

Biological Control of Alfalfa Snout Beetle with Persistent Entomopathogenic Nematodes:

Expanding a Single Farm's Success to an Area-Wide Biological Control Program

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Alfalfa snout beetle, *Otiorhynchus ligustici* (L.), (ASB) remains the most serious insect pest impacting alfalfa production in North America. Its minor status is directly related to its limited North American distribution of nine counties in northern New York State and a small portion of Ontario, Canada, across the St. Lawrence River from the U.S. infestation (>200,000 hectares; Fig. 1). Within its infested area, this root-feeding parthenogenic flightless insect of European origin frequently kills entire alfalfa stands in a single year. Emerging adult populations can frequently exceed 2 million beetles per hectare, and with a host range of more than 20 common plants, once an area is infested with this insect, it is considered permanently infested. An in-depth discussion of this insect's introduction to the U.S., as well as its distribution, life history, economic impact, and attempts at management were reported in an earlier *American Entomologist* article (Shields et al. 2009).



Shields et al. (2009) also reported on the first success in controlling this important insect on a single farm with persistent entomopathogenic nematodes (EPNs) native to New York. This article is focused on the steps necessary to turn a single farm's success into an ongoing area-wide biological control program against ASB using a single field inoculation of northern New York-adapted persistent EPNs for multi-season control of this insect.

A number of questions needed to be answered before the success on a single

farm could be scaled up for additional farms across the nine-county infested region. These questions will be addressed in the following sections.

Can a cost-effective EPN mass-rearing procedure be developed that is both farmer-friendly and low-labor while retaining the genes for persistence?

Two options for mass-rearing EPNs are available: in vivo and in vitro. In vivo requires rearing in host insects, usually utilizing the concept of the White trap (White 1927) with variations reported by various authors (Dutky et al. 1964, Poinar 1979, Woodring and Kaya 1988, Lindegren et al. 1993, Flanders et al. 1996, Kaya and Stock 1997, Shapiro-Ilan et al. 2002). In vivo mass-culturing of EPNs is expensive due to high labor costs and the use of live insects as production hosts. However, in vivo mass-rearing operations require minor capital outlay and nominal expertise to achieve good product quality, and

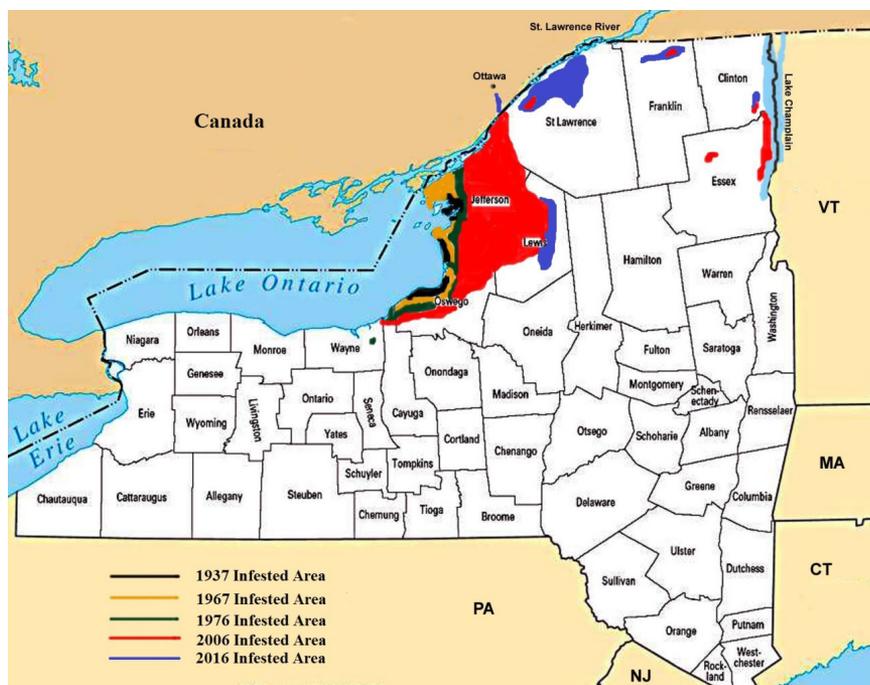


Fig. 1. Area of New York State and Canada infested with alfalfa snout beetle, *Otiorynchus ligustici*. This map represents the entire known North American infestation of this European insect.

the system is easily adapted to multiple EPN species. Without significant reduction of costs (primarily the cost of the host insect and labor) there is little cost benefit of scale (Shapiro-Ilan et al. 2014). The second option involved is in vitro rearing with the use of artificial media. This type of rearing requires highly technical expertise to produce the required monoxenic culture of the symbiont bacteria and the establishment of bacteria-free nematodes (Shapiro-Ilan et al. 2014).

