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Efficacy of commercially available entomopathogenic nematodes against insect pests of canola in Alberta, Canada

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Abstract

Certain entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are among the most studied biocontrol tools, some of which are commercially available against pest insects. Their use against foliar and subterranean insect pests is largely unexplored in the Canadian Prairies. We conducted a laboratory-based study to produce baseline information on the biocontrol potential of a few commercial EPN species. Percent mortality of flea beetles, diamondback moths (DBMs), lygus, cabbage root maggots, and black cutworms (BCWs) was assessed after 72 hours exposure to *Steinernema carpocapsae*, *S. kraussei, S. feltiae*, and *Heterorhabditis bacteriophora* at varying concentrations (25, 50, 100, and 200 infective juveniles (IJs) per larvae, pupae, or cm^2 of soil surface). Irrespective of concentration level, *S. carpocapsae* and *S. kraussei* caused significant mortality in DBM and BCW larvae compared with *H. bacteriophora*. *S. kraussei*, and *S. feltiae* were more efficient than *S. carpocapsae* in controlling root maggot larvae. *H. bacteriophora* caused zero mortality to root maggots at any concentration. Root maggot pupae were resistant to entry to EPN species tested, likely due to hard outer covering. Compared with root maggot pupae, a moderate level of mortality was observed in DBM pupae, suggesting differential ability of the tested EPNs in killing different life stages of certain pests. All nematode species tested caused low mortality (≤10%) in flea beetle adults. The findings of this investigation form fundamental data essential for carrying out field-based studies on canola and other related crops aimed at control and management of these pest species.

Introduction

The canola industry in Canada, along with other regions around the globe, faces significant threats from a variety of crop insect pests, with detrimental impacts on crop productivity, agro-economic sustainability, and ultimately, global food security (Arthey 2020). Among those pests are the diamondback moth (DBM), *Plutella xylostella* (Linnaeus 1758); several root maggot species, especially *Delia radicum* (Linnaeus 1758); larva of several noctuid moth species, including black cutworms (BCWs) (*Agrotis ipsilon* (Hufnagel 1766)); flea beetles (FBs), *Phyllotreta cruciferae* (Goeze 1777); *Phyllotreta striolata* (Fabricius 1801); and several species of lygus bugs, *Lygus* spp. (Hahn 1833), which have had considerable significance in Canada over the past years as major pests of canola, the majority being non-native, introduced from Europe or Asia (Arthey 2020; Fried *et al.* 2017). In Canada, the DBM, a foliar insect pest, routinely infests canola and other crops of the mustard family (Brassicaceae), reaching outbreak densities in certain years, thus causing severe crop losses (Dosdall *et al.* 2002). For instance, the Western Committee on Crop Pests estimated a cost of 45 to 52 million Canadian dollars for insecticide applications on controlling DBM populations in a geographic area of 1.25 million ha in Western Canada in 1995, whereas in 2001, they reported an outbreak on an even greater geographic area (approximately 1.8 million ha). On the other hand, the cabbage root maggot, a subterranean pest, feeds on small fibrous roots and tunnels into stems and large fleshy roots of cruciferous crops (Broatch 1993). Maggot infestations, depending on their intensity, can lead to halted blooming in canola and mustard (Government of Manitoba 2023). Additionally, these infestations can cause severe lodging and yield losses. The regions affected by maggot feeding serve as entry points for root rot fungi, leading to additional stress to the crop (Griffiths 1986). According to Griffiths (1986) and Soroka and Dosdall (2011), the estimated yield losses due to maggot damage may reach up to 50% in crops of *Brassica rapa* and 18% in *Brassica napus* across Alberta. In-furrow application of granular insecticides during seeding is reportedly an effective first-generation control measure to prevent root maggot larvae infestation (Ritcey *et al.* 1991). However, beyond the larvae stage of development, there is no known post-emergence pesticide control measure to combat this pest. Similarly, cutworm larvae and subterranean larvae of several other noctuid moth species (Lepidoptera: Noctuidae) can cause severe damage to crops, however, interestingly, with no negative impacts on crop productivity and yield observed at the adult or pupa stages of insect

development (Floate 2017; Knodel & Shrestha 2018). Most of the cutworm species, including BCWs (*A. ipsilon*), army cutworm (*Euxoa auxiliaris*), clover cutworm (*Anarta trifolii*), and red-backed cutworm (*Euxoa ochrogaster*), are polyphagous, an indication of their ability to feed off different types of crops, including canola (Floate 2017).

The most popular control method of these insect pests is the application of insecticides (Metcalf & Luckmann 1994). However, the issue of insects increasingly developing resistance to chemical control methods was reported at least five years ago (Bass & Jones 2018), signifying a steady decline in the number of viable insect pest control measures available today. A number of different insecticides are registered for controlling different insect pest species at various stages of development; however, the chemicals in the insecticides may also endanger other unrelated wildlife species, especially pollinators and other beneficial organisms (Chagnon *et al.* 2015). These tendencies support the need for the development of a diversity of integrated pest management (IPM) techniques, including alternative, non-chemical measures that can be implemented solely or in combination with chemical measures, to prevent below-ground and above-ground insect pest populations.

