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Montana Wheat and Barley 50 years

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MONTANA STATE UNIVERSITY 125 YEARS IN THE PEOPLE'S INTEREST

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INTRODUCTION

The information and data reported are a collaboration of ongoing or new research projects located at or near Western Triangle Agricultural Research Center (WTARC) of Montana State University, College of Agriculture, Conrad, Montana. Many projects are conducted in cooperation with faculty members, research associates and Post-doctoral fellows from the Depts. of Plant Science and Plant Pathology (PSPP) and Land Resources and Environmental Science (LRES) located on the campus of Montana State University (MSU), and Agricultural Research Centers: Central (CARC), Northern (NARC), Eastern (EARC), Northwestern (NWARC) Southern (SARC) and Western (WARC) of the Dept. of Research Centers.

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Low Energy Sprinkler Application Effect on Montana Malt Barley

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Background

Barley has been grown in North America over the years as a forage crop, feed crop and malt crop adapting to dry land and irrigated ecosystems, and it works well in rotation with sugar beets serving as a valuable source of malt in Montana. In spite of vast variation in response of barley varieties to soil types, rainfall, nutrient content and other environmental factors, Montana is second in barley production, producing 22% of the U.S. barley, with an average yield of 60 bu/ac. (USDA, 2015). Providing water requirements by choosing acceptable irrigation methods to have the proper moisture distribution, leads to maximized grain yield, malt quality and decreases weed growth and disease. Depending on the irrigation method, optimum yield that might be expected to happen when available soil moisture (ASM) is to retain above 50% of ASM. The center pivot system is a dominant irrigation method in Montana barley fields, and was designed to conserve water. The pivot should be correctly installed and managed to achieve high water conservation. It has been reported that a pivot system is the most common irrigation method in Montana, and applies water in a uniform distribution and efficient manner, but is not the best method to keep the soil moisture at proper levels during the barley reproductive period (Rogers et al., 2008).

Water limitation or inappropriate distribution leads to abundant morpho-physiological and biochemical changes in plants. There are not too many studies on plant physiology responses to using different irrigation methods in terms of seed quality, yield and water conservation. Therefore, the present study aims to investigate on:

- Compare Low Energy Sprinkler Application (LESA) irrigation system with regular or traditional irrigation methods in selected barley fields.
- Study the effect of irrigation methods on dry matter pattern and leaf area during the season.
- Compare the differences in yield components, plant height and physiological traits related to yield under different irrigation application methods.

Material and methods

Locations and Installment

In order to test LESA methods and compare their effect on malt barley in Montana, experiments were conducted at six sites in three different locations (2 sites at each location), Choteau, Conrad, and Valier. At these sites phenological events, agronomic traits and physiological responses under center pivot system with a single drop tube (Regular, hereafter identified as 'Control') and the 2-drop tubes sprinklers (hereafter LESA site) for LESA pressure reducers and spreader plates. For installing the two drop tubes the multiple drop tubes were split off the single outlet to create two drop tubes. In comparison to last year (in 2018 control, two drops, and three drops were compared), this year at the LESA site, the entire pivot was set up as 2 drop system.

One data loggers, four soil moisture sensors, and a manual rain gauge were installed at each site in Choteau, Conrad, and Valier locations. The soil moisture sensors were installed at four depths including, 24 inches, 18 inches, 12 inches and 6 inches below the soil surface after planting and removed from the fields when the plots were harvested. Soil moisture probes and data loggers were installed soon after seeding as practical, they were installed at the two sites at Choteau on May 31 and June 11, two sites at Conrad on May 23, 2019, two sites at Valier on May 30, 2019. Soil moisture samples were taken two times during the entire experiment, the first set of samples was taken while installing the moisture probes and the second set of soil samples were produced after harvesting. Soil samples were broken into six-inch increments for all depths. Water usage was measured throughout the cropping season to see if there is a difference in efficiency between the irrigation methods.

Seeds were planted on two sites at three locations on different dates, varied seeding rates and with variation in fertilizers as explained in Table 1.

Table 1: Seeding dates, crop varieties, seeding rates, and fertilizers at three locations in 2019.

Location	Control	LESA
Choteau	May 2, 2019 (Merit 57 barley); 100 lb/ac; top dressed 12 tons manure/ac	May 10, 2019 (Merit 57 barley); 100 lb/ac; top dressed 12 tons manure/ac
Conrad	September 15, 2018 (Keldin winter wheat)	May 15, 2019 (Voyager barley)
Valier	May 1, 2019 (Coors barley)	May 1, 2019 (Coors barley)

Data Collection

Plant sampling plots were along both sides of field transects in parallel with installed soil sensors. Plot size was measured at 5 ft x 20 ft. Row spacing was considered about 7.5 inches.

For measuring leaf area index (LAI C2200) was used to record plants leaf area weekly between the tillering and physiological maturity stages, during 9 a.m. to 3 p.m. Every week plant samples were cut at ground level, oven-dried at 70˚C for 72 hours to reach a constant weight and subsequent data was recorded. Biomass dry weight was also recorded. Every week plant height was measured using a ruler, depth of soil moisture using the brown probe was measured, and rain gauges were also checked and reset weekly.

A Wintersteiger Classic plot combine was used to collect harvest data for each sampling block along the transect. As post-harvest data, grain yield, test weight, seed protein, plump and thin kernels were collected. Kernel plumpness was assessed by sieving over a 6/64” slotted screen. Grain protein content and test weight were obtained at Western Triangle Agricultural Research Center, Conrad using the Near-Infrared Inframatic 9500 SW- Whole Grain Analyzer (Perten Instruments IM9500; Hägersten, Sweden).

Statistical Analysis

Data were processed for ANOVA in Microsoft Excel (2016). Means within the groups were obtained from ANOVA were used to analyze the Least Significant Differences by using the graphing calculator (TI-nspire CX II, CAS, Texas Instruments, Dallas, Texas, U.S.). Graphs were generated in Microsoft Excel (2016).

Results

Soil type at six sites varied and overall moisture percentage also varied. Out of six sites, five sites had barley grown, whereas the control site at Conrad had winter wheat. Therefore yields at Conrad are not compared however they are analyzed separately (Table 2). At Choteau, LESA site had

numerically higher yields compared to the control site however there was no statistically significant difference. Whereas at Valier, control site had greater yield compared to the Valier LESA site, but there was no significant difference. At both Choteau and Valier locations, at control sites higher test weight, plant height, and plumps recorded but both control sites had less protein compared to LESA sites (Table 2). The LESA site at Valier also had a patch of weeds that might have contributed to the low yields.

In terms of soil moisture recorded by ground soil sensors indicated greater soil moisture (cubic meter) at Valier LESA site at all four depths (6, 12, 18, and 24 inches). Whereas at Choteau and Conrad locations only at 12 inches, the LESA site showed greater moisture (Figure 1). Rainfall and irrigation water received at six sites also varied (Figure 2). The maximum amount of water was collected in July at all the locations and especially at the LESA site of Conrad (3.9 inches). A sudden increase in water collected in rain gauge at Conrad location could also be because of rainstorm encountered in this area in July 2019.

Soil moisture obtained by comparing dry and wet soil samples in spring (May) and fall (August) of 2019, indicated the use of water content from the soil during the crop production. Soil moisture at the soil depth of 6-12 inches indicated drop at all the six sites. Soil moisture at the depth of 0-6 inches also showed decrement at all the sites, other than the Valier control site (Figure 3). Also to indicate that Valier control had the maximum yield (Table 2).

In terms of performance of crop, LAI and plant matter was analyzed. Although no significant variations were observed in LAI, numerically greater LAI was measured for the Valier control site. Valier control site overall (June-August) had greater LAI measurement, followed by Choteau LESA>Conrad LESA. At two locations (Choteau and Conrad) greater LAI were obtained for LESA sites, whereas at Valier location it was reversed (Figure 4). In general the greater the LAI is an indication of higher yields, which is also shown in present data where maximum yield was recorded at Valier control. Average plant moisture was overall greater for Choteau LESA site, followed by Conrad LESA=Choteau control>Valier control>Valier LESA>Conrad control (Figure 5). Plant dry matter accumulation increased by leaf number and tiller development until anthesis

and then started to decline as the plant began to ripen, with a noted increase just before harvest at Valier Control (Figure 6).

Overall results indicate although not a significant but numerically greater moisture content, plant dry matter, and LAI were observed at LESA sites. In Valier, the control site performed better in terms of yield, which is supported by other datasets including plant moisture, soil moisture, and LAI measurements. Nevertheless, some impact of the presence of weeds at Valier LESA might also have played role in lowering the parameters at Valier LESA site. None of the sites had any significant effect on plant height, plumpness and thin. Greater protein content was generated at LESA sites. Also since Conrad control was winter wheat field we did not compare the yield data for Conrad location.

Recommendations

Sprinkler height affects the wetted area, water distribution and water productivity especially in windy areas like Montana's Western Golden Triangle region. Wind velocity also influenced water distribution and uniformity, once released from sprinkler irrigation systems. In the present study for most of the parameters, there was no significant difference in using the regular center pivot system and LESA application. Even though there are no significant differences in the grain data it appears that the LESA irrigation system has an impact on soil moistures and plant performance in terms of dry matter, LAI measurements. Chances of disease occurrence are there with greater moisture with LESA application however, no disease occurrence was observed at six sites in 2019. Nozzle size, height and drop numbers to meet plant water requirements might increase regular pivot system efficiency. Testing different varieties of barley, various crops, using precision application based on crop needs and soil profile in consecutive multi-location experiments would deliver scientists and farmers a better understanding of water- plant -soil relationships. Also, assessing the effect of different water regimes on saved water amount and plant response would be useful.

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Table 2. 2019 LESA irrigation, grain data by location. Data were processed for ANOVA in Microsoft Excel (2016). Means within the groups were obtained from ANOVA were used to analyzed the Least Significant Differences by using graphing calculator (TI-nspire CX II, CAS, Texas Instruments, Dallas, Texas, U.S.).

Location	Irrigation Type	Yield (bu/ac*)	Test Wt (lb/bu*)	Height (inch)	Plump (%)	Thin (%)	Protien (%)
Conrad	LESA	148.4	53.1	32.5	99.1	0.4	8.98
	Control**	133.7	61.3	31.5	NA	NA	11.42
Mean		NA	NA	NA	NA	NA	NA
LSD		NA	NA	NA	NA	NA	NA
C.V. (s/mean)*100		NA	NA	NA	NA	NA	NA
P- Value		NA	NA	NA	NA	NA	NA
Valier	LESA	149.6	52.6	25.8	95.2	1.7	11.12
	Control	200.9	55.7	29.5	98.9	0.4	10.58
Mean		175.3	54.2	27.6	97.0	1.0	10.85
LSD		ns	0.705	0.935	2.267	0.353	ns
C.V. (s/mean)*100		32.9	3.15	7.48	2.43	72.77	6.86
P- Value		0.2332	0.00004	0.00006	0.0065	0.000085	0.3421
Choteau	LESA	182.4	52.2	35.5	91.9	3.2	10.67
	Control	168.0	53.0	36.5	95.7	1.5	8.71
Mean		175.2	52.6	36.0	93.8	2.4	9.69
LSD		ns	ns	0.998	3.193	ns	1.571
C.V. (s/mean)*100		9.5	1.54	2.10	2.84	60.00	13.87
P- Value		0.2489	0.2021	0.0498	0.0262	0.0890	0.0225

* Yield and test weight are adjusted to 13% seed moisture.
** Conrad Control is winter wheat, hence means are not obtained and compared.
NA=not applicable since no plumps and thins were obtained from winter wheat crop

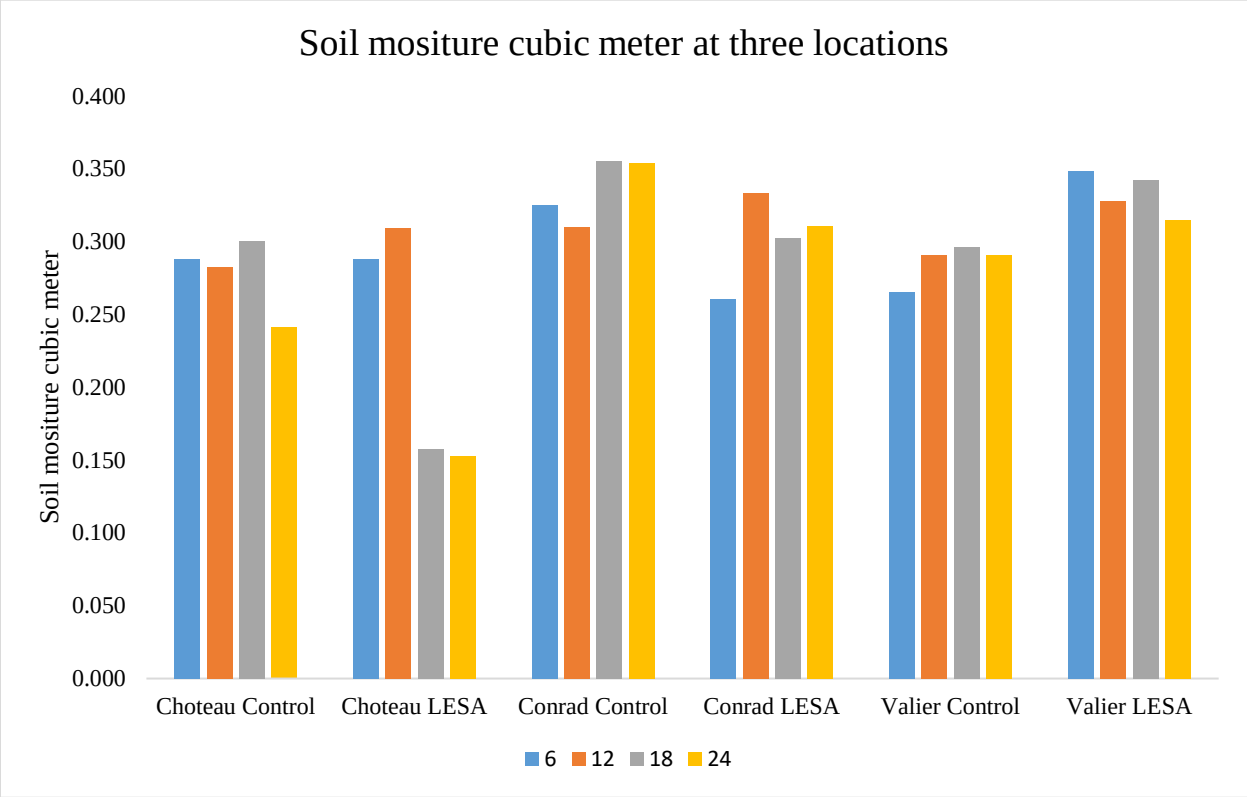

Figure 1: Soil moisture (cubic meter) recorded for three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites) with moisture sensors.

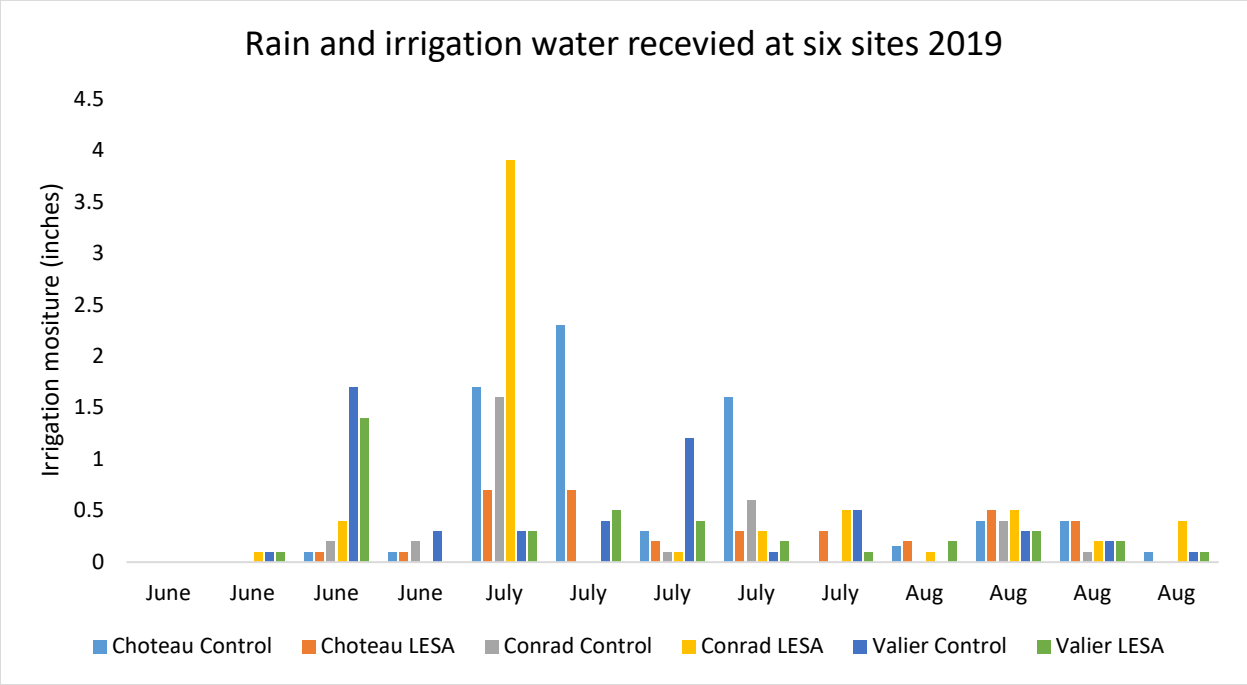

Figure 2: Water received (rainfall and irrigation) in three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites) measured with rain gauge.

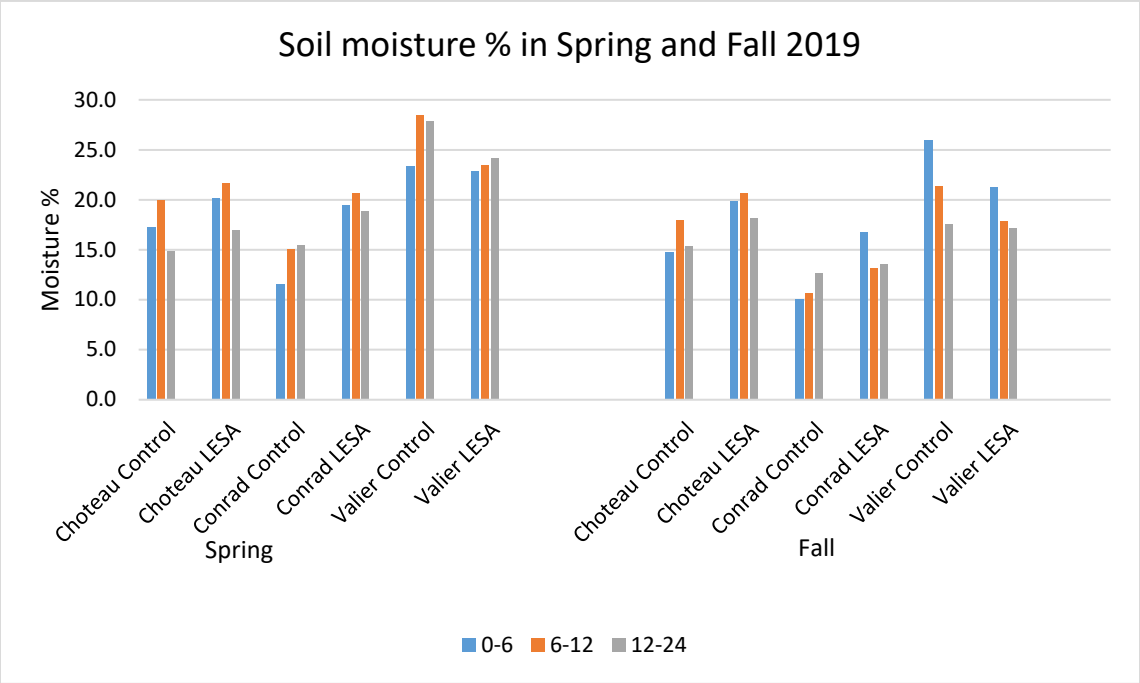

Figure 3: Soil moisture (%) measured at three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites) at the time of installation (spring) and harvesting (fall) at three depths (0-6, 6-12, 12-24 inches).

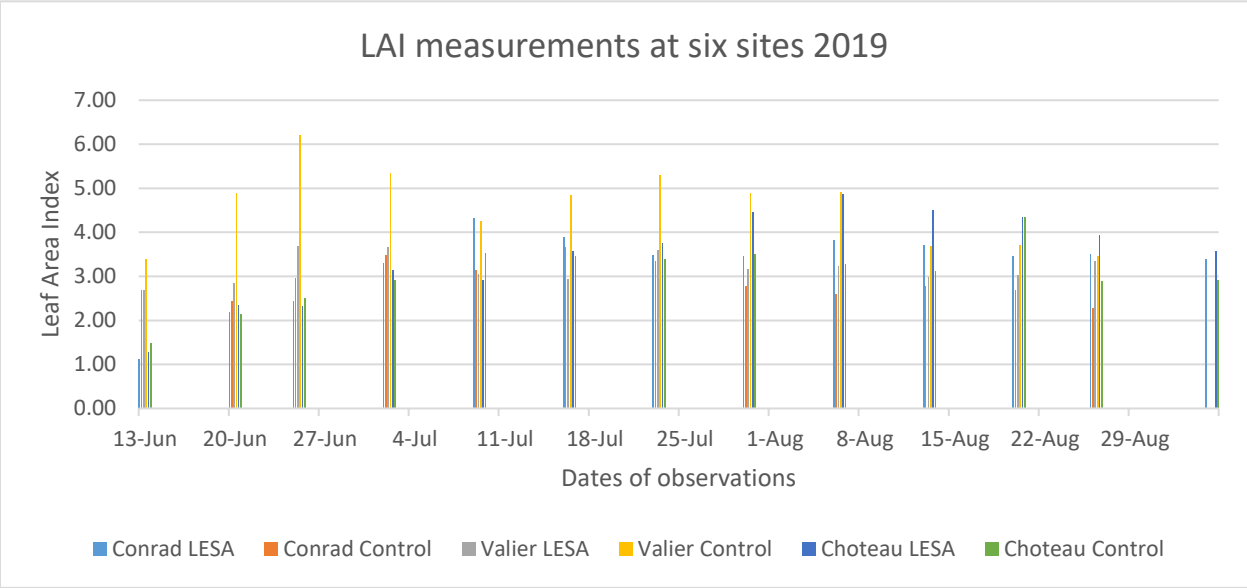

Figure 4: Leaf Area Index (LAI) measured at three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites) from June to August 2019.

