# Chapter 6
# Utilizing Persistent Entomopathogenic Nematodes in a Conservation or a More Classical Biological Control Approach

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## 6.1 Introduction

Entomopathogenic nematodes (EPNs) have been the focus of a significant amount of research since the early 1980s due to their many favorable attributes as biological control agents of potential pest insects (Kaya & Gaugler, 1993). As a group, EPNs have a broad host range, variation in foraging strategies and host associations which suggest the potential to control pest species with diverse life histories (e.g. Campbell & Gaugler, 1997; Grewal, Lewis, Gaugler, & Campbell, 1994, Lewis, Gaugler, & Harrison, 1992, 1993; Lewis, Ricci, & Gaugler, 1996; Wilson, Ehlers, & Glazer, 2012). The vast majority of the EPN research has been focused on important components required to utilize EPNs in an inundative release strategy or a biopesticide. These components include mass rearing techniques, isolating/propagating populations with the highest laboratory efficacy with little regard to ecological adaptation to the release environment, species/populations with the longest storage "shelf" life, and application technology. EPNs are used almost exclusively as a biological insecticide, typically at high densities to the soil ($2.5 \times 10^9$ per ha, 25 IJ/cm$^2$) with little concern for the fate of applied EPNs. Evaluation of EPN population is usually focused on pest reduction and is limited to a few days or weeks after application (Lewis, Campbell, & Gaugler, 1998). In these releases, little emphasis is placed on the long term establishment of the EPN in the soil profile for long-term pest suppression through pest recycling and the selection of an EPN population which retains the genetic coding for long-term persistence under low host density and unfavorable environmental conditions.

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In contrast, little research has been focused on the utilization of naturally occurring or adapted EPNs in the long-term suppression of pest outbreaks in managed systems, ranging from turf to agricultural fields. EPNs have been isolated from every inhabited continent, in virtually every type of soil habitat where concentrated effort has been made to find them (Adams et al., 2006; Hominick, 2002). Isolation records demonstrate the great diversity of habitats exploited by EPNs (Hara, Gaugler, Kaya, & Lebeck, 1991; Kaya & Gaugler, 1993). Natural populations are extremely common, though poorly understood and range from >1 to 100 % of soil samples collected (e.g. Campos-Herrera et al., et al., 2013; Gaugler, Campbell, Selvan, & Lewis, 1992; Mráček & Becvár, 2000). Native populations of *Steinernema carpocapsae* (Weiser) Wouts, Mráček, Gerdin & Bedding, (Rhabditida: Steinernematidae), *Steinernema feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) have been isolated from numerous samples of NY agricultural soil samples ranging in levels from 1 to 15 % (Shields, unpublished; see also Chap. 11).

The use of EPNs has been primarily focused on inundative releases, yet the origins and future use of EPNs is inoculative and conservation biological control (Lewis et al., 1998). After the discovery of *Steinernema glaseri* (Steiner) Wouts, Mráček, Gerdin & Bedding (Rhabditida: Steinernematidae) from scarab larvae in 1929, small plot field successes and the development of in vitro rearing, $10^9$ of IJs were reared and released in an inoculative control program between 1939 and 1942, across New Jersey, focused on Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabeidae), an introduced pest (Glaser, 1932, Glaser & Farrell, 1935, McCoy & Glaser, 1936). This program was largely unsuccessful due to the elimination of the bacterial symbionts by the use of antimicrobials in the artificial rearing media and *S. glaseri* was only re-isolated from southern New Jersey (Gaugler et al., 1992).

Long-term multigenerational survival and recycling through hosts is required for inoculative release programs. Kaya (1990) suggests that for an inoculative release program to be successful, three conditions are required: (1) moderately susceptible pests need to be present throughout most of the year, (2) pests should have a high economic threshold level, and (3) soil conditions should be favorable for nematode survival. These suggestions may be more appropriate for the tropical and subtropical regions where temperature conditions are favorable for continuous insect activity and nematode attack/recycling. In the more northern temperate regions, native EPNs must survive long periods of adverse condition including frozen soils and cold temperatures. In these areas, EPNs must have the ability to conserve their resources and pass significant time between host availability (Shields, Testa, Miller, & Flanders, 1999).

