Journal of Economic Entomology, XX(X), 2018, 1–7
doi: 10.1093/jee/toy258
Research

Long-term Persistence of Native New York Entomopathogenic Nematode Isolates Across Crop Rotation

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Subject Editor: Eric Riddick

Received 28 June 2018; Editorial decision 7 August 2018

Abstract

Entompathogenic nematodes are found worldwide in a wide array of soil habitats with a broad host range and significant variation in foraging strategies. The primary use of entomopathogenic nematodes (EPNs) in managed plant systems has been focused on inundative releases in a biopesticide strategy. Little effort has been placed in investigating the use of natural occurring or adapted EPN strains for long-term suppression of pest outbreaks in managed systems. This study examined the potential of EPN isolates from Northern New York (NNY), inoculated at a low level (250 million IJ/ha), which are climate adapted and their persistent characteristics preserved to maintain population levels in agricultural fields (N = 82) for multiple years and across crop rotation (alfalfa:corn:alfalfa). Persistence levels for *Steinernema carpocapsae* (Weiser) (Rhabditida:Steinernematidae) ranged between 8 and 12% of the soil cores assayed in continuous alfalfa and 1–14% of the soil cores assayed in continuous corn rotated from EPN treated alfalfa. *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) residual persistence level ranged between 17 and 32% in continuous alfalfa and 22–41% in continuous corn rotated from EPN treated alfalfa. Combined EPN level ranged between 27 and 43% of the soil cores in continuous alfalfa and 28–55% in continuous corn rotated from EPN-treated alfalfa. Inspection of individual fields suggested EPN populations established in prior years at the residual soil core level of 18–35% can respond positively to an increase of susceptible hosts in both alfalfa and corn, often increasing their presence to 100%.

Key Words: Persistent Entomopathogenic Nematodes, Persistence across crop rotation, EPN

Entompathogenic nematodes are found worldwide in a wide array of soil habitats with a broad host range and significant variation in foraging strategies (e.g., Hara et al. 1991, Kaya and Gaugler 1993, Hominick 2002, Adams et al. 2006). Natural populations range from <1 to 100% of collected soil samples, but are poorly understood (e.g., Gaugler et al. 1992, Campos-Herrera et al. 2013). These characteristics suggest the potential to control a wide array of soil insect pest species with wide-ranging life histories (e.g., Lewis et al. 1992, 1993; Grewal et al. 1994; Lewis, et al. 1996; Campbell and Gaugler 1997; Wilson et al. 2012).

Natural populations evolved within the native habitat recycling on available native hosts utilizing the local flora for their food source. When areas are converted to agriculture, the local entomopathogenic nematodes (EPNs) may not share the same soil niche (Ferguson et al. 1995) as the new potential host/insect pest attacking the agricultural crops or may not be virulent enough to overcome the immune system of the new potential hosts brought in with the agricultural ecosystem. This mismatch may be the factor preventing the native EPNs from suppressing the agricultural soil-borne pests.

The primary use of EPNs in managed plant systems has been focused on inundative releases in a biopesticide strategy (defined as flooding the soil with high numbers of EPNs for immediate pest suppression). Little effort has been placed in investigating the use of natural occurring or adapted EPN strains for long-term suppression of pest outbreaks in managed systems which range from turf to agricultural fields. It has been suggested that an inoculative (defined as establishing an adapted isolate in the soil at lower numbers and relying on recycling to build the population) and conservation approach is the future use of EPNs (Lewis et al. 1998).

It has been suggested that an inoculative release program would be successful if 1) susceptible host were present throughout most of the year, 2) agricultural commodity has a high economic threshold level, and 3) soil conditions are favorable for nematode survival

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(Kaya 1990). In the more northern climates, EPNs must survive long periods of cold temperatures and frozen soils, so EPNs would need to retain the genetic ability to conserve their resources and persist between host availability (Shields et al. 1999).

This study was focused on the potential of climate-adapted EPN isolates to persist across a common multi-year field crop rotation after a single inoculation. A 4-yr alfalfa and 4-yr corn rotation is a typical crop rotation sequence for the dairy producers in New York State (NYS). This research is part of a larger effort to develop an effective biological control program for the serious alfalfa pest, alfalfa snout beetle, *Otiorhynchus ligustici* L. (Coleoptera: Curculionidae) (Shields et al. 2009, Shields and Testa 2017). The mix of EPN species (*S. carpocapsae* + *S. feltiae*) used have been shown to be effective against alfalfa snout beetle in field studies (Neumann and Shields 2008). This combination of two compatible EPN species with distinct soil niche preferences also overlap the soil profile preferences of the common agricultural soil insect (Ferguson et al. 1995).