Because neither of these two systems met the goals of being farmer-friendly and low-labor, a new in vivo EPN rearing system was developed without utilizing the White trap concept. After many false starts, a cost-effective, low-labor rearing method was discovered from a last-ditch idea. In the U.S., wax moth larvae, *Galleria mellonella* L. (Lepidoptera: Pyralidae), are sold as fish bait by several companies and delivered in 500 ml plastic containers with small holes punched in the lid for ventilation, filled with either wood shavings or sawdust. Approximately 250 larvae are in each container. The lid is removed and 20 ml of water containing 15,000 EPN infective juveniles (IJs) is spread onto the wood shavings or sawdust in a circular pattern. The lid is then replaced and the container is incubated at room temperature (22–25°C). Death of the *Galleria* larvae is noted within 48–60 h and all of the dead larvae accumulate on top of the wood shavings or sawdust.

After 12–15 d, IJs emerge from the cadavers and enter the surrounding wood shavings or sawdust, where they survive in high numbers for several days due to improved oxygen exchange (compared to emerging in water as part of the White trap technique; Shields and Testa 2015, Testa and Shields 2017).

To separate the IJs from the wood shavings or sawdust and other biological material, the contents of the container are inverted onto a wire screen (20 mesh, 841 µm openings) and the IJs are washed into a lower container with non-chlorinated water. This solution of nematodes is then poured through a second, finer screen (40 mesh, 400 µm openings) to remove any remaining debris. The number of EPNs present and alive in the nematode wash solution can be estimated using a dissecting microscope and the standard serial dilution methodology. This solution of water and nematodes can then be dumped into a spray tank filled with water for field application. However, the sprayer needs to be cleaned and all screens and filters must be removed to allow EPN IJs to flow through the sprayer unimpeded.

Despite the fact that EPN IJ production yields are influenced by incubation temperatures, this method has been utilized by numerous northern New York dairy farmers to rear their own EPNs for release on their own farm. At 25°C incubation temperature, IJ yields of *S. carpocapsae* 'NY 001' and *H. bacteriophora* 'Oswego'

are about $2.5\text{--}3.0 \times 10^7$ IJs per container. *S. feltiae* 'NY 04' produces better at 20°C and yields about $1.5\text{--}2.0 \times 10^7$ IJs. This *Galleria*-based rearing method has been used by the Shields lab to rear more than 1.0×10^{11} IJs over the past five years for field release in an area-wide biological control program focused on alfalfa snout beetle. The cost of this rearing method is between \$250–\$300 USD per 1.0×10^9 IJs (excluding labor), and it is simple enough for on-farm rearing (Shields and Testa 2015, Testa and Shields 2017).

EPNs persistent in the environment utilize phased infectivity to bridge periods of environmental stress and host scarcity (Griffin 2012). The loss of field persistence in many commercial populations (Ferguson et al 1995, Shields et al. 1999) supports the idea that these survival mechanisms are genetically encoded and are easily lost under conditions of continuous rearing (Griffin 2012). Rearing strategies must be adapted to retain phased infectivity in the populations of persistent EPNs to continue to use the strategy of inoculation for multiple-year pest suppression. Several of these techniques are discussed by Shields (2015).

Can EPN application rates and techniques be adapted to low-value crops and typical commercial pesticide application equipment? Is timing of application also important?

The use of commercial EPN populations with an inundative release strategy usually requires a high volume of water followed by irrigation to assist IJs with soil penetration before they die from UV light exposure or desiccation (Gaugler and Bousch 1978; Gaugler et al. 1992). Rapid soil penetration under these conditions is important due to an active ongoing pest problem and the relatively short life of the applied EPNs. However, under the inoculative release strategy, where the focus is on the inoculation of the soil with an adapted persistent EPN population focused on multi-year control, potential existed for the use of a lower water application rate (Shields 2015).