An alternative biological IPM measure involves entomopathogenic nematodes (EPNs), which are soil-dwelling nematodes (Order: Rhabditida) that parasitize certain insect pests (Askary & Abd-Elgawad 2017). Infective juveniles (IJs) of EPNs penetrate the insect host through natural openings and, in some cases, directly through the insect cuticle (Campbell & Gaugler 1991; Hazir *et al.* 2003; Askary & Abd-Elgawad 2017). IJs release symbiotic bacteria (*Xenorhabdus* in Steinernematidae and *Photorhabdus* in Heterorhabditidae) inside the insect's hemocoel, resulting in septicemia that kills the insect within 24 to 48 hours (Grewal *et al.* 2005; Askary & Abd-Elgawad 2017). Thus, EPNs have been widely studied as biocontrol agents against a variety of insect pests, and a few species are commercially available in North America and Europe (e.g., Entonem, Koppert, Berkel en Rodenrijs, The Netherlands; Nemasys, Becker Underwood, Ames, IA, USA (acquired by BASF); and NemaShield, Bioworks, Fairport, NY, USA) (Kaya *et al.* 2006; Caamano *et al.* 2008). However, the use of EPNs against insect pests is largely unexplored in the Canadian Prairies. Thus, the main objective of this project was to explore the biocontrol efficacies of different commercially available EPN strains at different application rates against above-ground (foliar) and below-ground (subterranean) insect pests of canola under controlled laboratory conditions. The findings of this study are expected to provide grounds for evaluating EPNs against crop insect pests under field conditions in future research which would provide sustainable solutions to Canadian growers.

Materials and methods

A laboratory experiment was designed to evaluate four different commercially available EPN strains at four different application rates on five different insect pests up to three different stages of development of each insect pest (i.e. larvae, pupa, and/or adults).

Collection and purchase of insect pests

The DBMs and BCWs were purchased from the insect research laboratory Benzon Research, Carlisle, PA, USA. Cabbage root maggot larvae and pupae were collected from the infested fields at Lacombe Research and Development Centre, Lacombe, AB and the fields of Olds College of Agriculture & Technology, Olds, AB. Pupae were collected from canola plots early in the spring of 2018; larvae were collected late in the spring to early summer of the same year. FB adults and lygus nymphs were collected from Lethbridge, AB, Canada, from selected canola fields using sweep nets during the summer months.

Four available species of EPNs (*Heterorhabditis bacteriophora*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae*) were purchased from Biobest Canada. Prior to use, the nematodes that come as packs in an inert matrix were reconstituted as aqueous solutions by adding distilled water to revive the nematodes, as instructed in the packaging.

Laboratory bioassays on the efficacy of EPNs against insect pests

Laboratory experiments were carried out to evaluate the efficacy of *H. bacteriophora, S. carpocapsae, S. kraussei,* and *S. feltiae* against above-ground insect pests, including FBs, DBMs, and canola lygus, and below-ground pests, including cabbage root maggots and BCWs, under controlled laboratory conditions.

Nematode preparation at different concentration levels

The IJs of *H. bacteriophora*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae* were stored separately in sterilized distilled water in tissue culture flasks at 6–8°C for no more than two weeks before they were used. The number of nematodes at each desired concentration level was determined in the exact volumes (average number of nematodes in three drops of 100 µL of the solution) of the nematode solution using counting slides. Four nematode IJ concentrations ranging from low to high levels were tested in the bioassays for each nematode species at different development levels of the interested pest species (i.e. for DBM larvae, FB, lygus, and cabbage root maggot, nematode concentration levels of 25, 50, 100, and 200 IJs/larvae or pupae; 50, 100, 200, and 500 IJs/nymph; 200, 400, 1000, and 2000 IJs/adult; and 25, 50, 100, and 200 IJs/cm^2). For BCWs, a bioassay was first conducted at concentration levels of 25, 50, 100, and 200 IJs/cm^2. 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 fourth larval instar of BCWs. For lygus nymphs, only three *Steinernema* species were tested. Before application, EPNs were transferred from 8°C to room temperature for two hours for acclimatization (Sandhi *et al.* 2020). The viability of IJs (based on any movements) was checked under the microscope prior to inoculations.

Experimental setup for the pest hosts

To accommodate the availability of test insects, a total of 10 replicates, each with an individual insect pest of interest on a select test arena, were generated for each of the four concentrations across all four species of EPNs. Specifically, for DBMs, BCWs, and FBs, 10 replicates of the corresponding bioassays were prepared for each of the four EPN treatments. The relevant bioassays were repeated three times for DBMs and BCWs and two times for FBs. For cabbage root maggots, there were seven replicates and the bioassay was repeated two times, while the bioassay for lygus had eight replicates and was performed only once.

