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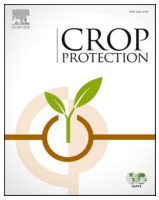

Evaluation of endemic entomopathogenic nematodes for managing Colorado potato beetle and tuber-damaging pests in potato

Pin-Chu Lai [a,b,*], Ramandeep Kaur Sandhi [a,c], Ollie Vetrovec [a], Tony Testa [d], Elson Shields [d], Brian A. Nault [a]

[a] Department of Entomology, Cornell University, Cornell AgriTech, 15 Castle Creek Drive, Geneva, NY, 14456, USA
[b] Department of Entomology, University of Nebraska-Lincoln, Panhandle Research, Extension, and Education Center, 4502 Avenue I, Scottsbluff, NE, 69361, USA
[c] FMC Corporation, Stine Research Center, 1090 Elkton Rd, Newark, DE, 19711, USA
[d] Department of Entomology, Cornell University, Comstock Hall, Ithaca, NY, 14853, USA

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ABSTRACT

Colorado potato beetle, *Leptinotarsa decemlineata*, wireworms and white grubs are important pests of potato that are challenging to manage. This study evaluated three endemic entomopathogenic nematodes (EPNs) (*Steinernema carpocapsae* 'NY01', *S. feltiae* 'NY04', and *Heterorhabditis bacteriophora* 'Oswego') as biocontrol agents against *L. decemlineata*, wireworms and white grubs in New York. The efficacy of individual EPN species and their combinations on *L. decemlineata* larval mortality was assessed via soil-based bioassays in the laboratory. Additionally, *L. decemlineata* survival and percentage of tubers damaged by wireworms and white grubs were evaluated in field trials in which combinations of pairs of EPN species were applied to the soil at least one month prior to initiating the experiment. Results from bioassays indicated that among the three EPN species, *L. decemlineata* late instars were most susceptible to *H. bacteriophora* 'Oswego'. Additionally, larval mortality was generally higher (14% on average) using pairs of EPN species compared to single species. Soil applications of pairs of EPN species prior to potato planting did not affect *L. decemlineata* adult survival either during the summer or following spring. However, tuber damage caused by wireworms and white grubs was reduced by 40% using a combination of *H. bacteriophora* 'Oswego' and *S. feltiae* 'NY04'. Overall, EPNs applied in soil have potential as biocontrol agents for soil-dwelling pests like wireworms and white grubs in potato, and their efficacy also may extend beyond a single cropping season, but do not seem to be an effective tool for *L. decemlineata* management in potato.

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is the most important insect defoliator of potato (*Solanum tuberosum* L.) (Alyokhin, 2008). Under high population pressure, adults and larvae can completely consume the entire above-ground portion of the crop, and this indirect damage can result in potato tuber losses up to 60% (Hare, 1980). *Leptinotarsa decemlineata* typically has one to two generations per year in the northeastern U.S. (Tauber et al., 1988; Voss et al., 1988). Adults overwinter in the soil and emerge in the spring when they feed, mate, and lay eggs on potato foliage. After completing four instars, the last instar enters the soil to pupate, and most summer adults emerge after 10–14 days (Hare, 1990). Late in the summer, *L. decemlineata* adults enter the soil to diapause and subsequently overwinter. Most overwintering adults emerge the following spring, but a small proportion will remain in the soil for longer periods (Alyokhin et al., 2022; Biever and Chauvin, 1990; Tauber and Tauber, 2002).

Wireworms (Coleoptera: Elateridae) and white grubs (Coleoptera: Scarabaeidae) are immature stages of click beetles and scarab beetles, respectively, which directly feed on potato roots and tubers. Wireworms create small circular holes and tunnel inside tubers, while feeding by white grubs creates large, shallow, and irregular gouges on tubers (Kroschel et al., 2019; Vernon and van Herk, 2022). Direct damage to the crop by wireworms and white grubs can downgrade tuber quality in up to 45% of tubers (Jansson and Lecrone, 1991; Keiser et al., 2012;

* Corresponding author. Department of Entomology, University of Nebraska-Lincoln, Panhandle Research, Extension, and Education Center, 4502 Avenue I, Scottsbluff, NE, 69361, USA.
E-mail address: plai3@unl.edu (P.-C. Lai).

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Kuhar et al., 2008). Wireworm species such as *Melanotus communis* Gyllenhal and *Conoderus vespertinus* (Fricius) are common pests of potato in the northeastern U.S.; with some variation by species, they have multiple instars and spend several years (2–5 years) feeding on hosts and developing in the soil before emerging as adults (Gill et al., 2014; Kuhar et al., 2008; Vernon and van Herk, 2022). White grubs such as *Popillia japonica* Newman, *Phyllophaga* spp., and *Cyclocephala* spp. are common in the northeastern U.S. and can damage potato crops (Frank, 2017; Kroschel et al., 2019; New York State Integrated Pest Management, 2013). In the spring, white grubs develop and feed on hosts in the soil as they typically go through three instars before emerging as adults (Frank, 2017; Ludwig, 1928; New York State Integrated Pest Management, 2013). As major pests of potato, wireworms, white grubs, and *L. decemlineata* all spend a portion of their lifecycles in soil, which provides an opportunity to manage them using entomopathogenic nematodes (EPNs).

EPNs are obligatory parasites and can be effective biological control agents for insect pests in crop production (Koppenhöfer et al., 2020). Infection occurs when EPN infective juveniles (IJs), a free-living stage, attack and kill hosts with assistance from symbiotic bacteria that release toxins into the host's hemolymph (Koppenhöfer et al., 2020). EPNs have been evaluated in the laboratory and field for controlling *L. decemlineata*, wireworms and white grubs, but efficacy has varied depending on EPN species and strains, environmental conditions, and release rates. Past studies have assessed the relative acute efficacy of EPNs against *L. decemlineata*, wireworms, and white grubs soon after application (Adel and Hussein, 2010; Ansari et al., 2009; Askar et al., 2023; Berry et al., 1997; Cantelo and Nickle, 1992; Chandel et al., 2019; Eski et al., 2022; Macvean et al., 1982; Nikoukar et al., 2021; Sandhi et al., 2020, 2021; Schalk et al., 1993; Toba et al., 1983; Trdan et al., 2009; Wraight et al., 2009; Wright et al., 1987). Efficacy of EPNs against these potato insect pests is not as well-documented in the long term, when EPNs have had months or years to establish in the soil after their application.