Methods and Materials

Source of EPNs

Our field study used two New York State (NY) EPN isolates. *Steinernema carpocapsae* (Weiser) (Rhabditida:Steinernematidae) ('NY 001'), was initially isolated from soil samples collected in 1990 from Oswego County, NY. *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) ('NY 04'), was initially isolated from soil samples collected from Jefferson County, NY in 2004. To maintain the ability of these strains to persist in NY conditions, each species was re-isolated from the field every second year beginning in 2007, and used to reinitiate the laboratory culture. The EPN strains used in this trial were re-isolated from field plots in 2007, 2009, and 2011 depending on the year of application. Greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), larvae (Woodring and Kaya 1988) were used as hosts to maintain the nematode cultures between field isolations. Culturing protocols have been modified to preserve the genes for persistence in the population during the 2 yr of laboratory culturing (Shields 2015). A Galleria based non-white trap rearing system (Testa and Shields 2017) was used for the production of infective juveniles (IJs) for field application.

Field Sites

A total of 82 different alfalfa/grass mixed fields spread across the six Northern NY alfalfa snout beetle infested counties (Clinton [*N* = 7], Essex [*N* = 7], Franklin [*N* = 16], Jefferson [*N* = 15], Lewis [*N* = 17], St Lawrence [*N* = 20]) (Shields et al. 2009, Shields and Testa 2017) were selected for the study. Selected fields were in their first full year of production after stand establishment the previous year. EPN IJs were applied to 4 fields in 2007, 8 fields in 2008, 48 fields in 2009, 14 fields in 2010, and 8 fields in 2011. Within each field, four sites were established with each site comprised of 0.10 ha. GPS coordinates were recorded for the perimeter of each field site to allow the return to the field site for soil sampling across the years of the study regardless of the crop in the field. Each field site was pre-sampled for the presence of native EPNs by collecting 100 soil samples, and conducting a laboratory bioassay for the presence of EPNs. All fields were within the range of 0–5% of EPN positive soil cores with 99% of the EPNs detected identified as *S. carpocapsae*, and 1% identified as Heterorhabditis bacteriophora Poinar (Rhabditida: Heterhabditidae). None of the pre-sample EPNs were identified as *S. feltiae*.

EPN Application

EPN IJs were applied to each field site through a truck mounted sprayer consisting of 2- to 190-liter tanks using 12-volt spray pumps to generate 275 kPa pressure and a 2.5-m spray boom with spray nozzles spaced at 56 cm. IJs were applied in 475 liters of water per ha onto the soil surface through fertilizer stream nozzles (TeeJet 0008) at the rate of 2.5 million for each species per ha (500 million IJ total per ha). IJs were applied 10–14 d after alfalfa harvest with 10–15 cm regrowth to provide ground shading. Applications were initiated 1 h before sundown to allow EPNs to enter the soil with minimal death from UV exposure.

EPN Sampling

All field sites were sampled for EPN establishment 30–45 d after application and subsequently once per year for the duration of the study. At each field visit, 25 soil cores were collected from each 0.10 ha site (total 100/field). Each soil core was collected with a probe with a 2 cm internal diameter inserted 20 cm into the soil. Each sample was divided, with the top 7 cm placed in a 120 ml container cup and the lower 13 cm placed in a 240 ml container. Soil cores were divided in this manner to isolate *S. carpocapsae* from *S. feltiae* for the assay (Ferguson et al. 1995). Each container had a tight-fitting lid. All soil samples were laboratory bio-assayed using *G. mellonella* larvae as indicator hosts (5 per 7 cm core, 10 per 13 cm core). Samples were incubated at room temperature (23°C), on shelves in the laboratory for 7 d. Dead *G. mellonella* were examined for nematode infection by observing the condition and color of the cadaver (Poinar 1984). Cadavers where death from EPNs were questionable were placed on moist plaster of Paris disks in Petri dishes (White 1927) and observed for IJ emergence. Subsamples IJs from the white trap emergence were used to verify EPN species/cadaver coloration by infecting *G. mellonella*, dissecting out the adult males and verifying the EPN species with the shape of the male spicule head (Neumann 2007).