All above-ground pests were exposed to EPNs in Petri dish setups: FBs were added to separate sterilized disposable Petri dishes (47-mm diameter), each with an absorbent cellulose pad. The dishes were sourced from Fisher Scientific. Two approximately

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similar-sized cotyledons of canola plants were added to each dish for the flea beetles to feed on. A similar experimental arena with similar-sized Petri dishes was prepared for the lygus nymph and DBM larvae. Two mature canola leaves cut into 2-cm^2 pieces, instead of cotyledons, were added to each Petri dish setup for lygus nymph and DBM larvae. DBM larvae and lygus nymphs were directly added to the Petri dish once the setups were arranged while adult FBs were cooled in a refrigerator immediately prior to transfer to the Petri dishes to reduce mobility and facilitate easy transfer.

All below-ground pests were exposed to EPNs in plastic cups filled with soil: plastic cups (30 mL) were filled with approximately 25 g of autoclaved sandy soil for BCWs and cabbage root maggots, both of which are below-ground pests (Sandhi *et al.* 2020). In each cup, a single larva of either pest was placed approximately 2 cm deep in the soil with two equal-sized freshly cut pieces of radish as food on the soil surface. The moisture level in each setup was maintained at 10% v/v by adding tap water. Lids were perforated to ensure proper ventilation (five holes in each lid). The fourth larval instar of BCWs and both larval and pupal stages of cabbage root maggots were used in the experiments.

EPN infectivity

All the test insect pests were acclimated for one hour in their corresponding experimental arena (i.e. Petri dishes for the FB, lygus nymph, and DBM larvae; plastic cups for fourth instar larvae of BCWs and both larval and pupal stages of cabbage root maggots) before they were exposed to nematodes. The corresponding concentrations of EPNs in 1-mL aliquots were then inoculated onto the cellulose paper in each Petri dish. In sand cup bioassays, two small holes (approximately 2 cm deep) were made into the sand to inoculate nematodes at corresponding concentrations. The controls of each setup (i.e. sand cups or Petri dishes) received 1 mL of tap water without any IJs of nematodes. Petri dishes were then incubated at 25°C and 80% relative humidity with a 12-hour photoperiod for 24 hours. After 24 hours, the insect larvae in Petri dishes were separately transferred to new, nematode-free Petri dishes with fresh, similar amounts of canola leaf disks (2 cm^2). The mortality of the insect larvae was assessed after 48 hours post-exposure. In sand cup bioassays, the evaluation of mortality was conducted 72 hours after the initial exposure. Throughout this period (i.e. 72 hours), the insect larvae or pupae remained buried in the soil, except at the time they surfaced to feed, in the sand cups until the assessment of mortality was performed. Sand cup bioassays were randomly arranged and placed in an incubator at 25°C and 80% relative humidity in the dark. The moisture content of each plastic container was maintained at 10% v/v after water-suspended nematodes were applied to the sand cup bioassays. Once the mortality based on visual assessments was confirmed (i.e. larval discoloration and lack of movement upon poking with a blunt needle), the dead larvae/pupae were collected directly from the bioassays (BCWs and cabbage root maggots from sand cup bioassays) or from post-exposure nematode-free Petri dishes (FB, lygus nymph, and DBM larvae). They were transferred separately into new Petri dishes and rinsed thoroughly with water to eliminate any extraneous nematodes attached to the cadavers. The rinsed cadavers were then transferred onto a clean glass slide or small petri dish where the nematode infections were confirmed by dissecting the test insect (larvae, pupae, or adult) in a few drops of distilled water. The cadavers were inspected under a dissecting microscope for the presence of adult and/or larval stages of the nematodes as evidence for the infection.

Data analysis

Percent mortality (means ± standard error) was calculated without using Abbott's formula (Abbott 1925), except for FBs, where the correction was applied. Lethal concentration for 50% mortality (LC_{50}) and 95% confidence interval (CI) were estimated using Probit Analysis in Minitab (version 13.0) software for all the tested pests under each nematode concentration, except for FBs, for which a minimal (or zero) mortality was observed even at the highest nematode concentration. Only the mean values are presented for lygus nymphs due to the limited availability of individuals for bioassay replication. Logistic regression models with a probit link function were performed on R software (version 4.3.0) through the RStudio integrated development environment (version 2023.06.2 +561) to compare the mortality of each pest species due to varying concentrations of the interested nematode species.

Results

Diamondback moth (DBM)

The efficacy at four concentrations (i.e. 25, 50, 100, and 200 IJs/larva) of the nematodes *H. bacteriophora*, *S. carpocapsae*, *S. kraussei*, and *S. feltiae* against third to fourth larval instar and pupae of DBMs was estimated after 72 hours of exposure. Mortality rates increased with increasing nematode concentrations (Figure 1), significantly at 100 IJs/larva (z = 4.36; df = 479; P < 0.05), and 200 IJs/larva (z = 5.83; df = 479; P < 0.05) compared with 25 IJs/larva. All three *Steniernema* species were virulent against the larvae of DBMs. *H. bacteriophora* caused low mortality at all concentrations (Table 1). Irrespective of the concentration level, the mortality caused by *H. bacteriophora* was significantly lower than that of *S. kraussei* (z =3.98; df = 479; P < 0.05) and *S. carpocapsae* (z =3.00; df = 479; P = 0.0027) but was not statistically any different from *S. feltiae*. LC_{50} was the least for *S. kraussei* (21 IJs) followed by *S. carpocapsae* (42 IJs) and *S. feltiae* (45 IJs). The percent mortality of DBM pupae was moderate irrespective of the nematode species (Table 2). Even at the high concentration level of 200 IJs, the mortality of DBM pupae was in the range of 50–70%. Contrary to the low mortality rates observed in DBM larvae, *H. bacteriophora* showed comparable results to those of other EPN species in causing mortality in pupae of DBMs.

