| Date Received |
| --- |
| For Administrative Use Only |

# PROJECT FINAL REPORT

**Instructions:**

- **Please note that making changes to the project without prior written consent from the funder(s) could constitute sufficient grounds for termination of funding.**
- This report must be a stand-alone report, *i.e.,* must be complete in and of itself. Scientific articles or other publications cannot be substituted for the report.
- A signed electronic copy of this report must be forwarded to the funders' representative on or before the due date, as per the investment agreement.
- A detailed, signed statement of revenues received and expenses incurred during the entire funding period of the project must be submitted along with this report, as per the investment agreement.
- For any questions regarding the preparation and submission of this report, please contact the funders' representative.

# Section A: Project overview

| | |
|---|---|
| **1. Project number:** 2019F054R | |
| **2. Project title:** Biocontrol potential of entomopathogenic nematodes (EPNs) against selected key insect pests of canola in Alberta. | |
| **3. Abbreviations:**<br>BCW- Black Cutworm<br>CRM- Cabbage Root Maggot<br>DBM- Diamondback Moth<br>EPN- Entomopathogenic Nematode | |
| **4. Project start date:** (2019/03/01) | |
| **5. Project completion date:** (2020/03/31) | |
| **6. Final report submission date:** (2020/03/31) | |
| **7. Research and development team data** | |
| **a) Principal Investigators:** | |
| **Name** | **Institution** |
| Dr. Paul Tiege | OCCI, Olds College Alberta, Canada |
| Dr. Shabeg Briar | OCCI, Olds College Alberta, Canada |
| **b) Research team members** | |
| **Name** | **Institution** |
| | |
| Hilke Beuck | OCCI, Olds College Alberta, Canada |

# Section B: Non-technical summary (max 1 page)

Crop losses and the economic impact caused by canola insect pests are substantial and resistance to chemical control is a growing problem as the number of options are shrinking over time. Reliance on chemical insecticide-based management increases the risk of development of pesticide resistance, and poses risk to beneficial insects. Therefore, there is a need for the development of alternative, environmentally friendly pest management techniques to manage both below and above ground insect pest populations effectively. Entomopathogenic nematodes, also known as predatory nematodes, are commercially available biocontrol agents. Their use against both foliar and below ground pests is largely unexplored in the Canadian Prairies. Our main objective for this short term laboratory based project was to produce base line information on the biocontrol potential of the predatory nematode species. These nematodes were tested at low to high concentrations under controlled laboratory conditions against foliar insect pests including Flea Beetles, Diamondback Moth and Lygus, and below ground pests including Cabbage Root Maggots and Black Cutworms using small petri dishes or plastic cups. Insect mortality was assessed after 72 hours of exposure to the nematodes and observed under the microscope to confirm nematode infection. Predatory nematodes belonging to *Steinernema* group provided significant mortality of Diamondback Moth, Lygus, Cabbage Root Maggots and Black Cutworms.

*Heterorhabditis bacteriophora* provided significant larval mortality for Black Cutworms and Diamondback Moth only. Moderate level of mortality to the Diamondback Moth pupae suggests even better outcomes as EPNs were effective on both larvae and pupae stages. Cabbage Root Maggot pupae appears to be resistant to entry to all EPNs likely due to hard shell covering. High efficacy of EPNs in causing significant mortality of Black Cutworms tested in the study proved to be encouraging as similar level of efficacy would be expected for other cutworm species. All nematode species tested showed very low mortality (10% or less) of flea beetles adults. Results of the current study provided base line information for conducting field application studies on canola for management of Diamondback Moth, Lygus, Cabbage Root Maggots and Black Cutworms. Exploration of locally adapted and virulent strains of EPNs, and further improvement in application technologies pertinent to the Prairie farming systems should be considered in future projects.

# Section C: Project details

## 1. Background

Crop losses and the economic impact caused by canola insect pests is substantial and resistance to chemical control is a growing problem as the number of options are shrinking over time. For example flea beetles control is primarily based on imidacloprid insecticide treated seeds, and further foliar applications are necessary when adult feeding injury levels reach 15-20% at the canola seedling stage (Lamb et al., 1982; Lamb, 1988; Antwi et al., 2007; Reddy et al., 2014). Diamondback moth (*Plutella xylostella* L) populations routinely infest crops of canola and mustard in Canada. In some years populations reach outbreak densities and substantial crop losses can occur (Canola Council). Several insecticides are registered for diamondback moth larvae control in canola but may pose risk to pollinators and other beneficial insects.

The below ground pest cabbage root maggot (*Delia radicum* L) feeds on small fibrous roots and tunnels into stems and large fleshy roots of cruciferous crops. Heavy maggot infestations in canola and mustard can halt blooming and cause severe lodging and yield losses. Maggot feeding damage also provides entry points for root rot fungi, causing further stress on the plant. Per Alberta Agriculture and Forestry data, canola yield losses of 20-50 per cent have been recorded in Alberta due to maggot damage. In-furrow application of granular insecticides with the seed only provide first generation maggot control while no pesticides are available for control later in the season. Similarly, subterranean pests, commonly considered as cutworms (larvae of several noctuid moth species) (Lepidoptera: Noctuidae) cause crop damage while the adults, eggs and pupa may have no impact on crop productivity and yield (Floate, 2017). Most of the cutworm species such as pale cutworm (*Agrotis orthogonia*), black cutworms (*A. ipsilon*), army cutworm (*Euxoa auxiliaris*), clover cutworm (*Anarta trifolii*) and red backed cutworm (*Euxoa ochrogaster*) are polyphagous and are capable of causing significant damage to various crops including canola in the Prairies (Floate, 2017).

Reliance on chemical insecticide-based management increases the risk of development of pesticide resistance and harm to beneficial insects (Knodel, 2017). Therefore, there is a need for the development of alternative, environmentally friendly Integrated Pest Management (IPM) techniques to manage both below and above ground insect pest populations effectively.