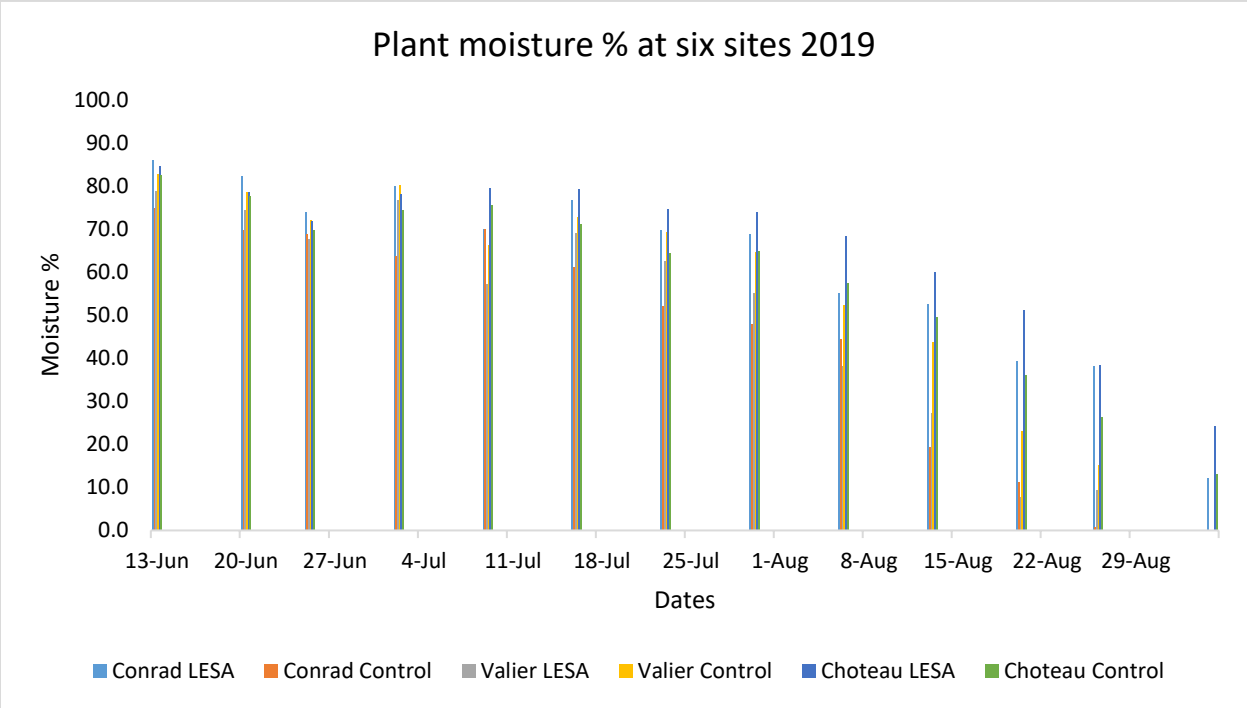

Figure 5: Plant moisture percentage measured from June 2019 to August 2019 for barley and winter wheat at three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites).

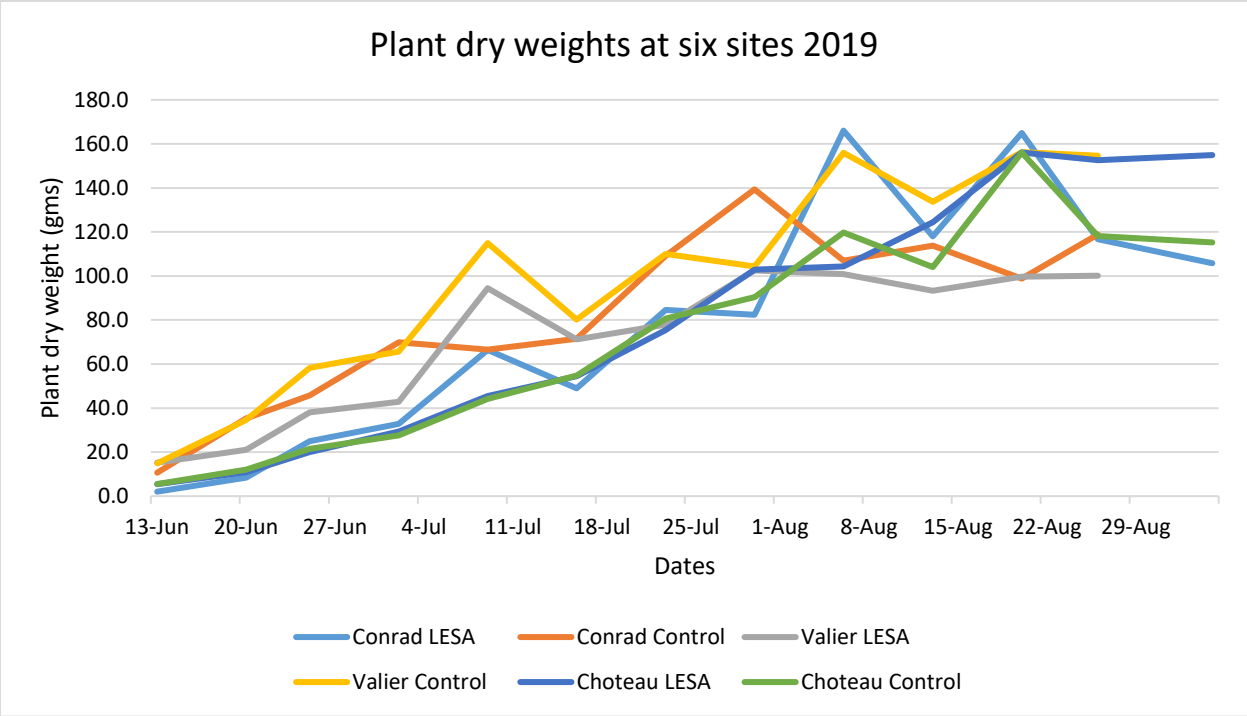

Figure 6: Plant dry weight measured for barley and winter wheat from June to August 2019 at three locations (Choteau, Conrad, and Valier) at six sites (3 control sites and 3 LESA sites).

Field Testing the Effect of Biopesticides against Wheat Stem Sawfly Management: Dose Response

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Aim of the Study

The aim of this study was: 1) to determine the effects of three biopesticides (Actigard, Xpectro and Neem) treatment application on wheat stem sawfly (WSS) management, using two doses (low and high concentration) of each product.

Materials and Methods

Locations of winter wheat fields used in field trials

The field experiments were performed at two locations: Knees (N 48°00'08.5 W 111°21'51.8) and Choteau (N 47°59'36.0 W 112°06'49.9), in the Golden Triangle, Montana, USA. Both experimental locations had moderate to high WSS infestations for several years. The experimental plots were seeded in September 2018 at a rate of 194 live seeds per m^2. The seeds were planted in four rows, with 30 cm between rows. Glyphosate (Roundup Powermax®) was applied at the rate of 2.5 L/ ha (the active ingredient of 540 g/L of acid glyphosate) prior seeding to control weed growth. Fertilizers N, P, and K at 224.2, 0, and 22.4 kg/ha were broadcasted while planting, and an additional application of 12.3, 25.2, and 0 kg/ha of these three nutrients were applied through the seed plot drill.

At each field location, treatments were arranged in a randomized complete block design (RCBD) with nine replicates per treatment. Plots for treatments were 3.6 × 1.2 m separated by 0.60 m buffer zones to avoid cross contamination of treatments.

Monitoring of wheat stem sawfly adults

Considering the ideal application time for biopesticides can be one of the important factors for WSS management. Currently, there is no established degree-day model for determining the precise timing of adult emergence. Two methods were used for monitoring the emergence of adults: 1) dissection of WSS-infested stubble to determine the stage of immature development and 2) sweep net sampling in the winter wheat fields to detect adults. Experimental plots and their adjacent winter wheat fields were scouted weekly from the last week of April – first week of June, 2019.

Application of chemicals

From 2017, WSS lab and field trials, we found that two-time application of two biopesticide products (Actigard and Neem) had some impacts on WSS management (Shrestha et al., 2018). In addition, 2016 WSS field trial study showed that two-time application of Xpectro biopesticide product had also some impact especially WSS larval mortality. The two-time applications refer to applications of chemicals when WSS eggs and larvae are expected to be present, respectively, inside stems. Therefore, these three chemicals were used for the study (Table 1). Since these

chemicals are relatively expensive than synthetic insecticides, it is important to test whether the repeated lower doses of potential biopesticide product may work for WSS management and thereby reducing costs for winter wheat producers. The rate of each chemical is presented in Table 1. These treatments were applied in 2018 and in 2019 they were tested again.
Treatment application were based on sawfly adult's emergence timing. First application were done on June 11 (Knees and Choteau locations) 2019. The second application was made on June 17 (Knees and Choteau locations) 2019. Treatments were applied using a SOLO backpack sprayer (SOLO, Newport News, VA) calibrated to deliver about 400 L of spray solution/ha based on nozzle flow and walking speed. Plants treated with water served as untreated control plots. At all field trial locations, chemicals were applied at the wheat stage with 4-6 nodes.

Table 1. Biopesticide products and rate of application in each treatment

Treatment	**Concentration**
T1: Water	-
T2: Actigard® High dose	1.50 g/L
T3: Xpectro® High dose	5.0 ml/L
T4: Azadirachtin® (Neem) High dose	5.76 ml/L
T5: Actigard® Low dose	0.75 g/L
T6: Xpectro® Low dose	2.5 ml/L
T7: Azadirachtin® (Neem) Low dose	2.88 ml/L

Collection of wheat stems

Wheat stems were sampled in all plots to determine the treatment effects during the growing season. Sampling was conducted 3 days before to treatment application (PT), and 10 and 50 days after treatments. Three random samples were collected from two central rows of each treatment plot, with five stems/sample. Wheat stems were cut from the base of plants with help of scissors, placed into one zipper-lock bag, and kept in picnic cooler. During the final sampling time, however, clumps of stems were pulled randomly from three sampling points of two middle rows of each plot with the help of shovel to collect entire matured plants. This technique was used mainly because the WSS diapausing larvae usually prefer to remain at the base of the wheat stem.

Samples were brought to the laboratory, where stems were dissected lengthwise with a fine bladed scalpel to determine the following parameters: 1) WSS stem infestation level; the presence of WSS immatures, parasitoid immature or frass inside dissected wheat stems, 2) WSS immatures population; the presence of eggs and larvae inside dissected wheat stems at each sampling time, 3) WSS larval mortality; the presence of dead larvae inside dissected wheat stems, 4) WSS larval body weight; body weight of diapausing larvae and 5) parasitism rate; presence of parasitoid cocoons inside stems parasitoid holes in stems.

Host and parasitoid adult populations: WSS and *Bracon* spp.

Three biopesticide products were also tested to examine whether they can repel WSS adults and their impact on WSS parasitoid adult population levels. A sweep net was used to assess insect population (WSS and parasitoid adults). Sweeping was done with a standard sweep net (180^{o} arc), collecting 15 sweeps from each treatment plot. Sampling was done one to two days before treatment application (PT) and, 10, 20 and 30 days after treatment application. Samples were stored in a freezer until examined in the laboratory and insects counted.

Stem lodging level at harvest

WSS larval feeding inside stems caused wheat stands fall into ground and thereby cause difficulty during harvesting. We examined that whether tested chemicals had any effects on plant stand levels during the wheat grain harvest. Wheat stems lodging measurements were made by visual classification rating scale of 1 to 10. The rating of 1 indicates that all plants in a plot were vertical and 10 for all plants in a plot were horizontal.

Yield and quality

To harvest the wheat grains from treatment plots, Hege 140 plot combine was used. The precaution was used to minimize the borders and any overlap of treatment effects on wheat yield and quality. Each plot length was measured, and the wheat grain threshed from each plot. Wheat grains were cleaned with a seed processor (Almaco, Nevada, IA) and weighed on a scale to determine yield. Test weight was measured on a Seedburo test weight scale. The protein and moisture percentages of seed were determined with NIR grain analyzer IM 9500 (Perten Instruments, Springfield, IL).

Statistical analysis

Analysis of variance (ANOVA) was carried out using the PROC Mixed procedure in SAS 9.4 (PROC Mixed, SAS Institute 2018). Data were pooled for each replicate, and treatments were considered as fixed effects while the block was considered a random effect. Normality of data was tested with a Univariate procedure (PROC Mixed). Estimates of least square means and differences of least square means were evaluated (Type 3 test of fixed effects F-test). Multiple comparisons among the treatments were made using Fisher's Least Significant Test (LSD) at $\alpha = 0.05$ by using the standard error generated in ANOVA.

Results

WSS infestation level

WSS infestation levels at different sampling time are presented at Table 2. This study showed that treatments had no significant impacts on WSS infestation levels (see Table 2 for statistical output). Overall, there was high variation in infestation levels at different time of sampling. However, Actigard high treatments had numerically lower infestation levels at both the sites compared with untreated control at 10 days after the treatments application, irrespective of location (Table 2).

Table 2. Effects of Neem, Xpectro and Actigard applications on wheat stem sawfly (WSS) infested stem % level (PT and 50 days), yield, and lodging (mean ± SE) in winter wheat fields at the two study location of Montana.

	Knees				Choteau			
	Yield	PT	50 DAT	Lodging	Yield	PT	50	Lodging
Control	3788±160a	20.8±8abc	98.3±7a	8.9±1.3a	4896±132a	13±3.8ab	102±6.4a	5.4±0.6a
Neem Low	3800±160a	29±8abc	101±7a	9.3±1.3a	4781±132a	12±3.8abc	110±6.4a	5.5±0.6a
Neem High	3722±160a	37±8ab	100±7a	6.8±1.3ab	4582±132a	8±3.8c	107±6.4a	6.3±0.6a
Xpectro Low	3974±160a	39±8a	95.6±7a	6.4±1.3ab	4719±132a	10±3.8abc	107±6.4a	6.5±0.6a
Xpectro High	3744±160a	15±8c	100±7a	6.6±1.3ab	4741±132a	8.7±3.8bc	107±6.4a	6.6±0.6a
Actigard Low	3560±160a	16.7±8bc	93±7a	6.8±1.3ab	4157±132b	9±3.8bc	110±6.4a	5.7±0.6a
Actigard High	3632±160a	12±8c	100±7.9a	5.4±1.3b	4010±132b	14±3.8a	93±6.4a	5.6±0.6a
	437 (0.61)	21 (0.09)	20 (0.97)	3 (0.16)	335 (<.0001)	4.8 (0.83)	17.9 (0.46)	1.5 (0.52)

PT, Pre Treatment; DAT, Days After Treatment Application

Wheat stem sawfly adults, and parasitoid adults and their parasitism level

In general, WSS adult populations were found higher at the Knees location compared with Choteau location. Regardless of location, treatments did not have a significant impact on WSS adult population, at any sampling time. Parasitism level was also not found significant however at Knees site Neem low had greater parasitoids population whereas at Choteau site Neem low and high, Xpectro high and Actigard high had greater parasitism levels.

WSS diapausing larval mortality

At Knees site not many diapausing larvae were found. At Choteau site Xpectro low and Actigard low showed numerically higher larval mortality (Figure 1).

Body weight of diapausing WSS larvae

Higher body weight of diapausing larvae were generally found at the Choteau site compared to Knees site (Figure 2). At Choteau site Neem low, Xpectro high and Actigard high showed greater larval weights.

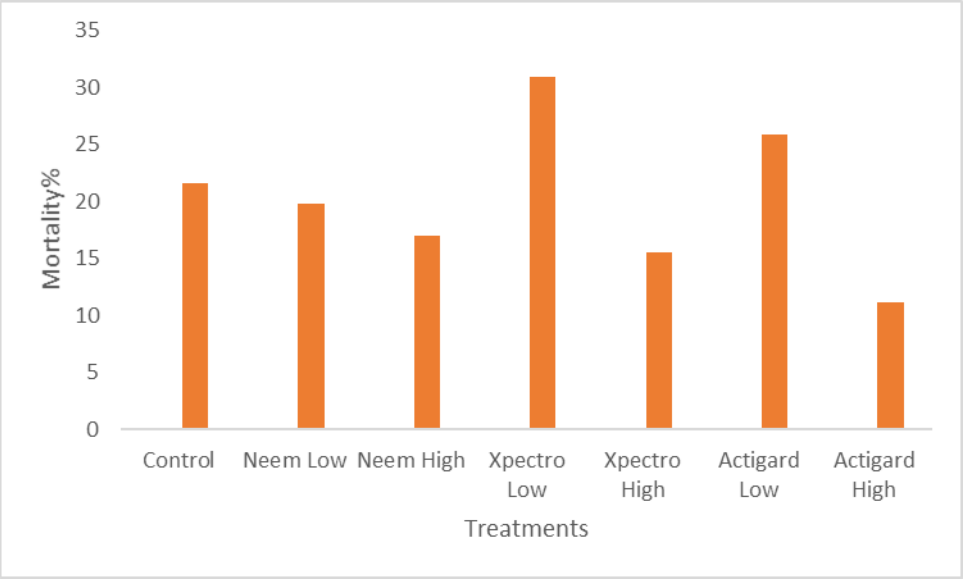

Figure 1: Mortality percentage at Choteau site in 2019.

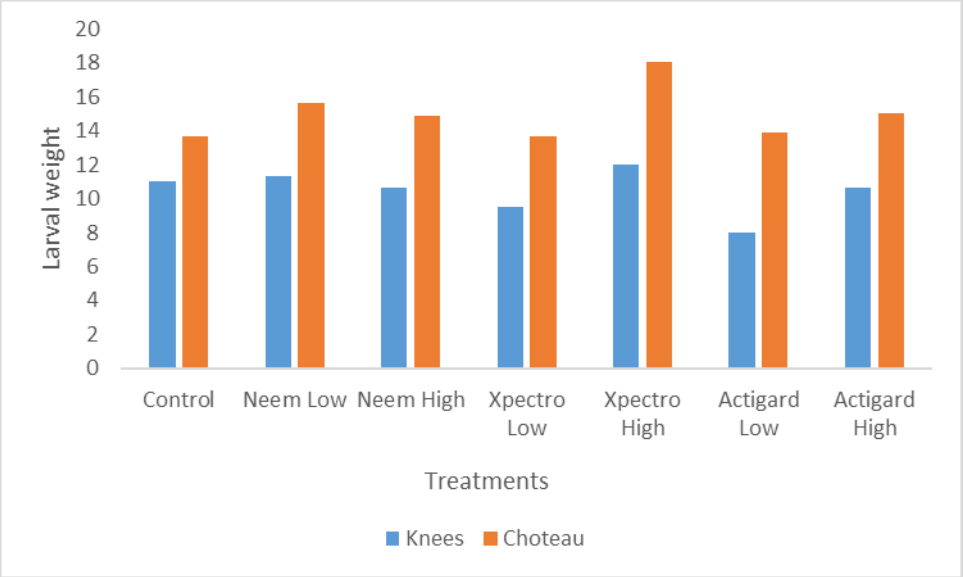

Figure 2: Larval body weight of diapausing WSS larvae at 50 DAT at Knees and Choteau locations in 2019.

Wheat stem lodging

No significant variation was found in lodging at both the sites Choteau (df = 48; F = 1.5; P = 0.5) and Devon (df = 24; F = 3; P = 0.16). However at Choteau site Neem low had less lodging whereas at Choteau site Actigard high had significant less lodging (Table 2).

Yield

In overall, higher average winter wheat yield was found in Choteau. At Knees site treatments had no significant impacts on winter wheat grain yield. At the Choteau site Actigard low and high both had significantly less yields compared to the other treatments (Table 2).

Acknowledgements

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Evaluation of the effectiveness of entomopathogenic fungus and trap crops for the management of wireworms on spring wheat

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Aim of the study

The aims of this study were: 1) to evaluate the effectiveness of trap crops for the management of wireworms and 2) to evaluate efficacy of entomopathogenic fungi for wireworm management under lab and field conditions.

Figure 1: Wireworms feeding on spring wheat and pupa of wireworm in the soil.

Material and Methods

Study sites

In 2019, two sites were selected for evaluating entomopathogenic fungus. Both sites were pre analyzed for presence of wireworms before commencing the experiments. One site was irrigated

(Choteau; N47^{o} 90.238' W112^{o} 23.802') and one site was non-irrigated (Pendroy; N48^{o} 56.009' W111^{o} 40.565'). The wireworm pressure was moderate to high in the selected sites.