In the laboratory under optimum conditions only 30–40 % of the IJs infect the hosts present (Fan & Hominick, 1991) and these results are independent of species and population. Multiple authors have reported that EPNs utilize phased infectivity to bridge periods of time of environmental stress and lack of host availability (Griffin, 2012). The loss of field persistence in some commercial populations

(Ferguson, Schroeder, & Shields, 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).

Sampling for native EPN populations in both agricultural fields and non-cultivated areas indicates that the population is clumped in distribution and highly variable in frequency (e.g. Cabanillas & Raulston, 1994; Campbell, Lewis, Yoder, & Gaugler, 1995; Campbell, Lewis, Yoder, & Gaugler, 1997). In natural areas, the distribution of the flora with its associated fauna are believed to contribute to the clumped EPN distribution as the nematodes attack and recycle in susceptible hosts feeding on the local flora. The lifecycle of the insects feeding on the local flora and their duration of soil contact along with EPN susceptibility also influences the population and distribution of the native EPN population. In addition, the lack of soil movement and the relative stability of the native areas also contribute to the observed clumped distribution (e.g. Campos-Herrera et al., 2008; Stock & Gress, 2006; Stock, Pryor, & Kaya, 1999). Over time as the flora and susceptible fauna change within a habitat in response to climatic cycles and pest outbreaks, the associated native EPN species, abundance and distribution also might change in response to the availability of susceptible hosts.

When these natural habitats become agricultural production areas, major changes in the historically susceptible fauna usually occurs, directly impacting the abundance and distribution of the native EPN species mix and populations. In the absence of subsequent EPN introductions, the EPN fauna in a geographical area is a historical remnant of the pre-agricultural native ecosystem where the EPN species complex evolved and maintained its population under the pre-agriculture succession of flora and fauna. A shift from natural areas to agricultural fields disrupts the historical relationship between the native EPN population and the availability of the array of their historical hosts. This disruption may force the native EPN to ecological extinction or reduce the population to the observed low levels in most agricultural fields (Campos-Herrera et al., 2008).

The intensity and type of agriculture following the conversion from native habitat has potentially a controlling impact on the native EPN population distribution, and size. For example, the establishment of semi-permanent crops (i.e. orchards, vineyards) with intertwining areas of grassy mixed species would be less detrimental to the native EPN population than a frequently rotated set of monoculture crops. These intertwining grassy mixed species areas are attractive to an array of susceptible EPN hosts, many of which may be native and historically served as susceptible hosts (Campos-Herrera et al., 2008; Duncan et al., 2013).

In the highly disturbed agricultural system, native EPN populations may remain clumped and may be relatively rare in abundance due to a lack of susceptible hosts or an ecological mismatch between the soil inhabiting herbivores and the EPN species present. However, soil tillage redistributes the resident EPN population. This EPN movement by tillage is illustrated in Fig. 6.1. In this experiment, two 10 m × 200 m strips of an established alfalfa field were treated with persistent native EPNs (*S. carpocapsae* 'NY 001' and *S. feltiae* 'NY 04') at a rate of $1.25 \times 10^8$ per species per ha (total = $2.5 \times 10^8$ IJs per ha, 2.5 IJ/cm$^2$) in 2009. Pre-sampling the field indicated