Entomopathogenic nematodes (EPNs, also known as predatory nematodes) are soil-dwelling round worms (Phylum: Nematoda, Order: Rhabditida) that specialize in parasitizing insects. Infective juveniles (IJs) of EPNs penetrate the insect host through natural openings and in some

cases directly through the insect cuticle (Campbell and Gaugler, 1991; Hazir et al., 2003). IJs release symbiotic bacteria (*Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae) inside the insect's hemocoel, resulting in septicemia that kills the insect 24-48 hours later (Grewal et al., 2005). EPNs have been widely studied as biocontrol and are commercially available for the management of variety of insect pests in North America and Europe. Although below ground insect stages are more susceptible, recent advancement in application technology has improved their bio-control effectiveness against foliar insect pests (Dito et al., 2016). Recently studies conducted in Montana have shown some success against foliar insect pests using chemical adjuvants such as the polyacrylate gel Barricade® (Antwi et al., 2016; Briar et al., 2018). EPNs use against foliar insect pests and below ground pests is largely unexplored in the Canadian Prairies. In this project, we explored four different commercially available EPN strains at different application rates against foliar insect pests including Flea Beetles, Diamondback Moth, canola Lygus and below ground pests including Cabbage Root Maggots and Black Cutworms under controlled laboratory conditions.

## 2. Objectives and deliverables

The main project objective was to assess the potential of using commercially available EPNs for the management of key insect pests (Diamondback moth, canola Lygus, Cabbage Root Maggot, Flea beetle and Black Cutworms).
Specific project objectives:
1) Develop laboratory methods to assess control of five insect pest species x EPN species;
2) assess infective threshold concentrations of EPN species to infect/kill insect pest species;
3) Determine effective dose (concentration ranges) of EPNs;
Long term objectives: Based on the finding of this project studies, a long term study will be proposed to evaluate EPNs against the selected insect pests under field conditions and provide sustainable solution to our growers.

## 3. Research design and methodology

### 3.1. Collection and purchase of insect pests

Diamondback Moth (DBM) and Black Cutworms (BCW) were purchased from the insect research lab Benzon Research Inc., 7 Kuhn Drive Carlisle, PA USA.

Cabbage Root Maggot larvae and pupae were collected from the infested fields at Lacombe Research Station and Olds College Research fields. Pupae were collected early in the spring from previous year canola plots while larvae were collected late in the spring to early summer from the maggot infested canola fields. Flea beetle (FB) adults and Lygus nymphs were collected from Lethbridge, Alberta from canola fields using sweep nets.

### 3.2. Purchase and initial preparation of EPNs

Four available species of EPNs were purchased from the Biobest Canada Ltd. Nematodes packaged in an inert matrix. Prior to use aqueous solutions were prepared by adding distilled water. Nematode concentration numbers were determined in exact volumes using counting slide.

### 3.3. Laboratory bioassays on efficacy of EPNs against insect pests

3.3.1. Scope of the experiment

Laboratory experiments were carried out to evaluate four different commercially available predatory nematode species including *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. kraussei* and *S. feltiae* against foliar insect pests including FB, DBM, canola Lygus and below ground pests including Cabbage Root Maggots and BCW under controlled laboratory conditions.

3.3.2. Nematode preparation and concentration levels

Nematodes packaged in an inert matrix were reconstituted with distilled water prior to use. Test solutions were prepared immediately prior to infection studies. The infective juveniles (IJs) were stored in sterilized distilled water in tissue culture flasks at 6-8 °C for no more than two weeks before they were used.

Four concentrations ranging from low to high levels were tested in the bioassays for each nematode and insect specie. For DBM (larvae), FB, Lygus, and Cabbage Root Maggot, nematode concentration levels of 25, 50, 100 and 200 IJs/larvae; 200, 400, 1000 and 2000 IJs/adult; 50, 100, 200 and 500 IJs/nymph; 25, 50, 100 and 200 IJs/ $cm^2$ respectively, were used for the bioassays. Same concentration levels were also used for pupae stage of DBM and Cabbage Root Maggot. For BCW, bioassay was first conducted at concentration levels of 25, 50, 100 and 200 IJs/ cm2. Due to high mortality (80-100%) observed even at the lower dose of 25 IJs/larva, the bioassay was later repeated at lower concentration levels with 5, 10, 50 and 100 IJs against 4th instar larvae. For Lygus nymphs only three *Steinernema* specie were tested.

The concentrations were prepared by counting out the desired number of IJs into 100 µl in a nematode counting slide under a compound microscope. Three counts were taken to arrive at a desired average concentration. Before application, EPNs were transferred from 8 °C to room temperature for 2 h for acclimatization (Sandhi et al., 2020). The viability of IJs was checked under the microscope prior to inoculations.

3.3.3. Experimental unit

For above ground pests, Petri dishes (47 mm) lined with thick cellulose paper were prepared by addition of two cotyledons of canola plants for flea beetle and mature canola leaves were added for Lygus nymph and DBM larvae. Immediately prior to transfer to Petri dishes, randomly selected adults of flea beetle were cooled in a refrigerator to reduce activity and facilitate transfer. DBM larvae and Lygus nymphs were directly added to the Petri dish.

For below ground pests (BCW and Cabbage Root Maggots), 30 mL plastic cups (approximate volume) were filled with 25 g of autoclaved sandy soil with surface area of 28 $cm^2$ (Sandhi et al., 2020). In each cup, a single larva was placed with two small pieces of freshly cut pieces of radish as food. Moisture was maintained at 10% v/v. The fourth larval instar (L4) was used for BCW and both larval and pupal stages were tested on Cabbage Root Maggots.

3.3.4. EPN infectivity procedures

Test insects were added to the plastic cup or the Petri dish experimental arena and allowed to adapt for one hour. EPN were then added on to the larvae using a pipette in 1 mL aliquots. In the case of plastic cup experimental arenas, two small holes were made into the sand to add the nematodes. The control cups or Petri dishes received 1 mL of water without any IJs.

Petri dishes were placed randomly in the controlled environment chamber at 25º C, 80% relative humidity and 12 hour photoperiod. After 24 hours, Petri dishes were removed and test insects were transferred to a clean Petri dish with new leaf disks and mortality assessments were made after 48 hours.

In case of below ground pests (sand cup bioassays) insects were left in the same plastic cups and mortality was assessed after 72 hours. Plastic cups were placed randomly in the controlled

environment chamber in the dark. The cups were placed in trays with approximately 5 holes in the lids for aeration and then placed in an incubator at 23 °C and 80 % RH in the dark. The moisture content of each plastic container was 10% (w/w) after water-suspended nematodes were applied to the containers that included one healthy larva. Then, the cups were sealed with a lid allowing air exchange.