Statistical Analysis

EPN population levels expressed in percent of soil samples with a positive bioassay for the presence of EPNs were transformed with Arcsine transformation before analysis. Significant differences in populations between years was tested with single factor analysis of variance (ANOVA) with post-hoc *t*-test applying Bonferroni correction (Systat Software Inc. 2009). Degrees of freedom were variable depending on the group of data analyzed (number of fields) and is listed in the results section with the other statistical results.

Results

EPN Levels in Alfalfa/Grass Mixed Fields

After inoculation, *S. carpocapsae* (NY-001) positive soil samples from the assay ranged from 7 to 13 ± 1–3%. Population levels were statistically similar, regardless of year ($F = 0.3$; df = 170; $P = 0.25$). *S. feltiae* (NY-04) positive soil samples from the assay ranged from 18 to 32 ± 2–6%. Population levels were statistically different between year 5 and year 6 ($F = 2.3$; df = 10; $P = 0.05$), but the remaining years did not differ statistically ($F = 0.4$; df = 170; $P = 0.23$). The combined level of both EPN species positive soil samples from the assay ranged from 27 to 43 ± 2–6% of the soil samples testing positive in the assay. Population levels were statistically different at the extreme values but not generally different from year to year ($F = 2.4$; df = 170; $P = 0.05$). The levels of *S. feltiae* were statistically

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higher than the levels of *S. carpocapsae* regardless of year ($F = 5.3$; df = 170; $P < 0.01$) (Fig. 1).

EPN Levels in Corn

All corn fields were rotated from alfalfa fields previously inoculated with EPNs 2–4 yr prior to the rotation. For comparison, the EPN population data from year 4 alfalfa was used as the initial starting population value since this represents the typical timing of rotation out of alfalfa and there was no significant difference in EPN levels in alfalfa between years 3 and 5 (Fig. 1). EPN populations of both species in first-year corn were not statistically different from the final year of alfalfa (Sc 8 ± 1% vs. 9 ± 1%, Sf 22 ± 3% vs. 23 ± 2%) ($F = 0.7$; df = 28; $P = 0.10$). In second-year corn, populations of *S. carpocapsae* increased numerically but the increase was not significant (9 ± 1% to 14 ± 2%) ($F = 1.3$; df = 32; $P = 0.10$). However, the *S. feltiae* population increased significantly over year 1 levels (23 ± 2% to 41 ± 5%) ($F = 4.3$; df = 32; $P < 0.01$).

Additionally, the combined level of both EPN species increased significantly over year 1 (31 ± 2% to 55 ± 6%) ($F = 4.1$; df = 32; $P < 0.01$). During corn years 3–6, *S. carpocapsae* levels dropped to less than 5% and *S. feltiae* populations returned to the year 1 levels (23–31 ± 1–4%), resulting in the combined EPN levels to drop to similar levels (28–32 ± 1–4%) (Fig. 2).

EPN Levels After Corn

After the corn fields were rotated back into alfalfa, the combined EPN and *S. feltiae* levels remained at the long-term residential levels (27–34% of the cores) and were not significantly different from the levels before the corn rotation. However, the *S. carpocapsae* levels remained lower than the pre-corn rotation levels (Fig. 3).