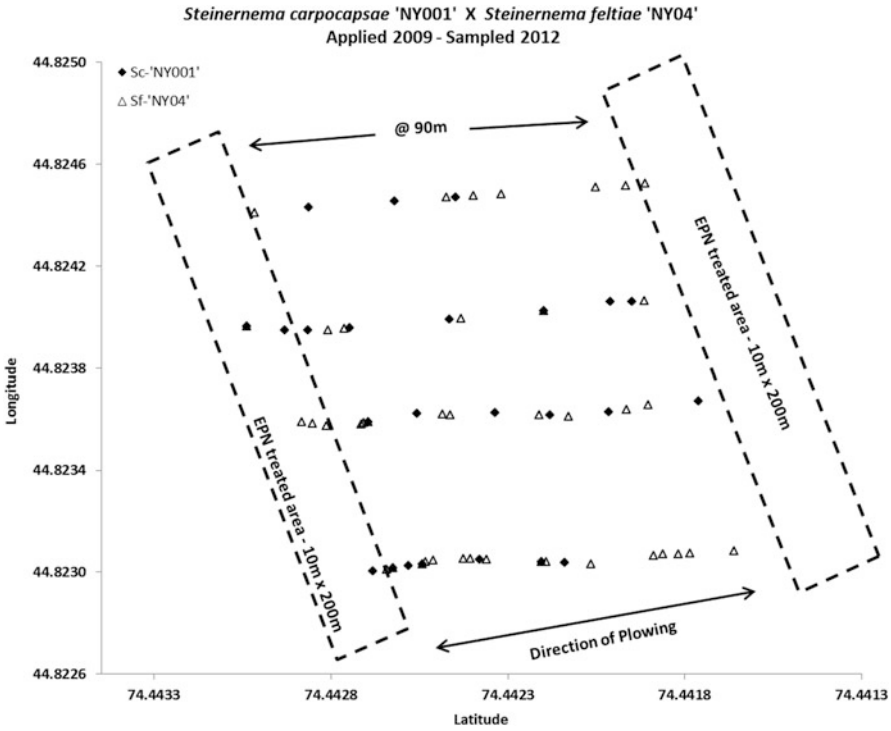


**Fig. 6.1** Areas within the treated areas were inoculated with *Steinernema carpocapsae* 'NY 001' and *Steinernema feltiae* 'NY 04' during July 2009 at $1.25 \times 10^8$ IJs per ha (1.25 IJ/cm$^2$) per species (total $2.5 \times 10^8$ IJs per ha, 2.5 IJ/cm$^2$) when the field was an alfalfa/grass mixture. The field was plowed and rotated to corn in 2010–2012 when it was sampled for EPN movement in June 2012. Both EPN species had been moved a minimum of 45 m between applications and subsequent

no detectable natural populations of EPNs. Orientation of the EPN treated strips was perpendicular to the direction of field tillage. The field was rotated to corn in 2010 and remained in corn through 2012. Each year during the corn rotation, the field was aggressively tilled before planting. In 2012, soil cores were collected in four transects between the two treated strips and the GPS coordinates of each soil core site was recorded. Each soil core was divided into the top 5 cm and the lower 15 cm and placed into individual containers. The soil cores were returned to the laboratory and bioassayed for the presence of EPNs using *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae as bait. After 7 days of incubation at 22 °C, the presence of EPNs and the species of EPN was recorded for each soil core. Figure 6.1 clearly shows that the EPNs applied in the two treated strips in 2009 were redistributed a minimum of 45 m into the untreated area between 2010 and 2012 as a result of three aggressive tillage operations. Similar results were recorded at seven other locations were a similar experimental area was established.

A classic example of this ecological mismatch between the native EPN and potential hosts is the ambush nematode, *S. carpocapsae* and its preference to the top 5 cm of the soil profile. Susceptible insects are exposed to attack until they move below the top 5 cm within the soil profile and significantly reduce the probability of attack (Ferguson et al., 1995). Under some conditions, *S. carpocapsae* utilizes plant roots as physical routeways and conduits to move deeper in the soil in response to feeding-associated stimuli and attack host deeper in the soil (Ennis, Dillon, & Griffin 2010). Given this soil ecological niche, *S. carpocapsae* is usually effective against susceptible adult insects hiding at the duff/soil interface and larvae moving across the soil surface and within the top 5 cm of soil. The cutworm complex (Noctuidae) would be easily attacked while the deeper soil insects like western corn rootworm (WCR) larvae *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) would be out of the zone of attack by this nematode species. In contrast, if the native nematode species was *S. feltiae*, its soil niche extends down from the surface to 20 cm and the deeper penetrating soil insects would be susceptible to attack until the insect moved below 20 cm (Ferguson et al. 1995).