A field study focused on water carrier

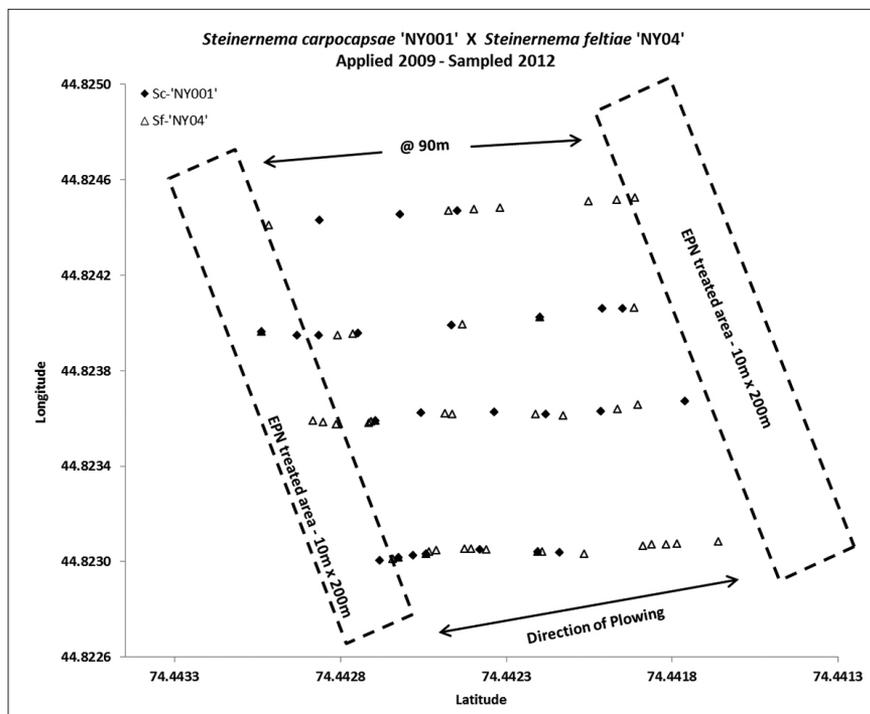


Fig. 2. Areas within the treated areas were inoculated with *Steinernema carpocapsae* 'NY 001' and *Steinernema feltiae* 'NY 04' during July 2009 at 1.25×10^8 IJs per ha (1.25 IJ/cm²) per species (total 2.5×10^8 IJs per ha, 2.5 IJ/cm²) when the field was an alfalfa/grass mixture. The field was plowed and rotated to corn in 2010–2012, and sampled for EPN movement in June 2012. Both EPN species had moved a minimum of 45 m between applications.

application rates was conducted. *H. bacteriophora* 'Oswego' at two rates (2.5 and 15×10^9 IJs/ha) was suspended in 1,000 L/ha (105 gpa), 2,000 L/ha (210 gpa) and 7,000 L/ha (740 gpa) and applied through fertilizer stream nozzles (type 0006) or flat fan nozzles (type 8006) onto the soil surface of a recently harvested alfalfa field. Results indicated that water carrier volume did not have a significant impact on EPN establishment at either EPN concentration tested. In addition, there was no significant difference in establishment between the stream nozzles and the flat fan nozzles used in the application. In all cases, EPN establishment ranged from 35–40% of the soil cores that tested positive. However, when applying EPNs through a plant canopy, the fertilizer stream nozzles penetrate the canopy better with very little spray residue remaining on the plant canopy, unlike applications with flat fan nozzles (Shields and Testa 2015). Subsequent research has indicated effective EPN establishment with the use of fertilizer stream nozzles and 500 L/ha (50 gpa) (Shields, unpublished data) and commercial applicators are effectively using liquid fertilizer drop tubes.

A small field trial was conducted to

investigate the possibility of reducing the EPN application rate while retaining an effective establishment rate. Three rates of *H. bacteriophora* 'Oswego' were used (2.5×10^9 /ha, 1.25×10^9 /ha and 0.63×10^9 /ha) applied in 1,000 L/ha water through fertilizer stream nozzles (type 0006) spaced 30 cm apart on the spray boom. The EPNs were applied to an *O. ligustici* infested alfalfa field, harvested 10 d prior to application with 15 cm regrowth to shade the soil surface. Application was initiated at sunset to allow time for the IJs to enter the soil without exposure to UV light. Sixty days after application, 35–40% of the soil cores tested positive for *H. bacteriophora* 'Oswego' independent of the rate of EPN application and all treatments were not statistically different ($P < 0.05$). Twelve months later, the incidence of *H. bacteriophora* 'Oswego' positive soil cores had increased to 50–60%. This increased incidence was believed to be a function of nematode recycling in hosts and a more uniform distribution from EPN movement into the areas between the strips of application through the stream nozzles (Shields and Testa 2015). These data also indicate that application timing is not critical because the EPNs persist in the

environment. Over the course of over 25 years of research using persistent EPNs against ASB, it has been determined that IJs can be applied whenever soil temperatures are above 15°C with no impact on soil establishment. Perhaps they can be applied on colder soils, but that has not been tested. In the absence of available hosts, the IJs persist using phased infectivity, waiting for the host to become available. At 20–25°C, these persistent strains remain infective for more than 365 d without recycling in a host (Shields 2015).

What is the best mix of EPN species to successfully attack ASB?

Research has shown that all three species of EPNs native to New York State successfully attacked and killed ASB larvae (Schroeder et al. 1994). In addition, *S. carpocapsae* 'NY 001' successfully attacked the adults (Neumann and Shields 2008). Mixing EPN species improved EPN effectiveness on ASB larvae and adults (Neumann and Shields 2008) due to the partitioning of the soil profile by the various species (Ferguson et al. 1995, Neumann and Shields 2006). Species mixes of *S. carpocapsae* and *S. feltiae* resulted in lower levels of root feeding and damage on alfalfa roots than *S. carpocapsae* and *H. bacteriophora* or any of the single species alone. These results suggested that *S. feltiae* attacked the ASB larvae at smaller larval instars than *H. bacteriophora*. Treatments with an EPN species mix of *S. feltiae* and *H. bacteriophora* resulted in an intermediate level of root feeding damage (Neumann and Shields 2008).