3.3.5. Replications

Bioassays were repeated depending upon the availability of test insects. For DBM, BCW and FB there were 10 replications for each of the four concentrations for all 4 EPN species. The bioassay was repeated total of three times for DBM and BCW, and two times for FB. For Cabbage Root Maggots there were 7 replicates and the bioassay was performed two times. In case of Lygus bioassay had 8 replicates and was performed only once.

3.3.6. Confirmation of mortality

Dead larvae were collected, transferred into a new Petri dish and rinsed with water in order to remove any nematodes attached to the cadavers. Cadavers were then transferred onto new clean glass slide and nematode infections were confirmed by dissecting the test insect (larvae, pupae or adult) with the scalpel in a few drops of distilled water. Nematode adults along with larval stages were observed under the microscope inside the dead insect to confirm infection.

### 3.4. Data analysis

For each species and concentration, the experimental unit was considered as all the test arenas with 1 test insect per dish or plastic cup for each concentration and insect specie. For example for DBM, experimental unit was 10 petri dish with 1 larvae in each dish, and the experiment consisted of total of 30 larvae per species tested per EPN concentration (10 $^{3}$per concentration). Concentration levels of EPNs for each insect species are provided in the results section. Statistical analysis was performed using Minitab (Version 13.0) statistical software package. Percent mortality (Means ± standard error) was calculated without being regulated by the Abbott formula since there was either no mortality or less than 3% in control plates except for flea beetles where the correction was applied (Abbott, 1925). Estimation of the lethal concentration required to kill 50% ($LC_{50}$) of the test population and the 95% confidence intervals (CI) for each nematode specie was calculated using Probit Analysis except for FB due to very low mortality only at the highest concentration. Due to lack of number of individuals available to repeat the bioassay for Lygus nymphs, only mean values are presented in the results section.

## 4. Results, discussion, conclusions and future directions

### 4.1. Results

Our main objective for this short term project was to collect information on the biocontrol potential of the commercially available EPN species against canola insect pests pertinent to the Prairies. Multiple canola insect pest species were sourced or collected from field and were tested using EPNs at low to high concentrations under controlled laboratory conditions. EPNs efficacy results for each insect pest specie are presented below.

#### 4.1.1. Diamondback moth (DBM)

Efficacy of four concentrations against 3rd-4th instar larva and pupae was estimated after 72 hours of exposure (Table 1 and 2). Mortality rates increased with increasing nematode concentrations.

Data clearly indicated that all three *Steniernema* sp were virulent against the larvae of DBM whereas *H. bacteriophora* caused low mortality at all concentrations (Table 1). $LC_{50}$ value was least for *S. kraussei* (21 IJs) followed by *S. carpocapsae* (42 IJs) and *S. feltiae* (45 IJs).

DBM pupae mortality was moderate levels irrespective of the nematode species (Table 2). Even at the high concentration level of 200 IJs/DBM pupae mortality was in the range of 50-70%. Interestingly, *H. bacteriophora* showed better results in causing pupae mortality relative to other species.

**Table 1:**

**Table 1: % mortality (Mean ± SE) and lethal concentrations to 50% mortality ($LC_{50}$) of diamondback moth (DBM) (*Plutella xylotstella* ) larvae exposed to different entomopathogenic nematode (EPN) sp. at four concentrations of infective juveniles (Ijs)/larva in petridish bioassays.**

| *EPN sp. | 25 Ijs | 50 Ijs | 100 Ijs | 200 Ijs | $LC_{50}$ ± SE | 95% CI[1] |
|---|---|---|---|---|---|---|
| | Mortality rates % (Mean ± SE) | | | | | Lower - Upper |
| HB | 33 ± 8.7 | 40 ± 9.1 | 60 ± 9.1 | 60 ± 9.1 | 80 ± 1.3 | 47 - 136 |
| SC | 40 ± 9.1 | 40 ± 9.1 | 90 ± 5.6 | 97 ± 3.3 | 42 ± 1.1 | 33 - 54 |
| SF | 43 ± 9.2 | 50 ± 9.3 | 67 ± 8.8 | 73 ± 8.2 | 45 ± 1.3 | 28 - 75 |
| SK | 63 ± 8.9 | 67 ± 8.8 | 70 ± 8.5 | 90 ± 5.6 | 21 ± 1.4 | 11 - 40 |

*HB: *Heterorhabditis bacteriophora;* SB: *Steinernema carpocapsae;* SF: *S. feltiae;* SK: *S. krausse.*

CI[1]: Confidence Interval; LC values calculated using Probit Analysis.

**Table 2:**

**Table 2: % mortality (Mean ± SE) and lethal concentrations to 50% mortality ($LC_{50}$) of diamondback moth (DBM) (*Plutella xylotstella* ) pupae exposed to different entomopathogenic nematode (EPN) sp. at four concentrations of infective juveniles (Ijs)/pupa in petridish bioassays.**

| *EPN sp. | 25 Ijs | 50 Ijs | 100 Ijs | 200 Ijs | $LC_{50}$ ± SE | 95% CI[1] |
|---|---|---|---|---|---|---|
| | Mortality rates % (Mean ± SE) | | | | | Lower - Upper |
| HB | 30 ± 10.5 | 35 ± 10.5 | 40 ± 11.0 | 70 ± 10.5 | 99 ± 1.4 | 55 - 179 |
| SC | 30 ± 10.5 | 35 ± 10.9 | 45 ± 11.4 | 55 ± 11.0 | 128 ± 1.5 | 57 - 291 |
| SF | 35 ± 10.9 | 35 ± 10.8 | 60 ± 11.0 | 65 ± 10.8 | 75 ± 1.3 | 43 - 137 |
| SK | 40 ± 11.0 | 50 ± 11.0 | 50 ± 11.4 | 50 ± 11.0 | 95 ± 1.6 | 38 - 236 |

*HB: *Heterorhabditis bacteriophora;* SB: *Steinernema carpocapsae;* SF: *S. feltiae;* SK: *S. krausse.*

CI[1]: Confidence Interval; LC values calculated using Probit Analysis.

### 4.1.2. Canola Lygus

Three *Steniernema* species were tested against the canola Lygus nymphs. *S. kraussei* and *S. carpocapsae* caused 87.5 % and 75% mortality respectively, at the concentration of 100 IJs while both were equally effective at 200 IJs level with mean mortality of 87.5%. *S. feltiae* caused maximum mortality of 62.5% even at the highest concentration of IJs/nymph (Figure 1).