Annual crop rotation often changes or disrupts the flow of susceptible hosts into fields, limiting the buildup of native EPN populations. In addition, EPN population buildup may be significantly limited by the mismatch of the EPN species and its preferred ecological niche in the soil profile with the movement in the soil by potential insect hosts.

This historical ecological perspective raises the following questions:

1. If native EPN species with the following characteristics were reintroduced into the agricultural system, would the EPN population maintain itself at a sufficient level to provide a significant impact on soil herbivores attacking the array of crops?
   - Persist across unfavorable conditions
   - Efficacious against the array of potential hosts in the agricultural system
   - Overlapped in soil profile preference with the potential host complex.
2. With the level of residual native EPN populations ranging from 1 to 50 % of individual soil cores testing positive for the presence of EPNs (Neumann & Shields, 2004), the question arises about the population level required to maintain EPNs in an agricultural situation where they can persist for long periods of time, yet be numerous enough to respond to insect invasion and reduce the economic losses from those invaders.

The reintroduction of a significant EPN population persisting across years into a cropping system would have at least two distinct benefits. The most obvious benefit would be a reduction in the frequency of economic outbreaks by the herbivores attacking the crop. In the case where herbivore populations build over several generations before the populations become large enough to cause economic damage, the addition of an additional mortality factor (residual EPNs) may have enough impact to prevent or at least delay the herbivore population increase and the

subsequent economic damage. A perfect example would be in fields with rotations from alfalfa/grass to a row crop (corn, soybeans). Native white grubs (*Phyllophaga sp.*) and wire worms (Elateridae) with multi-year lifecycles are attracted to the grass within the alfalfa fields and the populations continue to build over the 4+ years while the field is in alfalfa/grass. When the field is rotated to a row crop, the white grubs/wireworms in the soil then begin attacking the corn or soybean plants, requiring an insecticide seed treatment to prevent economic losses. If native persistent EPNs with a level of efficacy and overlapping soil profile preferences were reintroduced into the agricultural field, the EPNs would actively reduce the white grub/wireworm populations during the years in alfalfa/grass and thereby reduce or eliminate problems when the field was rotated to a row crop.

Four examples of this EPN interaction with a crop rotation are illustrated in Fig. 6.2. For this study, a subset of 51 fields was selected from 87 fields treated with EPNs. In each of the 51 fields, the field was inoculated a single time with a combination of EPNs species (*S. carpocapsae* 'NY 001' and *S. feltiae* 'NY 04') at a rate of $1.25 \times 10^8$ per species per ha (total $= 2.5 \times 10^8$ IJs per ha, 2.5 IJ/cm$^2$) in 2008 or 2009. Each field was sampled each year a single time during the growing season at approximately the same time of year by collecting 100 soil cores in a transect across the treated area of each field. The location of each soil core was recorded using GPS. Each soil core was divided into the top 5 cm and the lower 15 cm and placed into individual containers. The soil cores were returned to the laboratory and bioassayed for the presence of EPNs using *Galleria* larvae as bait. After 7 days of incubation at 22 °C, the presence of EPNs and the species of EPN was recorded for each soil core. The four fields illustrated in Fig. 6.2 are representative of EPN response across all the fields rotated to corn during the 6 years of the study. These data suggests that the persistent EPN field population is very dynamic with its response to insect invasion and residual EPN populations in the 10–15 % range (positive soil cores) is capable of a significant response to host invasion, even when the field is rotated to a different crop with a completely different array of insect herbivores.