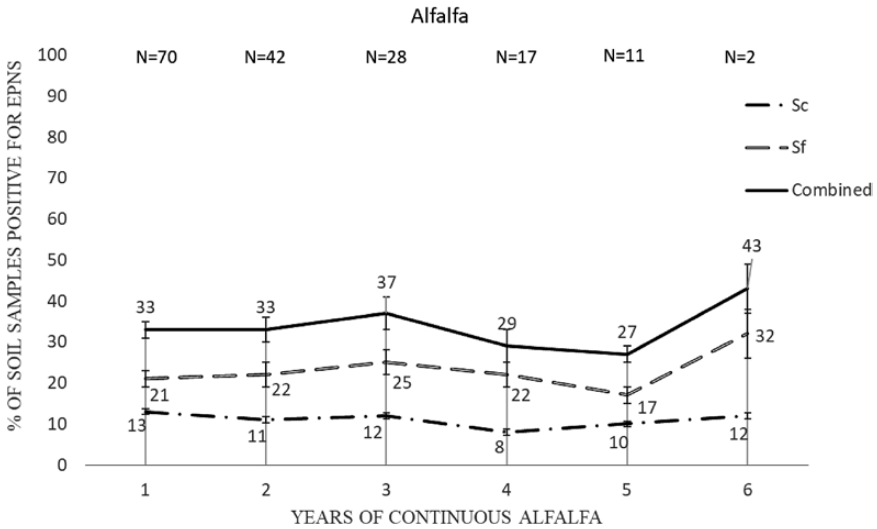

Fig. 1. Frequency of soil cores testing positive to the presence of EPNs in continuous alfalfa using a *Galleria* bioassay. Field areas were inoculated with EPNs once during the first year of alfalfa at the rate of 250 million IJs per species per ha. Graph values are: mean ± SE and the number of fields represented by each data point (*N* values) are indicated along the top of the graph.

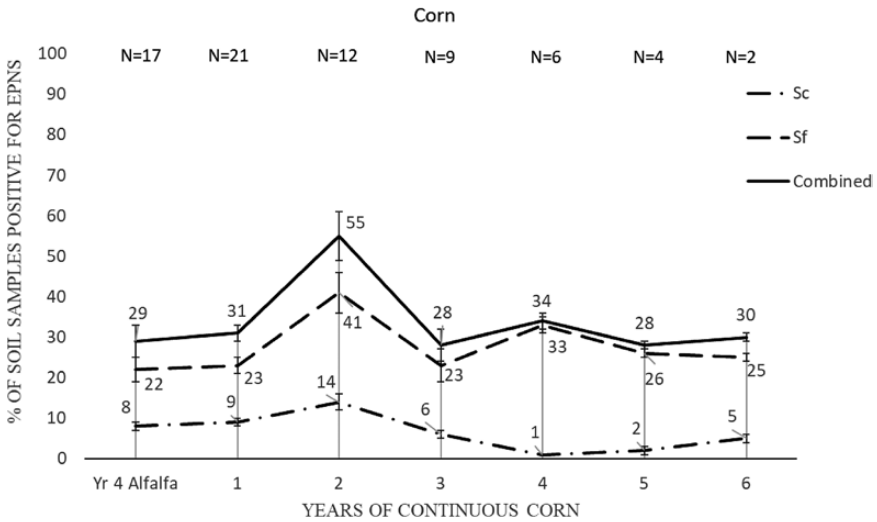

Fig. 2. Frequency of soil cores testing positive to the presence of EPNs in continuous corn using a *Galleria* bioassay. Field areas were inoculated with EPNs once during the first year of alfalfa at the rate of 250 million IJs per species per ha prior to rotation to corn. Graph values are: mean ± SE and the number of fields represented by each data point (*N* values) are indicated along the top of the graph.

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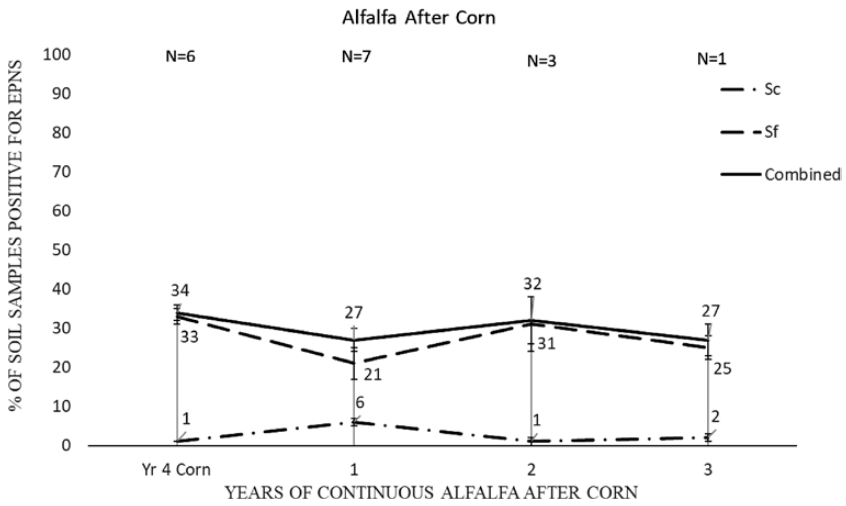

Fig. 3. Frequency of soil cores testing positive to the presence of EPNs in alfalfa following the continuous corn rotation using a *Galleria* bioassay. Field areas were inoculated with EPNs once during the first year of alfalfa prior to the corn rotation at the rate of 250 million IJs per species per ha prior to rotation to corn. Graph values are: mean ± SE and the number of fields represented by each data point (*N* values) are indicated along the top of the graph.