Experimental design

In 2017 and 2018, different fungal strains on nutritive carriers (polenta, millet, and couscous) were tested in the field conditions. In 2019, the selected high performing EPFs were tested based on 2017 and 2018 results (Sharma et al. 2019). In 2019, these EPFs were applied in the selected fields with conventional farming. The non-irrigated Pendroy site had barley grown and irrigated Choteau site had spring wheat. In both fields, we asked growers to follow conventional farming practice. After the seeds were sown, next day the EPFs were applied in the furrows along the seeds with the help of a harrow (at the rate of 5gms/plot Table 1). The fungus formulations were prepared by USDA ARS, Sidney MT.

The experiment used a Randomized Complete Block Design (for EPF experiment) with a total of 36 plots [including four replications. Each plot was 4×4 m. Buffer zones of 1.5 m were maintained between plots. The non-irrigated field (Pendroy) barley (Hockett) was shown on 9 May 2019 @20 seeds/ft^2 with a spacing of 10 inches. Dry fertilizers with drill were applied. Five gallons/acre nitrogen was applied. No pre-emergence herbicides were applied. Before seeding Roundup was applied (16-20 ounces). Seeds were treated with imidacloprid (Gaucho® 600, Bayer Crop Science). In the irrigated field (Choteau) spring wheat was seeded (Clear field) at 7.5 inches spacing on 10 May 2019. Fertilizers 15 gallons of 'thirty two' nitrogen, 20-10-5-10 (140 gallons/acre) and 25 tonn of manure (120 pounds/acre) were applied. Beyond and Wildcard were applied at label rates as herbicides. Non-irrigated sites did not receive any water. Irrigated sites received 5 cm of water via overhead irrigation once a week. The treatments were, millet, couscous, *Beauveria bassiana* GHA millet, *Beauveria bassiana* GHA couscous, *Beauveria bassiana* ERL836 millet, *Beauveria bassiana* ERL836 couscous, *Metarhizium robertsii* DWR2009 millet, and *Metarhizium robertsii* DWR2009 couscous. All EPF treatments were applied with seeds in furrows (at the rate of 5gms/plot Table 1). The fields were seeded at Pendroy (14 May and 16 May), Choteau and Valier (24 May) and Ledger (29 May 2018). The first irrigation took place within 30 days of planting in irrigated fields. The irrigated field was harvested on 29 August 2019 and the non-irrigated field was harvested on 12 September 2019.

For the trap crop experiment, in 2019 green house experiment was setup based on the results generated in 2017 (Sharma et al. 2017). Six plastic containers (60cmx50cmx50cm) were established in green-house conditions. In every container sandy loam soil from WTARC fields was filled. Once the soil was moisturized first the chickpea seeds 'Orion' variety. After 10 days spring wheat 'Duclair' variety were seeded. The chickpeas were planted on one side of the container (5 seeds) and spring wheat (25 seeds) were planted on another side of the container (Figure 2). After, 7 days 10 wireworms (*Limonius californicus*) were released in the middle of the containers. One container was kept as a control container where no wireworms were released. After every 7 days plant damage was recorded. In 45 days the experiment was dismantled by de-rooting the plants and the number of wireworms were recorded. The number of wireworms associated with chickpeas and spring wheat was reported. The experiment was established two times. The first experiment was established on 14th June 2019 and the second was established on 22nd July 2019.

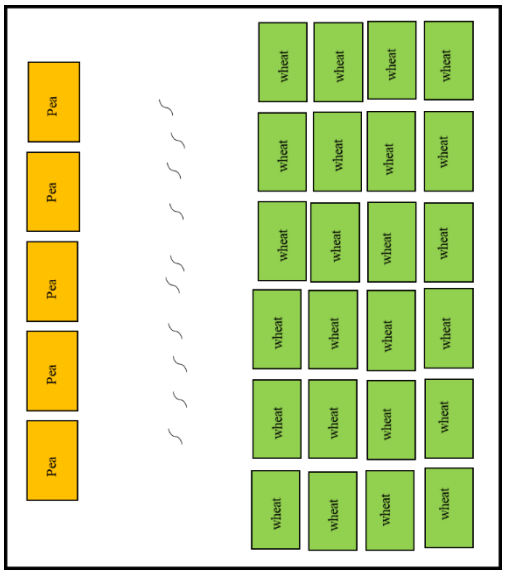

Figure 2: The schematic diagram of the trap crop experiment, where spring wheat seeds and chickpea seeds were planted to access the attractiveness of the chickpeas to wireworms.

Sampling for plant damage

To determine the level of crop damage from wireworms, the number of seedlings in each plot was measured randomly using the 1 m line intercept method. Two counts were taken from each plot, from the two middle rows. The first count was taken two weeks after planting. The starting and ending points of the sample areas (n = 2; each 1 m in length) were labeled with wooden stakes, so that the same group of seedlings could be recounted each time. Subsequent counts were taken at two-week intervals at both sites. At harvest, the height of these same marked plants was recorded using a wooden meter scale (Washington, USA).

Sampling for wireworm density

To determine the density of wireworm larvae, traps were established in each plot areas following the soil sampling bait-trap method of Reddy *et al.* (2014), which consisted of stockings filled with a mixture of wheat and barley. The traps were soaked for 24 hours to make the seeds sprout which is attractive to wireworms due to CO_2 release. The traps were buried in 8–15 cm deep hole and were covered with black plastic to provide an amenable environment to wireworms. Traps were then collected at 15 days and 30 days post-deployment for assessment of larval numbers. Traps with wireworms were brought to the Western Triangle Agricultural Research Center (WTARC), Conrad, Montana. At WTARC, traps were processed in Berlese Funnels (Bioquip products, California, USA; wooden stands set up for Berlese Funnel were built at WTARC) and wireworms were separately collected from each plot and identified using keys described by Etzler (2013).

Post-harvest data collection

Before harvesting, the plot's surface areas were calculated. After harvesting, wheat and barley from each plot were brought to the WTARC facility and cleaned using a seed cleaning machine (Almaco, Allan Machine Company, Iowa, USA). The plot and test weight was measured using a laboratory balance (Ohaus, Adventure™ Pro model AV8101). Wheat samples were processed

through a grain analyzer (Perten Instruments IM9500; Hägersten, Sweden) to determine grain moisture and protein.

Statistical analysis

Analysis of variance (ANOVA) was carried out using the PROC Mixed procedure in SAS 9.4 (PROC Mixed, SAS Institute 2018). Data were pooled for each replicate, and treatments were considered as fixed effects while the block was considered a random effect. Normality of data was tested with a Univariate procedure (PROC Mixed). Estimates of least square means and differences of least square means were evaluated (Type 3 test of fixed effects F-test). Multiple comparisons among the treatments were made using Fisher's Least Significant Test (LSD) at $\alpha = 0.05$ by using the standard error generated in ANOVA.

Results

Evaluation of efficacy of entomopathogenic fungus

In 2019, we applied selected EPFs based on the 2017 and 2018 results (Sharma et al. 2019). These EPFs were tested in irrigated and non-irrigated fields where seed treatment (imidacloprid) was also applied to the seeds. At Pendroy site with barley yield had no significant variation with any of the nine treatments (F=1008; df=24; P=0.96). Numerically higher yields were associated with Control plots. Whereas at Choteau site (irrigated site with spring wheat) four treatments (*Beauveria bassiana* ERL836 Couscous, *Beauveria bassiana* ERL836 Millet, *Metarhizium robertsii* DWR2009 Couscous, *Metarhizium robertsii* DWR2009 Millet) performed significantly better than other treatments and control (F=437; df=24; P=0.10). In terms of plant count and test weight of seeds no significant difference was found. At Pendroy site the greater wireworm pressure resulted in no yield with some treatments (Figure 3; Table 2).

Evaluation of the effectiveness of trap crops

In the trap crop experiment, according to last results (Adhikari and Reddy 2017; Sharma et al. 2018), pulses especially peas attracted wireworms towards them. In 2018 we established a spilt plot design to access the border cropping design and found no significant difference. However in 2018, at Northwest Agriculture Experiment Station MSU (Kalispell) it was recorded that in a variety trial experiment among different chickpea varieties wireworms preferred 'Orion' chickpea variety and fed on the Orion plots completely. Therefore in 2019 we decided to access the attractiveness of Orion chickpea with wheat plants. In two sets of experiments, the number of wireworms found associated with chickpeas and wheat plants were equal (n=50), however more damage was found with wheat plants (58%) in comparison to the chickpea plants (42%). When we compare with control container where no wireworms were released, in control container the germination and survival of chickpea plants was 80% and wheat plants was 76% and in treated containers (10) the survival rate of chickpea plants was 58% and wheat plant was 42.4% (Table 3; Figure 4).

Conclusion

In 2017, granular formulations of three EPFs, on polenta and millet spent substrate carriers, were applied in-furrow at planting, at two rates, against a water control and imidacloprid seed treatment in spring wheat in Montana, USA. The selected EPFs were *Beauveria bassiana* GHA, *Metarhizium robertsii* DWR356, *M. robertsii* DWR2009, applied as granular formulations at 11 kg ha−1 or 22

kg ha−1. In 2017, at Valier, DWR356, DWR2009 on millet carrier at 22.4 kg ha−1 provided greater yield, but all the treatments at the lower rate were still cost-effective. In 2018, *B. bassiana* GHA and *M. robertsii* DWR2009 were retested along with *B. bassiana* ERL836 and *M. brunneum* F52. Millet carrier alone, GHA and ERL836 on millet carrier obtained cost-effective results at irrigated and non-irrigated sites in 2018. However, these were less cost-effective than imidacloprid as a seed treatment. Earlier it was recorded that fungus along with seed treatment (imidacloprid) provide improved protection to the wheat plants (Antwi et al. 2018). Therefore in 2019, we selected high performing EPFs based on 2017 and 2018 results and applied in the fields where seed treatment was applied to the seeds. In 2019 at the non-irrigated site with barley crop no significant difference was observed with any EPF, rather control plots had a numerically higher yield. Nevertheless, in this field due to the high pressure of wireworms (<5 wireworms per trap) and a greater population of weeds, some of the plots had zero yield. Hence less yield cannot be directly related to the efficacy of EPFs. In the irrigated field with spring wheat crop B. bassiana ERL836 on couscous and millet carrier and *M. robertsii* DWR2009 on couscous and millet carrier both provided greater protection to the wheat plants.

In the trap crop experiment, the experiment was done in the green house to access the attractiveness of chickpea 'orion' variety to wireworms. This experiment indicated some degree of attractiveness if the wheat plants and chickpea plants are in close vicinity. An equal number of wireworms were associated with both the crops but greater damage was reported to the wheat plants.

Table 1: Materials, rates, and methods of application for treatments applied in study of wireworm control at non-irrigated (Pendroy) and irrigated site (Choteau), Montana in 2019. Plot size: 4x4 meters.

Treatment	**Material**	**Rate**	**Source**
T1:	Control (Water)		
T2:	Couscous	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T3:	Millet	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T4:	*Beauveria bassiana* GHA Couscous	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T5:	*Beauveria bassiana* GHA Millet	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T6:	*Beauveria bassiana* ERL836 Couscous	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T7:	*Beauveria bassiana* ERL836 Millet	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T8:	*Metarhizium robertsii* DWR2009 Couscous	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS
T9:	*Metarhizium robertsii* DWR2009 Millet	(10lb/acre) 18.5 gms/plot	Stefan T. Jaronski USDA ARS

Table 2: Impact of Entomopathogenic fungus treatments on spring wheat and barley performance in 2019 in terms of plant count, yield, and test weight. Standard error and least significant differences are calculated with the means generated by PROC MIXED analysis (α=0.05). The treatments were applied in Randomized Block Design (n=4); water was used as control.

Non-irrigated sites				Irrigated sites		
Pendroy				Choteau		
Treatments	Plant Count	Yield (kg/ha)	Test Weight (gms)	Plant Count	Yield (kg/ha)	Test Weight (gms)
Control (Water)	7.2±1.3a	1810±547a	0±15a	32±2.7a	1416±160ab	74±8.6b
Couscous	5.5±1.3a	1398±547a	17±15a	34±2.7a	1433±160ab	72.9±8.6b
Millet	5.3±1.3a	1279±547a	32±15a	34±2.7a	1346±160ab	72.3±8.6b
Beauveria bassiana GHA Couscous	6±1.3a	1412±547a	35±15a	34±2.7a	1374±160ab	71.9±8.6b
Beauveria bassiana GHA Millet	5.3±1.3a	1150±547a	33±15a	33±2.7a	1014±160b	98.5±8.6a
Beauveria bassiana ERL836 Couscous	5.6±1.3a	1408±523a	40±14a	33±2.7a	1559±143a	72.2±7.7b
Beauveria bassiana ERL836 Millet	5.2±1.3a	1310±547a	18±15a	33±2.7a	1606±160a	73±8.6b
Metarhizium robertsii DWR2009 Couscous	6.1±1.3a	1473±591a	0±18a	31±3.2a	1769±185a	72±10b
Metarhizium robertsii DWR2009 Millet	6±1.3a	1646±547a	0±15a	31±2.7a	1716±160a	73.4±8.6b
F (P)	2.2 (0.83)	1008 (0.96)	42 (0.39)	7.5 (0.99)	437 (0.10)	22 (0.45)

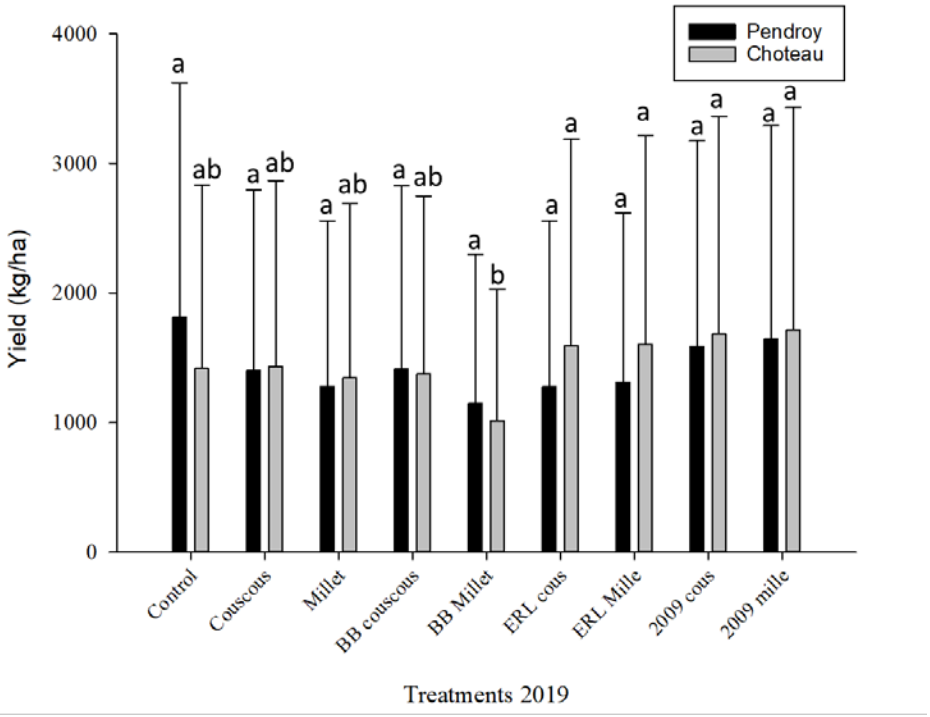

Figure 3: Mean yield (kg/ha) of wheat at irrigated field [Choteau; (□)] and barley at non-irrigated field [Pendroy; (■)] in 2019, [n=2]. Different letters above the bars indicate significant differences (α= 0.05). y-axis shows mean yield (mean yield+ SE) and x-axis indicates nine treatments.

Figure 4: Orion chickpea damaged by wireworms.

Table 3: Performance of 'Orion' chickpea and 'Duclair' spring wheat in containers.

	Container 1 Control		Container 2		Container 3		Container 4		Container 5		Container 6	
Experiment 1	No. of plants	No. of wireworms	No. of plants	No. of wireworms	No. of plants	No. of wireworms	No. of plants	No. of wireworms	No. of plants	No. of wireworms	No. of plants	No. of wireworms
Orion chickpea	4	0	1	3	3	2	3	7	4	5	4	5
Duclair wheat	18	0	8	7	16	8	9	3	8	5	8	5
Experiment 2												
Orion chickpea	4	0	2	5	2	6	4	4	3	6	3	7
Duclair wheat	20	0	10	5	15	4	11	6	9	4	12	3
Total chickpea	8	0	3	8	5	8	7	11	7	11	7	12
Total spring wheat	38	0	18	12	31	12	20	9	17	9	20	8

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Exploring the possibilities of entomopathogenic nematodes for wireworm management (Coleoptera: Elateridae)

Aim of the study

The different aims of the study were: (1) To test the efficacy of available EPN strains against wireworms in shade house and field, (2) To identify the native EPN strains in Golden Triangle Region of Montana, (3) To test the efficacy of native EPN strains against wireworms in shade house.

Materials and methods

Wireworm Collection: The larvae of different instars were collected from different locations (Conrad, Pendroy, and Kallispell fields). The wireworms were collected by using stocking traps. The stocking traps with soaked wheat seeds were placed in the different spots in soil and then covered with plastic sheets. After 15-20 days, the stocking traps were collected and brought back to the laboratory. These stocking traps were replaced every time we collected the old traps. The stocking traps with wireworms were placed in the Berlese Funnels for 12 hours and the wireworms were collected and categorized into small, medium and large based on their size. We found mainly three wireworm species viz. *Limonius californicus, Hypnoides bicolor,* and *Aeolus mellillus.* However, the wireworm specie, *L. californicus* is the dominant specie in this area, and subsequently used in further experiments. Wireworms were stored in 5 oz. plastic cups in sterilized sandy loam soil with wheat seeds as their food. These plastic cups were placed in an incubator at 8°C.

Objective 1

1.1 Efficacy of available EPN strains against wireworms in shade house and field

Material and methods

Ten nematode strains were obtained from Dr. David Shapiro (USDA ARS, Georgia). The list is given in Table 1 as follows:

Table 1. List of EPN strains (Source: Dr. David Shapiro, USDA-ARS, Georgia)

Entomopathogenic nematode species	Strain
Steinernema carpocapsae	All strain
	Cxrd strain
Steinernema feltiae	SN strain
Heterorhabditis bacteriophora	HP88 strain
	VS strain
Steinernema riobrave	355 strain
	7-12 strain
Heterorhabditis floridensis	K22 strain
Heterorhabditis georgiana	Kesha strain
Steinernema rarum	17 c + e strain

Four EPN strains (*S. carpocapsae* All and Cxrd strains and *S. riobrave* 355 and 7-12 strains) were found effective against *L. californicus* in the laboratory bioassay and shade house experiment in 2018. These four EPN strains were further tested against *L. californicus* in shade house experiment with modifications as well as field in 2019.

Nematodes rearing: Ten waxworm larvae were placed on filter paper in the petri dishes. 500-1000 Infective Juveniles (IJ)/ml for different strains were inoculated in the petri dishes with 100-200 IJ/waxworm larva. The petri dishes were left at room temperature for nematode infection. After 3-5 days, nematodes infected larvae were placed on the white traps for rearing. After 7 to 10 days, nematodes were collected from the white traps and stored in the tissue culture flasks.

Shade house screening: The experiment was conducted in a completely randomized block design with four EPN strains based on their efficacy in laboratory and shade house experiment in 2018. Plastic pots (16 cm diameter) were filled with approximately 2.4 kg of sterilized field-collected sandy loam soil (depth: 14 cm) with a surface area of 200 cm^2. The soil used was sandy loam soil (78% sand, 12% silt and 10% clay, pH 7.7, and 1.4% organic matter). Ten wheat seeds were planted in each pot and allowed to grow for 10 days. Ten wireworms were added to each pot after 10 days. After a further 24 hours, any larvae that did not enter the soil were replaced. Two concentrations [80,000 IJs/pot (400 IJs/cm^2) and 10,000 IJs/pot (50 IJs/cm^2)] were used for each of the four strains. These two concentrations were prepared according to Navon and Ascher (2000) and standardized as 4000 IJs/ml and 500 IJs/ml of tap water. The IJs, in 20 ml of water were inoculated into the pots with a pipette. The pots with the control treatment received 20 ml of plain water. There were five replicates (pots) for each concentration. The pots were placed in a shade house and watered daily (Figure 1). After 4 weeks, the pots were destructively sampled and the number of dead wireworm larvae observed. The dead larvae were dissected to confirm nematode infection. If a wireworm was not found, it was recorded as dead. The average air temperature in the shade house was 30°C (26-32°C) with average soil temperature and soil moisture of 20°C (11-35°C) and 21 ± 5%, respectively in pots. The whole experiment was conducted in two trials with an interval of 10 days in both trials. Plant damage, i.e. number of wheat seedlings damaged by wireworms, was observed in each pot at the end of the experiment and the average percentage of plant damage was recorded. The presence of wilted or dead central leaf and/or seedling death was the main criteria in observing plant damage.

Figure 1. Shade house screening of available EPNs against *L. californicus*

1.2 Efficacy of selected EPN strains against *L. californicus* in field

EPN source and production of cadavers

Greater wax moth larvae, *Galleria mellonella* L. (Lepidoptera: Pyralidae) were obtained from the Bassett's Cricket Ranch (CA, USA). The larvae were stored in the containers provided by the supplier at 10°C until used for culturing. EPN infected *G. mellonella* cadavers were used as a source of nematodes in field rather than aqueous suspensions to mimic the natural conditions. Ten *G. mellonella* larvae were exposed to approximately 200 freshly produced IJs of four selected EPN strains; *S. carpocapsae* (All and Cxrd strains) and *S. riobrave* (355 and 7-12 strains) in a 90 mm diameter Petri dish. These EPN strains were obtained from the USDA-ARS Entomopathogenic Nematode culture collection (Byron, GA). The petri dishes were held at room temperature (22°C) for 3-4 days. Overall, 80 cadavers were prepared for each EPN strain. The nematode infected cadavers were then transferred to individual White traps (Kaya and Stock 1997) to observe the initiation of IJs emergence for another 4-5 days at room temperature. The traps were checked daily for the initiation of IJs emergence from the cadavers. After 4-5 days, the cadavers that just started releasing IJs were used in field experiments to reduce the chances of variation due to initiation of emergence among replications.