EPNs can be found in soil for days, weeks, and even years after the initial soil application (Ferguson et al., 1995; Jansson et al., 1991; Klein and Georgis, 1992; Shields et al., 1999; Shields and Testa, 2021; Wright et al., 1993). Endemic strains of EPNs may establish more successfully and for longer periods than non-endemic strains of EPNs because they may be better adapted to the local soil environment, (Shields et al., 1999). However, the efficacy of endemic EPNs for killing insect pests compared with non-endemic ones has varied among studies (Berry et al., 1997; Dillon et al., 2006; Nikoukar et al., 2021; Sandhi et al., 2020; Shields et al., 1999).

Management of soil-dwelling pests using EPN species could be influenced by the EPN's foraging behavior and its range in the soil profile (Lewis et al., 2006). *Heterorhabditis bacteriophora* uses a cruising strategy to locate its prey, while *Steinernema carpocapsae* uses an ambushing strategy to find prey, and *S. feltiae* uses an intermediate strategy between the two (Grewal et al., 1994). Thus, EPN's distribution in the soil is likely influenced by its foraging behavior. For example, *S. carpocapsae* was often found closer to the soil surface (around 5 cm), while *H. bacteriophora* could be found up to 40 cm deep in the soil; *S. feltiae* was generally located within the top 15 cm of the soil profile and rarely moved deeper than 20 cm down the soil (Campbell et al., 1996; Dillon et al., 2006; Ferguson et al., 1995; Susurluk, 2009). Interspecific interactions between EPNs can cause positive (synergistic) and/or negative (competitive) effects on performance of host infection, which likely depends on their foraging behaviors and distribution in soil (Alatorre-Rosas and Kaya, 1990; Neumann and Shields, 2006, 2008).

Endemic EPNs isolated from northern New York have been used to successfully manage pest populations and reduce crop damage in New York. Examples include Japanese beetle grubs (*P. japonica*) in turf, alfalfa snout beetle larvae (*Otiorhynchus ligustici* L.) in alfalfa, black vine weevil larvae (*O. sulcatus*) in strawberries, and wireworms in sweet potato (Ferguson et al., 1995; Schroeder et al., 1993, 1994; Shields et al., 1999, 2009, 2022; Shields and Testa, 2017, 2021). In addition, populations of endemic EPNs from New York can successfully establish for multiple years in the soil (Shields et al., 2018, 2022), and combinations of two endemic EPN species reduced wireworm damage (a combination of *Limonius agonus*, *M. communis*, and *Glyphonyx inquinatus*) in sweet potato in New York (Shields et al., 2022).

The objective of this study was to evaluate the efficacy of endemic EPNs from New York against *L. decemlineata*, wireworms and white grubs in potato in New York. We assessed the susceptibility of *L. decemlineata* late instars to each of the three EPN species. We hypothesized that *L. decemlineata* larvae would be most susceptible to *H. bacteriophora*, as this species had the highest virulence against *P. japonica* and *O. ligustici* L. in a previous study (Schroeder et al., 1994). In addition, we compared the efficacy of individual and paired EPN species against *L. decemlineata* late instars; we hypothesized that pairs of EPN species would cause higher *L. decemlineata* mortality than individual species. Finally, we evaluated the impact of combinations of EPN species on *L. decemlineata* survival and tuber damage by wireworms and white grubs in the field; we hypothesized that the most efficacious combination of EPNs (*H. bacteriophora* and *S. feltiae*) against wireworms in sweet potato would also be to the best combination for reducing wireworm and white grub damage in potato (Shields et al., 2022). Because *L. decemlineata* pupae complete their development at soil depth between 5 and 12 cm and adults burrow down to 10–25 cm to overwinter, we hypothesized that all combinations of EPN species would significantly reduce *L. decemlineata* survival (Alyokhin et al., 2022; Ferguson et al., 1995).

2. Materials and methods

2.1. Source of endemic EPN species

Three EPN species that originated from soil collections in New York, USA were used in this study including *Heterohabditis bacteriophora* Poinar 'Oswego', *Steinernema carpocapsae* (Weiser) 'NY01', and *S. feltiae* (Filipjev) 'NY04'. All species used in this study were maintained in laboratory cultures at Cornell University in Ithaca, NY (Shields et al., 2022). As of September 2024, *Heterohabditis bacteriophora* Poinar 'Oswego' and *S. feltiae* (Filipjev) 'NY04' can be obtained from Persistent Biocontrol (Austin, TX). Infective juveniles (IJs) of the EPNs were reared using the greater wax moth larvae, *Galleria mellonella* (L.), as hosts in an *in vivo* production system (Testa and Shields, 2017).

2.2. Assessing susceptibility of L. decemlineata fourth instars to EPN species

Soil-based, dose-dependent laboratory bioassays were conducted to evaluate the susceptibility of *L. decemlineata* late fourth instars to the three EPN species. Six IJ concentrations (0, 20, 100, 500, 2500, 5000 IJs/larva) were used in the bioassay to calculate a median lethal concentration (LC_{50}) for each EPN species. High-concentration stock solutions of the three EPN species were prepared from laboratory colonies, and the working solutions for all concentrations were prepared by serial dilutions using deionized (DI) water. The bioassays were conducted using plastic deli cups (237 ml; 11.4 cm diameter, 4.4 cm height; 102.6 cm^2 surface area) filled with ~30 g of autoclaved potting soil. Each soil cup was first rehydrated with 40 ml of DI water before applying EPN solutions. EPNs in 10 ml of solution (IJ suspension in DI water) were inoculated on the soil surface of each cup with respective concentrations and EPN species. The total volume of liquid added to each soil cup was 50 ml. Three deli cups were used for the 0 IJ/larva concentration, while five cups were set up for each of the other five concentrations for each EPN species. *Leptinotarsa decemlineata* late fourth instars were collected from a non-insecticide treated potato field located on a research farm near North Rose, NY (43°11′30.1″N 76°55′21.5″W). Late fourth instars were collected based on their larger size, large head capsule width, and

beige color (in contrast to bright orange). On the same day as the *L. decemlineata* collection, the bioassay was initiated by placing five larvae into each cup. Soil cups were covered with lids and maintained in the laboratory at room temperature (23 °C). Larval mortality was assessed at 24-h intervals up to seven days after the EPN applications.