Discussion

Pre-Rotation Alfalfa

Climate-adapted EPN isolates persisted in alfalfa/grass mixed fields for multiple years after an initial inoculation (Fig. 1), and these data suggest that the alfalfa/grass mixed ecosystem is a stable system for EPN persistence. *S. carpocapsae* with its preferred niche in the top 6 cm of the soil profile maintained its population between 8 and 13% of the soil cores. *S. feltiae* with its preferred niche ranging from the surface to 20 cm deep, maintained its population across multiple fields between 21 and 32% of the soil cores. The combined species level of EPNs ranged from 33 to 43% of the soil cores. These levels were maintained for 6 production years after initial inoculation with 250 million IJ/ha of each species. This inoculation level is 10-fold lower than the commercial level of 2.5 billion IJs per ha. The average EPN level across multiple fields is important because it strongly suggests that this EPN combination of isolates is compatible with the array of agricultural soils, ranging from clay-loams to sandy-loams, used to grow alfalfa in Northern New York (NNY). In addition, persistence indicates that EPN isolates are well adapted to the climate and actively recycling in hosts present in the fields.

Our analysis across farms did not allow us to fully evaluate the ability of these EPNs to respond to insect invasion. However, inspection of individual fields indicated that these long-term resident EPN populations are responsive to an increase of insect hosts, which may be economically damaging species. Four individual fields (Fig. 4) are utilized to illustrate the potential of these long-term resident EPN populations to respond to insect invasion. In the first example (Fig. 4A), the high initial population level of EPNs 45 d after inoculation (70% of the cores) indicates an abundance of hosts in the field shortly after the initial inoculation compared to the expected level (33% of the cores, Fig. 1). In subsequent years, the resident EPN population decreased to the range of 14–27% soil cores, a long-term resident level with minimum number of host available. In the second example (Fig. 4B), EPN populations in the year of inoculation established at the multiple field average level (34% of the cores). In the third year after inoculation, EPN levels to exploded to 100% of the soil cores (Sc = 29%, Sf = 88%), suggesting an insect invasion before the population declined to the long-term resident range of 17–25% the following year. While the combined EPN curve in year 3 actually shows 117%, the value reflects soil samples which tested positive for both EPN species. In the third example (Fig. 4C), the EPN population responds to an insect invasion in year 4 after inoculation (81% combined), declines in year 5 to a lower level (24% combined) before rebounding in year 6. In the fourth example (Fig. 4D), the EPN population established at an unusually low level in year 1, increased to 16% combined in year 2, increased to 48% combined in year 4, and increased to 100% in year 5. These four fields clearly indicate a dynamic system where the EPNs have the ability to reside for several years at a lower level and respond to insect invasion. These data only track the EPN population and do not suggest if the invading insects were controlled before causing economic damage or losses.

Corn Rotation

During the first year of corn, EPN populations remained at the same level as the final year of alfalfa. However, in year 2 corn, the EPN population significantly increased to 55% of the soil cores averaged across all nine fields. The larger standard error suggests an interesting variation between the nine fields worth exploring. The two fields in Fig. 5 demonstrate the potential of a residual EPN population to respond to an insect invasion. In the first field (Fig. 5A), the EPN population was at a very low level in the final year of alfalfa (7% of the cores) and increased to 21% during the first year of corn. In the second year of the corn rotation, EPN populations exploded to 100% of the soil cores with most of the population increase being *S. feltiae*. In years 3 and 4, the EPN population dropped to the long-term residential level of 23–36%. In the second field (Fig. 5B), the EPN population was slightly higher in the final year of alfalfa (16%), increased to 43% in the first year of corn and exploded to 100% in year 2 corn before returning to the long-term residential level for years 3–6 (19–31%). While the combined EPN curve in both fields in year 2 actually shows values for the combined EPNs over 100%, these values reflect soil samples which tested positive for both EPN species.