Study sites

The field trials were conducted in a barley field (Pendroy: N48.04130°, W112.16945°) and a spring wheat field (Choteau: N47.9023°, W112.2330°) in Golden Triangle Region of Montana in 2019. Both the fields were selected on the basis of history of moderate to high wireworm pressure. The fields were tested to assure the presence/absence of naturally occurring EPN species and were found negative in respect to nematodes. According to NRCS (1999), the soils at Pendroy site had Rothiemay-Niart clay loams soil, with 0–4% slopes and Choteau site had Niart-Crago gravelly loams soil with 0–4% slopes (NRCS 1999).

Experimental design

The spring wheat (variety: Clear field) was seeded on May 9th 2019 and the barley field (Variety: Hockett) was seeded on May 10th 2019. The farmers at both the sites seeded the plots. The Choteau site (spring wheat) was managed as irrigated site and Pendroy site (barley) was managed under dryland farming practices. The row spacing was kept at 7.5 inch and 10 inch in spring wheat and barley fields, respectively. In spring wheat field, 15 gallons of '32' nitrogen, N-P-K (20-10-5) at the rate of 140 gallons/acre, and 25 tons of manure at the rate of 120 pound/acre were applied before seeding. The herbicides Beyond and Wildcard were applied at the label rate for weed control. Imidacloprid was used as seed treatment in both fields. In addition, Roundup at the rate of 16-20 ounces and liquid nitrogen at the rate of 5 gallon were applied before seeding.

The experiment was completely randomized block design including 33.7 m long and 17.6 m wide field plot with five 33.7 m × 1.52 m sections with 9 subplots (1.52 m × 1.52 m) in each plot. Each sub plot had four to five plant rows. There was 2.5 m buffer zone between each subplot in one row of nine subplots, to avoid the inter-specific competition between the EPN strains. Similarly, there was 2.5 m buffer zone between four rows of 33.7 m × 1.52 m sections to avoid effects from migration of EPNs and plot order was randomized at each location.

Two doses (3 and 6 cadavers per subplot) were tested with five replications. There were 5 replications for control subplots without any cadavers. Overall, there were 45 subplots (4 strains × 2 doses × 5 replications = 40 subplots + 5 subplots (Control)). A hand shovel was used to dig the soil and the cadavers were placed 5-8 cm beneath the soil surface and at least 10 cm away from each other five days after seeding. The holes with cadavers were covered with soil thereafter. In spring wheat field, cadavers were released in early morning at 9:00 AM in cloudy conditions with an average soil temperature of 5.5±4°C, average air temperature of 16°C, and average soil moisture percentage of 23.7%. However, in barley field, EPN cadavers were released in the evening at 7:00 PM in cloudy conditions with an average soil temperature of -6±2°C, average air temperature of 23°C, and average soil moisture percentage of 59%.

Plant Count

To observe the wireworm damage to wheat plants, number of seedlings in each plot were randomly counted using 1 m line intercept method. Two rows were selected from each plot and both ends of each row (1 m length) were marked with iron nails for plant counting. The first count from both rows (n=2) was taken three weeks after plant germination. The second and final plant count was made just before harvesting. At harvest, the height of these same marked plants was recorded using a wooden meter scale (Washington, USA).

Number of Wireworms/Wireworm sampling

Soil bait traps as explained by Reddy et al. (2014) were used to determine the wireworm density in the experimental plots. The traps were placed in the soil and collected back from the field in plastic bags, labeled and brought back to the Western Triangle Agricultural Research Center

(WTARC), Conrad laboratory for extraction. The larvae were sorted from the traps manually using Berlese funnels (Bioquip products, California, USA, built at WTARC). The collected wireworms were counted and identified using the Etzler (2013) key for wireworm identification. The traps were placed in both field twice a month starting 10 days after barley seeding in Choteau and 20 days after spring wheat seeding in Pendroy. The reason for delay in trap placement in Pendroy field was excessive rain in the field. The traps were replaced five times in both fields from June to August at two weeks intervals. Soil temperature and soil moisture were also recorded at the time of wireworm trap collection by using Soil Thermometer (Taylor, IL, USA) and soil moisture meter (Spectrum Technologies Inc., IL, USA), respectively. Air temperature was also checked every time the traps were replaced.

Emergence of IJs from cadavers

To determine IJ emergence rates from cadavers applied in the field, randomly selected 15 infected cadavers were removed from the treatment batches for all the four EPN strains and placed individually on separate white traps at room temperature. Emerging IJs were collected, washed with tap water for 2-3 times, counted by serial dilution method by following the procedure of Navon and Ascher (2000). The emerging IJs were collected and counted until emergence stopped (3 weeks).

IJs persistence

The IJs persistence or survival of EPN IJs was observed in the treatment plots in August 2019 (one month before harvesting). Five soil core samples (approx. 100 g each) were taken from each plot by a hand shovel and mixed together to make a composite sample. The hand shovel was washed with water and rinsed with 75% ethanol in between plots to avoid contamination. Overall, there were 45 composite samples from each plot. These composite samples were kept in plastic bags separately in a thermal cooler and brought back to WTARC laboratory. EPN IJs were recovered from the soil samples using the insect baiting technique (Bedding and Akhurst 1975). Approximately 300 g soil sample from each composite sample was transferred to a 500 ml plastic container with ten *G. mellonella* larvae in each cup. The containers were kept in the dark at room temperature (22 ± 2°C). After seven days of incubation, the dead larvae were removed and rinsed with tap water. The dead larvae that showed signs of infection with EPNs, i.e. placid soft odorless larvae with either pale yellowish to brown or black color were recorded as dead because of EPN infection. The dead larvae were also dissected to confirm the IJ presence. The dead *G. mellonella* larvae were averaged over the replication to observe the mean larval mortality.

Post-harvest data collection

After harvesting, wheat and barley grains from each plot were brought to the WTARC facility and cleaned using a seed cleaning machine (Almaco, Allan Machine Company, IA, USA). The plot and test weight were measured using a laboratory balance (Ohaus, Adventure™ Pro model AV8101). Wheat and barley samples were processed through a grain analyzer (Perten instruments IM9500; Hägersten, Sweden) to determine grain moisture and protein. About 300 g of sample for each plot was processed to obtain protein and moisture content. Plot weight and moisture were used to calculate yield.

Objective 2

2.1 To identify the native EPN strains in Golden Triangle Area of Montana

In 2018, soil samples were collected from 30 different fields from Pendroy, Choteau, Valier, Conrad, Kallispell, Knees, Brady, Collins, Dutton, Shelby, Sunburst, and Tiber areas were collected in Golden Triangle Area of Montana. These soil samples were observed for presence of naturally occurring EPNs in Montana. Different samples were found positive with nematodes.

However, the nematodes collected from some soil samples were not able to reproduce further indicating that these might be other bacterivore nematodes other than EPNS. Overall, three EPN species were found from all the soil samples collected. These isolates were sent to Monte L. Bean Museum, and Evolutionary Ecology Laboratories, Brigham Young University, Provo for molecular identification.

2.2 Molecular identification

For DNA extraction, pooled EPN IJs of each isolate were macerated with a plastic pestle in 1.5 ml centrifuge tube and genomic DNA was extracted using Qiagen DNeasy® Blood and Tissue kit (Waltham, MA) by following manufacturer's protocol. The extracted DNA was concentrated to 20 µl using Eppendorf Vacufuge Plus Vacuum Concentrator (Hamburg, Germany). A part of rDNA comprising the internal transcribed spacer regions (ITS), ITS1 and ITS2 including 5.8S were sequenced using two sets of primers. Primer set ITS-F (5'-TTGAACCGGGTAAAAGTCG-3 and ITS-R (5'-TTAGTTTCTTTTCCTCCGCT-3') was used to sequence the entire ITS1, 5.8S and ITS2 regions while primer set Fnema18S (5'-TTGATTACGTCCCTGCCCTTT-3') and rDNA1.58S (rev) (5'-ACGAGCCGAGTGATCCACCG-3') pair targeted the ITS1 region. Each PCR reaction was carried out in a total volume of 30 µl consisting of 9 µl of DNA template, 15 µl of JumpStart™ REDTaq® ReadyMix (Sigma-Aldrich, St. Louis, MO), 2.4 µl of each primer and 1.2 µl of molecular grade water. The PCR conditions included initial denaturation at 94^0C for 5 minutes, 40 cycles of denaturation at 94^0C for 30 s, 40 cycles of annealing at 48^0C for 30 s, 40 cycles of extension at 0.5^0C/sec for 90 s and a final extension at 72^0C for 5 minutes. The PCR products were analyzed for expected DNA band weights on 1% agarose gel run at 150V for 20 minutes. PCR products were treated with ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol to digest excess primers and nucleotides. The products were sequenced bidirectionally with their PCR primers using Bigdye reaction chemistry on an ABI ABI3730xl. Primer sequences were removed from chromatograms, aligned and edited manually in Geneious Prime 2019.2.1 (http://www.geneious.com). Each species was identified via BLASTn (NCBI; http://www.ncbi.nlm.nih.gov) against the nucleotide collection (nr/nt) database using default search parameters.

Overall, 18 samples out of 150 samples were found to have nematodes. However, we were able to culture only three samples. This might be due to a number of factors like *Galleria* as inappropriate host for some particular species, unfavorable environmental conditions in the laboratory, low number of nematodes present in the soil samples to infect the host. Three nematode species were named as KP, MLS1, and MLS2 and identified using morphological and molecular techniques. Both species named KP and MLS1 were found to be *Steinernema feltiae* and MLS2 was identified as *Heterorhabditis bacteriophora*.

Objective 3: To test the efficacy of native EPNs against wireworms in laboratory and shade house

3.1 Efficacy of native EPNs against medium sized *L. californicus* larvae (Laboratory concentration response assay)

Heterorhabditis bacteriophora, S. feltiae 1 and *S. feltiae* 2 were tested against medium sized *L. californicus* larvae in a laboratory bioassay. 500 ml plastic cups were filled with 150 gm of soil (soil surface area: 114 cm^2). The soil used was sandy loam soil (78% sand, 12% silt and 10% clay, pH 7.7, and 1.4% organic matter). Before use, the soil was autoclaved at 121°C for 1 hr and left at room temperature for one week for acclimatization. Five medium sized wireworm larvae were

introduced into each cup with eight to ten germinated wheat seeds as food. The larvae that did not enter the soil within 12 hrs were replaced. Four concentrations [3500 IJs/cup (30 IJs/cm^2), 7000 IJs/cup (60 IJs/cm^2), 14,000 IJs/cup (120 IJs/cm^2), and 28,000 IJs/cup (240 IJs/cm^2)] were tested for all three test EPN species. The concentrations were prepared by counting the desired number of IJs into 100 µl to 1 ml of tap water (depending on the concentration) in a nematode counting slide (Chalex, LLC, Park City, UT, USA) under a compound microscope by following Navon and Ascher (2000) formula. Ultimately, four doses for three species were standardized and adjusted as 5600, 2800, 1400, and 700 IJs per ml of water and stored in the tissue culture flasks at 8°C in an incubator. The IJs were used within 15 days of culturing.

Before application, EPNs were transferred from 8°C to room temperature for 12 h for acclimatization. The viability of IJs was checked under the microscope before application. The IJs were pipetted onto the soil surface in 1 ml of IJs suspension, and the control cups received one ml of tap water without any IJs. The final soil moisture was adjusted to 18% (v/w) that may be prevalent in Montana soils during EPN application. There were five replications for each of the four concentrations for all EPN species. The bioassay was conducted twice, on various dates from June to August 2018. The cups were placed in trays with approximately 10 holes in the lids and placed in an incubator at 22°C and 75% RH in the dark. 22°C temperature in the bioassay is an average temperature during the wireworm activity in the field and can be targeted for EPN application. Larval mortality was observed weekly for four weeks.

3.2 Effect of soil texture on the efficacy of native EPNs against *L. californicus* in laboratory

Steinernema feltiae 1 and *S. feltiae* 2 isolates were tested in this laboratory bioassay. Four different types of soils as described in Table 1 were used in this study. Different soils were sterilized in an autoclave at 121°C for 1 hr to kill natural nematode populations and other microorganisms. The soils were left at room temperature for at least two weeks for acclimatization. 500 ml plastic cups were filled with 150 gm of soil (soil surface area: 140 cm^2) for different soils. Five medium sized wireworm larvae were introduced into each cup with 8-10 germinated wheat seeds as food. The larvae that did not enter soil within 12 hrs were replaced. After 24 hrs, 7000 IJs/cup (50 IJs/cm^2) were inoculated in one ml of water into each cup. This dose was prepared by following the same procedure as mentioned in section 2.3. Control cups received only one ml water without IJs. The final moisture content was standardized at 18% (v/w) for all the soil types. The reason for standardizing the moisture was to compare the EPN efficacy at same moisture level. After inoculation, the cups were placed in an incubator at 22°C and 75% RH in dark conditions. The moisture was provided every two to three days to maintain the moisture in the cups. The wireworm mortality was assessed at weekly intervals for four weeks. There were five replications for each treatment. The experiment was conducted twice with an interval of two weeks between trials and different nematode cultures were used for both trials.

3.3 Efficacy of selected EPN strains against *L. californicus* larvae in shade house trial

The experiment was conducted in a completely randomized block design with *S. feltiae* 1 and *S. feltiae* 2 as treatments. Plastic pots (14 cm diameter) were filled with approximately 1.7 kg of sterilized field-collected sandy loam soil (depth 9 cm) with a surface area of 150 cm^2. The soil was 78% sand, 12% silt, and 10% clay, with pH 7.7 and 1.4% organic matter. Ten wheat seeds were planted in each pot and allowed to grow for 10 days. Five wireworms were added to each pot after 10 days. After further 24 hours, any larvae that did not enter the soil were replaced. Two concentrations [60,000 IJs/pot (400 IJs/cm^2) and 7,500 IJs/pot (50 IJs/cm^2)] were used for each of the two *S. feltiae* isolates. These two concentrations were standardized as 6000 IJs/ml and 750 IJs/ml of tap water. The IJs, in 10 ml of water were inoculated into the pots with a pipette. The

pots with the control treatment received 10 ml of plain water. There were five replicates (pots) for each concentration. The pots were placed in a shade house and watered daily. After four weeks, the pots were destructively sampled and the number of dead wireworm larvae observed. The dead larvae were dissected to confirm nematode infection. If a wireworm was not found, it was recorded as dead. Soil temperature and moisture were observed three times each week throughout the experiment using a soil moisture meter (Spectrum Technologies Inc., Illinois, USA) and soil thermometer (Taylor, Illinois, USA). The average air temperature in the shade house was 31°C (26-35°C) with average soil temperature and soil moisture in the pots was recorded as 22°C (11-35°C) and 21±5%, respectively. The whole experiment was conducted in two trials from May to August 2019 with an interval of 10 days in both trials. Plant damage, i.e. number of wheat seedlings damaged by wireworms, was observed in each pot at the end of the experiment and the average percentage of plant damage was recorded. The presence of wilted or dead central leaf and/or seedling death was the main criteria in observing plant damage.

Statistical Analysis: GLM with binomial/quasibinomial distribution was used for the laboratory and shade house experiments in case of both available and Montana native EPNs. The data for EPN efficacy against wireworms were analyzed separately for both field because crops were different in both fields. The data regarding IJs emergence in the laboratory, number of wireworms collected, yield, plant count, plant height, test weight, moisture and protein content were subjected to Analysis of variance. Wireworm number data in Pendroy site was normalized using log transformation. The data with number of *Galleria mellonella* larvae infected with EPNs in the soil samples taken from the field were analyzed using GLM with quasibinomial distribution to avoid the overdispersion problem. Tukey-Kramer test was used to get the significant differences between the treatments. Data were analyzed using the software statistical package R 2.15.1 (R Development Core Team, 2017).

Results and discussion

1.1 Efficacy of available EPN strains against wireworms in shade house in 2019

The wireworm mortality proportions differed significantly between the two trials ($\chi^2 = 26.37$, df = 1, $P < 0.0001$), but interaction between trail and strain was not significant ($\chi^2 = 2.795$, df = 4, $P = 0.59$) and therefore the data for two trials were pooled for further analysis. EPN strains had a significant impact on wireworm mortality ($\chi^2 = 91.89$, df = 4, $P < 0.0001$). Wireworm mortality also differed significantly among concentrations ($\chi^2 = 8.43$, df = 1, $P = 0.004$). However, there was no significant interaction between EPN strain and concentration ($\chi^2 = 9.36$, df = 4, $P = 0.052$). There were no significant differences among EPN strains in terms of wireworm mortality (Figure 2). The wireworm mortality due to EPN strains ranged from 34 to 56% when applied at 80,000 IJs/pot with 56% and 50% mortality caused by *S. riobrave* 355 and *S. carpocapsae* All strains, respectively. The mortality percentage was 35-40% when EPNs were applied at the rate of 15,000 IJs/pot. However, the wireworm mortality was significantly lower in control (13%).

The two trials in 2019 differed significantly in regards to the rate of plant damage ($\chi^2 = 22.99$, df = 1, $P < 0.0001$), and were therefore analyzed separately. In trial one, plant damage was not significantly affected by EPN strain ($\chi^2 = 7.42$, df = 4, $P = 0.12$) or concentration ($\chi^2 = 1.02$, df = 1, $P = 0.312$). No significant interaction was found between EPN strain and concentration ($\chi^2 = 1.04$, df = 4, $P = 0.904$). None of the four EPN strains tested differed significantly from that of the control, all being in the range of 34-52%, the highest level (52%) being caused when S. *carpocapsae* Cxrd was applied (Figure 3A). However, in trial two, EPN strain and concentration both had significant effects on plant damage (EPN strain: $\chi^2 = 19.29$, df = 4, $P = 0.0007$; Concentration: $\chi^2 = 4.29$, df = 1, $P = 0.038$). In addition, a significant interaction was observed

between EPN strain and concentration (χ^2 = 10.69, df = 4, *P* = 0.030). *Steinernema riobrave* 7-12 strain was associated with the highest plant damage at 44% followed by 40% plant damage in the control treatments (Figure 3B). For *S. carpocapsae* All, *S. carpocapsae* Cxrd, and *S. riobrave* 355 plant damage did not exceed 28%.

1.2 Efficacy of selected EPN strains against *L. californicus* in field

In June, the Pendroy (barley) and Choteau (spring wheat) sites had soil temperature varied from 6-14±5°C with 15-20°C air temperature. In July, both sites were observed with 15-22±5°C soil temperature and 22-27°C air temperature. In beginning of August, the soil temperature was higher in Pendroy site (20±5°C) with 16±2°C soil temperature at Choteau site. However, the soil moisture varied between two sites. At Pendroy site, the soil moisture content was almost twice as compared to Choteau site. The soil moisture content at Pendroy site was 55.8-56.2±3%, 43-48±5%, and 55.38±5% in June, July, and first week of August, respectively. However, the soil moisture content at Choteau site was 22-29±5%, 25.88-32.24±7%, and 31.02±8% in June, July, and August, respectively. The IJs emerged from one cadaver in the laboratory varied significantly among four EPN strains (F=8.55, df=3, p<0.0001). EPN strains *S. carpocapsae* Cxrd, *S. riobrave* 7-12 and *S. riobrave* 355 produced significantly higher number of IJS (248,500 to 270,270) as compared to 181,860 IJs produced by *S. carpocapsae* All strain (Figure 4).

The data were analyzed separately for both sites, as the crops were different at both sites. At Pendroy site, all the three wireworm species were found with *L. californicus* as the dominant species with greater numbers (~75%) followed by *A. mellilus* (~15%) and *H. bicolor* (~10%). However, at Choteau field site with barley, only *H. bicolor* was observed. Wireworm larvae found at both the field sites were of almost all the instar stages. The data regarding total number of wireworms collected throughout the season were statistically analyzed. However, the data for number of wireworms collected at different time intervals are provided in Table 2. The wireworm pressure was high in Pendroy site as compared to Choteau site (Figure 5 and Table 2). Overall, in Pendroy, wireworm number trends remained almost same from June to July but more wireworms were collected in the beginning of August (Table 2). However, the number of wireworms collected remained same throughout the collection time in Choteau.

At Pendroy site, total mean number of total wireworms (log transformed) collected in the season did not differ significantly (Figure 5) among EPN strains (F=0.62, df=4, p=0.65) and dose (F=0.11, df=1, p=0.74). The interaction between EPN strains and dose was also non-significant (F=0.12, df=4, p=0.97). Similar trend was seen at Choteau site where wireworm number did not vary significantly due to EPN strains (F=1.20, df=4, p=0.33) and dose (F=1.78, df=1, p=0.19). The interaction between EPN strain and dose was also not significant for wireworm numbers (F=0.46, df=4, p=0.76). There was a major problem of weeds and volunteer plants at both the sites and plant count done at three weeks after planting could not be considered accurate further. Therefore, data for plant count before harvesting is being analyzed further. At Choteau site, no parameters (yield, test weight, plant count, plant height, moisture, and protein) varied significantly due to different treatments and doses (Table 3). Similarly, none of the post-harvest and other parameters (yield, test weight, plant count, plant height, moisture and protein) differed significantly among EPN strains and doses (Table 4).