During each evaluation, larvae or pupae were removed from the cup and recorded as live (any movement detected within 30 s) or dead under a stereo microscope (Zeiss Stemi 2000, 10-50X). Larval mortality data at two- and seven-day post EPN application were subjected to probit analysis to calculate the concentration that caused 50% mortality (LC_{50}) for each EPN species using PROC PROBIT procedure in SAS Studio version 3.81 (Enterprise edition 2022, SAS Institute Inc., Cary, NC, USA). The LC_{50} values were compared among EPN species and considered significantly different if the 95 % confidence interval (CI) did not overlap.

2.3. Assessing L. decemlineata larval morality using single and pairs of EPN species

Soil-based, laboratory bioassays were conducted to evaluate the efficacy of individual and pairs of EPN species against *L. decemlineata*. This bioassay included seven treatments: *H. bacteriophora* 'Oswego' (Hb) alone, *S. carpocapsae* 'NY01'(Sc) alone, *S. feltiae* 'NY04' (Sf) alone, Sc plus Sf, Sc plus Hb, Sf plus Hb, and a non-treated control. The bioassay used plastic deli cups (1000 ml; 11.4 cm diameter, 14 cm height; 102.6 cm^2 surface area) filled with ~950 g of autoclaved sandy soil, which was collected from the non-insecticide treated potato field where *L. decemlineata* larvae were collected. The soil in 1000 ml-cups was 14 cm deep, which allowed EPN species to locate themselves at their preferred depth in soil profile. EPNs were extracted from laboratory colonies and diluted with DI water to obtain the desired rates. EPNs in 1 ml aliquot suspension containing 2500 IJs (157.2 IJs/cm^2) were inoculated on the soil surface. In treatments that included two EPN species, 1250 IJs (78.6 IJs/cm^2) of each species were inoculated in a soil cup. Fourth instars were collected from the same potato field as previously mentioned on the same day when the bioassay was initiated. Five *L. decemlineata* fourth instars were introduced into each cup immediately after EPN application, and soil cups were covered with lids. Soil cups were maintained in the laboratory at room temperature (23 °C) for seven days, and larval mortality from EPN infection was evaluated and recorded as described in section 2.2. The bioassay was conducted twice with ten and four replications (one soil cup represents one replication) in the first and second trial, respectively.

The effect of EPN species on *L. decemlineata* larval mortality was analyzed by generalized linear mixed models using PROC GLIMMIX procedure in SAS. Larval mortality data were tested for normality using the Shapiro-Wilk test. Because data were not normally distributed, a square-root transformation (i.e., $\sqrt{x} + 3/8$) was used before analysis. EPN treatment was a fixed factor, while replication and trial were random factors in the analysis. Least square means (LS-means) were used for post hoc comparisons with Tukey Studentized Range (HSD) Test when the fixed factor was significant at $\alpha = 0.05$ for mean separation.

2.4. Assessing L. decemlineata larval mortality using recommended field rates of pairs of EPN species

A soil-based bioassay was conducted to evaluate efficacy of the three pairs of EPN species, Sc plus Sf, Sc plus Hb, and Sf plus Hb, against *L. decemlineata* fourth instars at recommended field rates (1211 million IJs per hectare; 12.1 IJs/cm^2) for high value crops such as potato. In addition, soil used in the bioassay was sourced from the field site where the field trials were located (described in the next section). Soil was collected from an undisturbed area 3 m (10 ft) away from the field trials in the same field using step soil probes (40" Plated One-Piece Step Probe, AMS, Inc., American Falls, ID, USA). The soil probe cut a 1.6 × 20.3 cm^2 (5/8 x 8 in^2) soil core sample. Two soil core samples (65 cm^2) were placed in each 540 ml deli cup (11.4 cm diameter, 7.6 cm height; 102.6 cm^2 surface area). Treatments included the three pairs of the EPN species (Sc + Sf, Sc + Hb, Sf + Hb) and a non-treated control, and each treatment had four replications. Five soil cups were used for each treatment in each replicate. EPNs were inoculated to the soil surface using the recommended field rate and scaled down to the top surface area of the deli cup (~1200 IJs per cup; 11.7 IJs/cm^2). Each EPN species was applied to soil cups with the respective treatment in 1 ml of EPN solution, while 2 ml of deionized water were applied to control cups. *Leptinotarsa decemlineata* fourth instars were collected from the non-insecticide treated potato field (described in section 2.2) on the same day when EPNs were applied to the soil cups, and five larvae were added to every soil cup. Soil cups were covered with lids and maintained in the laboratory at room temperature (23 °C) for seven days. Afterward, *L. decemlineata* mortality and EPN infection were evaluated using the method described in section 2.2. Statistical analyses were performed using the same method described in the previous section.

2.5. Assessing L. decemlineata survival and tuber damage by wireworms and white grubs in potato plots treated with pairs of EPN species

2.5.1. Field site, experimental design, and EPN applications

Combinations of EPN species were evaluated for their impact on *L. decemlineata* survival and tuber damage by wireworms and white grubs in potato plots with Honeoye loam soil at the Cornell University's AgriTech Research South Farm near Geneva, New York (USA) (42°52′08.6″N 77°02′11.98″W). The field site had been fallow for over 10 years and the only maintenance was occasional mowing. The experiment followed a Latin square design with four treatments and four replications. Treatments included the same three pairs of the EPN species as described previously, which were Sc plus Sf, Sc plus Hb, and Sf plus Hb, with the addition of a non-treated control. Each EPN species was applied at a rate of 1211 million IJs per hectare (12.11 IJs/cm^2). Combinations of EPNs were suspended in water (38 L or 10 gallon per plot; plot dimensions described in next section) contained in trash cans (121 L or 32 gallon) and were applied to the soil surface using a garden hose and garden hose nozzle with a portable, gasoline-powered water pump (Honda brand, 50 cc). The hose, nozzle, and trash cans were thoroughly washed between treatment applications.