Canola lygus

Three *Steniernema* species (i.e. *S. kraussei*, *S. carpocapsae*, and *S. feltiae*) were tested against the canola lygus nymphs at 50, 100, 200, and 500 IJ nematode concentration levels. At all concentration levels, *S. kraussei* and *S. carpocapsae* caused significant mortality to lygus nymphs compared with the control with no nematodes (P = 0.0099 and P = 0.024, respectively); especially at 100 IJs, they caused, respectively, 87.5% and 75% mortality to lygus nymphs. Both nematode species were equally effective at 200 IJs level with a mean mortality of 87.5%. In contrast, *S. feltiae* caused maximum mortality of 62.5% at the higher concentration levels of IJs/nymph (200 and 500 IJs) (Figure 2).

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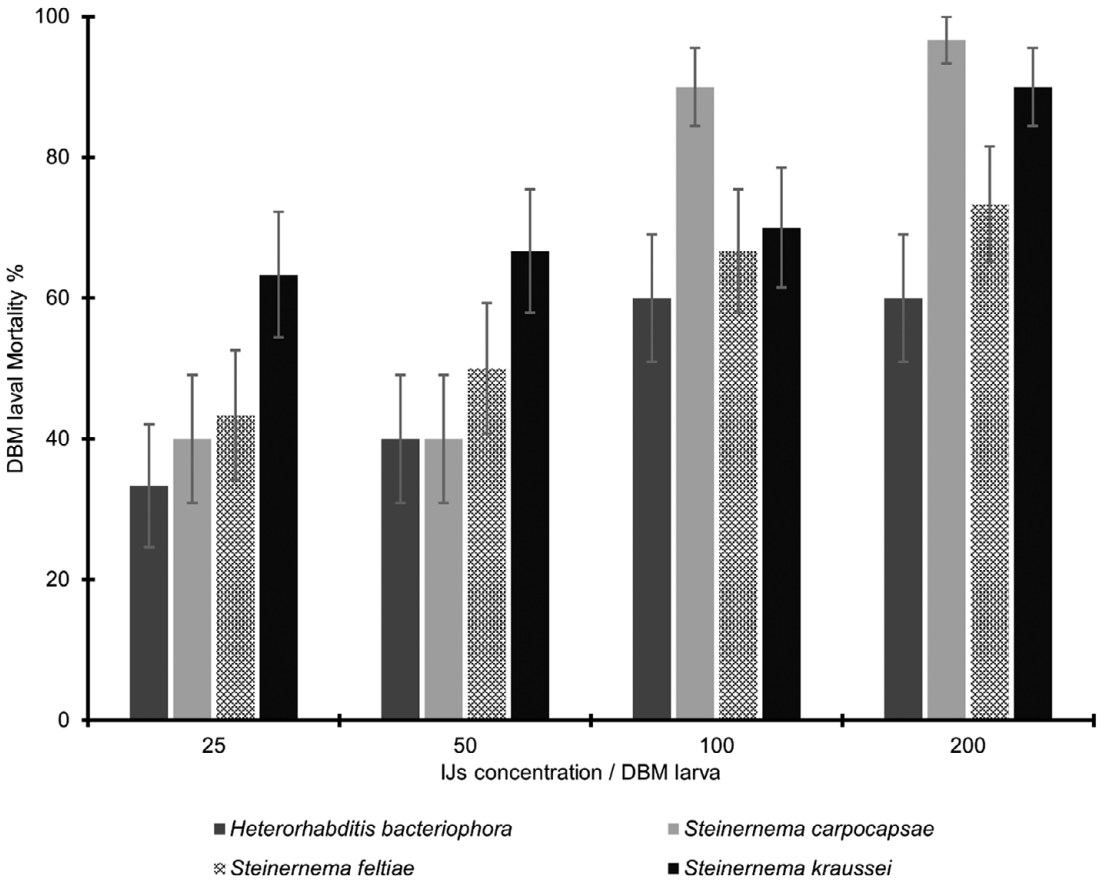

Figure 1. Mortality (± standard error) of diamondback moth (DBM) larvae exposed to commercial formulations of entomopathogenic nematodes (EPNs) infective juveniles at 25, 50, 100, and 200/insect larva. LC_{50}: lethal concentration for 50% mortality.