The data regarding number of *Galleria mellonella* larvae infected in soil samples taken were analyzed separately for both fields. At Pendroy site, EPN dose had a moderate level of effect on the *Galleria* mortality (χ^2=3.52, df=1, p=0.06). However, EPN strain alone and the interaction of EPN strain and dose did not have a significant effect on the *Galleria* mortality (EPN- χ^2=6.94, df=4, p=0.14; EPN:Dose- χ^2=3.34, df=3, p=0.34). However, at Choteau site, EPN strains had

significant effect on the *Galleria* mortality (χ^2=17.30, df=4, p=0.002). The interaction between EPN strain and dose was also significant (χ^2=16.44, df=3, p<0.0001). However, dose did not have significant effect on the *Galleria* mortality (χ^2=1.50, df=1, p=0.22). Overall, the *Galleria* percentage mortality was very low at Choteau site as only 25% average mortality was observed in samples collected from plots with *S. carpocapsae* infected cadavers (Figure 6). However, at Pendroy site, the Galleria mortality was observed to be higher than Choteau site. There were no significant differences among EPN strains in terms of percentage larval mortality at Pendroy site. The percentage *Galleria* mortality ranged from 30-45% at Pendroy.

Overall, EPNs applied in the form of infected cadavers were observed not to prevent wireworm damage in crops as well as protecting yields at both sites.

Objective 3. To test the efficacy of native EPNs against wireworms in laboratory and shade house

3.1 Efficacy of native EPNs against medium sized *L. californicus* larvae (Laboratory concentration response assay)

No three-way interaction was found between EPN strain, dose, and time to mortality (χ^2=14.81, df=27, p-value=0.97), and therefore this factor was also removed from the model. There were significant differences among four EPN strains (χ^2=445.38, df= 3, p-value<0.0001), dose (χ^2=59.13, df= 3, p-value<0.0001), and time (χ^2=171.22, df= 3, p-value<0.0001) with respect to wireworm larval mortality. The interaction between EPN strain and dose (χ^2=24.26, df= 9, p-value=0.004) and EPN strain and time to mortality (χ^2=16.96, df= 9, p-value=0.04) had significant effect on the wireworm larval mortality proportions. However, no significant interaction was detected between dose and time (χ^2=2.13, df= 9, p-value=0.98) on the wireworm larval mortality. No wireworm mortality was observed in the control treatment. On average, wireworm larval mortality increased with higher nematode concentrations, from 3500 to 28,000 IJs/cup. After one week, the larval mortality did not even exceed 15% in all the three tested EPNs at all the concentrations applied (Figure 7). This mortality trend remained almost the same after two weeks with mortality ranging from 12 to 30% with 30% mortality caused by *S. feltiae* 1. However, the larval mortality increased after three weeks of treatment. When EPNs were applied at the rate of 28,000 IJs/cup, *Steinernema feltiae* (1 and 2) caused significantly higher (48-50% mortality) as compared to only 24% mortality caused by *H. bacteriophora* after four weeks of treatment. *Steinernema feltiae* 1 and 2 isolates and *H. bacteriophora* did not differ significantly in terms of wireworm mortality when applied at 14,000 IJs/cup with 52% mortality caused by *S. feltiae* 1.

3.2 Effect of soil texture on the efficacy of native EPNs against *L. californicus* in laboratory

No significant differences observed between two trials in terms of *L. californicus* larval mortality [(trial: χ^2=0.99, df= 1, P=0.318); (trial:strain: χ^2=0.07, df= 2, P=0.97)], therefore the data were pooled together for further analysis. The interaction between EPN strain and time on wireworm mortality was not significant (χ^2=1.55, df= 6, P=0.96), therefore data for cumulative wireworm mortality after 4 weeks of treatment is being analyzed further. Two EPN strains had significant effect on *L. californicus* mortality (χ^2=74.29, df=2, P<0.0001). However, wireworm mortality caused by different EPN strains did not differ significantly among four soil types (χ^2=0.36, df= 3, P=0.95). In addition, no significant interaction was observed between EPN strain and four soil types (χ^2=2.54, df= 6, P=0.86). No wireworm mortality was observed in the control treatment. Similarly, the wireworm mortality caused by two isolates of *S. feltiae* did not differ significantly from control (P>0.05) in different soil types (Figure 8). Overall, the wireworm mortality caused by *S. feltiae* did not exceed 25%.

3.3 Efficacy of selected Montana native EPN strains against *L. californicus* larvae in shade house trial

There were no significant differences observed between two trials repeated in time in respect to *L. californicus* larval mortality (χ^2=0.621, df= 1, P=0.430), therefore the data were pooled together for further analysis. EPN strains had significant effect on the wireworm mortality proportions (χ^2=6.64, df= 2, P=0.04). However, two doses did not differ significantly in regards to wireworm mortality proportions (χ^2=1.20, df= 1, P=0.27). Additionally, no significant interaction was detected between EPN strains and dose (χ^2=0.65, df= 2, P=0.72) on wireworm mortality. *Steinernema feltiae* 1 and *S. feltiae* 2 did not differ significantly from control in regards to *L. californicus* mortality (Figure 9). Overall, *L. californicus* mortality ranged from 15 to 25% when EPNs were applied at the rate of 60,000 IJs/pot and 7500 IJs/pot.

Two trials repeated in time did not differ significantly in respect to plant damage caused by *L. californicus* (χ^2=3.67, df= 1, P=0.06). Similarly, the interaction between EPN strains and trials was not significant (χ^2=3.77, df= 2, P=0.15), therefore the data were pooled together for further analysis. EPN strains did not have significant effect on plant damage (χ^2=0.14, df= 2, P=0.93). *L. californicus* larval mortality did not differ between two doses applied (χ^2=0.63, df= 1, P=0.43). No significant interaction was detected between EPN strains and the doses applied (χ^2=1.27, df= 2, P=0.53). None of the two isolates of *S. feltiae* tested differed significantly from control treatment in respect to plant damage (Figure 10). The plant damage caused by *L. californicus* in the presence of *S. feltiae* ranged from 30-40%. *Limonius californicus* larvae were able to cause 36% plant damage in control treatment as well.

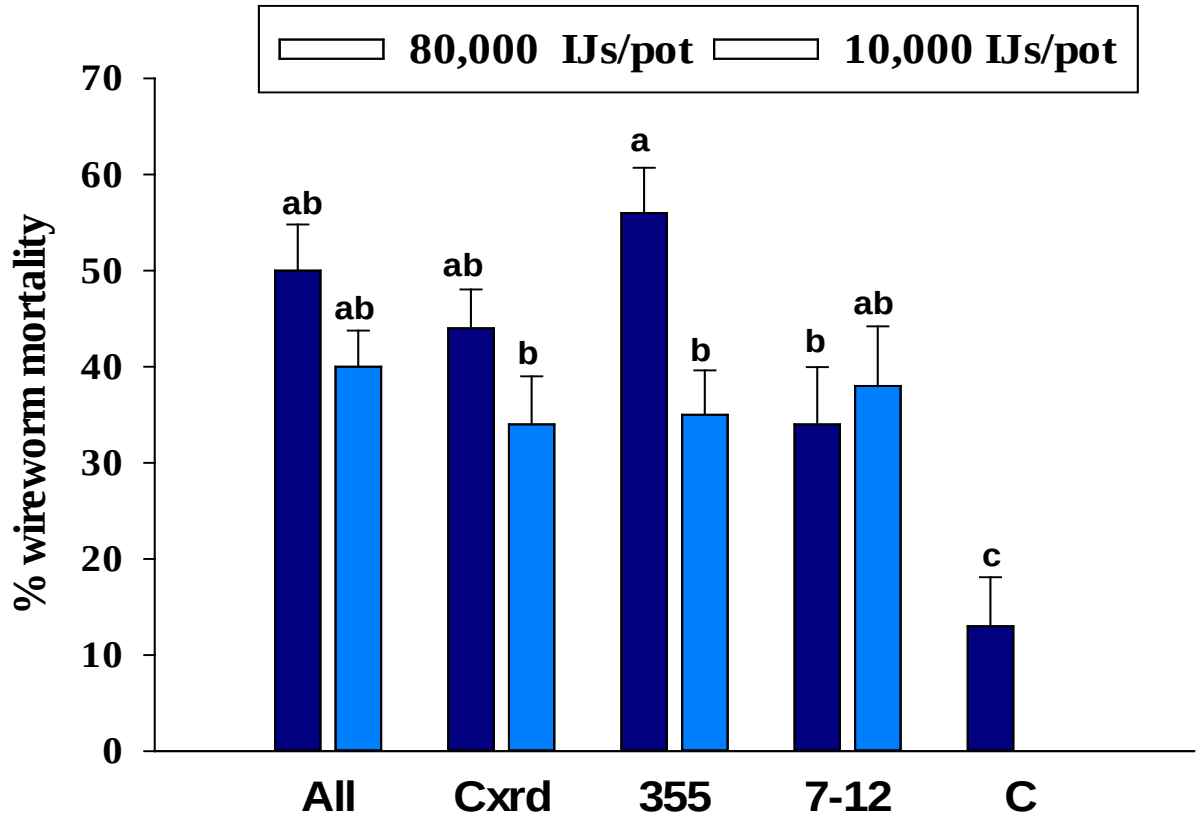

Figure 2. Average percentage mortality of larval *Limonius californicus* after exposure to entomopathogenic nematodes at 80,000 Infective Juveniles (IJs)/pot and 10,000 IJs/pot in shade house in 2019. All = *Steinernema carpocapsae* All; Cxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12; Con = Control. Different letters above bars indicate statistical significance ($P \leq 0.05$, Tukey-Kramer test).

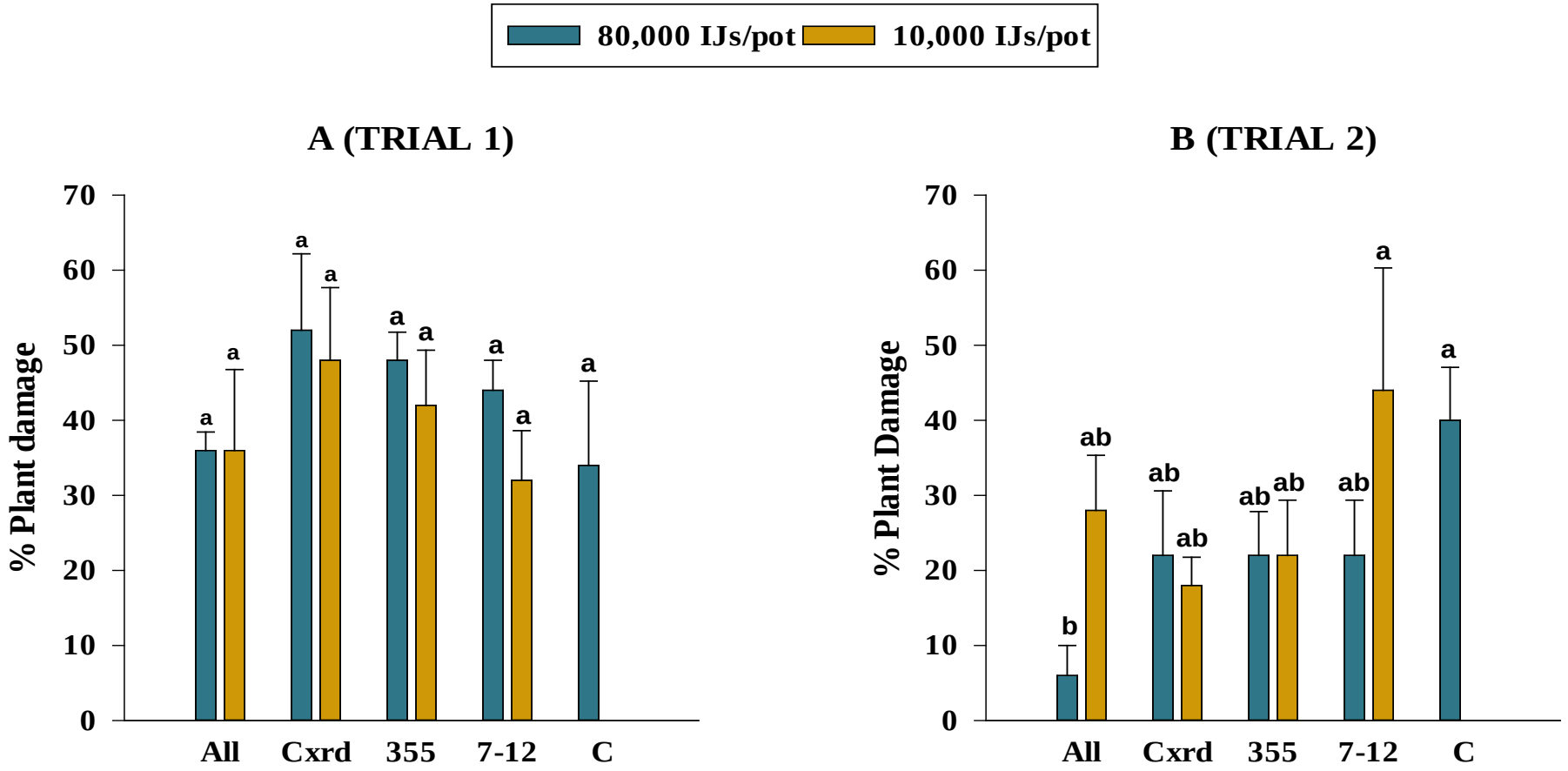

Figure 3. Average percentage plant damage by larval *Limonius californicus* after exposure to entomopathogenic nematodes at 80,000 Infective Juveniles (IJs)/pot and 10,000 IJs/pot in trial 1 (A) and trial 2 (B) in shade house in 2019. All = *Steinernema carpocapsae* All; Cxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12; Con = Control. Different letters indicate statistical significance ($P \leq 0.05$, Tukey-Kramer test).

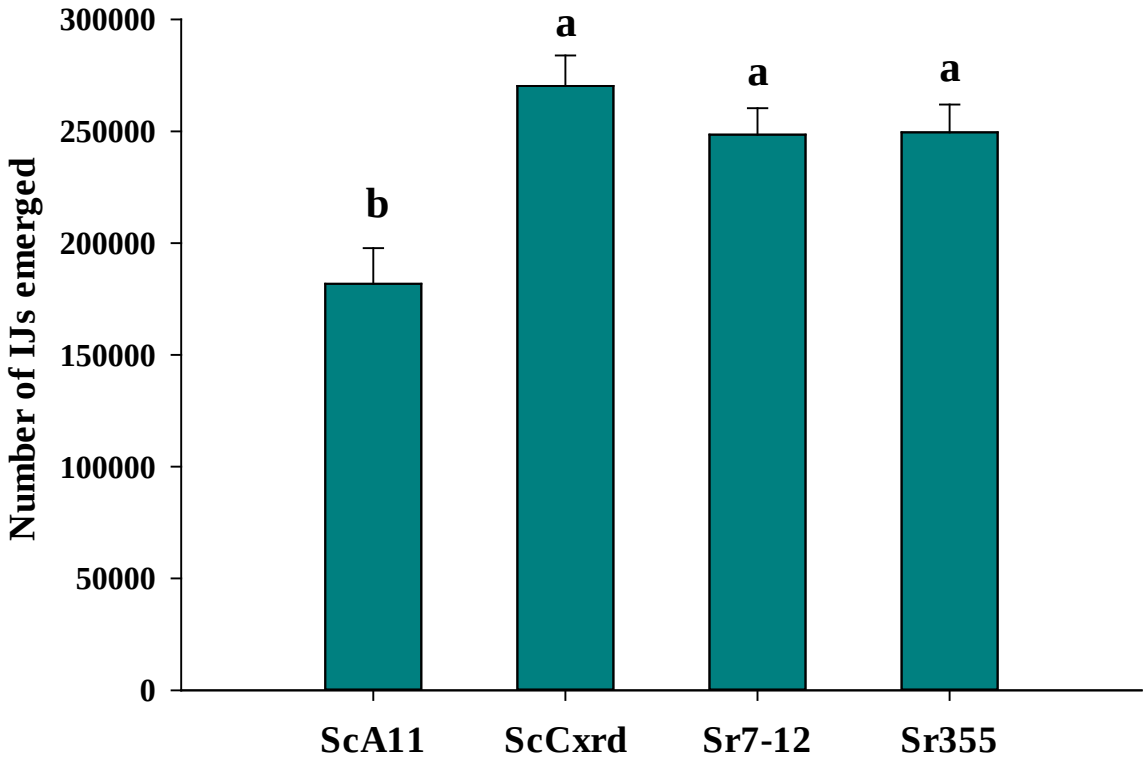

Figure 4. Mean number of IJs emerged from a cadaver infected with different EPN strains where where ScAll = *Steinernema carpocapsae* All; ScCxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12.

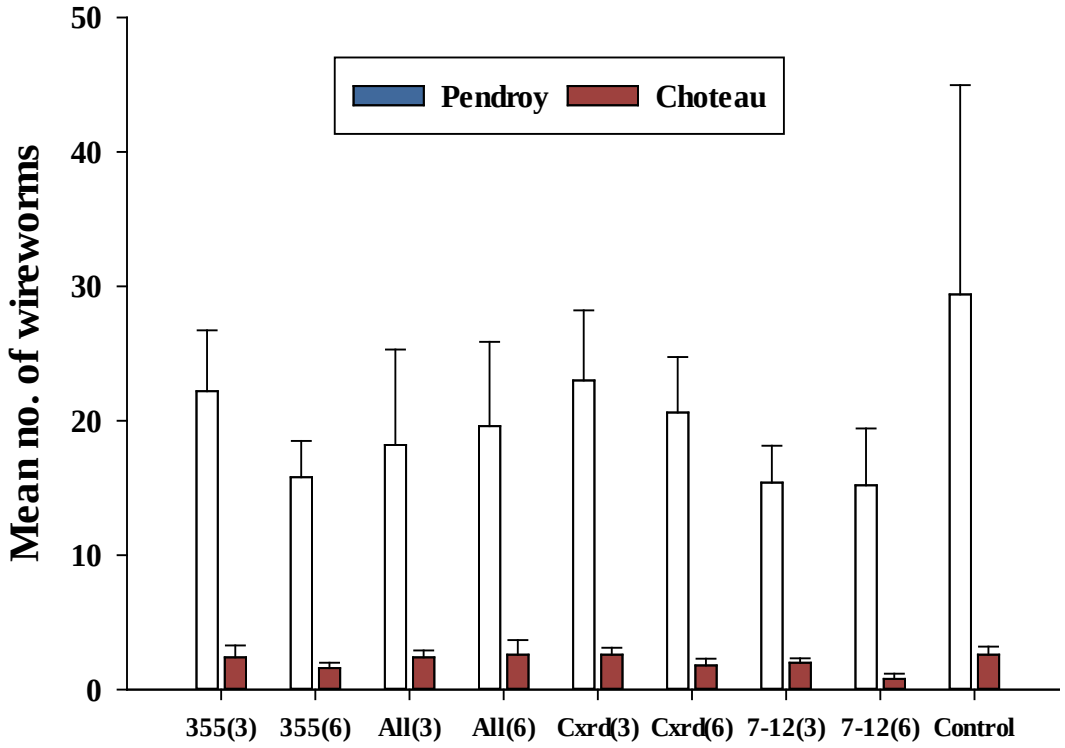

Figure 5. Total mean numbers of wireworms collected in bait traps after 75 days in 2019 [n=5]. No significant difference was found between treatments (α = 0.05, Tukey-Kramer test), where ScAll = *Steinernema carpocapsae* All; ScCxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12.

Table 2. Number of wireworms collected from treatment plots in 2019.

Pendroy (Barley)*						
Treatment	12 June 2019	27 June 2019	10 July 2019	25 July 2019	9 August 2019	**Total**
Sr7-12(3)	12	9	10	16	30	77
Sr7-12(6)	9	15	9	21	22	76
Sr355(3)	3	26	16	30	36	111
Sr355(6)	13	14	5	12	35	79
ScAll(3)	13	17	13	24	24	91
ScAll(6)	7	23	9	21	38	98
ScCxrd(3)	2	12	9	35	57	115
ScCxrd(6)	9	21	22	25	26	103
Control	18	33	14	26	56	147
Choteau (Spring wheat)						
Treatment	3 June 2019	18 June 2019	3 July 2019	17 July 2019	31 July 2019	**Total**
Sr7-12(3)	4	0	2	0	4	10
Sr7-12(6)	0	0	1	2	1	4
Sr355(3)	7	0	1	2	2	12
Sr355(6)	0	2	3	1	2	8
ScAll(3)	2	4	2	3	1	12
ScAll(6)	3	4	0	2	4	13
ScCxrd(3)	1	6	3	0	3	13
ScCxrd(6)	2	4	1	1	1	9
Control	2	3	3	3	2	13

***At Pendroy site, wireworm sampling was late for nine days as compared to Choteau site because of heavy rain at Pendroy during that nine days period.**

Table 3. Plant count, plant height, moisture, protein, seed test weight, and yield in the EPN treated plots (Mean ± *SE*) in Choteau (Spring wheat) in 2019.