How well will the adapted persistent EPNs persist across typical soil types and cropping rotations found within the northern New York ASB-infested area?

On the farm where the original research on control of ASB with EPNs was conducted, the movement of soil by farm equipment was observed to aid in the redistribution of EPNs and the subsequent collapse of ASB, leading to the inoculation strategy of treating strips perpendicular to the direction of field tilling (Shields et al. 2009). Unassisted EPN movement in the alfalfa system has been reported by Neumann and Shields (2011) and a subsequent experiment has documented a longer-distance movement associated

with the movement of soil during tillage (Fig. 2)(Shields and Testa 2015, Shields 2015). This application strategy was also a method to reduce the cost of the field inoculation for the farmers and allow the EPNs to spread throughout the field over a period of two to three years. One progressive farmer, an early adopter of the EPN biological control program against ASB, developed a far better and more efficient method that will be discussed in a later section.

In 2008 and 2009, a total of 87 fields were inoculated, distributed across six of the nine ASB-infested counties. EPNs were applied to each field through a sprayer mounted on a pickup truck. Nozzle spacing was 60 cm and the nozzles used were fertilizer stream nozzles (type 0006). At each location, the EPN species mix used was *S. carpocapsae* 'NY001' × *S. feltiae* 'NY04', applied at a rate of 1.25×10^8 IJs/species/ha in 500 L water/ha (2.5×10^8 IJs total IJs). At each field location, four areas were inoculated (2,000 m² comprising areas of 10 m × 200 m, total = 8,000 m² per field). The length of all plots (200 m) was aligned perpendicular to the direction the field was tilled or plowed, so subsequent tillage operations could

assist EPN movement throughout the field. GPS locations were recorded for the inoculation zone at the time of the EPN application, allowing those areas to be relocated for subsequent sampling in the following years. Fields were not sampled prior to EPN inoculation because previous research has indicated that if EPNs are naturally present in these soils, they will be present in under 5% of positive soil cores.

Each field was sampled annually between 1 June and 15 October by taking fifty 2 cm diameter soil cores to a depth of 20 cm on two different transects within the EPN-treated zones (total/field = 100 individual samples). Each sample was divided and placed in two different containers. The top 5 cm of the soil core was placed into a 130 ml container with a lid and the lower 15 cm of the soil core was placed in a 260 ml container with a lid. The samples were then returned to the laboratory and tested for the presence of EPNs using the *Galleria* bait method (Bedding and Akhurst 1975, Fan and Hominick 1991).

The year following inoculation, the number of soil cores positive for EPNs had increased to 25–70% at all sites,

indicating that establishment and recycling on the host had occurred in the fields. Fifty-one of these 87 fields have been continuously sampled each year since the initial inoculation, and the EPNs have persisted in all fields through 2015. EPN populations in each field fluctuated between 15% and 85% of soil cores testing positive for EPNs, depending on the year, crop, and soil insects present. Field populations increased or decreased in response to insect invasion. Examples of these responses are shown in Figs. 3–5.

Will a single application of EPNs under the inoculative strategy provide enough mortality to ASB populations to reduce population levels to sub-economic?

Shields et al. (1999) showed that one of the native EPN species (*H. bacteriophora* 'Oswego') could successfully reduce large ASB larval populations by 81% while reducing alfalfa stand loss by 46%. Due to the large number of hosts present, the EPN levels remained at 70–90% of the soil cores testing positive for the first growing season (60 d) dropping to 60–80% at 328 d and 50–60% at 708 d. In a subsequent study, Neumann and Shields