The second but less obvious benefit would be the introduction of a second mortality factor for soil insects attacking crops with Plant Incorporated Protectants (PIP) (i.e. BT-corn). Herbivores attacking plants with incorporated toxins often experience a longer larval stage, increasing their vulnerability to attack by other mortality factors. A second mortality factor working with the PIP could have a significant impact on reducing the risk and speed of resistance development to the PIP (Pertzold-Maxwell, Jaronski, & Gassmann, 2012). The EPN response in Fig. 6.2 during the corn rotation years was probably due to EPN recycling in corn rootworm (*D. virgifera*). However, is it realistic to expect native EPN populations to persist at a high enough level in our intensive agricultural systems to have a significant impact on invading insects with part of their lifecycle at the soil interface or within the soil profile? In the case of a highly migratory and invasive insect such as common army worm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae), residual EPNs would have minimal impact because of the large number of invaders and the relatively slow EPN recycling time.

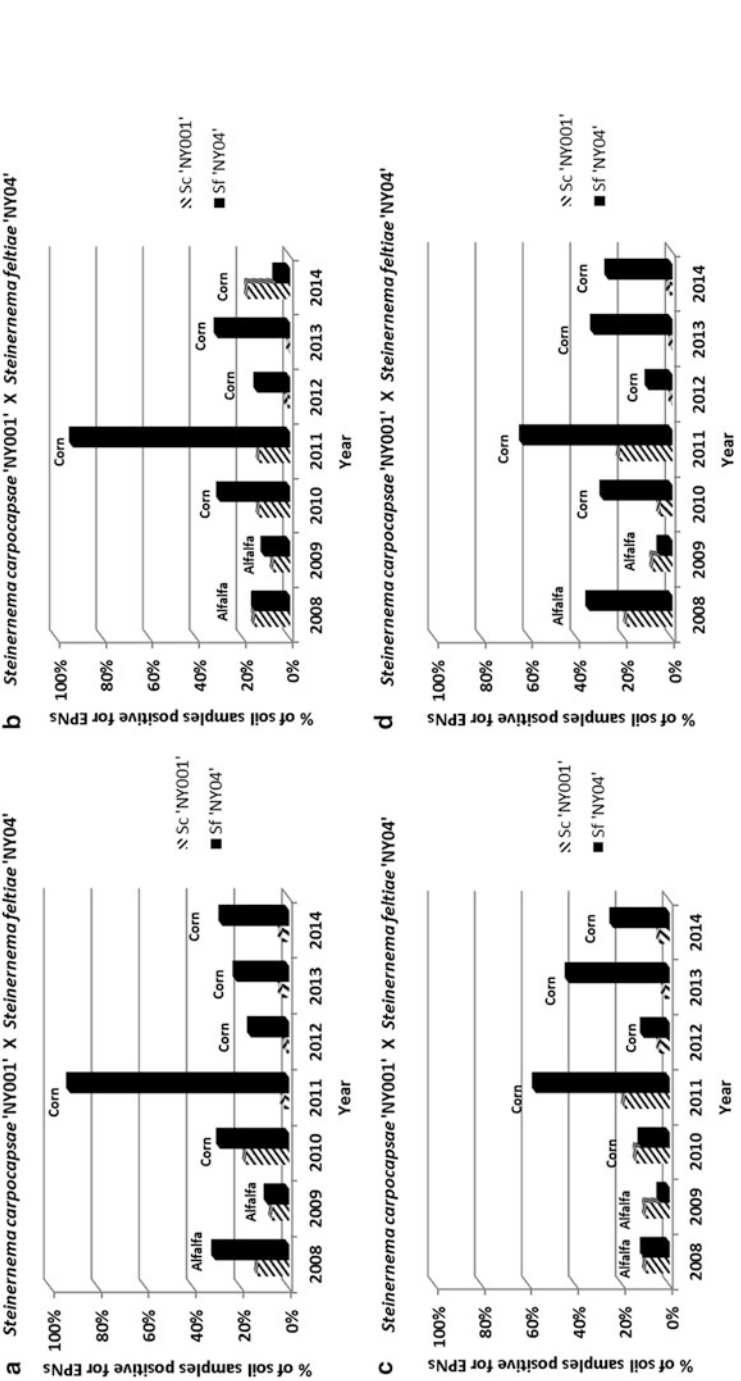


**Fig. 6.2** Four different fields in Northern NY where native EPNs were applied once in 2008, inoculated with *Steinernema carpocapsae* 'NY 001' and *Steinernema feltiae* 'NY 04' during July 2009 at $1.25 \times 10^8$ IJs per ha (1.25 IJ/cm$^2$) per species (total $2.5 \times 10^8$ IJs per ha, 2.5 IJ/cm$^2$) when the field was an alfalfa/grass mixture. Entomopathogenic nematodes (EPNs) population frequency was measured once per year during the growing season (200 samples/field/year). Each column represents percent of the 200 samples positive for EPNs in a *Galleria*-based laboratory bioassay. EPN recycling on western corn rootworm (WCR), *Diabrotica virgifera virgifera* larvae during the corn years is inferred based on insect biology but not directly measured.

## 6.2 Matching Entomopathogenic Nematodes to the Cropping System and Pests

In our own research using native persistent EPNs in the alfalfa/grass (4 years) rotated to corn (4 years) system, fields inoculated once at a rate of approximately $1.25 \times 10^8$ per species per ha (total $= 2.5 \times 10^8$ IJs per ha, 2.5 IJ/cm$^2$) had stable EPN populations become established and remain in the field for multiple years across a corn rotation. EPN numbers maintained themselves at 10–25 % of the soil cores positive for EPNs and responded to insect invasion with the percentage of soil cores testing positive for EPNs increasing from 40 to 80 % (Figs. 6.2 and 6.3).

Since native EPN populations in agricultural soils are a remnant of the pre agricultural ecosystem and agriculture is usually comprised of non-native plants with its associated non-native insect pests, an ecological or efficacy mismatch between the native EPNs and the available non-native hosts frequently exists. This mismatch prevents the residual native EPN population from suppressing the non-native pest insect complex below damaging levels. This mismatch is illustrated by the examples presented below.