Treatment	Plant Count	Plant height (cm)	Moisture (%)	Protein (%)	Test weight (kg/ha)	Yield (kg/ha)
Sr7-12(3)	33.8±2.3[a]	81.7±1.52 [a]	11.89±0.30 [a]	12.32±1.92 [a]	77.89±1.17 [a]	2050.15±330.91 [a]
Sr7-12(6)	37±2.07 [a]	69.6±3.31 [a]	11.79±0.06 [a]	11.06±0.44 [a]	81.19±1.83 [a]	1525.90±160.81 [a]
Sr355(3)	34.5±2.80 [a]	69.6±5.01 [a]	11.42±0.16 [a]	13.89±0.85 [a]	76.49±1.69 [a]	1495.58±265.91 [a]
Sr355(6)	36±3.5 [a]	76.1±5.28 [a]	11.82±0.23 [a]	11.69±1.30 [a]	79.05±0.98 [a]	1598.19±371.44 [a]
ScAll(3)	34.6±2.5 [a] 8	74.1±3.59 [a]	11.78±0.20 [a]	11.90±1.27 [a]	80.42±1.14 [a]	1818.65±353.65 [a]
ScAll(6)	30.9±1.6 [a]	70.3±3.52 [a]	11.69±0.32 [a]	13.28±1.61 [a]	78.61±0.64 [a]	1845.98±249.22 [a]
ScCxrd(3)	31.8±1.54 [a]	68.0±3.84 [a]	12.16±0.16 [a]	10.70±0.67 [a]	78.68±2.65 [a]	1540.48±247.68 [a]
ScCxrd(6)	39.3±2.12 [a]	79.7±2.66 [a]	11.54±0.30 [a]	14.73±1.61 [a]	76.24±0.99 [a]	1969.97±237.57 [a]
Control	38.7±1.68 [a]	74.4±3.65 [a]	11.80±0.30 [a]	12.05±1.44 [a]	77.07±1.37 [a]	1689.78±106.56 [a]
Treatment	F_4=1.74, p=0.16	F_4=0.26, p=0.90	F_4=0.18, p=0.95	F_4=0.26, p=0.90	F_4=1.22, p=0.32	F_4=0.37, p=0.83
Dose	F_1=1.41, p=0.24	F_1=0.04, p=0.85	F_1=0.26, p=0.61	F_1=0.18, p=0.68	F_1=0.13, p=0.72	F_1=0.002, p=0.97
Treatment:Dose	F_4=1.65, p=0.18	F_4=3.03, p=0.03*	F_4=0.95, p=0.45	F_4=1.49, p=0.22	F_4=1.51, p=0.22	F_4=0.88, p=0.49

Where ScAll = *Steinernema carpocapsae* All; ScCxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12. "3" and "6" represents number of cadavers. None of the treatment had significant effect on different parameters (α=0.05, Tukey-Kramer test).

Table 4. Plant count, plant height, moisture, protein, seed test weight, and yield in the EPN treated plots (Mean ± *SE*) in Pendroy (Barley) in 2019.

Treatment	Plant Count	Plant height (cm)	Moisture (%)	Protein (%)	Test weight (kg/ha)	Yield (kg/ha)
Sr7-12(3)	8.6±1.70 [a]	79.3±6.82 [a]	9.70±0.19 [a]	12.3±0.72 [a]	60.28±1.55 [a]	1623.96±261.86 [a]
Sr7-12(6)	8.0±1.80 [a]	79.4 ±4.92 [a]	9.67±0.19 [a]	12.59±0.89 [a]	61.27±1.19 [a]	2169.25±315.46 [a]
Sr355(3)	8.2±1.50 [a]	72.6±5.81 [a]	9.99±0.34 [a]	12.71±0.59 [a]	59.22±1.17 [a]	1737.86±304.71 [a]
Sr355(6)	8.3±1.51 [a]	71.8±2.91 [a]	10.18±0.34 [a]	12.54±0.80 [a]	59.26±1.23 [a]	1617.07±343.27 [a]
ScAll(3)	11.2±1.33 [a]	84.3±5.01 [a]	9.81±0.27 [a]	12.64±0.53 [a]	59.41±2.34 [a]	2135.89±387.31 [a]
ScAll(6)	8.5±0.97 [a]	80.0±5.77 [a]	9.79±0.27 [a]	11.94±0.48 [a]	57.40±2.39 [a]	1812.46±351.64 [a]
ScCxrd(3)	11.6±1.34 [a]	91.5±4.23 [a]	9.61±0.26 [a]	11.84±0.54 [a]	61.49±2.08 [a]	1879.59±383.32 [a]
ScCxrd(6)	9±2.21 [a]	76.8±8.48 [a]	10.30±0.44 [a]	13.39±1.12 [a]	58.58±2.09 [a]	1531.80±495.99 [a]
Control	9±1.88 [a]	71.9±6.60 [a]	10.13±0.12 [a]	12.88±0.56 [a]	58.83±1.72 [a]	1860.02±261.80 [a]
Treatment	F_4=0.62, p=0.65	F_4=1.83, p=0.14	F_4=1.04, p=0.40	F_4=0.05, p=0.99	F_4=0.56, p=0.70	F_4=0.28, p=0.89
Dose	F_1=1.24, p=0.27	F_1=1.11, p=0.29	F_1=0.87, p=0.36	F_1=0, p=0.99	F_1=0.47, p=0.50	F_1=0.15, p=0.70
Treatment:Dose	F_4=0.35, p=0.84	F_4=0.57, p=0.69	F_4=0.63, p=0.65	F_4=0.67, p=0.61	F_4=0.40, p=0.81	F_4=0.59, p=0.67

Where ScAll = *Steinernema carpocapsae* All; ScCxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12. "3" and "6" represents number of cadavers. None of the treatment had significant effect on different parameters (α=0.05, Tukey-Kramer test).

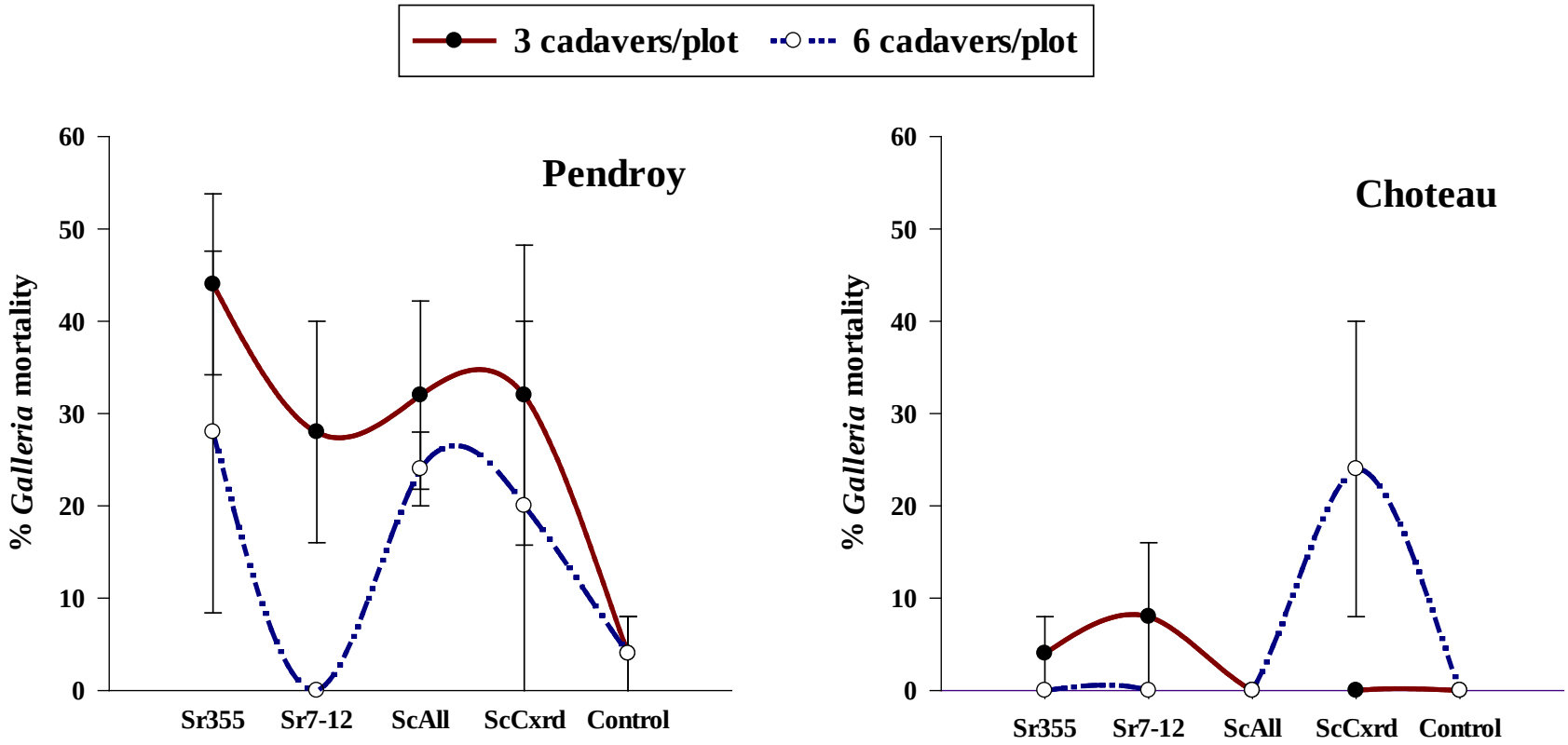

Figure 6. Average percentage of *Galleria mellonella* infected with EPNs in collected soil samples. ScAll = *Steinernema carpocapsae* All; ScCxrd = *Steinernema carpocapsae* Cxrd; Sr355 = *Steinernema riobrave* 355; Sr7-12 = *Steinernema riobrave* 7-12. No significant differences were observed among the treatments (α=0.05, Tukey-Kramer test with GLM)

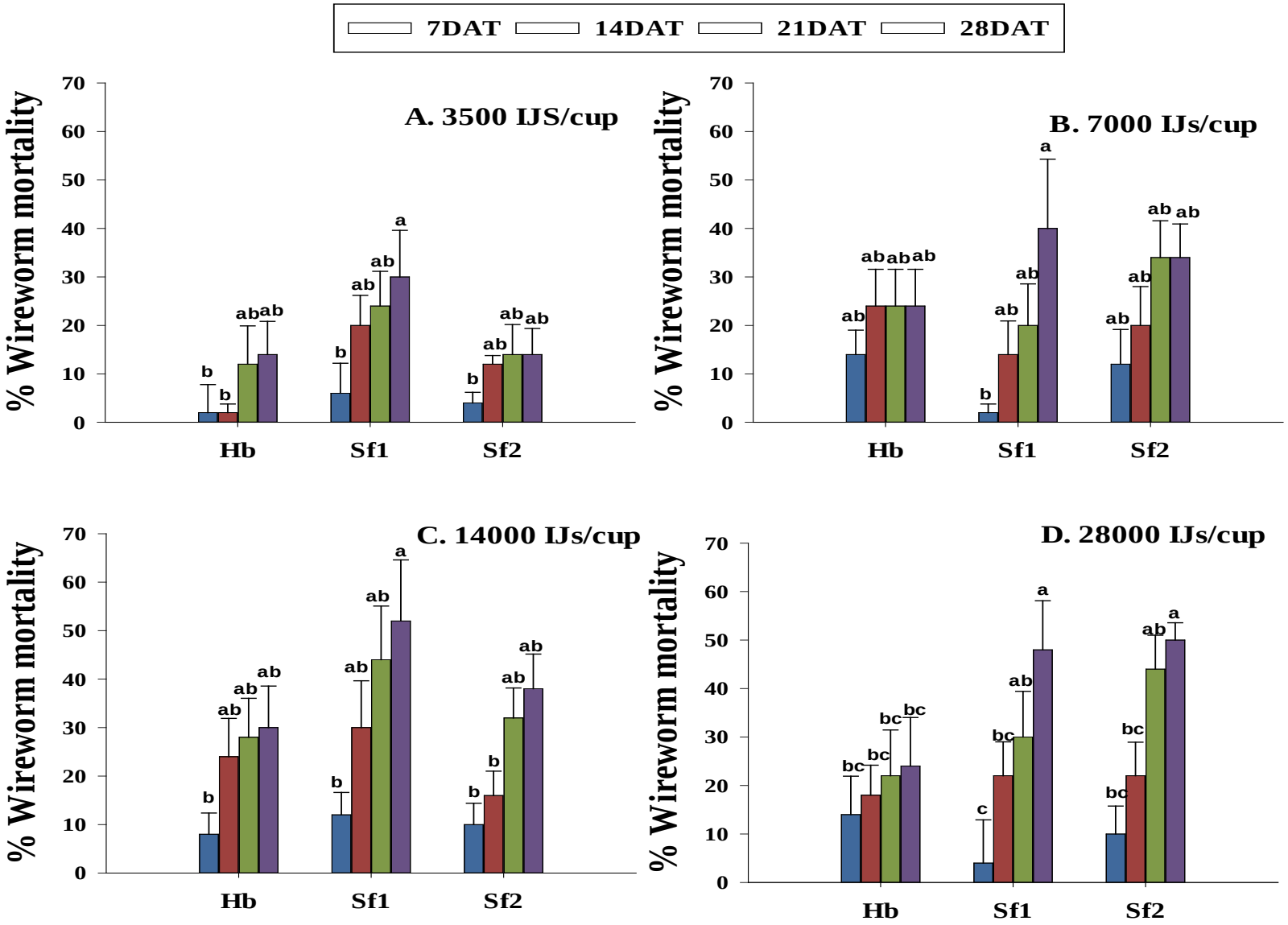
7DAT
14DAT
21DAT
28DAT
A. 3500 IJS/cup
B. 7000 IJs/cup
C. 14000 IJs/cup
D. 28000 IJs/cup
% Wireworm mortality
0
10
20
30
40
50
60
70
Hb
Sf1
Sf2

Figure 7. Average percentage mortality of larval *Limonius californicus* after exposure to entomopathogenic nematodes at 3500 (A), 7000 (B), 14000 (C), and 28000 (D) infective Juveniles/cup. Sf1 = *Steinernema feltiae* 1; Sf2 = *Steinernema feltiae* 2; Hb = *Heterorhabditis bacteriophora*. Different letters above bars indicate statistical significance (P≤0.05, Tukey-Kramer test); DAT=Days after Treatment; No larval mortality was observed in the control.

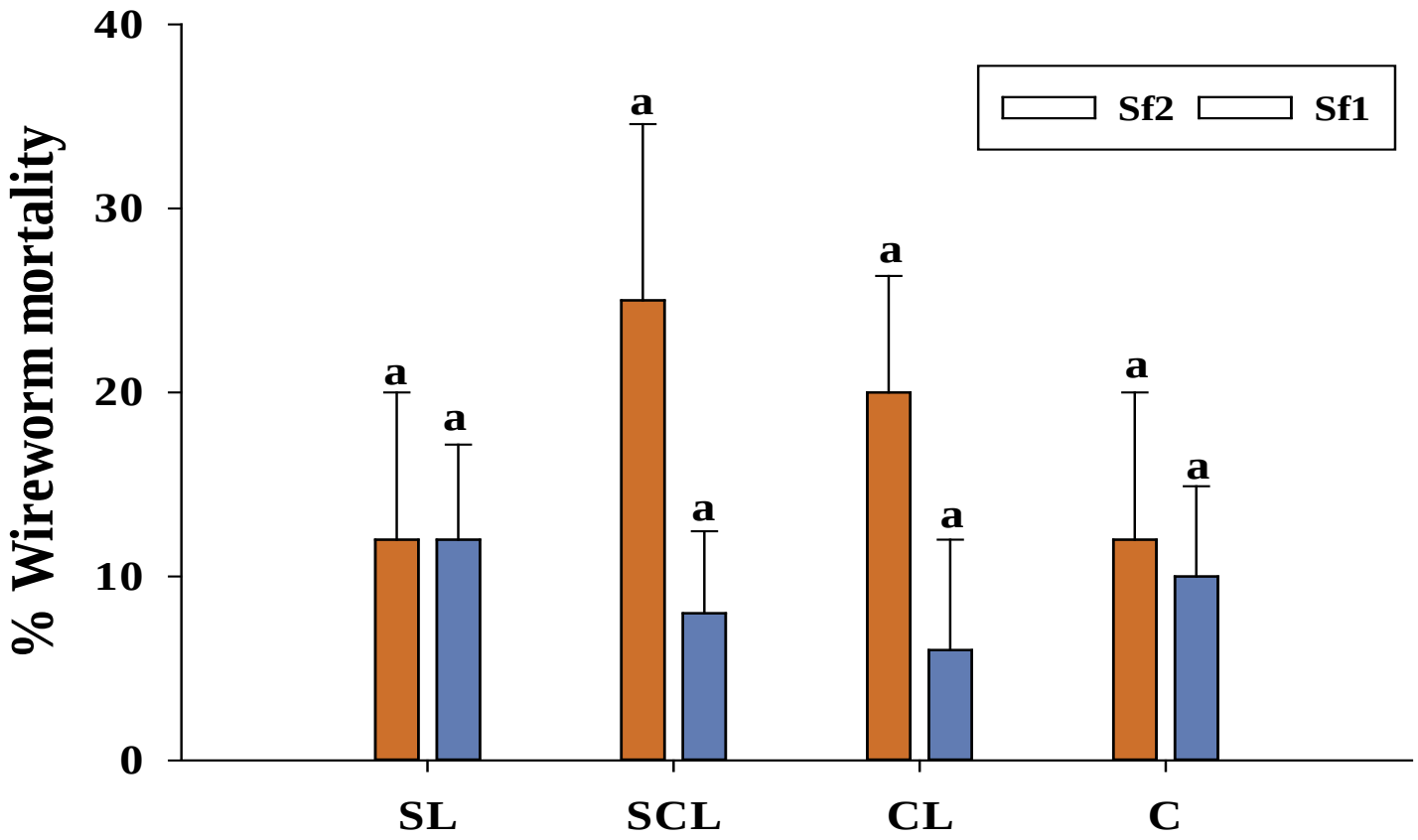

Figure 8. Average percentage mortality of larval *Limonius californicus* after exposure to entomopathogenic nematodes at 7000 infective Juveniles (IJs)/cup in shade house in 2019. Sf1 = *Steinernema feltiae* 1; Sf2 = *Steinernema feltiae* 2. Different letters above bars indicate statistical significance among EPN strains (*P* ≤0.05, Tukey-Kramer test); SL = Sandy loam; SCL = Sandy clay loam; CL = Clay loam; C = Clay. No larval mortality was observed in control treatments.

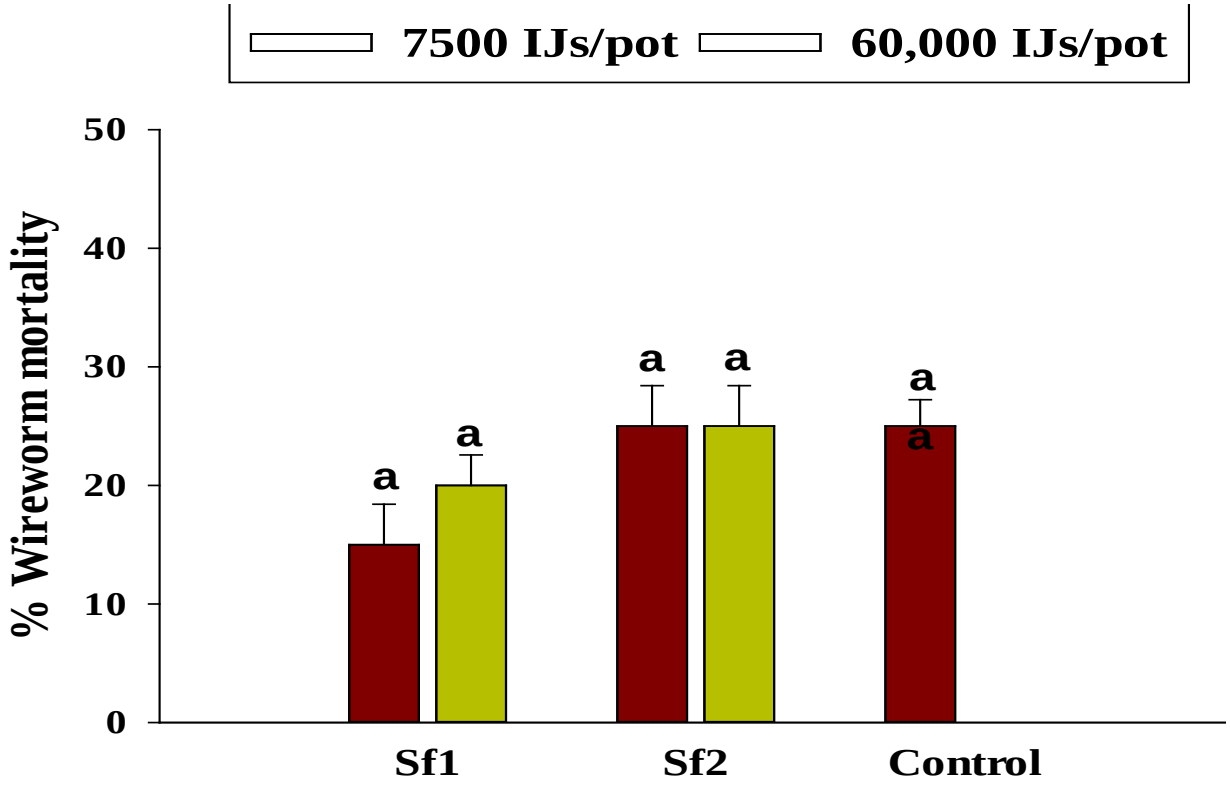

Figure 9. Average percentage mortality of larval *Limonius californicus* after exposure to entomopathogenic nematodes at 60,000 infective Juveniles (IJs)/pot (A) and 7500 IJs/pot (B) in shade house in 2019. Sf1 = *Steinernema feltiae* 1; Sf2 = *Steinernema feltiae* 2; Control= Control treatment. Different letters above bars indicate statistical significance among EPN strains ($P \leq 0.05$, Tukey-Kramer test).

