) Temporary Help	$ 9,800	$ 9,420
(11) Fringe Benefits	$ 898	$ 1,111
(20) Travel	$ 4,600	$ 4,721
(30) Other Expenses	$ 5,200	$ 5,200
(40) Capital Outlay >$5k	$ -	$ -
(45) Capital Outlay <$5k	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -
TOTALS	$ 30,522	$ 30,476

TOTAL BUDGET REQUESTED FOR FY2023:	$ 30,476

BREAKDOWN FOR MULTIPLE INDEXES:

Budget Categories	*Rashed*	*Liang*	*Popova*	*Marshall*
(10) Salary *(staff, post-docs, et*	$ 10,024		$ -	$ -
(12) Temporary Help	$ 5,520	$ 2,400	$ 1,500	$ -
(11) Fringe Benefits	$ 776	$ 206	$ 129	$ -
(20) Travel	$ 3,920	$ -	$ -	$ 801
(30) Other Expenses	$ 3,400	$ 600	$ 700	$ 500
(40) Capital Outlay >$5k	$ -	$ -	$ -	$ -
(45) Capital Outlay <$5k	$ -	$ -	$ -	$ -
(70) Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ 23,640	$ 3,206	$ 2,329	$ 1,301
			Total Sub-budgets	$ 30,476

ANNUAL REPORT

Grant Code: AN5477
Title: A survey of central and eastern Idaho wireworm species and evaluating ecological and chemical approaches to maximize cereal production
Personnel: Arash Rashed, Associate Professor
Atoosa Nikoukar (report preparation), Graduate Student
Xi Liang, Associate Professor
Inna Popova, Assistant Professor
J. M. Marshall, Professor
Address: Arash Rashed, University of Idaho, Moscow, ID 83844; 208-885-5972; arashed@uidaho.edu

Accomplishment: To identify the damaging wireworm species in Idaho, more than 200 fields (wheat and barley) have been monitored for wireworm presence and numbers statewide since 2015. Among the fourteen wireworm and click beetle species identified, sugar beet wireworm (*Limonius californicus*) has been the most common species in Idaho. However, *L. infuscatus* (a close relative of *L. californicus*) is also widely present in northern Idaho. In collaboration with researchers from AgriFood Canada (British Columbia), the University of California, USDA-ARS (South Carolina), and the University of Illinois, we have tested several synthetic pheromones to identify effective compounds in attracting click beetles of different species. Lures have been deployed in multiple geographical locations in Idaho. We collected sugar beet wireworms from southeastern, southcentral, and northern Idaho fields to identify endosymbiont bacteria associated with wireworms. Although our approach to DNA isolation was unsuccessful in 2020, we resolved the issue in 2021, extracted sufficient quantities of bacterial DNA, and submitted the samples for sequencing. Following our 2020-21 success in greenhouse bioassays in reducing sugar beet wireworm numbers by applying the newly developed brown mustard concentrated extract, we designed field trials in southern and northern Idaho wheat fields to validate our findings. This project is in close collaboration with producers in Idaho and eastern Washington. We also initiated laboratory assays to evaluate the effects of temperature on wireworm survival.

Objective 1: *Wireworm/ adult click beetles species survey. L. californicus, L. infuscatus,* and *Aeolus mellillus* constituted most of our collected samples. Like previous years, sugar beet wireworm (*L. californicus*) was the most common wireworm species in both southern and northern Idaho. This year we also emphasized testing pheromone lures to attract male click beetles. Using pitfall traps (18 per location), we deployed pheromone traps in the infested fields in three geographical locations of northern Idaho. Traps were placed during April 2021 and monitored monthly through July and August, depending on field location. Collected beetles were identified to the species level. *L. californicus*, *L. infuscatus*, and *Dalopius asellus* were the predominant click beetles collected (Fig.1). A few *Agriotes* sp. beetles were also