Alfalfa snout beetle (ASB), *Otiorhynchus ligustici* (L.) (Coleoptera: Curculionidae) larvae move through the top 45–60 cm of soil during their root feeding stage. Within the infested regions of New York, *S. carpocapsae* can frequently be found in the alfalfa fields infested with snout beetle and at relatively high numbers. In spite of the high *S. carpocapsae* numbers, enough ASB larvae survive to completely destroy the alfalfa stand within 1–2 growing seasons. In regions of Hungary where ASB is native and at very low population levels, native populations of *S. carpocapsae* are found to co-exist with native populations of *S. feltiae*. In these Hungarian alfalfa fields, ASB is a non-economic pest, alfalfa roots show few ASB feeding scars and alfalfa stands last for more than 4 years (Neumann & Shields, 2004). Within the New York ASB infested area, fields inoculated with a combination of native NY *S. carpocapsae* 'NY001' with native NY *S. feltiae* 'NY 04' demonstrate the same impact on ASB larval populations as observed in Hungary (Neumann & Shields, 2004). The explanation to this example is suggested in Ferguson et al. (1995) when the authors show the different EPN species have different preferred, sometimes overlapping soil niches. Interactions between nematode species when coexisting was demonstrated in the laboratory using sand columns where *S. carpocapsae* dominated the upper 5 cm, pushing both *S. feltiae* and *H. bacteriophora* to the lower portions of the sand column (Neumann & Shields, 2006).

A second insect which illustrates this potential mismatch with native nematodes is *D. v. virgifera* (WCR) which feeds on corn roots in the upper 20–30 cm of the soil profile. When only *S. carpocapsae* is present, this nematode usually restricts itself to the top 15 cm and often to the top 1–2 cm (Georgis & Poinar, 1983; Moyle & Kaya, 1981; Schroeder & Beavers, 1987) when surface applied. A significant amount of CRW larval feeding occurs under the "carpocapsae layer" and a significant number of the larvae are not attacked when below the "carpocapsae layer". Although, some

authors have reported *S. carpocapsae* utilizing root channels to move deeper in the soil following host cues (Ennis et al., 2010). However, adults laying eggs on the soil surface and within the upper few cm of soil and newly hatched larvae would be vulnerable and attacked by *S. carpocapsae* when present. The addition of a second nematode species like *S. feltiae* which ranges deeper in the soil profile adds additional mortality pressure on CRW larvae deeper in the soil as shown by Fig. 6.2 and indicated by the increase in the frequency of *S. feltiae* compared to *S. carpocapsae*.