2.5.2. Approach and data collection

Two independent field trials (Experiment I & II) were conducted adjacent to each other following the same experimental design, but with different application timing of EPNs. In Experiment I, the field site was tilled before planting potato, *S. tuberosum* 'Dark Red Norland', on 18 May 2021. Plots were 6.1 m (20 ft) long and 3.7 m (12 ft) wide with 6.1 m of undisturbed ground surrounding each plot. Potato seeds were planted in four rows with 0.9 m (2.8 ft) row spacing in each plot with 0.3 m (1 ft) plant spacing within row. EPNs were applied on 20 May 2021 late in the afternoon to minimize UV exposure that can be harmful to EPNs. Two canopy tents (0.3 x 0.3 m/10 × 10 ft Coleman Screened Canopy Sun Shelter Tent with Instant Setup, The Coleman Company, Inc., Chicago, IL, USA) were placed over the rows of potatoes in each plot and served as field cages; one 'summer emergence cage' and one 'overwintering cage' in each plot. The bottom flaps along the circumference of each tent were buried below the soil surface to ensure that *L. decemlineata* released within the tents could not escape, nor could *L. decemlineata* outside the tent enter. On 1 July 2021, two hundred *L. decemlineata* fourth instars were collected from the non-insecticide treated potato field described in section 2.2 and released in the 'summer emergence cage' in each plot to evaluate the effect of pairs of EPN species on *L. decemlineata* survival as determined by adult emergence. Adult *L. decemlineata* started to emerge two weeks after larvae were released in the cages. Emerged adults were collected and counted from the 'summer emergence cage', and then moved to the 'overwintering cage' in the same plot (less than 200 adults in all cases; ranged from 50

to 140 adults). This procedure was repeated every other day for several weeks until no more adults emerged from the 'summer emergence cage'. Adults in the 'overwintering cage' had time to feed on potato foliage and most entered the soil to diapause without reproducing. However, for those that did, all egg masses and small larvae generated from these adults were removed from plants by hand in all cages and destroyed to ensure that all adults accounted for were from the original larvae used to initiate the experiment. At the end of the season on 9 September 2021, potato tubers in the center two rows of both tents in each plot were harvested, graded by size (minimum tuber diameter: Grade A- 4.8 cm; Grade B- 3.8 cm), and examined for wireworm and white grub damage. Afterward, the exact position of each tent was marked and then all tents were discarded. In the following spring before adults began emerging from the soil, six greenhouse-grown potato plants (20–30 cm tall) were transplanted in each plot on 13 May 2022 before new tents were placed in the same spots as those during the previous season on 17 May. Three potted plants were placed in the center of each tent in each plot. These plants had two purposes: 1) to facilitate counting and collecting overwintered *L. decemlineata* adults by attracting them as they emerged, and 2) to assess tuber damage by wireworms and white grubs at the end of the season. Overwintering adults began emerging on 23 May. Emerged adults from both tents in each plot were collected and counted two days per week until mid-June when no more adults were found. The near absence of overwintered adults emerging from the 'summer emergence cage' indicated that most adults had been removed successfully and adults did not enter diapause in the 'summer emergence cage' the previous summer. Potato plants were maintained in the tents until the end of the season. Potato tubers were harvested, graded by size, and examined for wireworm and white grub damage on 31 August 2022. Wireworm feeding creates circular holes and tunnels inside tubers, while white grub feeding generates large, shallow, and irregular gouges on tubers (Kroschel et al., 2019; Vernon and van Herk, 2022).

In Experiment II, the field site was mowed, but soil was not disturbed before EPN applications. Experimental design, plot size, treatments, and EPN applications were identical to those in Experiment I. Plots were marked and then combinations of pairs of EPN species were applied to the ground late in the late afternoon on 14 September 2021. The field was undisturbed after EPN applications until the following spring. Individual plots were rototilled on 10 May 2022. Non-treated control plots were tilled first. To avoid contamination of plots with EPNs not assigned to those plots, the rototiller and boots for those walking in the plots were thoroughly rinsed and cleaned with 10% sodium hypochlorite between treatments. On 11 May, potato tubers were manually planted in a similar fashion as in Experiment I. Two tents (i.e., 'summer emergence cage' and 'overwintering cage') were erected within each plot as described in Experiment I on 17 May. On 23 June, two hundred *L. decemlineata* fourth instars were collected from the non-insecticide treated potato field in North Rose (as described in section 2.2), and all were released into the 'summer emergence cage' in each plot. In July, all emerged adults from the 'summer emergence cage' were counted and moved to the 'overwintering cage' in the plot as described in Experiment I. Adults fed on foliage and then entered diapause in the 'overwintering cage'. Egg masses and larvae were removed by hand in all cages to prevent a confound in data with newly generated adults. Potato tubers were harvested, graded, and examined for wireworm and white grub damage on 7 September 2022. Afterward, the precise locations for all tents were marked before discarding tents. On 9 May 2023, new tents were erected in the locations where tents had been placed the previous year. On 15 May, six greenhouse-grown potato plants were transplanted in each plot (three per tent) for the same purposes described in Experiment I. Overwintered adults began emerging within the tents on 18 May and the total number was recorded. No adults emerged from the 'summer emergence cage' in this experiment. Potato plants were maintained in the tents until 5 September when potato tubers were harvested, graded, and examined for wireworm and white grub damage.

2.5.3. Statistical analyses for field data

The effect of endemic EPN species combinations on adult *L. decemlineata* survival including emergence in summer and overwinter was analyzed using generalized linear mixed models with PROC GLIMMIX procedure in SAS Studio version 3.81 (Enterprise edition 2022, SAS Institute Inc., Cary, NC, USA). Data were analyzed separately for Experiment I and Experiment II. Percent survival data for summer adult emergence in both experiments and for overwintering adult emergence in Experiment II were normally distributed as tested using the Shapiro-Wilk test, so the Gaussian distribution with the identity linked function were used. Percent survival data for overwintering adult emergence in Experiment I was not normally distributed, hence the beta distribution with the logit linked function was used as it was the best model fit based on Akaike information criterion (AIC) value. The combination of EPN species was the fixed factor while replication was the random factor in the analyses.

The effect of the combination of EPN species applied in the soil on marketable-sized potato tubers (Grade A & B) damaged by wireworms and white grubs was also analyzed using generalized linear mixed models with PROC GLIMMIX procedure in SAS Studio version 3.81. Four data sets (two harvests for each experiment) were first analyzed independently and then combined for an additional analysis to increase the statistical power. The data set of percent marketable-size tubers damaged by wireworms and white grubs was not normally distributed based on the Shapiro-Wilk test, and the binomial distribution with the logit linked function was identified as the best model fit based on AIC values and was used in the analysis. The combination of EPNs and harvest time (first and second year) were the fixed factors while replication was the random factor in the analysis.

For all response variables, least square means (LS-means) were used for post-hoc comparisons with Tukey Studentized Range (HSD) Test when the fixed factor was significant at $\alpha = 0.05$ for mean separation.