**Figure 1:**

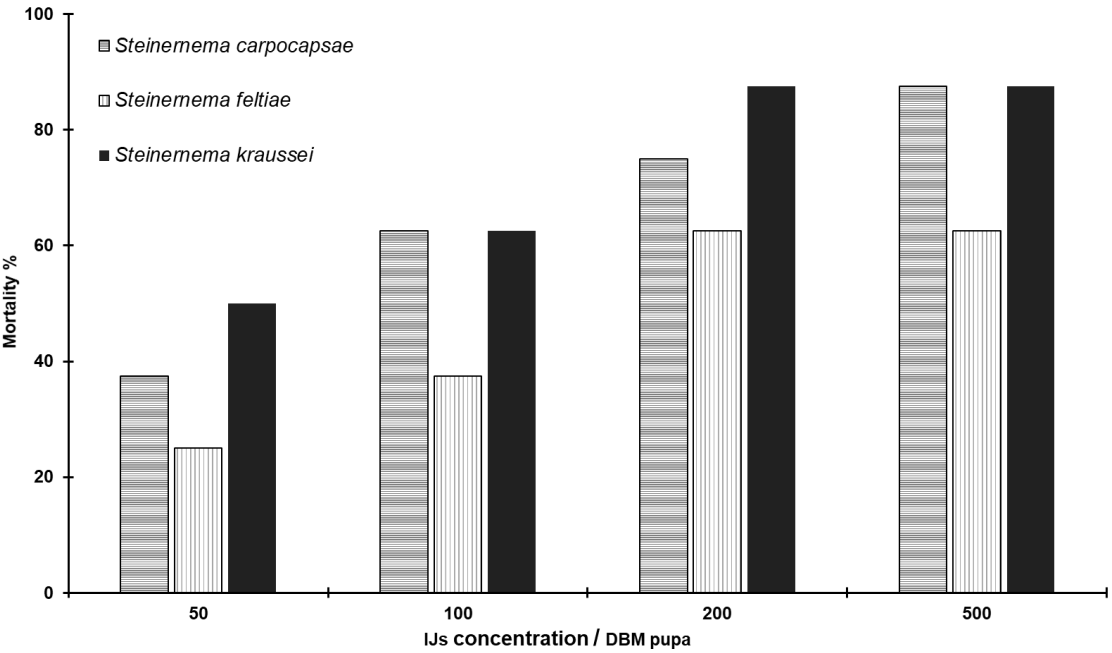



**Fig 1** : Mortality of canola Lygus bug sp. (nymph) exposed to commercial formulations of Entomopathogenic Nematodes (EPNs) infective juveniles (IJs) at 50, 100, 200 & 500/nymph.

### 4.1.3. Flea beetle (FB)

Results showed very low mortality (10% or less) of FB adults at the highest concentration levels only.

### 4.1.4. Black Cutworms (BCW)

*H. bacteriophora* provided an average mortality of 95% only at the highest concentration (100 IJs) while other species were effective even at 10 IJs/ larvae. (Table 3) (Figure 2). Estimated $LC_{50}$ value for *Steniernema spp* was in the range of 3-9 IJs compared to *H. bacteriophora* which was 14 IJs/cm$^2$. At the 95% confidence interval, the $LC_{50}$ value of all *Steniernema* species were significantly lower than those of *H. bacteriophora*.

**Figure: 2**


Black Cutworm larva Infected with Entomopathogenic nematodes
Photo credit S.S. Briar, OCCI, Olds College, AB

**Table 3: % mortality (Mean ± SE) and lethal concentrations to 50% ($LC_{50}$) of Black Cutworms (*Agrotis ipsilon*) larvae exposed to different entomopathogenic nematode (EPN) sp. at four concentrations of infective juveniles (Ijs)/$cm^2$ in sand cup bioassays.**

| *EPN sp. | 5 Ijs | 10 Ijs | 20 Ijs | 50 Ijs | $LC_{50}$ ±SE | 95% CI[1] |
|---|---|---|---|---|---|---|
| | Mortality rates % (Mean ± SE) | | | | | Lower - Upper |
| HB | 20 ± 9.1 | 30 ± 10.5 | 55 ± 11.4 | 95 ± 5.0 | 14 ± 1.2 | 11 - 20 |
| SC | 70 ± 10.5 | 95 ± 5.0 | 100 ± 0.0 | 100 ± 0.0 | 4 ± 1.2 | 3 - 6 |
| SF | 10 ± 6.8 | 70 ± 10.5 | 95 ± 5.0 | 95 ± 5.0 | 9 ± 1.1 | 7 - 10 |
| SK | 80 ± 9.1 | 85 ± 8.2 | 85 ± 8.1 | 100 ± 0.0 | 3 ± 1.3 | 2 - 6 |

*HB: *Heterorhabditis bacteriophora;* SB: *Steinernema carpocapsae;* SF: *S. feltiae;* SK: *S. krausse.*
CI[1]: Confidence Interval; LC values calculated using Probit Analysis.

### 4.1.5. Cabbage Root Maggots

Root maggot larvae were exposed to EPNs at four concentration levels 25, 50, 100 and 200 IJs/ $cm^2$ in a sand cup bioassay. Both *S. kraussei* and *S. feltiae* caused more than 80% mortality while

*S. carpocapsae* showed only a low level of mortality, in the range of 25%, at the highest level of application (Table 4) (Figure 3). No larval mortality was recorded with *H. bacteriophora*. Pupal stage of root maggots appeared to be resistant to all the EPN species of nematodes used in this study. Nematodes showed no host penetration of the pupa stage and consequently mortality estimation were not possible.