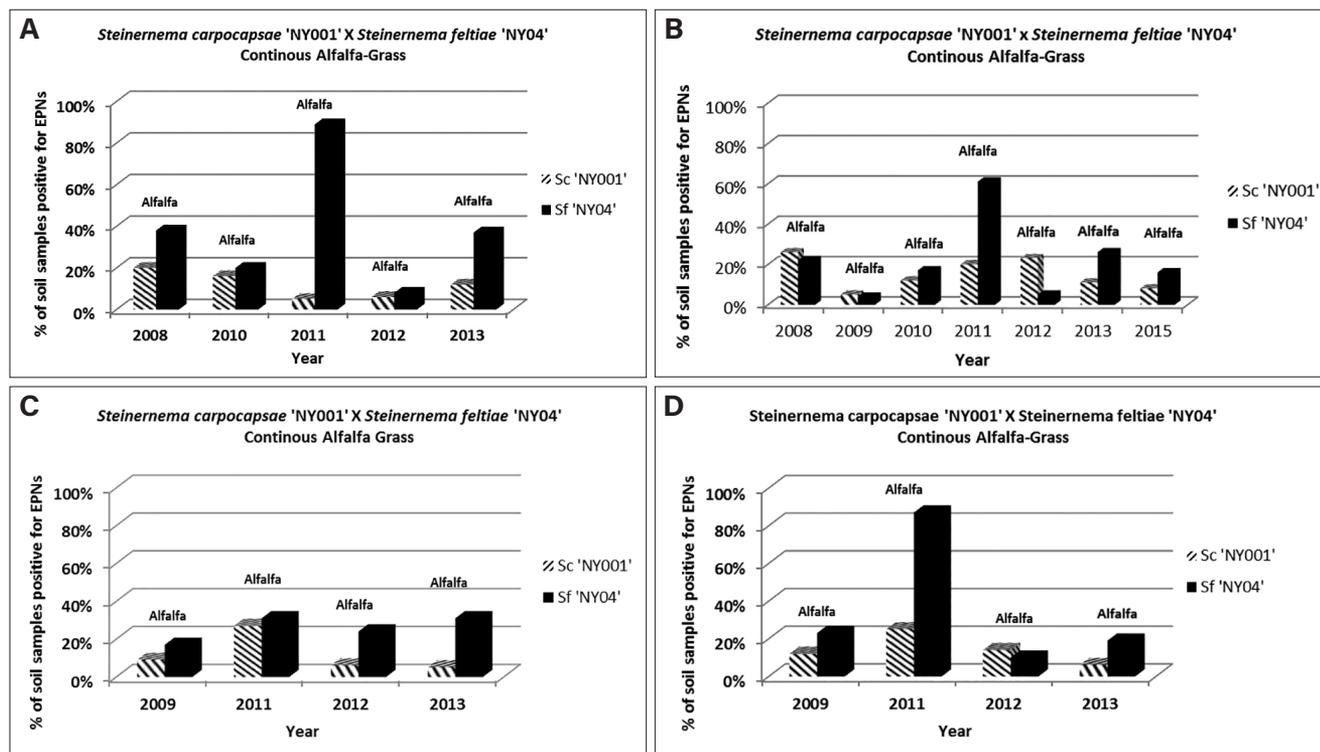


Fig. 3. Four different fields in northern NY. Fields A & B were inoculated in 2008 with 1.25×10^8 IJs of *Steinernema carpocapsae* 'NY 001' and 1.25×10^8 IJs of *Steinernema feltiae* 'NY 04' per hectare. Fields C & D were inoculated with the same EPN rate in 2009. EPN population frequency was measured once per year during the growing season. EPN population appears stable and responds to invasion of host insects.