2.5.4. Assessing L. decemlineata larval mortality using EPNs applied at varying periods before exposure

In the summer of 2022, a laboratory bioassay was conducted to investigate the effect of pairs of EPN species and their period of establishment in the field on *L. decemlineata* fourth-instar mortality. This experiment was designed as a two-way factorial with the combination of EPN species and the period after EPN application prior to exposing *L. decemlineata* late instars to treated soil serving as the two main factors. Soil samples were collected on 27 to 29 June in plots in Experiments I & II, respectively. In each plot, two soil core samples were taken by step soil probes and placed in a 540 ml deli cup. On 30 June, fourth instars were collected from the non-insecticide treated potato field described in section 2.2, and five larvae were added to each soil cup. Soil cups were covered with lids and maintained in the laboratory at room temperature (23 °C) for seven days. Afterward, *L. decemlineata* mortality and EPN infection was evaluated following the same procedure as described in the previous sections.

The impact of the combination of EPN species and the period after EPN application prior to exposing *L. decemlineata* late instars to treated soil on mortality were analyzed using generalized linear mixed models with PROC GLIMMIX procedure in SAS. The combination of EPN species and the period after EPN application prior to *L. decemlineata* exposure were the fixed factors while replication was the random factor. Percent mortality data were subjected to a square-root transformation (i.e., $\sqrt{x} + 3/8$) to remedy large variances in several treatments and zero-inflated data, and the Gaussian distribution with the identity linked function was used. LS-means were used for post hoc comparisons with Tukey Studentized Range (HSD) Test when the fixed factor was significant at $\alpha = 0.05$ for mean separation.

2.5.5. Soil sampling and EPN presence

Soil core samples were collected to estimate the presence of EPNs prior to the initiation of field trials, and three times post EPN application

Table 1
Susceptibility of *Leptinotarsa decemlineata* fourth instars to endemic EPN species from New York, *Steinernema carpocapsae* (Sc), *S. feltiae* (Sf), and *Heterorhabditis bacteriophora* (Hb), in a soil-based bioassay.

Species	N	Slope (SE)	LC_{50} (IJs/larva)[a]	95% CI	χ^2 (df)
Day 2[b]					
Sc	125	1.40 E+19 (0.00)	29600 **a**	27160–32260	0.00 (3)
Sf	125	667.42 (0.94)	23335 **ab**	12390–48235	0.14 (3)
Hb	120	50.41 (0.30)	8890 **b**	5760–14295	1.42 (3)
Day 7[b]					
Sc	125	37.35 (0.46)	2216 na[c]	27–137125[c]	14.02 (3)[c]
Sf	100	17.05 (0.18)	1323 **a**	703–2454	1.03 (2)
Hb	120	59.28 (0.29)	555 **a**	352–868	4.26 (3)

[a] Lethal concentration 50 (LC_{50}) was calculated based on probit analyses. LC_{50} value followed by different letters within each evaluation day indicates a significant difference based on nonoverlap of 95% confidence interval (CI).
[b] Two and seven days after EPN applications.
[c] A poor fit of the data to the probit analysis and therefore not included in the LC_{50} comparison.

in each experiment (42, 145, 459 days post application in Experiment I; 28, 267, 618 days post application in Experiment II). *Galleria mellonella* larvae were used as indicator hosts to bait EPNs in the soil in laboratory bioassays. Prior to initiation of field trials, 78 soil core samples were collected across the experimental area using step soil probes on 6 October 2020, while ten cores were collected from each plot in all bioassays conducted after EPN application. The top 5.1 cm (2 inches) of the soil core sample was placed in a 59.1 ml (2 oz) deli cup (small cup), while the bottom 15.2 cm (6 inches) of the soil core sample was placed in a 540 ml (18.3 oz) deli cup (big cup). All soil samples were taken to the laboratory for the bioassays. Ten and five *G. mellonella* larvae (Speedy Worm, Alexandria, MN, USA) were added to the big cup with the bottom samples and the small cup with the top sample, respectively. Dry soil samples were misted before adding *G. mellonella* larvae. Samples were incubated at room temperature (23 °C) in the laboratory for seven days. Nematode infection of *G. mellonella* larvae was examined based on the condition and color of the cadaver (Poinar, 1983). Cadavers that turned light brown, dark brown, and brick red were considered infected and killed by *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*, respectively (Shields et al., 2022). In instances when EPN infection could not be determined by cadaver coloration, white traps were set up to collect IJs for further species identification (Shields et al., 2022; White, 1927).

3. Results

3.1. Susceptibility of L. decemlineata larvae to EPN species

Two days after EPNs were inoculated in the soil, the LC_{50} for *L. decemlineata* exposed to *H. bacteriophora* 'Oswego' was significantly lower than the LC_{50} for *L. decemlineata* exposed to *S. carpocapsae* 'NY01', while the LC_{50} for *L. decemlineata* exposed to *S. feltiae* 'NY04' was intermediate between *H. bacteriophora* 'Oswego' and *S. carpocapsae* 'NY01' (Table 1). Seven days after EPNs were inoculated in the soil, the LC_{50} for *L. decemlineata* exposed to *H. bacteriophora* 'Oswego' was numerically lower than the LC_{50} for *L. decemlineata* exposed to *S. feltiae* 'NY04', but the difference was not statistically significant (Table 1). The LC_{50} for *L. decemlineata* exposed to *S. carpocapsae* 'NY01' was numerically higher than the others, but the probit model fit for *S. carpocapsae* 'NY01' was poor with an extremely wide range of 95% CI, precluding the opportunity to compare statistically (Table 1).

3.2. Leptinotarsa decemlineata larval morality using single and pairs of EPN species

When EPNs were applied to soil either alone or in pairs, mortality of *L. decemlineata* was significantly affected by the EPN treatment ($F = 14.36$, $df = 6, 78$; $P < 0.0001$). All treatments caused significantly higher mortality than the non-treated control, except *S. carpocapsae* alone. Larval mortality in treatments that included *H. bacteriophora* alone, *S. feltiae* alone, and *S. feltiae* plus *H. bacteriophora* was significantly greater than larval mortality in *S. carpocapsae* alone and the non-treated control (Fig. 1). Larval mortality in treatments that included *S. carpocapsae* plus *S. feltiae* and *S. carpocapsae* plus *H. bacteriophora* was significantly higher than the non-treated control, but not different from the other treatments (Fig. 1). All three pairings of EPN species caused significantly higher larval mortality than the non-treated control.