**Figure: 3**

Cabbage Root Maggots Infected with Entomopathogenic nematodes
Photo Credit S.S. Briar, OCCI, Olds College, AB

## Table 4:

**Table 4: % mortality (Mean ± SE) and lethal concentrations to 50% ($LC_{50}$) of Cabbage Root Maggots (*Delia radicum*) larvae exposed to different entomopathogenic nematode (EPN) sp. at four concentrations of infective juveniles (IJs)/$cm^2$ in sand cup bioassays.**

| *EPN sp. | 25 Ijs | 50 Ijs | 100 Ijs | 200 Ijs | $LC_{50}$ ±SE | 95% CI[1] |
|---|---|---|---|---|---|---|
| | Mortality rates % (Mean ± SE) | | | | | Lower - Upper |
| HB | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0.0 | _ | _ |
| SC | 0 ± 0 | 8 ± 8.3 | 25 ± 13 | 25 ± 13.0 | 399 ± 2.2 | 87 - 182 |
| SF | 17 ± 11.2 | 50 ± 15 | 67 ±14 | 83 ± 11.2 | 61 ± 1.2 | 40 - 95 |
| SK | 8 ± 8.3 | 42 ± 14.8 | 50 ± 15 | 83 ± 11.2 | 81 ± 1.2 | 55 - 121 |

*HB: *Heterorhabditis bacteriophora;* SB: *Steinernema carpocapsae;* SF: *S. feltiae;* SK: *S. krausse.*

CI[1]: Confidence Interval; LC values calculated using Probit Analysis.

## 4.2. Discussion

The ability of infective juveniles to cause pest insect mortality varied among the EPN species investigated in the current study. Although multiple factors influence the efficacy of EPNs, host-finding behaviour, symbiotic bacterial species hosted in them, and insect host behaviour such as life stage, evasive behaviour, and physical barriers to nematodes entry appears (Grewal e l. 20005) to be the most probable reasons that may be attributed for the differences among the species in inducing mortality.

According to the host-finding mechanisms, EPNs belongs to two main groups: cruisers and ambushers. *Heterorhabditis* species are characterized as cruisers as they search for the host in a cruiser strategy, and are therefore efficient in infecting non-mobile hosts. In contrast ambushers lift their body into the air for nictation or exhibit jumping behavior to attach to moving insects. *Steinernema* species varies among both cruisers and ambushers (Labaude and Griffin 2018; Grewal et al., 2005). Bacterial symbiont species associated with the nematodes is another important determinant in terms of host infection (Hazir et al., 2003). Mutualistic bacteria associated with Steinernematidae and Heterorhabditidae are *Xenorhabdus* and *Photorhabdus* respectively. These bacteria are released by the nematodes into the insect's hemocoel and induce septicemia to help kill the host (Hazir et al., 2003).

This study was primarily aimed at collecting baseline information to compare effectiveness of representative EPN species to cause mortalities in the five pest insect species investigated, with the intention to assess their biocontrol potential under field conditions. All three *Steniernema spp* provided high larval mortality of DBM whereas *H. bacteriophora* was less virulent at all concentrations. In contrast, *H. bacteriophora* was as effective as *Steniernemas* in terms of pupae mortality. The cruiser strategy was likely helpful in terms of locating the immobile pupal stages. For example, *H. bacteriophora* was more efficient relative to other species at infecting non-mobile hosts *Galleria mellonella* larvae maintained in cages compared to mobile hosts (Bal and Grewal, 2015). Results of our study are in general agreement with other research studies where both locally isolated strains and commercial EPNs provided high larval and moderate pupae mortality (Baur et al., 1995).

In addition to DBM, our study results also indicate that EPNs have significant potential of managing Lygus at nymph stage. Two EPN species, S. *kraussei* and S. *carpocapsae* caused high mortality of Lygus nymphs. To our knowledge there is no published research study in which EPNs were exploited for the management of canola Lygus bugs in the prairies. High efficacy of EPNs against DBM and Lygus bugs would be particularly more interesting especially when multiple generations of both pests occur in some geographical regions of the prairies and lifecycle overlap during the same crop stage. It appears that better outcomes would be expected with foliar applications as the current study showed that the EPNs were effective on more than one lifecycle stage, i.e. on larvae and pupae of DBM. We also hypothesize that a single application of EPNs under field conditions would be helpful in managing populations of both DBM and Lygus bugs as indicated by efficacy of EPNs under lab conditions.

Crucifer flea beetle (FB), *Phyllotreta cruciferae*, adult stage does not appear to be easy target of any of the EPN species tested in this study. Adults of FB cause major crop injury at the cotyledons stage of canola (Lamb, 1988). Multiple generation can occur in the field and larval feeding on the canola root hairs cause minor crop damage (Thomas, 2003). FB adults are highly mobile and possess thick cuticular layer thereby creating a physical barrier. Therefore, the likely hood of EPNs coming in contact and further host penetration is expected to be low. In, under field conditions, Antwi and Reddy (2016) found that EPNs provided some level of reduction in FB damage of canola seedlings. Lab bioassays conducted by Xu et al (2010) found significant larval mortality using different isolates of EPNs. Similarly, in small scale field study on Chinese cabbage by Yan et al (2012) found that EPNs were capable of reducing populations of the soil-dwelling larval stage of striped FB (*P. striolata*) thereby leading to a reduction of the adult populations. This may explain as to why Antwi and Reddy (2016) observed reduction in crop damage in their study results. However, neither adults nor larval counts were recorded in their study to further support their conclusions. Based on the above discussion, at present we expect a slim potential of EPNs for controlling FB at the adult stage and especially at a large scale under prairie farming system. Further exploration on either direct soil or foliar applications of EPNs targeting larval populations particularly at the overwintering sites, however, appears to be practical with the expectation of reduction in adult FB population migrating to the neighbouring fields.

For BCW (*Agrotis ipsilon*) EPNs provided 80% or higher control with the exception of *H. bacteriophora* being effective only at higher level dose of 50 IJs/cm$^2$. Cutworm is a common name given to the larvae of several noctuid moth species (Lepidoptera: Noctuidae). Although according to AAFC researchers canola crop in the prairies does not appear to be primary host of BCW specie *A. ipsilon* (Floate, 2017), tested in the current study, it is reported to cause significant damage to canola and other cruciferous plants in other parts of the world (Mahmoud et al., 2016). Other similar polyphagous species such as pale cutworm (*A. orthogonia*), army cutworm (*Euxoa auxiliaris*), clover cutworm (*Anarta trifolii*) and red backed cutworm (*Euxoa ochrogaster*) are capable of causing significant damage to various crops including canola in the Prairies. High efficacy of EPNs in causing significant mortality of BCW specie *A. ipsilon* appears to be encouraging, as similar level of efficacy would be expected for other cutworm species. Studies conducted in Québec, Canada found that commercial EPNs and indigenous isolate of *S. carpocapsae* caused high mortality of *A. ipsilon* both under lab and pot studies (Bélair et al., 2012). However, in their study an isolate of *S. feltiae* caused low cutworm mortality. In contrast high efficacy of *S. feltiae* against *A. ipsilon* in our results was likely due to better virulence of commercially available strain tested in our study.