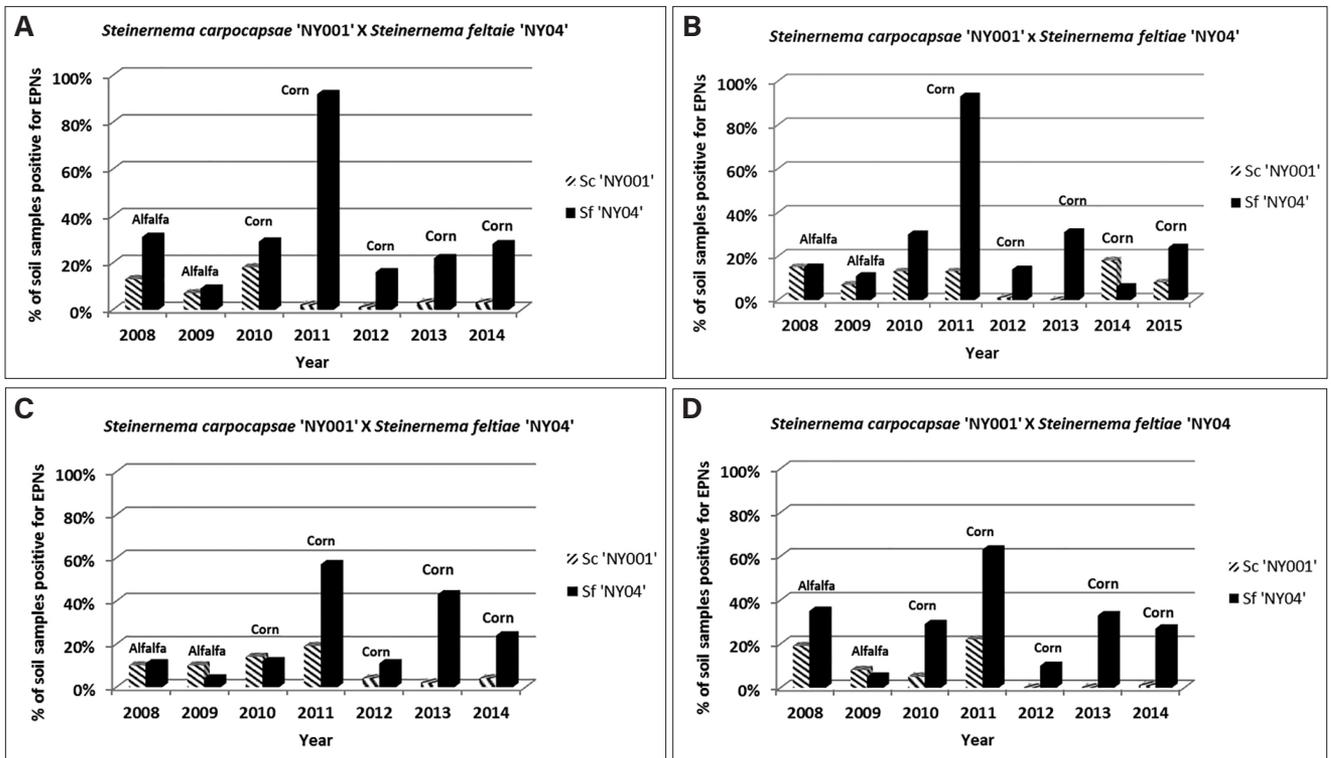


Fig. 4. Four different fields in northern NY inoculated in 2008 with 1.25×10^8 IJs of *Steinernema carpocapsae* 'NY 001' and 1.25×10^8 IJs of *Steinernema feltiae* 'NY 04' per hectare. EPN population frequency was measured once per year during the growing season. EPN recycling on *Diabrotica virgifera virgifera* (corn rootworm) larvae during the corn years is inferred based on insect biology but not directly measured.

(2008), testing EPN combinations, reported similar results under a more moderate ASB larval population. The presence of native EPNs in a plot reduced the alfalfa stand loss from ASB larval feeding by 88%, while EPN persistence fluctuated between 25–50% of soil cores testing positive at 142 d and 15–25% testing positive 357 d after application. EPN persistence was tracked for more than 400 d after application, along with EPN movement into plots where particular species of EPNs were not applied (Neumann and Shields 2011). The EPN species which moved most frequently and the greatest distance was *S. carpocapsae* 'NY 001,' an "ambush" EPN. This species has been shown to infect adults, and we suggest that this movement was the result of infected adults that moved before death (Neumann and Shields 2011).

These studies indicated that persistent native New York populations remained in the soil for multiple years, and their frequency ranged from 15% to >50% of the soil samples testing positive for EPNs more than two years after the initial inoculation. The level of EPNs persisting 60 d (35–40%) and 365 d (50–60%) after inoculation appeared independent of the initial application rate (2.5×10^9 IJs/ha, 1.25×10^9

IJs/ha, and 0.63×10^9 IJs/ha; Shields and Testa 2015).

Fifty-one fields were selected from the 87 total fields inoculated with EPNs in 2007–2009 to track EPN persistence across years and crop rotations. The selected fields represented a wide array of soil types ranging from clay loams to sandy soils. These fields also represented different crop rotation ranging from continuous alfalfa/grass mixture to an alfalfa/row crop rotation (4 years; corn or soybeans). Because it is more realistic to sample and monitor EPN population levels than to monitor soil insect densities across 51 fields, we chose to collect multiple soil samples from each field a single time each year, bioassay those samples for EPNs, and infer levels of hosts in the field from the relative level of EPNs. Our previous data suggests that EPN population levels of 10–35% of the soil cores testing positive for EPNs indicate a long-term persistence level for EPNs, maintaining the population on a low level of soil insects; EPN levels of 35–60% indicate response to a moderate level of soil insects; and >60% of soil cores testing positive for EPNs indicate a high level of soil insects being attacked.

As expected, EPN populations

fluctuated in each field across years, influenced by insect availability and crop rotation. In addition, a wide variation in EPN populations was observed across fields. In all fields, EPN populations persisted from the year of application (2008 or 2009) until the final sampling date covered by this paper (2014 or 2015). Several examples of EPN population fluctuations are shown in Figs. 3–5, covering continuous alfalfa, alfalfa–corn rotation, and alfalfa–corn–alfalfa rotation.

The four fields illustrated in Fig. 3 demonstrate the range of EPN response typical of all fields sampled, which remain in a continuous cropping of alfalfa–grass. Two important points to draw from the graphs are that EPNs from this single introduction persisted across multiple years in a continuous cropping of alfalfa–grass, recycling in the multitude of hosts invading the alfalfa–grass ecosystem, and that EPN populations rise and fall in response to the various levels of insect host availability. These graphs suggest that the residual population of EPNs maintain a population in the range of 10–20% of the soil cores testing positive for EPNs. At that level, the EPN population appears to be stable, persists long-term, and is capable