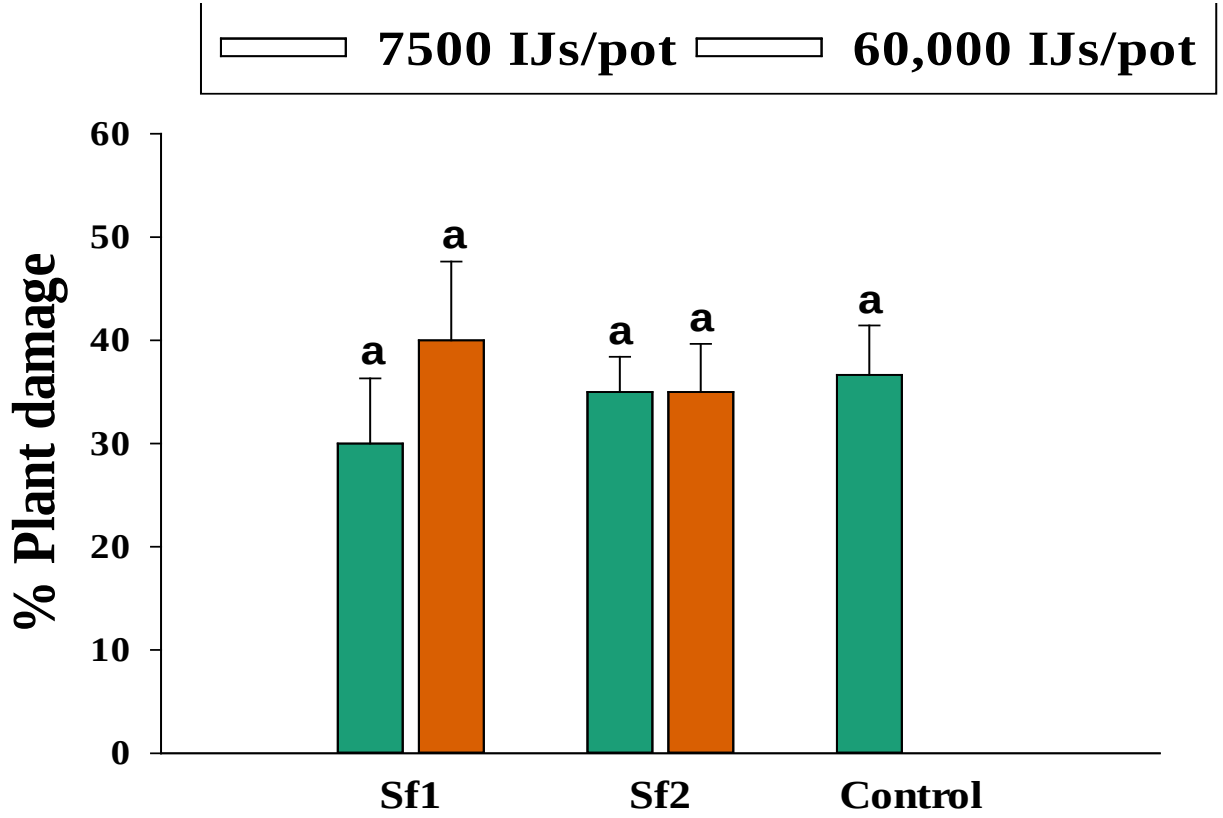

Figure 10. Average percentage plant damage by larval *Limonius californicus* after exposure to entomopathogenic nematodes at 60,000 Infective Juveniles (IJs)/pot and 7500 IJs/pot in shade house in 2019. Sf1 = *Steinernema feltiae* 1; Sf2 = *Steinernema feltiae* 2; Control= Control treatment. Different letters indicate statistical significance ($P \leq 0.05$, Tukey-Kramer test).

Acknowledgements

This work was supported by Montana Wheat and Barley Committee. We would like to thank all the farmers (Garrett Grub, John Stultz and Mike Lyne) for letting us use their fields for studies. We are thankful to Anamika Sharma, Ramadevi Gadi, Jonathan Blanchard, Shad Chrisman, and Julie Prewett for their help in different aspects of this study.

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Monitoring of Wheat Midge and its Parasitoids *Macroglenes penetrans* and *Platygaster tuberosula* in Irrigated and Dryland Spring Wheat in Golden Triangle, Montana

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Project personnel: Ramadevi L. Gadi and Anamika Sharma

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Aim of the study

The objectives of this study were: 1) to assess the wheat midge population trend in Golden Triangle, Montana, 2) the wheat midge population dynamics in irrigated and dryland spring wheat fields and 3) to monitor the wheat midge parasitoid *M. penetrans* and *Platygaster tuberosula* population in spring wheat fields.

Materials and methods

Wheat midge populations

Western Triangle Agricultural Research team installed 16 delta traps baited with wheat midge pheromone lures (2S, 7S)-nonadiyl dibutyrate in spring wheat fields (dryland and irrigated) in multiple locations of Pondera, Toole, Teton and Chouteau Counties of Golden Triangle, Montana (Table 1). Pheromone traps were installed on June 07, 2019. Traps were checked at 1-3 days interval in Pondera (Valier and WTARC trap sites) and Toole (Ledger), while at 15 days interval in Teton and Chouteau Counties locations. The monitoring work was wrapped up on August 13, 2019.

Parasitoids *Macroglenes penetrans* and *Platygaster tuberosula* population levels

The parasitoids adult population levels were monitored using the sweet net at spring wheat fields located at Valier, WTARC and Ledger locations. For sweep net sampling, 150 sweeps were made per field and the sampling was done at least 15 m from inside fields. The sweep net sampling was begun on June 24 and completed in July 23, 2019. Parasitoid adults were monitored at 15 days interval throughout the wheat midge adult activity period. The collected samples were stored at -20 °C until processing. The parasitoids were identified under a microscope.

Results

Wheat midge population levels in the Golden Triangle, Montana

Total cumulative midge count observed in our trap established locations are shown in Table 1.

Table 1. Total cumulative wheat midge count per trap observed in Pondera, Toole, Teton and Chouteau Counties of Montana in 2019.

County	Field name	Lat.	Lng.	Total cumulative count/trap	Parasitoid observed
Pondera	Kyle Dean-Rock city-irrigated 1	48.410	-112.1956	499	Yes
Pondera	Kyle Dean-House-irrigated 2	48.399	-112.2194	453	Yes
Pondera	Kyle Dean-Dean Rd-dryland	48.3954	-112.1942	67	Yes
Pondera	Cory Crawford-Crestview Rd-irrigated 1	48.2998	-112.1430	1247	Yes
Pondera	Cory Crawford-Highway- irrigated 2	48.3072	-112.1889	1618	Yes
Pondera	Cory Crawford-Hill top-dryland	48.3084	-112.0638	111	Yes
Pondera	Jodi Hobel-Swanson Rd-irrigated	48.3741	-112.2300	533	Yes
Pondera	Jodi Hobel-Sullivan bridge-dryland 1	48.4424	-112.2075	52	Yes
Pondera	Jodi Hobel-Beaver head-dryland 2	48.3663	-112.2010	158	Yes
Pondera	WTARC-irrigated	48.3064	-111.9229	126	Yes
Pondera	WTARC-dryland	48.3043	-111.9245	73	Yes
Toole	Terry Peters-Saint olaf-dryland 1	48.2487	-111.6381	9	Yes
Toole	Terry Peters-Saint olaf-dryland 2	48.2479	-111.5928	40	Yes
Toole	Terry Peters-Ledger Rd-dryland 3	48.2633	-111.6370	14	Yes
Teton	Scott Inbody-Choteau-dryland	47.9120	-112.0450	8	No
Chouteau	Knees-dryland	47.9589	-111.3828	9	No

In 2019, wheat midge populations were monitored in seven counties (Liberty, Toole, Teton, Chouteau, Glacier, Cascade and Pondera) at the Golden Triangle, Montana. A portion of the wheat midge count data was extracted from PestWeb Montana. The total number of wheat midge pheromone traps installed in wheat fields was 32 in 2019. Among the seven counties, the highest wheat midge population level per trap was observed in Pondera County (Fig. 1). The second highest wheat midge populations were noticed at Liberty County followed by Toole, Cascade and Teton Counties (Fig. 1). Compared to the last year, wheat midge population was low in Liberty County but higher in Pondera and Toole Counties.

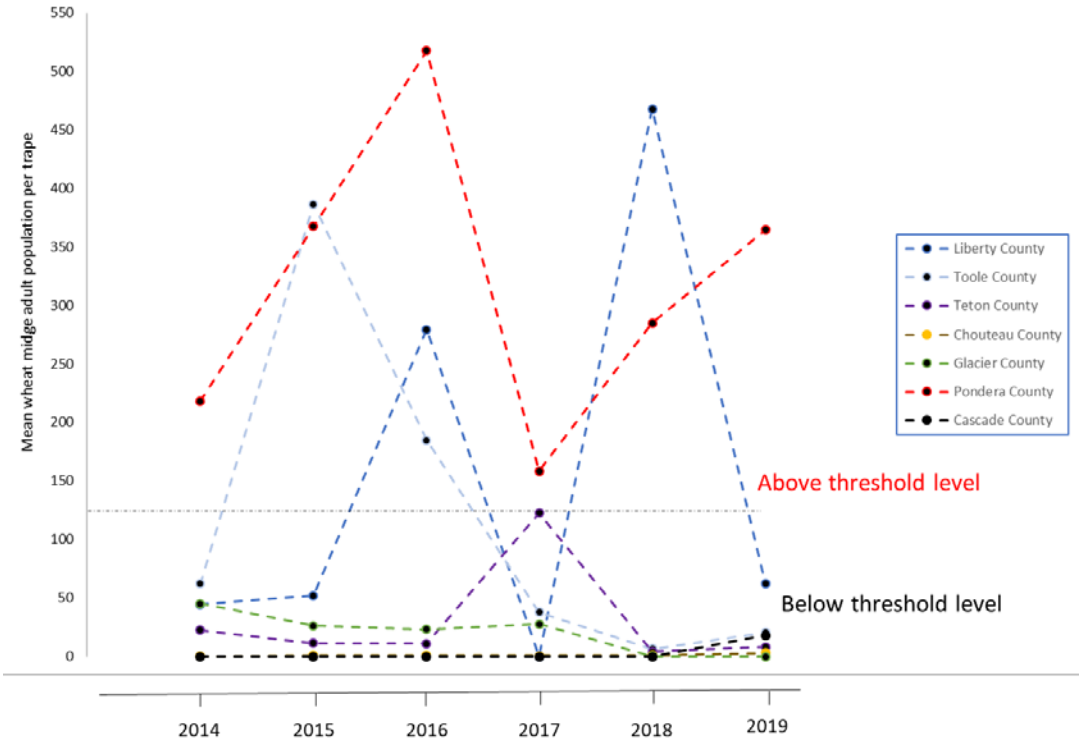

Figure1. Wheat midge population levels in the Golden Triangle, Montana from 2014-2019.

Wheat midge population level: Irrigated vs. dryland spring wheat fields

In overall, the flight activity of wheat midge adults began about one and half weeks earlier (June 19-June 26) in 2019 when compared to 2018 with emergence occurred on June 30-July 5 in Pondera County (Fig. 2). The midge adult activity reached a peak on August 04, 2019 both in dryland and irrigated fields and which is similar to 2018 (Fig. 2). In 2019, we had similar results as in 2018 regarding wheat midge population levels in irrigated and dryland wheat fields. Wheat midge populations were relatively at higher levels in irrigated fields compared to dryland spring wheat fields. However, it is interesting to report that wheat midge population levels were nearly twelve-fold higher in irrigated compared to dryland fields in 2019. The total cumulative numbers of male adults captured per pheromone trap were: 746 and 65 in irrigated and dryland fields, respectively (Fig. 2). Similarly in 2018, wheat midge population levels were fourteen folds higher in irrigated compared to dryland fields. The total cumulative numbers of male adults captured per pheromone trap were: 700 and 50 in irrigated and dryland fields, respectively (Fig. 2). Environmental factors could be the main triggering factors for wheat midge population dynamics in irrigated and dryland spring wheat fields in Montana.

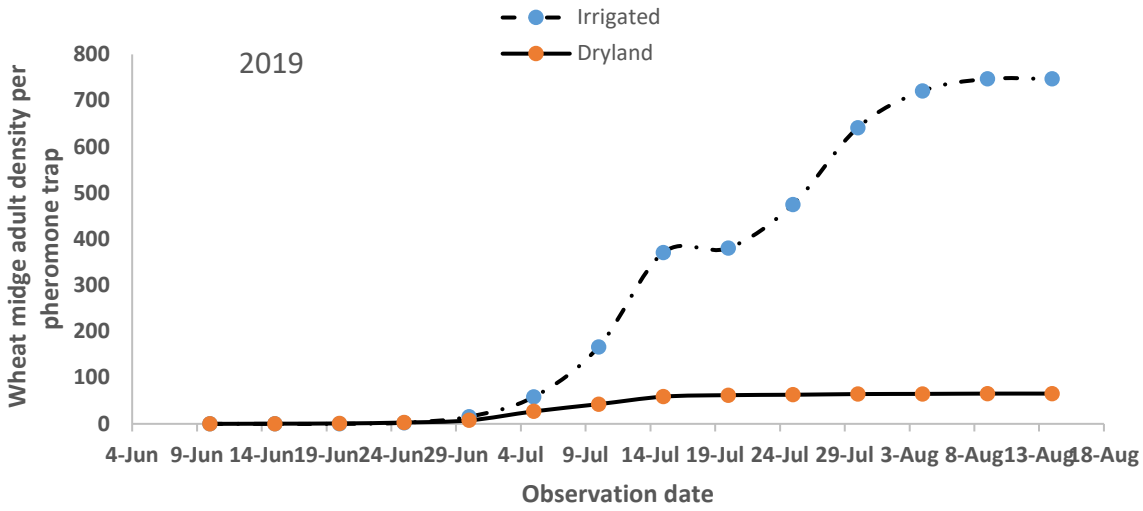

Figure 2. Wheat midge adult activity based on pheromone trap catch in dryland and irrigated spring wheat fields (2018-2019).

Parasitoids:

Sweep net samples collected on June 24 did not show any parasitoids in Valier and WTARC locations but *P. tuberosula* was present (6) in samples collected from dryland Ledger locations. We have observed some *M. penetrans* and *P. tuberosula* on midge trap sticky liners collected from all location on June 28. Later collected sweep net samples on July 08 and 22 contain both *M. penetrans* (40) and *P. tuberosula* (18) in Valier, WTARC and Ledger locations. In 2019, the total cumulative parasitoid numbers per 150 sweeps were 51 and 14 compared to 48 and 12 in 2018 in irrigated and dryland fields, respectively.

M. penetrans has been established with adequate population level and *P. tuberosula* has been seen in some of the spring wheat fields. To improve *P. tuberosula* and *E. error* population establishment, 20,000 wheat heads will be collected again from spring wheat fields of Saskatchewan, Canada during the second week of August, 2019 and will be transported to WTARC. The same procedures will be followed for storing the wheat midge larvae, rearing and release of the parasitoids during the spring of 2020.

Acknowledgements

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"Development of Management strategies of Pulse Insect Complex in Montana"

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Project collaborators: Dr. Kevin Wanner and Dr. G.V.P Reddy

Project personnel: Pragya Kiju and Dr. Anamika Sharma

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Aim of the study

The different aims of this study were: 1. To develop the management strategies for pulse insect pest complex in Montana. ; 2. To perform larval and adult damage assessment of different pea varieties at two different sites. ; 3. To compare damage level by pea leaf weevil on different pea varieties. ; 4. To evaluate and establish efficacy of biopesticides. ; and 5. To develop pheromone-based low-cost technology "Attract and Kill Method".

Location

Study sites

The assessment was done at two sites, the Western Triangle Agriculture Research Center (WTARC) Conrad (48.307˚N, 111.917˚W), and Northern Agriculture Research Center (NARC), Havre (48.32˚N, 109.40˚W). A total of 44 (32 at SW,12 at WR) varieties were assessed at WTARC and 54 varieties at NARC were assessed which were planted in a random block design for Statewide (SW) and Western Regional (WR) spring pea variety trails. Similarly, For the pheromone based low cost technology that is attract and kill method; the experiment was conducted on three sites; both of the above sites and at the Arthur Post agronomy farm, Bozeman (45.676998˚N, 111.042931˚W).

Materials and methods

1. Adult and larval damage assessment of different spring pea varieties:

A. Adult damage assessment

Figure 1. Adult pea leaf weevil feeding on pea leaves

Adult pea leaf weevil feeds upon the leaf margins and the growing point of legume seedling and produce a characteristic distinct notch (Otani, 2013).The adult weevils feed on the leaves, chewing a half-moon shaped notch in the leaf edge (Fig.1) (Wanner, 2016). For the adult assessment, 10 individual plants are randomly selected from the plots of pea that were assigned according to the Random Block Design (RBD). The distinct number of notches made by pea leaf weevil are noted and recorded. Along with the notches, the nodal stage of pea and the number of leaves of the respective pea plant were also noted. The process was repeated for every plot. In WTARC, this process of recording was done from 23rd May to 28th May in both SW and WR spring pea variety. Meanwhile in NARC, the adult assessment was done from 29th May to 7th June.

B. Larval damage assessment

Pea leaf weevils lay eggs in the soil and the larva develop under the soil. They are "c" shaped milky white in color with the distinctive dark brown head capsule. After hatching, larvae enter the root nodules of the pea plant where they consume the contents of the nodules that is rhizobium bacteria of the host plant (Otani, 2013). Due to this feeding on the root nodules, it results in the complete or partial inhibition of nitrogen fixation and which therefore result in less yield due to poor growth.

Figure 2. Root nodules of pea (left panel) and b. the larva found inside the root nodule (right panel).

In both of the sites WTARC and NARC, the larval assessment was carried out with the following steps:

- 5 plants were dug from each plot in a random way.
- In WTARC, Conrad the process of taking out the samples was carried out from 22nd June to 23rd June whereas in NARC, Havre, the process was carried out from 9th July to 12th July.
- Plants along with the soil intact were kept in the bags. All the samples were stored in a cool dry place.
- From each bag, sample plants were taken out and put into respective buckets.
- The buckets with the sample were filled with water. The plants were soaked to allow the soil to soften.

- Inspection on respective bucket was done for floating larva. If larvae were present, they were scooped out with a net. The scooped larvae were taken out with the help of forceps and therefore were transferred into a labeled container.
- The plants were rinsed through cold water until the soil intact was removed, thus exposing the root nodules. The roots with exposed root nodules were cut.
- The cut root nodules were placed into the container and marked. Soil in the respective container were dumped on the sieve; sieve was placed above any sink or portable plastic tubs. The soil left on the screen was rinsed with the cold water.
- The remnant on the sieve was sorted through. Root nodules or roots were accessed meanwhile. As not all the larvae float on the top of the bucket; when soil is caught in the bottom of sink or tubs, larvae if present were seen as floating on the water. Nets were used to scoop the floating larvae and were transferred to respective container. The deposits of soil were washed away. The screens, sinks, tubs were all cleaned completely. All the cut root nodules were stored in the refrigerator. The number of root nodules was counted and recorded in every plant. Each root nodules was cut and checked for larvae under a Microscope. The larvae if present were recorded and stored in 70 % ethyl alcohol.

Results

Table 1. Average number ($\bar{x}$) of notches/leaf with the respective average number of larvae per plant ± Standard Error (SE) in the spring pea-variety trial of Western region at NARC, Havre.

Location: NARC, Havre (WR)		
Variety	Notches/Leaves($\bar{x}$) ±SE	Larvae ($\bar{x}$) ±SE
PS08101004	1.74± 0.17	2.50± 1.89
PS17100022	1.62± 0.15	2.00± 1.00
PS071000925	1.57± 0.11	3.50± 3.50
PS1710NZ0016	1.49± 0.08	5.00± 1.25
PS08101022	1.47± 0.01	2.25± 1.31
PS16100096	1.46± 0.08	2.25± 1.93
PS1710NZ0116	1.46± 0.13	3.75± 2.17
PS1410B0073	1.44± 0.23	6.00± 5.02
DS Admiral	1.43± 0.21	3.75± 1.89
PS17100239	1.38± 0.29	1.75± 0.85
Hampton	1.34± 0.13	3.00± 2.12
PS16100102	0.95± 0.23	3.25± 2.29

The variety with the highest to lowest number of Notches/Leaf along with the respected number of larvae are arranged in the above Table 1. PS08101004 seems to have highest average number of Notches/Leaf that is 1.74 ± 0.17 standard error along with the respective average larvae of 2.50 ± 1.89. PS16100102 has the lowest average number of Notches/Leaf that is 0.9 with 0.23 standard error with average larvae of 3.25 with the standard error of 2.29.

Table 2. Average number ($\bar{x}$) of notches/leaf with the respective average number of larvae per plant ± Standard Error (SE) in the spring pea-variety trial of Western region at WTARC, Conrad.

Location: WTARC, Conrad (WR)		
Variety	Notches/Leaves($\bar{x}$) ± SE	Larvae ($\bar{x}$) ± SE
PS071000925	4.28±0.41	10.75± 4.37
PS10100207	4.00±0.41	22.50± 5.38
PS08101022	4.00±0.50	19.00± 4.51
PS1710NZ0124	3.90±0.54	21.00± 2.74
PS1410B0073	3.81±0.24	20.75± 6.77
PS1710NZ0002	3.68±0.35	16.50± 8.88
Hampton	3.63±0.54	11.50± 3.30
PS08101004	3.47±0.32	19.25± 6.33
PS1710NZ0016	3.44±0.19	9.50± 5.39
PS1710NZ0116	3.38±0.31	19.25± 9.57
PS16100102	3.13±0.29	16.50± 8.88
DS Admiral	3.06±0.33	11.25± 2.39

The variety with the highest to lowest number of Notches/Leaf along with the respected number of larvae are arranged in the above Table 2. PS071000925 seems to have highest average number of Notches/Leaf that is 4.28±0.41 standard error along with the respective average number of larvae 10.75± 4.37. DS Admiral has the lowest average number of Notches/Leaf that is 3.06 with 0.33 standard error with average larvae of 11.25 with the standard error of 2.39.