Figures 2, 4 and 5 clearly indicate a dynamic system where the EPNs have the ability to reside for several years at a lower level and respond to insect invasion. These data only track the EPN population and do not suggest if the invading insects were controlled before

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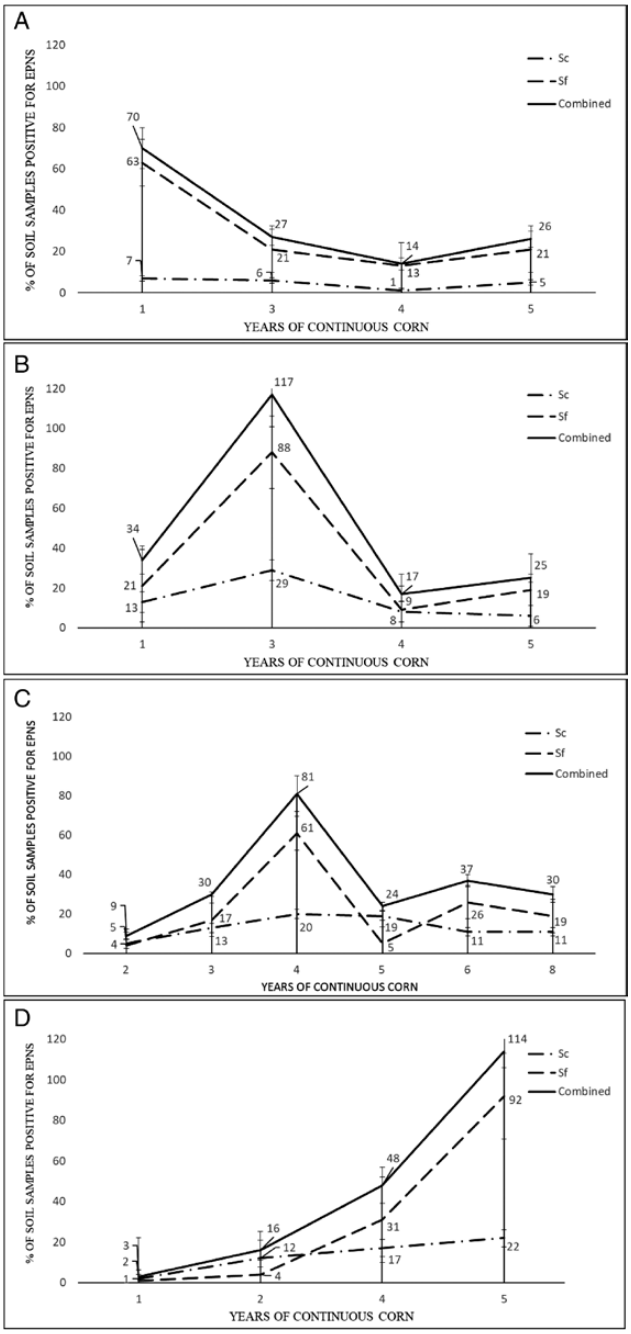

Fig. 4. Frequency of soil cores testing positive to the presence of EPNs in continuous alfalfa using a *Galleria* bioassay. These single fields (A–D) were inoculated with EPNs once during the first year of alfalfa at the rate of 250 million IJs per species per ha. Graph values are: mean ± SE.

causing economic damage or losses. The large increase of EPNs in year 2 corn is suggested to be a result of recycling on corn rootworm larvae, *Diabrotica virgifera*, an economically important insect in year 2–4 continuous corn and a reported host. Validation of this suggestion will require a follow-up study.

Another interesting study would be to expand on the data in Fig. 3. During the alfalfa portion of the rotation after EPN inoculation, the *S. carpocapsae* population remains between 8 and 13% of the soil cores and appears stable across 6 yr after establishment. The data from the corn years would suggest that this crop is not favorable to retaining the *S. carpocapsae* portion of the EPN population with the level dropping from the 8–14% range to 1–6% range in corn years 3–6. Figure 3 illustrates the EPN levels after the fields are rotated out of corn and back into alfalfa. In these fields, the *S. carpocapsae* populations remain very low and appear to not rebound to the pre-corn levels. This raises questions regarding the long-term survival of *S. carpocapsae* in the alfalfa-corn rotation.