Table 1. Percentage mortality (mean ± SE) and LC_{50} of DBMs (*Plutella xylotstella*) larvae exposed to four different EPN species each at four different concentrations of IJs/larva in Petri dish bioassays

EPN species	25 IJs	50 IJs	100 IJs	200 IJs	LC_{50} (95% CI)
	Mortality rates % (mean ± SE) of DBM larvae				
HB	33 ± 8.7	40 ± 9.1	60 ± 9.1	60 ± 9.1	80 (47–136)
SC	40 ± 9.1	40 ± 9.1	90 ± 5.6	97 ± 3.3	42 (33–54)
SF	43 ± 9.2	50 ± 9.3	67 ± 8.8	73 ± 8.2	45 (28–75)
SK	63 ± 8.9	67 ± 8.8	70 ± 8.5	90 ± 5.6	21 (11–40)

CI: confidence interval; DBM: diamondback moth; EPN: entomopathogenic nematode; HB: *Heterorhabditis bacteriophora*; IJ: infective juvenile; LC_{50}: lethal concentration for 50% mortality; SC: *Steinernema carpocapsae*; SE: standard error; SF: *S. feltiae*; SK: *S. krausse*; LC values calculated using Probit Analysis in Minitab (version 13.0) software.

Table 2. Percentage mortality (mean ± SE) and LC_{50} of DBM (*Plutella xylotstella*) pupae exposed to four different EPN species at four different concentrations of IJ pupa in Petri dish bioassays

EPN species	25 IJs	50 IJs	100 IJs	200 IJs	LC_{50} (95% CI)
	Mortality rates % (mean ± SE) of DBM pupae				
HB	30 ± 10.5	35 ± 10.5	40 ± 11.0	70 ± 10.5	99 (55–179)
SC	30 ± 10.5	35 ± 10.9	45 ± 11.4	55 ± 11.0	128 (57–291)
SF	35 ± 10.9	35 ± 10.8	60 ± 11.0	65 ± 10.8	75 (43–137)
SK	40 ± 11.0	50 ± 11.0	50 ± 11.4	50 ± 11.0	95 (38–236)

CI: confidence interval; DBM: diamondback moth; EPN: entomopathogenic nematode; HB: *Heterorhabditis bacteriophora*; IJ: infective juvenile; LC_{50}: lethal concentration for 50% mortality; SC: *Steinernema carpocapsae*; SE: standard error; SF: *S. feltiae*; SK: *S. krausse*; LC values calculated using Probit Analysis in Minitab (version 13.0) software.

Flea beetle (FB)

We observed low mortality (maximum 10%) in FB adults even at the highest concentration levels (2000 IJs/adult) of all nematode species applied. Zero mortality was reported in FB adults at 200 and 400 IJ concentration levels regardless of the nematode species.

Black cutworms (BCWs)

H. bacteriophora provided an average mortality of 95% only at the highest concentration (50 IJs), while other species (i.e. *S. kraussei*, *S. carpocapsae*, and *S. feltiae*) were effective even at 10 IJs/larvae (Table 3). The estimated LC_{50} value for *Steniernema* spp. was in the range of 3–9 IJs compared with *H. bacteriophora*, which was 14 IJs/cm^2. At a 95% CI, the LC_{50} value of all *Steniernema* spp. was significantly lower than those of *H. bacteriophora.*

Cabbage root maggots

Root maggot larvae were separately exposed to *H. bacteriophora, S. kraussei*, *S. carpocapsae*, and *S. feltiae* at four concentration levels 25, 50, 100, and 200 IJs/cm^2 in sand cup bioassays. Even at the highest

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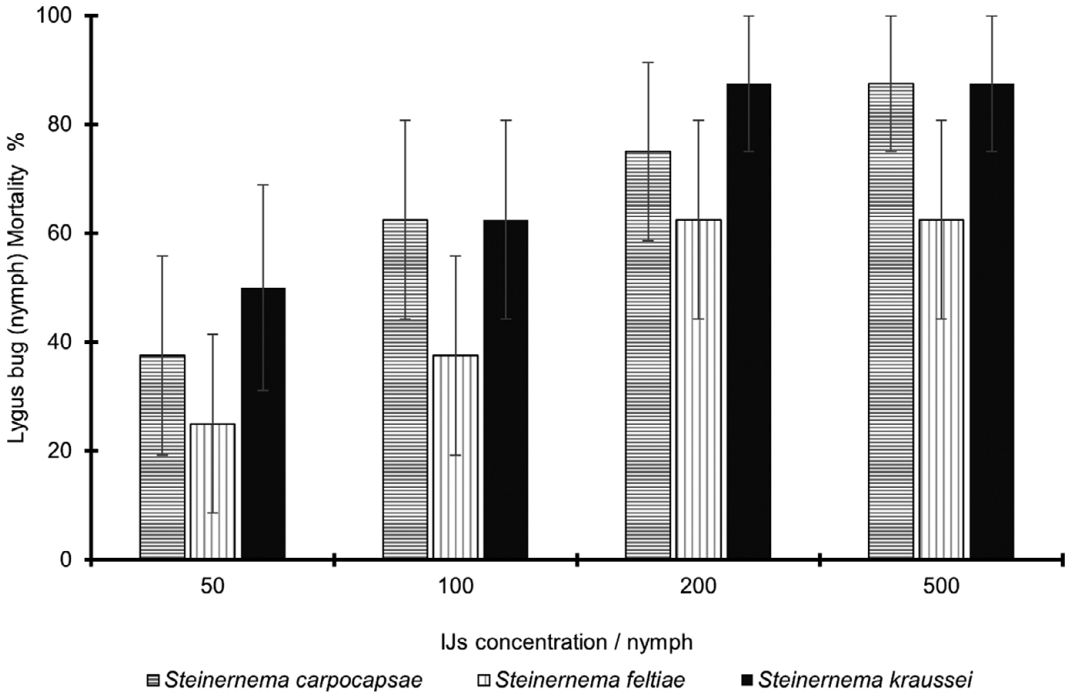