EPNs showed moderate to high effectiveness on Cabbage Root Maggots tested in the current study. Our study confirmed the results of previous research conducted by Chen and Moens (2003) where *S. feltiae* was highly virulent to Cabbage Root Maggots while *S. carpocapsae* was partially effective. These authors also observed that the thick cuticle of root maggots was likely the reason for low penetration of *H. bacteriophora* and consequently lead to low level mortality. Bioassays conducted in the current study showed *H. bacteriophora* was unable to cause even low level of mortality to the larvae. This minor difference form the other study results was likely due to small surface area of well plates where nematodes were in much close contact to the host, as opposed to the current bioassay where root maggots were exposed in relatively larger sand cups. Overall from their and our study results we conclude that *H. bacteriophora* may not be a good candidate while *Steniernema* species particularly *S. feltiae* appears to possess better efficacy against Cabbage Root Maggots. Further, our observation that the EPNs were unable to enter into the pupae (likely due to harder shell covering) suggests that nematode application may provide better results only if the target is on susceptible larval stage.

No chemical is registered for Cabbage Root Maggots control in canola. Even in a small scale vegetable production where chemical application is permitted, the expected challenge is that the chemicals applied form lesser contact with the partially hidden larvae inside the root system. We hypothesize that the virulent EPN species like *S. feltiae* may relatively serve as an effective biocontrol provided that the timing of application also coincides with the susceptible larval stage.

### 4.3. Conclusions

Efficacy in terms of insect mortality varied among the EPN species. *Steniernema* spp tested in this study provided moderate to high mortality of insects in general while *H. bacteriophora* was relatively less effective. We also found moderate level of mortality to the DBM pupae with all EPN spp tested while Cabbage Root Maggot pupae appears to be resistant to entry to all EPNs likely due to hard shell covering. Our bioassays showed almost no mortality of flea beetle adults likely due to high mobility and hard covering of adults which may have prohibited nematodes to make effective contact, gain entry and cause host mortality. Therefore, we expect a slim potential of EPNs for controlling FB at the adult stage. Further exploration on applications of EPNs targeting only at the overwintering sites appears to be practical with the aim of reduction in FB adult population migrating to the neighbouring fields. Although, current study provided encouraging base line information for conducting field application studies with commercially available species on canola for management of multiple insect pests including DBM, canola Lygus, Cabbage Root Maggots and Black Cutworms, exploration of locally adapted and virulent strains of EPNs pertinent to the prairies should also be considered in the future projects.

### 4.4. Future work directions

The purpose of this work was to test the efficacy of infective juvenile stage of multiple entomopathogenic nematode species to control important pest insects collected from Alberta populations under lab conditions. Our research studies demonstrated favourable evidence that suggest further studies. Specifically, some EPN species were effective in inducing mortality in Diamondback Moth and Lygus nymph suggesting the potential for foliar sprays, and in inducing mortality in below ground pests i.e. Cabbage Root Maggot and Black Cutworm suggesting the potential for targeted soil application (soil drenching). We intend to develop future funding proposals to investigate the effectiveness of selected EPN species identified in this work outside laboratory environment under greenhouse and field conditions.

## 5. Literature cited


Antwi, F., Olson, D., Knodel, J. (2007). Comparative evaluation and economic potential of ecorational versus chemical insecticides for crucifer flea beetle (Coleoptera: Chrysomelidae) management in canola. Journal of Economic Entomology, 100:710-716.

Antwi, F.B., Reddy, G.V.P. (2016). Efficacy of entomopathogenic nematodes and sprayable polymer gel against crucifer flea beetle (Coleoptera: Chrysomelidae) on canola. Journal of Economic Entomology, 109:1706-1712.

Bal H.K., Grewal, P.S. (2017). Entomopathogenic nematodes for management of insect pests of canola and other oilseed crops: In, Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops, G.V.P. Reddy (ed), pp. 130-146. Oxfordshire: CABI

Bélair, G., Simard, L., Dionne, J. (2013). Canadian entomopathogenic nematode isolates: virulence against black cutworm (Lepidoptera: Noctuidae). Phytoprotection, 93, 43-46.

Briar, S.S., Antwi, F., Shrestha, G., Sharma, A., Reddy, G.V. (2018). Potential biopesticides for crucifer flea beetle, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) management under dryland canola production in Montana. Phytoparasitica, 46:247-254.

Baur, M.E., Kaya, H.K., Thurston, G.S. (1995). Factors affecting entomopathogenic nematode infection of Plutella xylostella on a leaf surface. Entomologia Experimentalis et Applicata, 77, 239-250.

Canola Council of Canada: https://www.canolacouncil.org.

Campbell, L., Gaugler, R., 1991. Mechanisms for exsheathment of entomopathogenic nematodes. Int. J. Parasitol. 21, 219-224.

Chen, S., Han, X., Moens, M. (2003). Biological control of *Delia radicum* (Diptera: Anthomyiidae) with entomopathogenic nematodes. Applied entomology and zoology, 38, 441-448.

Dito, D.F., Shapiro-Ilan, D.I., Dunlap, C.A., Behle, R.W., Lewis, E.E. (2016). Enhanced biological control potential of the entomopathogenic nematode, Steinernema carpocapsae, applied with a protective gel formulation. Biocontrol Science and Technology, 26:835-848. Entomologist, 116:269-280.

Floate, K.D. (2017). Cutworm pests on the Canadian Prairies: Identification and management field guide. Agriculture and Agri-Food Canada, Lethbridge, Alberta.

Grewal, P.S., Ehlers, R.U., Shapiro-Ilan, D.I., (Eds.) 2005. Nematodes as biocontrol agents. CABI Publishing, Cambridge, MA USA.

Hazir, S., Kaya, H.K., Stock, S.P., Keskin, N. (2003). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. Turkish J. Biol. 27, 181-202.

Knodel, J.J. (2017). Flea Beetles (*Phyllotreta* spp.) and Their Management: In Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops, G.V.P. Reddy (ed), pp. 1-12. Oxfordshire: CABI.