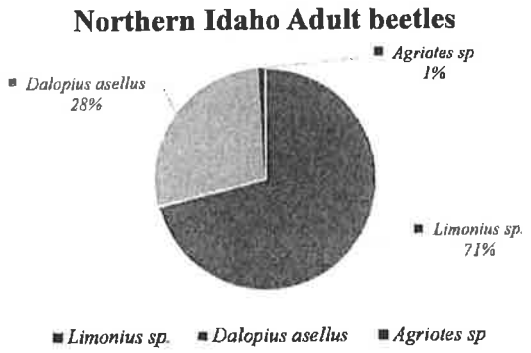

Fig.1: Proportion of different click beetle species collected in the northern Idaho in 2021, using experimental pheromone lures.

collected. We also collaborated with Canadian colleagues to test the newly developed limoniic acid lures to collect *Limonius* spp. Traps were deployed in selected fields in northern and southern Idaho. These traps collected thousands of click beetles throughout the season and retain their effectiveness for at least a year in the field. This product is at the final stages of approval and is expected to be released in the spring of 2022.

Objective 2: *Mutualistic endosymbionts of wireworms.* We overcame our 2020-21 obstacle and used whole wireworms to extract sufficient amounts of DNA (including endosymbiont DNA) instead of each body section. Wireworms were collected from 4 geographical locations. The total DNA of each wireworm was extracted using the DNA extraction kit ZymoBIOMICS (Zymo Research, Irvine, CA). Using 16S universal primers, two-step PCR was run to recover prokaryote endosymbiont communities associated with individual wireworms collected from different locations. The 16S amplicon prepared from PCR2 products was sent to IBEST for sequencing, bacterial identification, and quantification.

Objective 3: *Impact of mustard concentrated seed meal extracts in wireworm control.* To validate our last year's greenhouse results on the efficacy of the newly developed brown mustard extract on wireworm populations, we selected three heavily infested fields in southern and northern Idaho and eastern Washington. Canola (as a control), yellow and brown mustard were planted in early September 2021 and disk incorporated in early November. The yellow and brown mustard seed meals and brown mustard concentrated seed meal extract were applied and incorporated into the soil in November. All plots will be planted with spring wheat in 2022. Wireworm numbers were estimated in each plot before planting by using solar bait traps and will be monitored again prior to our spring planting to estimate the impact of each treatment on wireworm numbers. In addition, plant stand counts will be used to determine the effectiveness of our treatments compared with nontreated controls.

Objective 4: *Quantify among-cultivar differences in susceptibility to wireworms, in relation to tiller number (**completed**) and root structure in low and high organic matter soil (new focus).* This objective will start in the early growing season.

Objective 5: *Wireworm response to heat and cold stress in different species.* In the first year, we examined the survivorship of *L. infuscatus* at -18°C, 0-1°C, and 18°C (ambient temperatures), 5, 14, and 21 days after exposure. All wireworms died within two hours of placement in the -18°C chamber at soil temperatures of -5.7°C (±0.25). The greatest wireworm survival was associated with the air temperature ranging between 0 and 1°C (soil temperature of 6.5°C (±0.1)), in which 100, 70, and 10% of survivorship was recorded 5, 14, and 21 days after placement in the chamber. Survivorship of 100% was recorded for wireworms after 5 days at 18°C, however, in the absence of moisture, survivorship dropped to 30 and 0%, 14 and 21 days after placement in the chamber. It is important to note that our thermal chambers lacked adequate humidity controls. Wireworm mortality at 18°C is likely due to soil drying during the experiment.

Projections: We have completed analyses of our 6-year data (not reported) and are planning to develop a manuscript for publication in 2022-23. We will continue to sample wireworms in both southern and northern infested fields to collect wireworms for laboratory assays. The role of endosymbiont bacteria in wireworm susceptibly to neonicotinoids is expected to be tested in the greenhouse as soon as we receive the sequencing results and collect sufficient *L. californicus* during the season. Field trials for evaluating the effect of brown mustard seed meal concentrated extract on sugar beet wireworm population will be completed in 2022. All treatments

have been incorporated into the field, and spring wheat will be planted in the April of 2022. Cultivar screening for resistance against wireworms will be initiated in 2022.

Publications:

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