Since *S. carpocapsae* was present at a 1–5% level in most of the fields before a subsequent inoculation with the exact same *S. carpocapsae* strain, it is difficult to know if the post inoculation increased presence of *S. carpocapsae* is due to the inoculation of additional EPN IJs, the addition and continued presence of a second EPN species (*S. feltiae* 'NY 04') or some unknown factor. However, since *S. feltiae* was never found in any of the pre-samples and is rare in widespread sampling across NYS (Shields unpublished), the continual persistence of *S. feltiae* for multiple

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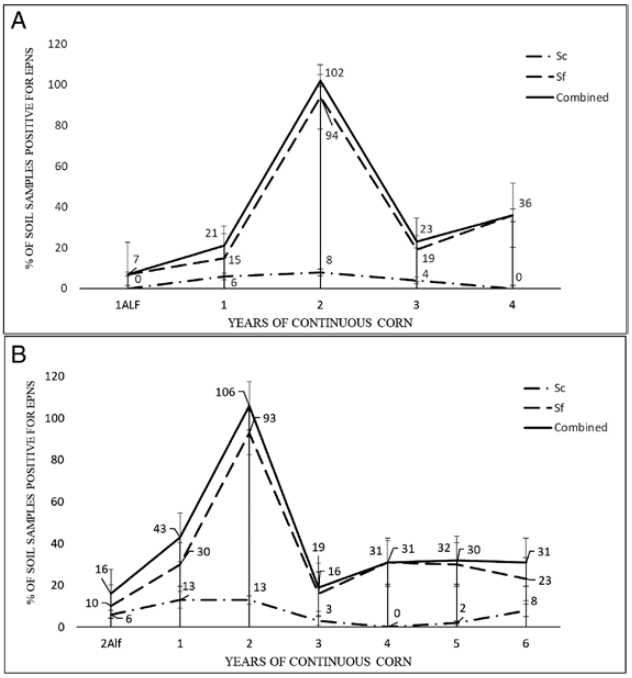

Fig. 5. Frequency of soil cores testing positive to the presence of EPNs in continuous corn using a *Galleria* bioassay.These single fields (A and B) were inoculated with EPNs once during the first year of alfalfa at the rate of 250 million IJs per species per ha before rotation to corn. Graph values are: mean ± SE.

years after a single inoculation strongly suggests the introduction of a complementary EPN species into the agricultural environment. The continued persistence of *S. feltiae* at a significant level and its response to insect invasion in both the alfalfa and corn portions of the crop rotation strongly suggests the potential for biological control of soil insect pests. Follow-up studies will be required to parse out the soil insects being used as hosts in each of these cropping environments, identify population reductions and its impact on economic damage reduction.

Acknowledgments

We thank the Northern NY Agricultural Development Program, NY Farm Viability Institute and Cornell University for funding this research. We also thank the 37 summer student field assistants who helped collect and bioassay more than 50,000 soil cores for EPNs between 2007 and 2016 for this study.

References Cited

Adams, B. J., A. Fodor, H. S. Koppenhöfer, E. Stackebrandt, S. P. Stock, and M. G. Klein. 2006. Biodiversity and systematics of nematode-bacterium entomopathogens. Biol. Control 37: 32–49.

Campbell, J. F., and R. Gaugler. 1997. Inter-specific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? Fund. Appl. Nematol. 20: 393–398.

Campos-Herrera, R., E. Pathak, F. E. El-Borai, R. J. Stuart, C. Gutierrez, and J. A. Rodriguez-Martin. 2013. Geospatial patterns of soil properties and the biological control potential of entomopathogenic nematodes in Florida citrus groves. Soil Biol. Biochem. 66: 163–174.

Ferguson, C. S., P. C. Schroeder, and E. J. Shields. 1995. Vertical distribution, persistence and activity of entomopathogenic nematodes (Nematoda: Heterorhabditidae and Steinernematidae) in alfalfa snout beetle (Coleoptera: Curculionidae) infested fields. Environ. Entomol. 24: 149–158.

Gaugler, R., J. F. Campbell, S. Selvan, and E. E. Lewis. 1992. Large scale inoculative releases of the entomopathogen, *Steinernema glasseri*: assessment 50 years later. Biol. Control 2: 181–187.