Figure 2. Mortality of canola lygus bug (nymph) exposed to the commercial formulations of the entomopathogenic nematode species *Steinernema kraussei*, *S. carpocapsae*, and *S. feltiae* each at 50, 100, 200, and 500/nymph.

Table 3. Percentage mortality (mean ± SE) and LC_{50} of BCW larvae exposed to different EPN species at four concentrations of IJs/cm^2 in sand cup bioassays

EPN species	5 IJs	10 IJs	20 IJs	50 IJs	LC_{50} (95% CI)
	Mortality rates % (mean ± SE) of BCW larvae				
HB	20 ± 9.1	30 ± 10.5	55 ± 11.4	95 ± 5.0	14 (11–20)
SC	70 ± 10.5	95 ± 5.0	100 ± 0	100 ± 0	4 (3–6)
SF	10 ± 6.8	70 ± 10.5	95 ± 5.0	95 ± 5.0	9 (7–10)
SK	80 ± 9.1	85 ± 8.2	85 ± 8.1	100 ± 0	3 (2–6)

BCW: black cutworm (*Agrotis ipsilon*); CI: confidence interval; EPN: entomopathogenic nematode; HB: *Heterorhabditis bacteriophora*; IJ: infective juvenile: LC_{50}: lethal concentration for 50% mortality; SC: *Steinernema carpocapsae*; SE: standard error; SF: *S. feltiae*; SK: *S. krausse*; LC values calculated using Probit Analysis in Minitab (version 13.0) software.

Table 4. Percentage mortality (mean ± SE) and LC_{50} of cabbage root maggots (*Delia radicum*) larvae exposed to four different EPN species at four different concentrations or IJs/cm^2 in sand cup bioassays

EPN species	25 IJs	50 IJs	100 IJs	200 IJs	LC_{50} (95% CI)
	Mortality rates % (mean ± SE) of root maggot larvae				
HB	0	0	0	0	—
SC	0 ± 0.1	8 ± 8.3	25 ± 13	25 ± 13.0	399 (87–182)
SF	17 ± 11.2	50 ± 15	67 ± 14	83 ± 11.2	61 (40–95)
SK	8 ± 8.3	42 ± 14.8	50 ± 15	83 ± 11.2	81 (55–121)

CI: confidence interval; EPN: entomopathogenic nematode; HB: *Heterorhabditis bacteriophora*; IJ: infective juvenile; LC_{50}: lethal concentration for 50% mortality; SC: *Steinernema carpocapsae*; SE: standard error; SF: *S. feltiae*; SK: *S. krausse*; LC values calculated using Probit Analysis in Minitab (version 13.0) software.

level of nematode application, *S. carpocapsae* showed a significantly lower level of mortality (25%) compared with *S. kraussei* and *S. feltiae* ($z = -2.82$; $df = 35$; $P = 0.005$), both of which caused more than 80% mortality (Table 4). No larval mortality was recorded with *H. bacteriophora*. The pupal stage of root maggots appeared to be resistant to all the EPN species of nematodes tested. At the pupa stage, the nematodes did not show any host penetration consequently, mortality estimation was not possible.

Discussion and Conclusions

In this study, we compared the efficacy of four commercial EPN species in causing mortality in a few insect pest species common to Canadian Prairies. EPN species varied in their ability to cause mortality to different pest insect species and in some pests at their different life stages; thus, certain explored commercial EPNs in this study might offer host- or host life stage–specific control opportunities against the tested insect pests in Canadian Prairies. All three *Steniernema* spp. provided high larval mortality to DBMs whereas *H. bacteriophora* was less virulent at all concentrations; however, *H. bacteriophora* was as effective as *Steniernema* in terms of pupae mortality of DBMs. The most probable factors that might have attributed to this differential level of success in infection, ultimately causing death to their host, may include but are not limited to host-finding behavior of the EPNs, symbiotic bacterial species housed within the host, insect host behavior at different life stages, evasive behavior, and/or physical barriers of the host to EPNs (Grewal *et al.* 2005). *Heterorhabditis* spp. are characterized as cruisers because they actively search for the host in soil and therefore are efficient in infecting non-mobile hosts (Bal & Grewal 2015). Bal & Grewal (2015) reported that *H. bacteriophora* was more efficient at infecting non-mobile hosts *Galleria mellonella*