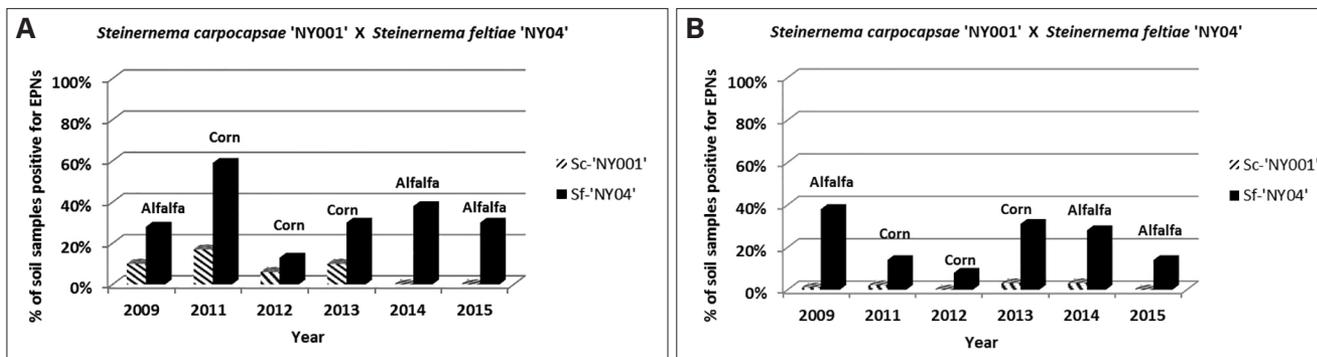


Fig. 5. Two different fields in northern NY where native EPNs were applied once in 2009. EPN population frequency was measured once per year during the growing season. EPN recycling on corn rootworm larvae during the corn years is inferred based on insect biology but not directly measured. EPN populations during the corn portion of the crop rotation maintain their levels, appear to be stable, and are present at a significant level when the field is rotated back into alfalfa. An ASB invasion would be met by a significant level of persisting EPNs.

of responding to host invasion.

Multi-year EPN persistence within a continuous alfalfa-grass cropping system was expected due to the wide array of susceptible hosts feeding within that cropping system, but high EPN persistence was not expected across a corn rotation due to the more limited number of hosts supported within the corn ecosystem. However, when rotated to corn, the EPN population responded to insect invasion within the corn-cropping years (Fig. 4). During the second year of corn production, a large increase in EPN numbers was observed, which was thought to be a response to the higher level of corn rootworm larvae, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), found in second-year corn fields. These results, representative of numerous fields, indicate that our preconceived idea of EPN loss during corn culture was in error. Three conclusions can be drawn from these graphs: 1) native EPN populations inoculated in the alfalfa-grass ecosystem easily persisted during multiple years of a corn crop growing in rotation with alfalfa-grass; 2) EPN populations responded to the corn-specific herbivore invasion; and 3) EPN populations were equal to or higher after four to five years of corn than the final year of the alfalfa-grass. The higher level of EPNs in alfalfa following a corn rotation is illustrated in Fig. 5. When ASB enters the field either in the seeding year (year one) or in year two, the higher level of EPNs carrying over from the corn rotation will be poised and ready to attack the invading ASB larvae.

Will farmers embrace an area-wide biological control program against ASB utilizing native persistent

EPNs even though impact after application may not be seen for two to four years?

Farmers within the ASB-infested area were interested in expanding the field-scale biological control research to farm-scale so that they could begin treating their own fields. The focus of this program was to inoculate fields a single time with persistent native EPNs adapted to the northern New York climatic conditions. The best nematode combination for the area-wide biological control program was determined to be *S. carpocapsae* × *S. feltiae*. This combination of EPNs infects adults in the spring and the larvae before a significant amount of root feeding could occur. If root feeding by ASB larvae was reduced, direct stress to the alfalfa plant from root loss was reduced, and the reduction of feeding wounds reduced the entrance zones for plant pathogens.

On-farm research in 2007–2009 indicated that EPN rates could be reduced to much lower levels than those used in the inundative release strategy (2.5×10^9 IJs per ha). In the field, no significant difference in establishment was observed between 1.25×10^8 IJ/ha and 2.5×10^8 IJ/ha. To reduce EPN costs, the recommendation for field application was 1.25×10^8 IJ/ha per species × 2 species for a total application of 2.5×10^8 IJ/ha. The EPN costs were about \$75/ha if farmers reared the EPNs themselves and \$150/ha if the EPNs were purchased from Cornell University. It was recommended that both species of EPN IJs could be mixed in the spray tank and be applied to the soil surface in 500 L/ha (50 gpa). To enhance IJ deposition on the soil surface, typical spray nozzles were replaced with fertilizer stream nozzles (type 0010) or liquid

fertilizer drop tubes, and it was recommended that field application occur 10–14 d after alfalfa harvest. In this manner, sufficient plant regrowth provided significant shading of the soil surface to reduce EPN IJ death from UV light exposure, but allowing the streams of EPN-containing water from the fertilizer stream nozzles to penetrate the plant canopy and deposit the IJs on the soil surface in a concentrated band. This recommended application strategy resulted in concentrated bands of IJs separated by 0.55m applied to the soil surface. With the combination of EPN IJ movement and spray splash, areas between the application bands would fill in with IJs within 30 d.