3.3. Leptinotarsa decemlineata larval mortality after exposure to recommended field rates of pairs of EPN species

When EPNs were applied in pairs at recommended field rates, larval mortality was significantly affected by the combination of EPN species ($F = 37.39$, $df = 3, 9$; $P < 0.0001$) (Fig. 2). Mortality of fourth instars for all three pairings of EPN species was significantly greater than mortality in the non-treated control, which was zero (Fig. 2). In addition, larval mortality was significantly higher in the *S. carpocapsae* plus *H. bacteriophora* treatment than in the treatments that included *S. carpocapsae* plus *S. feltiae* and *S. feltiae* plus *H. bacteriophora* (Fig. 2).

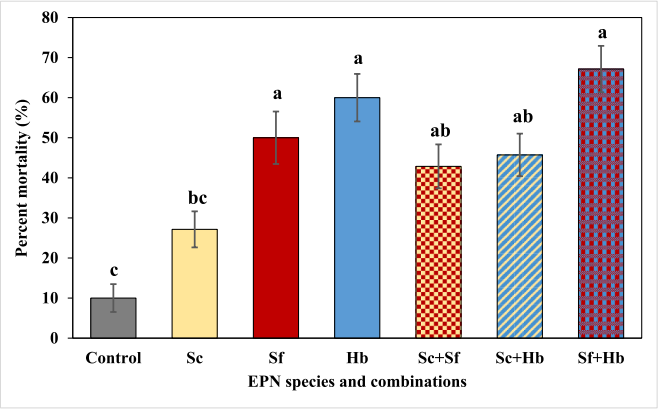

Fig. 1. Mean (±SE) percent mortality of *Leptinotarsa decemlineata* fourth instars after seven days incubation in sterilized sandy soil containing either one or two species of EPNs. EPN species abbreviations are as follows: Sc- *Steinernema carpocapsae*; Sf- *S. feltiae*; Hb- *Heterorhabditis bacteriophora*. Least square means with different letters indicate significant differences in percent mortality of *L. decemlineata* among EPN treatments (Tukey-HSD; $P < 0.05$; $n = 14$).

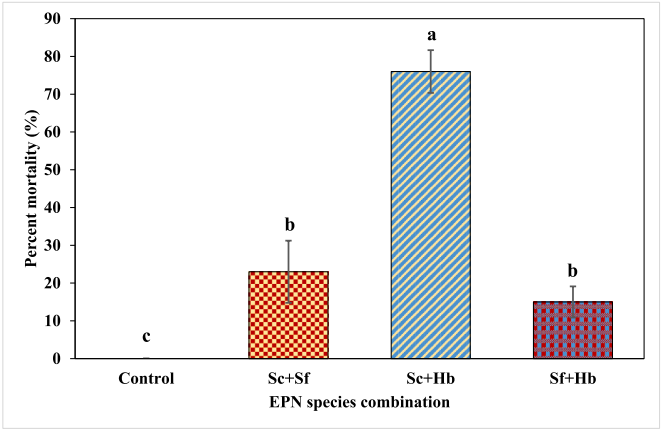

Fig. 2. Mean (±SE) percent mortality of *Leptinotarsa decemlineata* fourth instars after seven days incubation in field soil inoculated with field rates of EPNs. EPN species abbreviations are as followed: Sc- *Steinernema carpocapsae*; Sf- *S. feltiae*; Hb- *Heterorhabditis bacteriophora*. Least square means with different letters indicate significant differences in percent mortality of *L. decemlineata* among EPN treatments (Tukey-HSD; $P < 0.05$; $n = 4$).

3.4. Survival of L. decemlineata and tuber damage by wireworms and white grubs in potato plots treated with pairs of EPN species

No EPNs were detected in soil at the field site based on the pre-inoculation soil baiting bioassays. In Experiment I, all inoculated EPN species were detected in the respective treatments in bioassays conducted 42- and 145-days post inoculation, but nearly none were detected in the bioassays conducted 459 days post inoculation (Table S1). In Experiment II, 80% of inoculated EPN species were detected in the respective treatments in bioassays conducted 28- and 267-days post inoculation, while all inoculated EPN species were detected in their respective treatments in the bioassays conducted at 618 days post inoculation (Table S1). Overall, the detection rates ranged from 0 to 52.5% (Table S1).

3.4.1. Survival of L. decemlineata exposed to combinations of EPN species

Leptinotarsa decemlineata adult emergence in the summer was not affected by the EPN treatment in either Experiment I or Experiment II (Experiment I: $P = 0.802$, Fig. S1A; Experiment II: $P = 0.609$, Fig. S2A). Similarly, the number of overwintered adults was not affected by the EPN treatment in either Experiment I or Experiment II (Experiment I: $P = 0.468$, Fig. S1B; Experiment II: $P = 0.520$, Fig. S2B).

3.4.2. Larval mortality from pairs of EPN species that had previously established in soil for various periods

In laboratory bioassays using soil collected from field plots in Experiment I and II, larval mortality was not affected by the EPN establishment period prior to conducting the bioassays ($P = 0.173$; Fig. S3A) or the EPN treatment ($P = 0.304$; Fig. S3B). Larval mortality also was not significantly impacted by the interaction between the EPN establishment period and EPN treatment ($P = 0.781$; Fig. S3C).

3.4.3. Tuber damage by wireworms and white grubs in plots treated with pairs of EPN species

Potato tubers were damaged by both wireworms and white grubs in our field trials. Across all four tuber yield evaluations, 86% of tubers were damaged by wireworms, while 14% of the tubers were damaged by white grubs. When analyzed separately by harvest in each trial, percent potato tubers damaged by wireworm and white grubs was not affected by the EPN treatment (Fig. S4). However, when data were combined across trials and harvests, percent marketable-sized tubers damaged by wireworms and white grubs was affected by the EPN establishment period (marginal significance) ($F = 5.27$, $df = 1, 7$; $P = 0.055$; Fig. 3A)