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(greater wax moth) larvae maintained in cages compared with mobile hosts. Thus, we suspect that the cruiser strategy of *H. bacteriophora* was the most likely reason attributed in terms of locating and successfully invading the immobile pupal stages of DBMs in our assays. In contrast to *H. bacteriophora, Steinernema* spp. vary in their host-seeking behavior (Labaude & Griffin 2018; Grewal *et al.* 2005). The genus accommodates not only cruisers but ambushers capable of lifting their bodies into the air for nictation or exhibiting jumping behavior to attach to mobile insect hosts (Labaude & Griffin 2018; Grewal *et al.* 2005). Further, the observed drastic differences in host mortality by *H. bacteriophora* at different life stages of DBMs (i.e. mobile larvae and immobile pupae) are interesting in the sense that they provide insights into the complexity of the host–parasite interactions of EPNs. We observed a high level of virulence in each of the tested *Steinernema* spp. against larvae yet failed to see a similar mortality at the pupa stage of the host. Thus, *Steinernema* may offer promising control opportunities only at the larval stages of DBMs. The results of our study are further in general agreement with other research by Baur *et al.* (1995), in which they observed that commercial EPNs (and locally isolated EPN strains) provided high larval and moderate pupae mortality. Since the larval stage of DBMs is considered the most pestiferous life stage, *Steinernema* spp. might be the most tempting biocontrol agent against DBMs in Canadian prairies compared to *H. bacteriophora*. Thus, we suggest extending research on using *Steinernema* under field conditions to see if they would be as efficient as they were under laboratory conditions. In addition to DBM, our study results also indicate that EPNs have significant potential for managing lygus at their nymph stage. Two EPN species, *S. kraussei* and *S. carpocapsae*, caused high mortality to the lygus nymphs. To our knowledge, there is no published research in which EPNs were exploited for the management of canola lygus bugs in the prairies. Therefore, this study serves as the first report to use EPNs against canola lygus bugs with proven efficacy in control at nymph stages of this host under laboratory conditions. High efficacy of EPNs against DBMs and lygus bugs would be particularly more interesting, especially when multiple generations of both pests occur in the same geographical regions of the prairies and life cycles overlap during the same crop stage. It appears that better outcomes would be expected with foliar applications of the EPNs, because the current study showed that the EPNs were effective on more than one life cycle stage (i.e. on larvae and pupae of DBMs). We hypothesize that a single application of EPNs, especially *Steinernema* spp., under field conditions would be sufficient to effectively manage populations of both DBMs and lygus bugs, as indicated by the observed host mortality, even at low nematode concentrations. So far, the results of this study suggest *S. kraussei* and *S. carpocapsae* be the most efficient among commercially available *Steinernema* spp. against larvae of DBMs and nymphs of lygus bugs; however, it is important to note that our treatments only involved a maximum of three days of exposure of the pest hosts to the nematodes in a confined space, such as a Petri dish or a sand cup, under laboratory conditions. Therefore, we recommend conducting greenhouse pot screening for the EPNs followed by further testing under field conditions to confirm their efficacy before implementing them in practical applications.

Any of the tested EPN species did not appear to be effective in the adult stage of the crucifer FBs, *P. cruciferae*. Adult FBs cause major crop injury at the cotyledon stage of canola (Lamb 1988) whereas larvae seem to cause minor crop damage on the root hairs of canola (Thomas 2003). FB adults are highly mobile, and their bodies are physically protected by a thick cuticular layer, creating a physical barrier. This barrier makes it more challenging for EPNs to make contact through cruising or ambushing and subsequently penetrate the FB adults, unlike the soft-bodied larval stages. As a result, the likelihood of EPNs coming into contact with and penetrating FB adults is expected to be low. Regardless, studies have demonstrated the potential use of EPNs in managing FBs. For instance, Antwi & Reddy (2016) conducted a study under field conditions and found that the application of EPNs resulted in reduced damage to canola seedlings by adult FBs, while in another small-scale field study on Chinese cabbage, Yan *et al.* (2013) demonstrated that S. *carpocapsae* and *H. indica* (LN2) were capable of reducing populations of the soil-dwelling larval stage of striped FBs (*P. striolata*), ultimately reducing the adult populations. This explains why Antwi & Reddy (2016) observed a reduction in crop damage in canola seedlings by adult FBs. However, neither adults nor larval counts were recorded in Yan *et al.* (2013) study to further support their conclusions. Thus, we expect a slim potential of EPNs in controlling FBs at the adult stage, especially at a large scale under the prairie farming system. Further exploration on either direct soil or foliar applications of EPNs targeting larval populations, particularly at the overwintering sites, appears to be practical, with the expectation of reduction in adult FB population migrating to neighboring fields. Further, laboratory bioassays conducted by Xu *et al.* (2010) demonstrated significant FB larval mortality under 20 isolates of *Steinernema* and *Heterorhabditis*. Therefore, exploring opportunities to use the same commercial EPNs on the larvae of FBs is suggested in future studies to compare their efficacy under laboratory conditions followed by greenhouse pot experiments and field experiments in prairies in Alberta.