Knodel, J.J., Olson, D.L. (2002). Crucifer flea beetle: biology and integrated pest management in canola. NDSU Coop. Ext. Serv. Publ. E1234. NDSU, Fargo, ND.

Labaude, S., Griffin, C.T. (2018). Transmission success of entomopathogenic nematodes used in pest control. Insects, doi:10.3390/insects9020072.

Lamb, R. (1983). Phenology of flea beetle (Coleoptera: Chrysomelidae) flight in relation to their invasion of canola fields in Manitoba. The Canadian Entomologist, 115:1493-1502.

Lamb, R. (1988). Assessing the susceptibility of crucifer seedlings to flea beetle (Phyllotreta spp.) damage. Canadian Journal of Plant Science, 68, 85-93.

Mahmoud, M.F., Mahfouz, H. M., and KM, M. (2016). Compatibility of entomopathogenic nematodes with neonicotinoids and Azadirachtin insecticides for controlling the black cutworm, *Agrotis ipsilon* (Hufnagel) in canola plants. IJRES, 2, 11-18.

Reddy, G.V.P., Tangtrakulwanich, K., Wu, S., Miller, J.H., Ophus, V.L., Prewett, J. (2014). Sustainable management tactics for control of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) on canola in Montana. Journal of Economic Entomology, 107, 661-666.

Sandhi, R.K., Shapiro-Ilan, D., Sharma, A., Reddy, G.V. (2020). Efficacy of entomopathogenic nematodes against the sugarbeet wireworm, *Limonius californicus* (Mannerheim) (Coleoptera: Elateridae). Biological Control, 104190.

Shapiro-Ilan, D.I., Cottrell, T.E., Mizell, R.F., Horton, D.L. (2016). Efficacy of Steinernema carpocapsae plus fire gel applied as a single spray for control of the lesser peachtree borer, Synanthedon pictipes. Biological Control, 94, 33-36.

Thomas, P. (2003). Canola growers manual. Canola Council of Canada, Manitoba, Canada.

**6. Project team (max ½ page)**
**Describe the contribution of each member of the R&D team to the functioning of the project. Also describe any changes to the team which occurred over the course of the project.**

Dr. Paul Tiege wrote the co-funding Mitacs proposal and co-wrote the grant proposal, managed the project including the financial oversight, assisted in developing the experimental design, collecting insect pests from field locations, and preparing the final report.

Dr. Shabeg Briar is the project subject matter specialist and played a lead role in writing the current grant proposal and preparing research protocols, data collection, data analysis and preparing final report of this project. Dr. Briar managed sourcing and identification of nematode species, helped in sourcing or collection and identification of test insect pests and conducted entomopathogenic nematode bioassays in the Olds College Center for Innovation lab facilities. Dr. Briar also took a lead role on managing summer student helpers and guiding student intern in the Mitacs program.

Dr. Ken Fry extended his help in sourcing and identification and rearing of the test (pest) insects. Dr. Fry also helped in reviewing writing grant proposal, finalizing research protocols.

Research technician Hilke Beuck, extended help with student employee(s), and entering experimental data.

**7. Benefits to the industry (max 1 page; respond to sections *a)* and *b)* separately)**

**a) Describe the impact of the project results on the Alberta or western Canadian agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).**

Resistance to registered pesticides is increasing amongst certain insects and control options are becoming limited as even the registered insecticides such as neonicotinoids are under scrutiny by the Pest Management Regulatory Agency's (PMRA) and Environmental Protection Agency (EPA). Results of our laboratory bioassays provided encouraging base line information for conducting field application studies with commercially available species on canola for management of multiple insect pests including DBM, canola Lygus, Cabbage Root Maggots and Black Cutworms. With the help of field studies our long term goal hope is that producers would have a control option that might ultimately be used as a foliar treatment for leaf insect pests as well as a timed drench for multiple soil-dwelling stages of the pest insects such as root feeding larvae of maggots and subterranean cutworm species. Future key benefit of developing effective foliar-applied EPN preparations is compatibility with existing equipment and practices, and may become an important component of IPM strategies.

**b) Quantify the potential economic impact of the project results (*e.g.*, cost-benefit analysis, potential size of market, improvement in efficiency, etc.).**

This short term study aimed at providing only base line information and establish our in-house capabilities, and conduct future studies. Therefore, no cost-benefit analysis was possible and no information at present can be provided on potential size of the market.

From the positive results of this screen, we intend to identify the most economically impactful potential application(s) and to request funding for further study of these scenarios. The purpose will be to address the question of whether this alternative approach to pest insect control in canola can provide a meaningful or realistic alternative to pest control with favourable resistance-management characteristics for Alberta producers. However, information generated from this project for our future projects will help in market improvement efficiency.

## 8. Contribution to training of highly qualified personnel (max ½ page)

**Specify the number of highly qualified personnel (*e.g.*, students, post-doctoral fellows, technicians, research associates, etc.) who were trained over the course of the project.**

Student intern in Mitacs program (Darius Ramrattan), other summer students, and Research Technician (Emily Johnstone) all assisted during the experiments and became proficient in manipulating the various insects and in administering the EPN solutions to the test insect pests. The students helped to assemble the Petri dish and sand cup experimental units, source and feed the insects and gained valuable knowledge in the design of lab experiments. Student intern in the Mitacs program was trained in basic operation of the microscope in order to learn basic information on dead insects, and was taught theoretical aspects of nematode lifecycle, and mutualistic relationship between nematode and the bacterial symbiont. Although project investigators were ultimately responsible for using the identification keys to positively identify insects and to determine mortality and ascertain nematode life cycle stages for positive confirmation, Mitacs program student and other summer student helpers gained valuable experience and understanding of these areas and follow protocols.

**9. Knowledge transfer/technology transfer/commercialisation (max 1 page)**
**Describe how the project results were communicated to the scientific community, to industry stakeholders, and to the general public. Please ensure that you include descriptive information, such as the date, location, etc. Organise according to the following categories as applicable:**

**a) Scientific publications (*e.g.*, scientific journals); attach copies of any publications as an appendix to this final report.**

Manuscript entitled "Efficacy of commercially available entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the selected insect pests of Canola in Alberta" is currently under preparation and will be submitted to journal related to Biological Control. Acknowledgement to the contribution of each of the funders of the project will be listed in the manuscript.