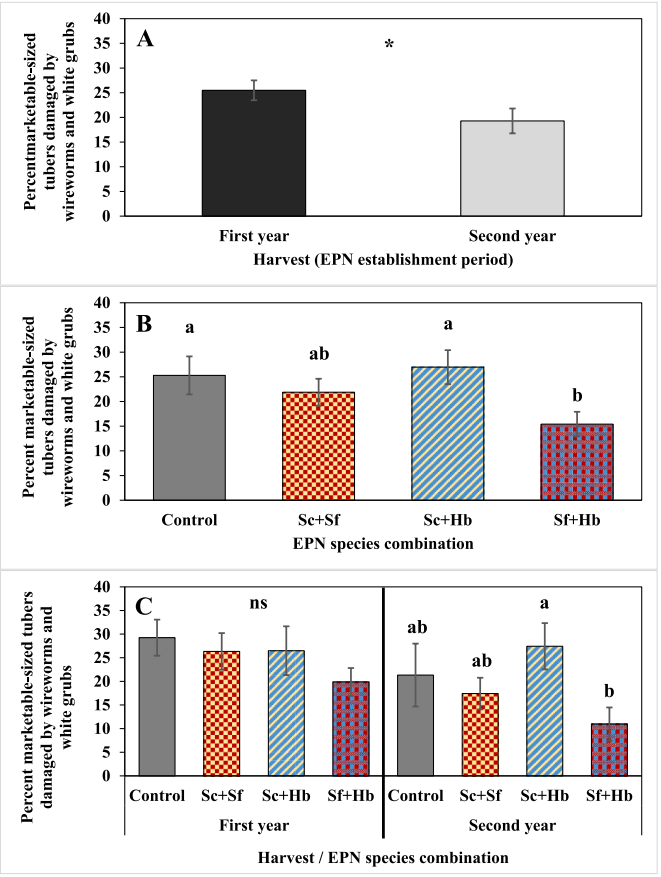

Fig. 3. Mean (±SE) percent marketable-sized tubers damaged by wireworms (most common was *Melanotus communis*) and white grubs. Percent tubers damaged affected by **(A)** harvest time (EPN establishment period), **(B)** EPN treatment, and **(C)** the interaction effect between harvest time and EPN treatment. EPN species abbreviations are as followed: Sc- *Steinernema carpocapsae*; Sf- *S. feltiae*; Hb- *Heterorhabditis bacteriophora*. Least square means with different letters within each graph indicate significant differences in percent damaged tubers among EPN treatments (Tukey-HSD; $P < 0.05$; $n = 32$ **(A)**; 16 **(B)**; 8 **(C)**); while 'ns' indicates no significant differences ($P > 0.05$). The asterisk in **(A)** indicates marginal significance ($P = 0.055$) of harvest time (EPN establishment period).

and the EPN treatment ($F = 4.11$, $df = 3, 21$; $P = 0.019$; Fig. 3B). In addition, the interaction effect between the EPN establishment period and EPN treatment was nearly significant ($F = 3.04$, $df = 3, 21$; $P = 0.052$; Fig. 3C).

Across all EPN treatments, percent marketable-sized tubers damaged by wireworms and white grubs was lower in the second-year harvest (i.e., longer EPN establishment period) than in the first-year harvest (i.e., 12 months shorter of EPN establishment period) (Fig. 3A). Across all harvest evaluations, percent marketable-sized tubers damaged by wireworms and white grubs was significantly lower in the *S. feltiae* plus *H. bacteriophora* treatment than in the *S. carpocapsae* plus *H. bacteriophora* treatment and the non-treated control, while percent damaged marketable-sized tubers in the *S. carpocapsae* plus *H. bacteriophora* treatment was not different from the non-treated control (Fig. 3B). Percent marketable-sized tubers damaged by wireworms and white grubs in the *S. carpocapsae* plus *S. feltiae* treatment was intermediate and not statistically different from the other three treatments (Fig. 3B). The treatment of *S. feltiae* plus *H. bacteriophora*, which was the best treatment combination, reduced tuber damage by 40% when compared with damage in the non-treated control (25 % and 15 % tuber damaged in the control and the best treatment, respectively) (Fig. 3B).

Considering the interaction effect between the EPN establishment

period and EPN treatment on tuber damage, percent marketable-sized tubers damaged was only affected by the EPN treatment in the second-year harvest (first year harvest: $P = 0.092$; second year harvest: $F = 4.34$, $df = 3, 21$; $P = 0.016$) (Fig. 3C). In the second-year harvest, percent marketable-sized tubers damaged by wireworms and white grubs was significantly lower in the *S. feltiae* plus *H. bacteriophora* treatment than in the *S. carpocapsae* plus *H. bacteriophora* treatment. Percent marketable-sized tubers damaged in the *S. carpocapsae* plus *S. feltiae* treatment and the non-treated control were intermediate between the others (Fig. 3C).

4. Discussion

Leptinotarsa decemlineata and tuber-damaging pests such as wireworms and white grubs are important insect pests in potato production that can cause significant yield loss, and using EPNs as biocontrol agents to manage them has been challenging. Endemic EPNs have the potential to establish in soil and provide below-ground insect pest management in potato for longer periods. Using soil-based bioassays, we demonstrated that three species of EPNs that are endemic to New York caused mortality in *L. decemlineata* late instars. However, in our field studies, combinations of these EPNs applied to soil did not impact adult *L. decemlineata* populations. In contrast, the combination of *S. feltiae* 'NY04' and *H. bacteriophora* 'Oswego' significantly reduced tuber damage by wireworms and white grubs in the field with the effect more prominent in the second year after the initial EPN application.

Several *Steinernema* spp. and *Heterorhabditis* spp. have been effective in causing *L. decemlineata* mortality in the laboratory and field, suggesting the potential for EPNs to be used as biocontrol agents (Adel and Hussein, 2010; Cantelo and Nickle, 1992; Eski et al., 2022; Trdan et al., 2009). Among the three EPN species evaluated in our study, *L. decemlineata* larvae were most susceptible to *H. bacteriophora* 'Oswego'. Similar results were shown that *H. bacteriophora* 'Oswego' caused the highest mortality in *P. japonica* and *O. ligustici* larvae in a previous study (Schroeder et al., 1994). Our results, along with previous research findings, indicated that *H. bacteriophora* 'Oswego' likely has the highest virulence to common Coleopteran pests in the region among the endemic EPN species that have been isolated and evaluated.