In BCWs (*A. ipsilon*), the tested EPNs provided 80% or higher control with the exception of *H. bacteriophora*. *H. bacteriophora* was effective as other EPNs tested only at a higher-level dose of 50 IJs/cm^2. Although canola in the prairies does not appear to be the primary host plant for BCW species (Floate 2017), it reportedly causes significant damage to canola and other cruciferous plants in other parts of the world (Mahmoud *et al.* 2016). The efficacy of EPNs in causing significant mortality in BCWs appears to be encouraging as a similar level of efficacy would be expected against the rest of other cutworm species. Bélair *et al.* (2013) compared the virulence of ten indigenous and two commercial isolates of EPNs against BCWs and found that none of the Canadian EPN isolates (isolated from the provinces of Quebec and Ontario), except one (*S. carpocapsae* (6Sc)) outperformed the two commercial formulations tested. Additionally, they demonstrated in the same study that the virulence of *S. feltiae* consistently exhibited lower levels compared with *S. carpocapsae* isolates, aligning with previously published research on BCW larvae susceptibility to EPNs. The local *S. carpocapsae* (6Sc) displayed similar or higher virulence than the commercial isolate against BCW larvae (Baur *et al.* 1997; Ebssa & Koppenhöfer 2011; Bélair *et al.* 2013). In our study, both *S. feltiae* and *S. carpocapsae* proved their significant efficacy in killing BCWs, but, based on the mean percentage mortality of BCWs, we further confirm that *S. feltiae* exhibits lower virulence compared with *S. carpocapsae* across all tested nematode concentrations we tested. The current study, along with the above studies, therefore further highlights the importance of isolating, identifying, and testing native strains, such as *S. carpocapsae* (6Sc), for their virulence in Prairies.

We noted similar efficacy of *S. feltiae,* and *S. kraussei* on killing cabbage root maggots; however, the mortality of cabbage root maggots by *S. carpocapsae* was not prominent compared with the

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other two *Steinernema* spp. The results are comparable with a similar previous study in which *S. feltiae* had higher virulence on cabbage root maggots compared with *S. carpocapsae* (Chen *et al.* 2003). *H. bacteriophora*, the other EPN species they tested, had limited success in causing mortality to root maggots. The low penetration success through the thick cuticle of root maggots was likely the reason for the insufficient penetration of this nematode, consequently resulting in low levels of mortality. We did not see any mortality in root maggots by *H. bacteriophora* unlike the low, yet positive virulence observed in the previous study by Chen *et al.* (2003). The observed differences between the two studies are suspected to be attributed to the limited space provided in the previous study, as opposed to our study, where we used relatively larger sand cups instead of well plates. The use of larger sand cups in our study does not allow immediate contact between the nematodes and the host unlike in well plates, potentially leading to less chances for the host encounters and subsequent nematode penetration, hence no mortality. Thus, we conclude that *H. bacteriophora* may not be a good candidate for root maggot control while *Steniernema* spp., particularly *S. feltiae* and *S. kraussei*, appear to be efficient against cabbage root maggots. Based on our observations regarding the difficulty of penetration into the pupae of root maggots, it was noted that the EPNs were unable to enter the pupae, most likely due to the harder shell covering. This suggests that nematode application may yield better results if the target is in the susceptible life stage, specifically the larval stage. No chemical is registered for cabbage root maggots control in canola. Even in small-scale vegetable production where chemical application is permitted, the expected challenge is that the chemicals might not form sufficient contact with the partially hidden larvae inside the root system, which might not be the case with EPNs. Further, provided that the timing of application also coincides with the susceptible larval stage, we hypothesize that the virulent EPN species, such as *S. feltiae*, may relatively serve as effective biocontrol agents against root maggots.

Although the current study provided encouraging baseline information for conducting field application studies with commercially available species on canola for the management of multiple insect pests, including DBMs, canola lygus, cabbage root maggots, and BCWs, exploration of locally adapted and virulent strains of EPNs pertinent to the prairies should also be considered in the future projects. Considering Canada's cold climate and the limited cold tolerance of most imported EPNs, there is a compelling need to evaluate and compare the locally adapted EPNs with their commercially imported counterparts to better understand the pros and cons associated with developing native strains for pest management at the local level. Notably, native strains are believed to possess cold-tolerance capabilities, setting them apart from the commercial EPNs. This distinction opens the possibility of a wide range of field-based applications of native strains in the context of pest management. In addition, we recommend the investigation of IPM strategies combined with the application of EPNs as a potential solution for controlling resilient pests like adult FBs. By adopting IPM practices and using EPNs, the objective is to reduce the pest population and minimize their migration to nearby fields, thus mitigating potential damage and promoting sustainable pest management approaches.

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Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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