Grewal, P. S., E. E. Lewis, R. Gaugler, and J. F. Campbell. 1994. Host finding behavior as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology 108: 207–215.

Hara, A. H. R., R. Gaugler, H. K. Kaya, and L. M. Lebeck. 1991. Natural populations of the entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian Islands. Environ. Entomol. 20: 211–216.

Hominick, W. M. 2002. Biogeography, pp. 115–143. *In* R. Gaugler (ed.), Entomopathogenic nematology. CABI, Wallingford, United Kingdom.

Kaya, H. K. 1990. Soil ecology, pp. 93–116. *In* R. Gaugler and H. K. Kaya (eds.), Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, FL.

Kaya, H. K., and R. Gaugler. 1993. Entomopathogenic nematodes. Ann. Rev. Entomol. 38: 181–206.

Lewis, E. E., R. Gaugler, and R. Harrison. 1992. Entomopathogenic nematode host finding: response to contact cues by cruise and ambush foragers. Parasitology 105: 309–315.

Lewis, E. E., R. Gaugler, and R. Harrison. 1993. Response of cruiser and ambusher entomopathogenic nematodes (Steinernematidae) to host volatile cues. Can. J. Zool. 71: 765–769.

Lewis, E. E., M. Ricci, and R. Gaugler. 1996. Host recognition behaviour predicts host suitability in the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida:Steinernematidae). Parasitology. 113: 573–579.

Lewis, E. E., J. F. Campbell, and R. Gaugler. 1998. A conservation approach to using entomopathogenic nematodes in turf and landscapes, pp. 235–254. *In* P. Barbosa (ed.), Conservation biological control. Academic Press, San Diego, CA.

Neumann, G. 2007. Dual-strategy biological control of the alfalfa snout beetle, *Otiorhynchus ligustici* L. (Coleoptera: Curculionidae), using persistent entomopathogenic nematodes in a single vs multispecies natural enemy approach. Ph.D Thesis. Cornell University, Ithaca, NY. pp. 113.

Neumann, G., and E. J. Shields. 2008. Multiple-species natural enemy approach for the biological control of the alfalfa snout beetle, *Otiorhynchus ligustici* L. (Coleoptera: Curculionidae), using entomopathogenic nematodes. J. Econ. Entomol. 101: 1533–1539.

Poinar, G. O. 1984. The natural history of nematodes. Prentice-Hall, Englewood Cliffs, NJ.

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on 28 August 2018

Shields, E. J. 2015. Utilizing persistent EPNs in a conservation or a more classical biological control approach, pp. 165–184 (Ch 6). *In* R. Campos-Herrera (ed.), Nematode pathogenesis of insects and other pests – ecology and applied technologies for sustainable plant and crop protection, series: sustainability in plant and crop protection, Vol. 1. Springer, Neuchatel, Switzerland.

Shields, E. J., and A. Testa. 2017. Biological control of alfalfa snout beetle with persistent entomopathogenic nematodes: expanding a single farm's success to an area-wide biological control program. Am. Entomol. 63: 216–223

Shields, E. J., A. Testa, J. M. Miller, and K. L. Flanders. 1999. Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* 'Oswego' and *H. bacteriophora* 'NC' on alfalfa snout beetle larvae (Coleoptera: Curculionidae). Environ. Entomol. 28: 128–136.

Shields, E. J., A. Testa, G. Neumann, K. L. Flanders, and P. C. Schroeder. 2009. Biological control of alfalfa snout beetle with a multi-species application of locally-adapted persistent entomopathogenic nematodes: the first success. Am. Entomol. 55: 250–257.

Systat Software Inc. 2009. SigmaPlot 11.2. User's guide, part 2 statistics. Systat Software Inc., San Jose, CA.

Testa, A. M., and E. J. Shields. 2017. Low labor "in vivo" mass rearing method for entomopathogenic nematodes. Biol. Control 106: 77–82.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science. 66: 302–303.

Wilson, M. J., R. U. Ehlers, and I. Glazer. 2012. Entomopathogenic nematode foraging strategies: is *Steinernema carpocapsae* really an ambush forager? Nematology 14: 389–394.

Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques. Southern cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station. Fayetteville, AR.

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