Results from all of our bioassays were similar when comparing larval mortality caused by a single EPN species. While *S. carpocapsae* 'NY01' alone did not cause significantly higher *L. decemlineata* mortality than the non-treated control, the addition of an EPN species with higher virulence, either *S. feltiae* 'NY04' or *H. bacteriophora* 'Oswego', increased *L. decemlineata* mortality to levels that were significantly higher than the non-treated control while keeping the same total amount of EPNs applied constant. These results indicated an advantage of adding a more virulent strain of EPN to *S. carpocapsae* 'NY01'. However, it would only be beneficial if *S. carpocapsae* 'NY01', a less virulent strain, was chosen over other more virulent strains because of other considerations. Performance of multiple species of EPNs in soil could be affected by complex interspecific interactions including possible positive effects from increasing soil distribution and negative effects from niche competitions (Alatorre-Rosas and Kaya, 1990; Neumann and Shields, 2006, 2008; Stuart et al., 2015). Because data generated from our study was not adequate to conduct a formal test for interactions (synergy, additivity, or antagonism), the interaction between NY endemic EPN strains should be considered in the future. Additional studies also should consider expanding the experimental period in lab bioassays to provide sufficient time for EPNs to adapt to their natural niche in soil, which could potentially increase the possibility of detecting significant interactions.

All combinations of pairs of EPN species resulted in significantly higher *L. decemlineata* mortality than the control in lab bioassays using soil from our field site. However, none of the endemic EPN species combinations impacted *L. decemlineata* survival in our field study. We propose four possibilities that may explain these results. First, it is likely that a proportion of the EPNs experienced high mortality after field inoculation due to biotic and abiotic conditions in the environment, so the EPN population could have been too low to impact *L. decemlineata*. Second, the distribution of *L. decemlineata* and EPNs in the soil may not have overlapped. For example, most *L. decemlineata* pupate within the top 5–12 cm of soil profile, whereas the range of activities for *H. bacteriophora* 'Oswego' likely would be much deeper. Third, while *L. decemlineata* adults overwinter deeper in the soil, which potentially increases the chance of encountering *H. bacteriophora* 'Oswego', adults are generally less susceptible to EPNs than larval and pupal stages (Stewart et al., 1998; Trdan et al., 2009). Fourth, the soil type in our experiment, Honeoye loam, is not a typical soil type for growing potatoes and might have affected the movement of *L. decemlineata* and EPNs. For instance, *H. bacteriophora* might be better finding *L. decemlineata* in sandy soils that are common for potato production because it is a larger nematode that could move through larger soil pores in sandier soils. One or more of these possibilities might explain the lack of EPN efficacy against *L. decemlineata* in our field study.

The combination of *S. feltiae* 'NY04' and *H. bacteriophora* 'Oswego' significantly reduced tuber damage by wireworms and white grubs. Our results are consistent with the findings by Shields et al. (2022) that the same EPN combination significantly reduced tuber damage caused by wireworms in sweet potatoes. Perhaps, the distribution of wireworms and white grubs in the soil is similar to the distribution of these EPNs in the soil. Unlike *L. decemlineata*, wireworms and white grubs spend more time during their lifecycle in the soil and are distributed over a wider range within the soil profile (Chandel et al., 2019; Vernon and van Herk, 2022). Lower levels of tuber damage in the second year compared with the first year suggests that EPNs may need a longer period to increase their populations to have an impact on soil pests. Fluctuations in infectivity of EPNs that can be induced by adverse environmental conditions (phased infectivity) likely also contribute to EPNs being detected long after initial application (Griffin, 2012; Shields and Testa, 2017). Although tuber damage was significantly reduced by the treatment of *S. feltiae* 'NY04' plus *H. bacteriophora* 'Oswego', levels of tuber damage were not economically acceptable, indicating that relying on endemic EPNs as a standalone management tool for these potato insect pests may be challenging and insufficient in some situations.

Soil types are known to affect EPN persistence, and survival rates of EPNs are generally higher in sand or sandy loam than in clay or clay loam (Kung et al., 1990). Endemic EPNs may provide better pest protection in sandy soil compared with heavier soil types because they are likely to move more easily through sandy soil. While the soil in our field trials (Honeoye loam) was not an optimal soil type for EPN persistence, endemic EPNs inoculated in the field were detected up to 618 days after inoculation in our study, and this phenomenon is consistent with previous studies (Shields et al., 2022, 2018; Shields and Testa, 2021). The lack of EPN detection at 459 days after inoculation in our Experiment I was unexpected as previous studies showed that endemic EPNs released in NY were detected several years after application (Ferguson et al., 1995; Shields et al., 1999; Shields and Testa, 2021). The very dry conditions during soil sampling at that time likely affected EPN detection. EPNs detected in non-treated plots indicated the existence of very low population of endemic EPNs, which could have increased variability in tuber damage by wireworms and white grubs across experimental treatments and control plots. *Steinernema carpocapsae* is the most common EPN species in NY, so detection of *S. carpocapsae* at a low level in non-treated plots was not surprising. However, low levels of *S. feltiae* and *H. bacteriophora* detected in control plots on a few sample dates may have been caused by either human contamination or from natural EPN movement from the undisturbed natural area surrounding individual field plots. Overall, EPN survival and movement can be affected by soil type, water content, and other field conditions such as slope and uniformity, which increase the challenges of field research on EPN persistence and efficacy.

5. Conclusions

This study demonstrated the potential of using endemic EPNs to protect potato tubers from wireworms and white grubs, but the failure of EPNs to manage *L. decemlineata*. We highlighted the complexity of ecological interactions between EPNs and different insect pests, which could explain the effectiveness of EPNs as biocontrol agents. Our findings suggested that endemic EPNs applied to the soil might have more success reducing the threat of soil pests that live in the soil for extended periods (months to years) than pests spending relatively shorter periods in soil for pupation and overwintering. Regardless, using endemic EPNs alone to provide sufficient protection from insect pests in a high value crop like potato will be challenging. Because endemic EPNs can be detected years after application in fields that have been rotated with several crops (Shields et al., 2018), endemic EPNs could contribute to managing wireworms in fields that are rotated with corn, small grain, and potato. Overall, potential contribution of endemic EPNs in pest suppression could reduce the reliance of insecticides and possibly reduce selection pressure for pests to develop resistance to insecticides. Additional research is required to assess the compatibility of using endemic EPNs with other management tools. Future studies should also investigate the maximum soil capacity of EPNs and whether re-application is required under certain conditions.

CRediT authorship contribution statement

Pin-Chu Lai: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ramandeep Kaur Sandhi:** Writing – review & editing, Methodology, Investigation. **Ollie Vetrovec:** Writing – review & editing, Investigation. **Tony Testa:** Writing – review & editing, Investigation. **Elson Shields:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Brian A. Nault:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106980.

Data availability

Data will be made available on request.

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