Table 3. Average number ($\bar{x}$) of notches/leaf with the respective average number of larvae per plant ± Standard Error (SE) in the spring pea-variety trial of Western region at NARC, Havre (SW)

Location: NARC, Havre (SW)		
Variety	Notches/Leaves ($\bar{x}$) ± SE	Larvae($\bar{x}$) ± SE
CDC Spectrum	0.83± 0.28	0.00± 0.00
Pro 121-7126	0.80± 0.09	0.00± 0.00
Delta	0.71± 0.27	0.25± 0.25
LG Amigo	0.65± 0.20	0.50± 0.50
Pro 141-6258	0.64± 0.15	0.50± 0.29
Salamanca	0.51± 0.16	0.25± 0.25
Bridger	0.50± 0.17	0.25± 0.25
NDP121587	0.49± 0.10	0.00± 0.00
Durwood	0.48± 0.13	0.00± 0.00
DL Apollo	0.48± 0.12	0.00± 0.00
PS07100925	0.48± 0.09	0.00± 0.00
PS08101022	0.47± 0.18	0.00± 0.00

Aragorn	0.47± 0.14	0.00± 0.00
Pro 093-7410	0.47± 0.10	0.50± 0.50
Nette 2010	0.46± 0.10	0.50± 0.50
CDC Inca	0.45± 0.12	0.25± 0.25
CDC Amarillo	0.45± 0.14	0.00± 0.00
CDC Saffron	0.45± 0.14	0.00± 0.00
Greenwood	0.43± 0.13	0.00± 0.00
Pro 133-6243	0.43± 0.14	0.00± 0.00
AC Earlystar	0.41± 0.09	0.00± 0.00
CDC Greenwater	0.40± 0.08	0.00± 0.00
Spider	0.40± 0.14	0.25± 0.25
Majoret	0.39± 0.11	0.00± 0.00
Pro 143-6236	0.39± 0.12	0.00± 0.00
Pro 131-7123	0.38± 0.05	0.25± 0.25
Korando	0.36± 0.03	0.50± 0.50
Jetset	0.36± 0.09	0.00± 0.00
Banner	0.35± 0.08	0.00± 0.00
Navarro	0.35± 0.09	0.25± 0.25
LG Sunrise	0.35± 0.08	0.25± 0.25
DS-Admiral	0.35± 0.06	0.00± 0.00
AAC Comfort	0.35± 0.07	0.00± 0.00
AAC Profit	0.33± 0.09	0.00± 0.00
Hampton	0.33± 0.01	0.50± 0.50
MT457	0.32± 0.06	0.00± 0.00
AC Agassiz	0.32± 0.07	0.33± 0.25
Ginny	0.30± 0.05	0.00± 0.00
Hyline	0.27± 0.05	0.25± 0.25
PS0877MT076	0.24± 0.06	0.75± 0.48
AAC Carver	0.24± 0.02	0.00± 0.00
PS0877MT632	0.23± 0.10	0.50± 0.50

The variety with the highest to lowest number of Notches/Leaf along with the respected number of larvae are arranged in the above Table 3. CDC Spectrum seems to have highest average number of Notches/Leaf that is 0.83± 0.28 standard error along with the respective average number of larvae 0. PS0877MT632 has the lowest average number of Notches/Leaf that is 0.23 and with 0.11 standard error with average larvae of 0.50 with the standard error of 0.5.

Table 4. Average number ($\bar{x}$) of notches/leaf with the respective average number of larvae per plant ± Standard Error (SE) in the spring pea-variety trial of Statewide at WTARC, Conrad

Location: WTARC, Conrad (SW)		
Variety	Notches/Leaves($\bar{x}$) ± SE	Larvae($\bar{x}$) ±SE
PS0877MT076	0.47± 0.08	6.00± 5.02
NDP121587	0.46± 0.09	0.75± 0.75

AC Agassiz	0.46± 0.14	1.25± 0.95
PS0877MT632	0.44± 0.07	1.50± 0.87
Korando	0.43± 0.13	0.75± 0.25
Bridger	0.43± 0.12	3.50± 3.50
Hampton	0.42± 0.11	3.00± 2.12
CDC Saffron	0.41± 0.10	1.00± 0.71
MT457	0.41± 0.04	1.75± 0.75
LG Amigo	0.39± 0.08	2.25± 1.93
Pro 141-6258	0.39± 0.03	0.75± 0.48
AAC Comfort	0.38± 0.12	3.75± 1.89
Delta	0.38± 0.02	0.33± 0.33
CDC Inca	0.36± 0.05	0.25± 0.25
Spider	0.35± 0.06	2.50± 1.89
Hyline	0.35± 0.12	1.75± 0.85
Salamanca	0.34± 0.03	0.75± 0.48
Pro 131-7123	0.32± 0.03	1.75± 1.11
Navarro	0.32± 0.03	1.25± 0.63
AAC Profit	0.31± 0.03	1.50± 0.87
LG Sunrise	0.31± 0.07	2.75± 1.03
AAC Carver	0.30± 0.05	0.50± 0.50
Majoret	0.29± 0.02	1.00± 0.71
Nette 2010	0.28± 0.02	0.25± 0.25
AC Earlystar	0.27± 0.03	0.25± 0.25
Jetset	0.27± 0.05	3.75± 2.17
CDC Greenwater	0.26± 0.06	2.25± 1.31
PS07100925	0.26± 0.04	1.50± 1.19
CDC Amarillo	0.26± 0.05	3.25± 2.29
CDC Spectrum	0.26± 0.03	0.50± 0.29
Durwood	0.26± 0.04	0.25± 0.25
PS08101022	0.24± 0.05	1.25± 0.95
Aragorn	0.20± 0.03	0.00± 0.00
DS-Admiral	0.20± 0.02	0.75± 0.25

The variety with the highest to lowest number of Notches/Leaf along with the respected number of larvae are arranged in the above Table 4. PS0877MT076 seems to have highest average number of Notches/Leaf that is 0.47± 0.08 standard error along with the respective average number of larvae 6.00± 5.02. DS-Admiral has the lowest average number of Notches/Leaf that is 0.20 and with 0.02 standard error with average larvae of 0.75 with the standard error of 0.25.

2. Development of pheromone -based monitoring and mass trapping for pea leaf weevil

Materials and methods

- Insecticide (Deltamethrin) (0.03 gram)
- Aggregation Pheromone (4–methyal-3,5-heptanedione) (used in pellet form and rubber

septa form, Manufactured from ChemTica International, Costa Rica)
- Soil sampler
- Stakes

Figure 3. Pellet in the pit and fall trap (left panel); b. granular insecticide used (right panel).

Figure 4. Demonstration of attract and kill method strategy in NARC, Havre

The main principle of this project is to attract mass number of pea leaf weevils and then kill through the insecticide. The trial was laid out with a Complete Randomized Block Design (CRBD) with 5 Treatments and 8 Replications in both WTARC, NARC. At every 10 m of distance, one treatment was set. Also, the same experiment was carried on the Arthur Post agronomy farm MSU, Bozeman.

Table 5. Number of treatments with the materials used

1	1 pellet and 0.03 gm insecticide
2	3 pellets and 0.03 gm insecticide
3	5 pellets and 0.03 gm insecticide

4	Only insecticide without lure or insecticide
5	Lure and 0.03 gm insecticide

For every treatment (Table 5), 2-inch deep hole was dug. At every hole, same weight of insecticide was spread evenly. With respective design in (Table 6) each treatment was completed. Long stakes were put at every treatment for identification. At intervals of 7-8 days, observations were recorded.

Experimental Design

Table 6. Design for "Attract and Kill" project in NARC, Havre

T3R7	T5R7	T1R7	T2R7	T4R7	T3R8	T1R8	T5R8	T2R8	T4R8
T5R5	T1R5	T2R5	T3R5	T4R5	T2R6	T4R6	T3R6	T1R6	T5R6
T3R3	T4R3	T5R3	T1R3	T2R3	T4R4	T5R4	T1R4	T2R4	T3R4
T1R1	T2R1	T3R1	T4R1	T5R1	T2R2	T3R2	T4R2	T5R2	T1R2

Results

No result for this experiment was obtained. At this later date pea leaf weevil migration and attraction to aggregation pheromone may vary. No pea leaf weevil was collected during this experiment at either of the sites. This experiment will be repeated in 2020 earlier in the spring season when pea leaf weevils are actively migrating into pea fields.

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Evaluation of commercially available bio-pesticides and pheromone traps for management and monitoring of crucifer flea beetles *Phyllotreta cruciferae*

Principal Investigator: Gadi V.P. Reddy, Michael A Ivie
Project Personnel: Anamika Sharma, John H. Miller, and Julie Prewett

Aim of the study

The first objective of this study was to test the impact of five bio-pesticides and compared them with conventional pesticide Mustang Maxx® (Zeta-cypermethrin). The bio-pesticides selected were Entrust WP® (spinosad 80%), Aza-Direct® (azadirachtin), BoteGHA® (*Beauveria bassiana* GHA), Xpectro® (*Beauveria bassiana* GHA and Pyrethrum), BeetleGONE® (*Bacillus thuringiensis* galleriae) on adults of crucifer flea beetles (*Phyllotreta cruciferae*) (Table 1). As second objective pheromone-traps (allyl isocyanate 10 mg in a nonvolatile carrier with antioxidant in a membrane release device) were installed to access the population of adult *Phyllotreta cruciferae* in various regions of Golden Triangle Area of Montana.

Material and Methods

Study sites

Pheromone traps were installed at five sites in Conrad (WTARC; 48º 18.627'N, 111º 55.402' W), Cut Bank (48º 30.77'N, 111º 92.55' W), Cooley (48º 56.009' N, 111º 40.565' W), Havre (Northern Agriculture Research Center; 48º.32.52' N, 109º.40 W), and Chester (48º 44.006'N, 110º 56.805' W). The traps were installed in the fields on different dates depending on the seeding and germination of the canola crop. The installation of traps began in the last week of May 2019 and data were collected until the last week of August 2019.

Experimental design and application

The pheromone traps were installed at two heights (0.4 meters from the ground and 0.8 meter from the ground). At every site four traps were installed, two at each height. The traps were installed at a distance of 10-15 meters. The traps were collected every 15 days and new traps were installed.
For bioassay, adult flea beetles were collected from the WTARC field as and when the experiment was set up. Five bio-pesticides were tested along with conventional pesticide Mustang Maxx (Table 1) against adult flea beetles. The solutions were prepared at low and high rates as per label description and applied on flea beetles directly and indirectly.

For direct application, the treatments were applied directly as a topical solution on the adult flea beetle's body surface using 750-ml hand-held sprayers. A total of 1 ml of solution was applied in each cup after flea beetles were released in the deli plastic cups (7 cm diameter and 3.5 cm deep; 10 adults in every cup). The canola leaves were supplied to the adults. For indirect spray, the treatments were sprayed directly on the leaves (3–4 cm in length). For every treatments 15–20 leaves were sprayed and dried for 10 minutes. About 3 leaves were used in each cup and adults were released (10 adults were released in each cup). The cups were kept in the incubator (55–60% moisture and 20–22º C). The mortality was checked in 5 and 10 days.

Table 1: Materials and rates of application in each treatment applied on flea beetles directly and indirectly in 2019.

Treatment	**Active Ingredient**	**Low Rate**	**High Rate**	**Source**
Control	Water			
Entrust®	Spinosad (a mixture of spinosyn A and spinosyn D)	0.114 gms/3.78 liter	0.65 gms/3.78 liter	Dow AgroSciences
BoteGHA®	*Beauveria bassiana* GHA	474 ml/7.57 liter	947 ml/7.57 liter	Certis
Xpectro®	*Beauveria bassiana* GHA and Pyrethrum	474 ml/378.5 liter	947 ml/7.57 liter	LAM international
BeetleGone®	*Bacillus thuringiensis* galleriae	113 gms/3.78 liter	227 gms/3.78 liter	PhyllomBioProducts
Aza-Direct®	Azadirachtin	5.32 ml/3.78 liter	7.99ml/ 3.78 liter	Gowan Company
Mustang Maxx®	Zeta-cypermethrin	0.028 ml/liter	-	FMC corporation

Table 2: Mean numbers of crucifer flea beetles collected in each trap installed at two different heights at five sites in 2019.

Treatment	WTARC		Cooley		Cut Bank		Chester		Havre	
Distance of traps from the ground	**0.4**	**0.8**	**0.4**	**0.8**	**0.4**	**0.8**	**0.4**	**0.8**	**0.4**	**0.8**
1st collection (June 1–June 15)	251	89	33	49	-	-	-	-	-	-
2nd collection (June 15–June 30)	213	69	54	55	30	36	221	204	-	-
3rd collection (June 30–July 15)	662	122	64	61	29	23	34	28	52	46
4th collection (July 14–July 30)	1511	372	50	45	108	70	10	18	44	44
5th collection (July 30–August 15)	357	698	56	148	545	307	305	70	858	1218

Table 3: Mean mortality of adult flea beetles in two set of experiments where 12 treatments were applied indirectly

Indirect application					Direct application	
	1st experiment		2nd experiment		1st experiment	
	5 days	8 days	5 days	8 days	5 days	8 days
Control	0.5	2.3	0.8	1.1	4.9	6.9
Entrust low	1	2.4	0.6	2.7	9.1	9.4
Entrust high	2	3.1	0.7	2.2	9.8	9.8
BoteGHA low	1	4.5	0.6	2.1	9.6	9
BoteGHA high	1.3	3.6	1.5	2.7	9.6	9.4
Xpectro low	1.7	2.6	1.7	1.5	9.9	9.7
Xpectro high	1.2	2.1	1.6	1.7	9.1	9.2
BeetleGone low	1.6	2.2	0.5	1.4	8.4	8.9
BeetleGone high	1.8	2.9	0.5	1.4	9.9	10.3
Aza-Direct low	0.9	1.6	0.7	2	3.6	6.7
Aza-Direct high	1.7	3.4	1.5	1.8	9.8	9
Mustang	1.8	2.6	0.4	1.1	9.9	9.7

Results

At the selected five sites, the maximum population of flea beetles were captured at WTARC, where no seed treatment was applied. The maximum adults were collected from 10 July to 15 August 2019. During spring and fall (from 25 May to 30 June; from 25 July to 15 August 2019) more adults were captured by the trap at 0.8 meters height. Whereas, during summer (from June to July 2019) more numbers were captured by the traps at 0.4 meters height (Table 2; Figure 1). In the mortality assessment, in the direct application method the mortality was maximum for BeetleGone at a high rate, however, the mortality was greater for all the treatments in direct application compared to the indirect application. In indirect application, maximum mortality was caused by BoteGHA high, Aza-direct high, and Entrust low and high at 8 days. At 5 days the maximum mortality was recorded by Entrust high, Xpectro low, BoteGHA high, and Mustang (Table 2, Figures 2,3).

Conclusion

In 2018, in field experiments, Entrust caused the maximum mortality and also provided greater yield. In 2019, we tested twelve treatments in laboratory bio-assay, which included two rates (low and high) of five bio-pesticides and a single rate of Mustang Maxx. The direct application did cause greater mortality, however, we do believe that although applied in small amounts, the direct application of treatments might have caused chocking mortality to the flea beetles. In lab-bioassay, the best treatment was BoteGHA at a high rate. Other treatments including Entrust and Aza-direct

also performed well. After 8 days greater mortality is observed. Xoectro low, entrust low and high, and Mustang performed better at 5 days compared to the other treatments. For flea beetle trapping, we have tested pheromone traps at two heights. It seems the newly emerged flea beetles hop more and more numbers were collected at 0.8 meters height. A distance of 10-15 meters between traps worked well for the flea beetle pheromones.

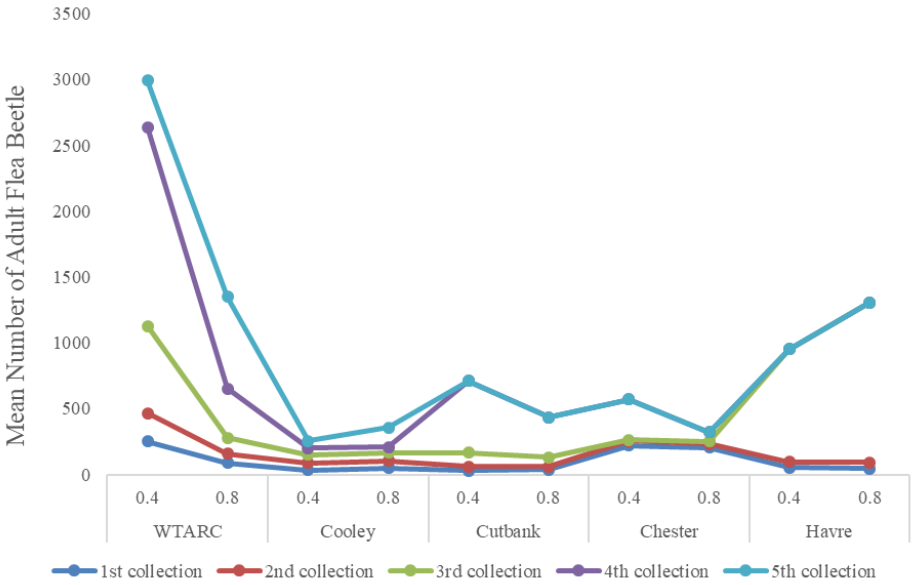

Figure 1. Mean number of *Phyllotreta cruciferae* trapped in the traps at 0.4 m and 0.8 m height at five sites in 2019 (June 2019–August 2019).

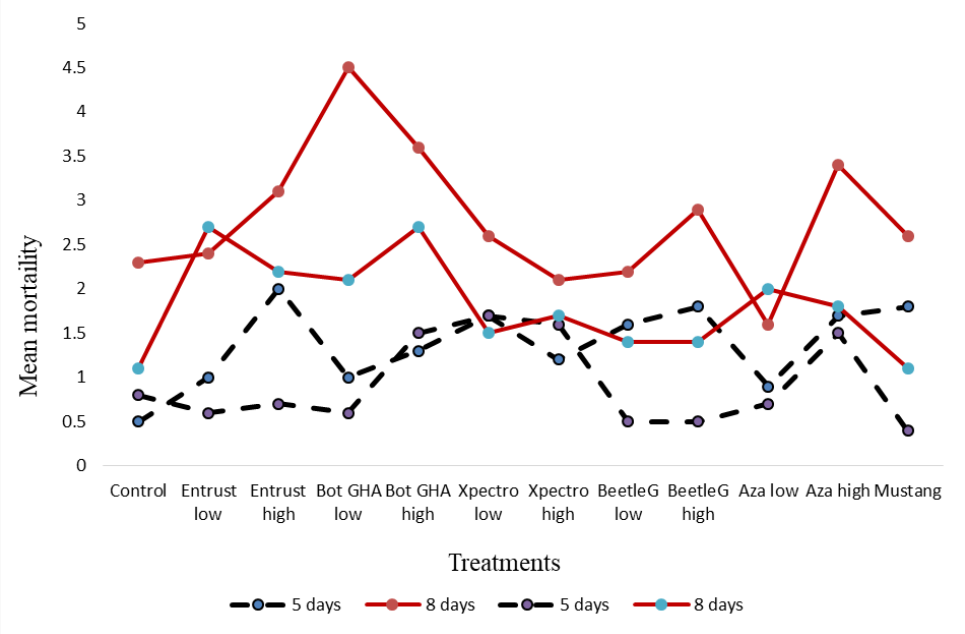

Figure 2. Mean mortality of adult *Phyllotreta cruciferae* by indirect application of twelve treatments (n=2). The mortality observed on 5th (----) and 8th day (—).

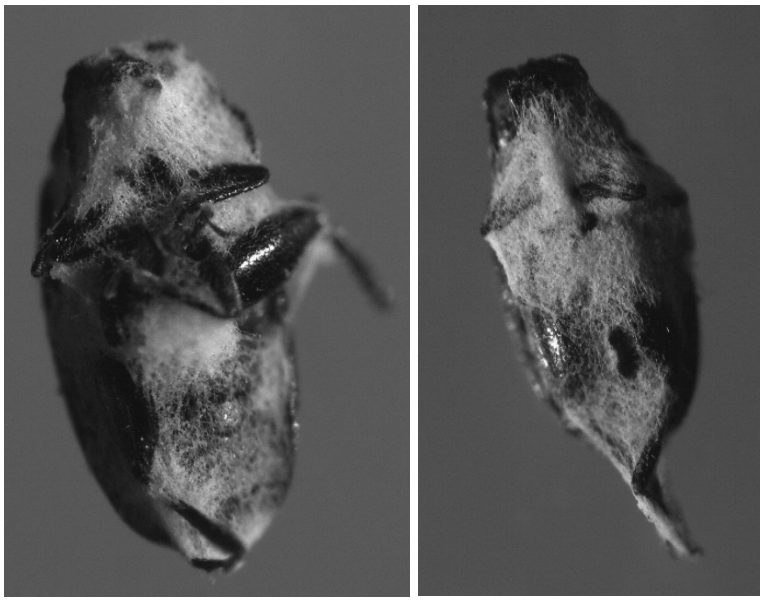

Figure 3. Fungus growth on the adult flea beetles. 1. Adult *Phyllotreta cruciferae* treated with BoteGha 2. Adult *Phyllotreta cruciferae* treated with Xpectro.

Acknowledgements
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