A farm-scale program was initiated in 2010 with 10 farmers applying EPNs to 20 fields (≈ 80 ha), seven farmers applying EPNs to 13 fields (≈ 120 ha) in 2011, and seven farmers applying EPNs to 10 fields (≈ 160 ha) in 2012. EPNs were applied in multiple strips oriented perpendicularly to the direction of tillage in most of these fields. Research has indicated that tillage will move EPNs into untreated strips with the movement of soil (Fig. 2). Soil sampling for EPN establishment in all of these fields documented EPN establishment ranging between 25–40% of soil cores indicating the presence of EPNs, a level similar to the results from field research plots.

One progressive farmer, after listening to an extension presentation that included information about EPN IJs moving 1–2 m/year, adapted our recommended application to reduce EPN costs. In 2011 and 2012, the farmer chose to apply IJs in bands separated by 3.4 m in the equivalent amount of water per nozzle. This application was achieved by having every seventh

spray nozzle apply the EPNs and blocking the six nozzles in between. When driving the entire field, the EPN application rate was 16% compared to the full-field application, but the amount of water out of the nozzle and the concentration of IJs in the spray remained the same. In addition, the farmer reared his own EPNs, which (in combination with reducing EPN application numbers) cut inoculation costs to \$12/ha. Subsequent soil sampling in these fields documented an EPN level of establishment of 30–40% of soil cores positive one year following application, without knowledge of the actual location of the concentrated application stream. After treating a number of alfalfa fields, the ASB populations on the farm have crashed to a very low level within three years of the first application. A subsequent fall ASB larval survey failed to find a single larva in any of the EPN-treated fields.

In 2013, this skip-nozzle application strategy was recommended and adopted by both farmers and commercial applicators for EPN application in the area-wide biocontrol program against ASB. We recommended the following procedure: in fields with moderate to heavy ASB pressure, every third nozzle should be left open, resulting in concentrated EPN streams separated by 1.65 m. When the whole field is driven, EPNs are applied to 33% of the field, and the nematodes only need to disperse 0.80 m from each stream to be present in the entire field. EPNs occupied the zones between the applied streams within 60 d (Bal et al. 2014a, Bal et al. 2014b). EPN costs are reduced by 66% from a full-field application and run between \$25–\$50/ha, depending on whether the EPNs are reared on the farm or purchased from Cornell University. In fields with light to moderate ASB infestations, many farmers chose to use the 16% strategy, reducing EPN costs 50% further.

Farmer adoption of the program has continued to grow. In 2013, 10 growers applied EPNs to 520 ha, and in 2014, 18 farmers applied EPNs to 370 ha. Many neighboring farmers watched these EPN applications with interest, and participating farmers reported a significant reduction of ASB damage in their fields. In 2015, the program surged in size, with 50 growers participating and 1,720 ha inoculated with EPNs. In 2016 and 2017, an additional 1000 ha were inoculated each year. Between 2010 and 2017, it is

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estimated that 7,600 ha located on 100 farms have been inoculated with New York-adapted persistent EPNs effective against ASB. This is an excellent start on the area-wide program, but with 200,000 ha infested, the program has a significant task ahead.

How will the biocontrol program be sustained for the long term?

Considering that ASB has a host range of 20 commonly occurring plants in northern New York, once an area is invaded, the insect will be present in the ecosystem for the long term, ready to rebound to an economically damaging level if anything happens to reduce the influence of the biological control organism. Additionally, with more than 200,000 ha infested, a continuing focused effort to inoculate fields with persistent EPNs will be required for a number of years to reduce the insect to a sub-economic status across its nine-county range.

Our eight years of field data suggest that a single inoculation in a field will establish these persistent native New York EPNs for multiple years—perhaps decades—and that the EPN field populations remain at a level where they can respond to insect invasion.

To date, commercial EPN producers have not expressed any interest in rearing a population of EPNs in a manner conducive for the retention of the persistence trait (phased infectivity) and the adaptations for the northern New York field environment. A single inoculation strategy with a multiyear persistent EPN suggests a retail market with few long-term return customers. The lack of commercial availability for these adapted persistent strains provides an opportunity for one to three

small businesses to establish and supply the adapted persistent EPNs to the agricultural community. Using the low-labor EPN-rearing procedure developed by this project, an individual can mass-rear EPNs for field inoculation on a seasonal basis with a minimum of capital outlay. The low-labor rearing procedure has been held in the public domain for use by any interested party. Mass-rearing can be set up using an enclosed area (3 m × 3 m) with tables or shelves for good air circulation and an ability to keep the air temperature around 22°C (such as a window AC unit). Nine square meters could sequentially rear several billion EPN IJs for field release over a five-month application season.

Currently, a single individual has established a business to rear EPNs for the 2016 growing season and has linked with a commercial applicator for field application. The next challenge will be to develop a system for independent EPN propagators to store EPNs throughout the winter months, ensuring that the EPN populations retain their phased infectivity and northern New York environmental adaptations.

Acknowledgements

We thank Cornell University, the Northern New York Agriculture Development Project, and the New York Farm Viability Institute for their long-term funding of this project.

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DOI: 10.1093/ae/tmx046

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