**b) Industry-oriented publications (*e.g.*, agribusiness trade press, popular press, etc.); attach copies of any publications as an appendix to this final report.**

**c) Scientific presentations (*e.g.*, posters, talks, seminars, workshops, etc.); attach copies of any presentations as an appendix to this final report.**

Dr. Shabeg Briar presented poster entitled "Biocontrol potential of entomopathogenic nematodes (EPNs) against selected key insect pests of canola in Alberta" at the Sustainability of Canadian Agriculture: Farming for Solutions on March 12 & 13, 2020, Saskatoon, SK.

One in house presentation was also delivered by the student under the seminar series at Center for Innovation at Olds College, Alberta.

**d) Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.); attach copies of any presentations as an appendix to this final report.**

A Presentation was delivered to Alberta Canola Council by the student intern in Mitacs program.

**e) Media activities (*e.g.*, radio, television, internet, etc.)**

**f) Any commercialisation activities or patents**

No.

*N.B.: Any publications and/or presentations should acknowledge the contribution of each of the funders of the project, as per the investment agreement.*

# Section D: Project resources

1. **Provide a detailed listing of all cash revenues to the project and expenditures of project cash funds in a separate document certified by the organisation's accountant or other senior executive officer, as per the investment agreement. Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage (CDL); and overhead (if applicable).**

Separate financial report will follow.

2. **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**

Included in the financial report.

3. **Resources:**
   Provide a list of all external cash and in-kind resources which were contributed to the project.

NOTE: Dr. Ken Fry contributed time to the project specifically related to developing the proposal, developing and reviewing protocols, and advising. His time is donated as in-kind but is not captured in the form of a contract at the College and so is not auditable and is therefore not included in the following table as Other government sources: In-kind. Approximate in-kind contribution (time) = $1500.

| **Total resources contributed to the project** | | |
|---|---|---|
| **Source** | **Amount** | **Percentage of total project cost** |
| Agriculture Funding Consortium | $ 36,600 | 67.6% |
| Other government sources: Cash (Mitacs) | $ 7500 | 13.9% |
| Other government sources: In-kind | $ 0 | 0% |
| Industry: Cash | $ 10,000 | 18.5% |
| Industry: In-kind | $ 0 | 0% |
| **Total Project Cost** | $ 54,100 | **100%** |

| **External resources (additional rows may be added if necessary)** | | |
|---|---|---|
| **Government sources** | | |
| Name (no abbreviations unless stated in Section A3) | Amount cash | Amount in-kind |
| Mitacs | $7500 | |
| | | |
| **Industry sources** | | |
| Name (no abbreviations unless stated in Section A3) | Amount cash | Amount in-kind |
| Alberta Canola Producers Commission | $10000 | |

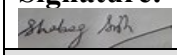
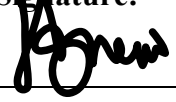
## Section E: Research Team Signatures and Authorised Representative's Approval

**The Principal Investigator and an authorised representative from the Principal Investigator's organisation of employment MUST sign this form.**

**Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.**

By signing as an authorised representative of the Principal Investigator's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

## Principal Investigator

| **Principal Investigators** | |
|---|---|
| **Name:**<br>Dr. Paul Tiege | **Title/Organisation:**<br>Research Scientist<br>Olds College, Alberta Canada |
| **Signature:**<br>  | **Date:**<br>03/31/2020 |
| **Name:**<br>Dr. Shabeg Briar | **Title/Organisation:**<br>Research Associate<br>Olds College, Alberta Canada |
| **Signature:**<br>  | **Date:**<br>03/31/2020 |
| **Principal Investigator's Authorised Representative's Approval** | |
| **Name:**<br>Dr. Joy Agnew | **Title/Organisation:**<br>Director of Applied Research |
| **Signature:**<br>  | **Date:**<br>03/31/2020 |

## Research Team Members (add more tables as needed)

| 1. Team Member | |
|---|---|
| **Name:**<br>Hilke Beuck | **Title/Organisation:**<br>Research Technician, OCCI Olds College Alberta, Canada |
| **Signature:** | **Date:**<br>**03 March 31** |
| **Team Member's Authorised Representative's Approval** | |
| **Name:**<br>**Paul Tiege** | **Title/Organisation:**<br>**Research Scientist**<br>**Olds College** |
| **Signature:**<br>  | **Date:**<br>**31 March 2020** |
| | |

| 2. Team Member | |
|---|---|
| | |
| **Signature:** | **Date:** |
| **Team Member's Authorised Representative's Approval** | |
| **Name:** | **Title/Organisation:** |
| **Signature:** | **Date:** |

## Section F: Suggested reviewers for the final report

Provide the names and contact information of four potential reviewers for this final report. The suggested reviewers should not be current collaborators. The Agriculture Funding Consortium reserves the right to choose other reviewers. Under *Section 34* of the *Freedom of Information and Protection Act (FOIP)* reviewers must be aware that their information is being collected and used for the purpose of the external review.

**Reviewer #1**
Name: Dr. Swaroop Kher
Position: Research Scientist (Entomologist)
Institution: Corteva Agri Science
Address: Edmonton, Canada
Phone Number: 780-709-3037
Email Address: swaroop.kher@corteva.com

**Reviewer #2**
Name: Dr. Harvinder Bennypaul
Position: Research Scientist (Nematologist, Virologist)
Institution: Canadian Food Inspection Agency
Address: 8801 East Saanich Road North Saanich V8L 1H3 British Columbia, Canada
Phone Number: 250-363-6650( Ext 236)
Email Address: harvinder.bennypaul@canada.ca

**Reviewer #3**
Name: Dr. Héctor Cárcamo
Position: Research Scientist (Entomologist, IPM Specialist)
Institution: AAFC
Address: PO BOX 3000, 5403 1st Avenue South, Lethbridge, T1J 4B, Canada
Phone Number: 403-317-2247
Email Address: hector.carcamo@canada.ca

**Reviewer #4**
Name: Dr. Govinda Shrestha (Entomologist, IPM Specialist)
Position: Postdoctoral Scholar
Institution: Oregon State University
Address: Hermiston Agricultural Research and Extension Center
2121 South 1st, Street Hermiston, OR 97838
Phone Number: 541- 567-6337 Ext. 117
Email Address: govinda.shrestha@oregonstate.edu