Our research suggests that an effective EPN biocontrol program works best with a two species EPN mix to cover the typical profile inhabited by most potential hosts. Depending on the cropping system and array of potential insect hosts, any two species combination of *S. carpocapsae*, *S. feltiae* or *H. bacteriophora* persist well together and do not drive the other species to extinction. When all three species are applied together, *S. feltiae* is squeezed between *S. carpocapsae* in the upper layer and *H. bacteriophora* and forced to extinction (Newmann & Shields 2006, 2008, 2011). When *S. feltiae* or *H. bacteriophora* is matched with *S. carpocapsae*, the second species occupies the area of the soil profile below the top 5 cm. Interestingly, when *S. feltiae* is matched with *H. bacteriophora*, *S. feltiae* is moved to the upper layers of the soil profile whereas *H. bacteriophora* occupies the soil profile below 10 cm (Neumann & Shields, 2006, 2008, 2011).

## 6.3 Techniques for Preserving Persistence Genetics in Culture

Organisms reared in the laboratory adapt to those laboratory conditions and lose traits which help them survive under field conditions. This laboratory adaptation also occurs in EPNs when cultured continuously in the laboratory. As reported earlier, Fan and Hominick (1991) found that even under optimum laboratory conditions only 30–40 % of the IJs infect the host. In many cases, the continuous culture of EPNs in the laboratory or commercial production facility utilizes only this 30–40 % of the population, the IJs immediately infective upon emergence from the host cadaver in their rearing. Continuous culture utilizing only these immediately infective IJs narrows the gene pool to those individuals who do not carry the coding for any delayed infectivity and its associated benefit of the ability to persist across unfavorable conditions. This loss of field persistence is reported between a commercial and native population of *H. bacteriophora* (Ferguson et al., 1995, Shields et al., 1999) and can be demonstrated in the laboratory in less than 15 generations in native populations of *S. carpocapsae* 'NY 001' and *H. bacteriophora* 'Oswego' (Shields, unpublished).

Since the inoculative approach requires the EPNs used to retain their adaptation for field survival, several approaches can be utilized to help to retain these genes in laboratory culture.

### 6.3.1 Re-isolation from the Field

We re-isolate the native NY populations from a number of areas in Northern NY every 2 years and these isolations are used to completely restart our cultures. We have been successful in retaining a high level of field adaptation and persistence in our cultures across all three native NY species that we have worked with over the past two decades by restarting our cultures every 2 years. We have noticed that the initial few cycles of these new cultures in the laboratory produce reduced yields of IJs until some adaptation to laboratory conditions and rearing host are made. Mixing of new genetic material from re-isolated individuals with ongoing laboratory cultures only delays the loss of persistence.

### 6.3.2 Establishing "Wild Populations" in Easily Accessible Areas

A second strategy we currently use as a backup plan to maintain EPN populations adapted to NY conditions is to utilize areas around the Cornell University campus as "natural storage areas". Every university and many commercial buildings are surrounded by ornamental plants and significant grassy areas, which are invaded by insect hosts. We have selected individual areas which are isolated and inoculated these areas with individual populations of NY native species of EPN. Subsequent sampling has documented persistence of the inoculated populations/species in these "wild" areas.

### 6.3.3 Laboratory Culturing to Retain Persistence

A third strategy we have recently initiated is to change our laboratory culturing methodology to help preserve the persistence genetics. We inoculate 200 g of moistened autoclaved loam soil placed in a plastic container with a fitting lid with 1,000 IJs of a species/population and store it at room temperature (20–22 °C) on a dark shelf in a cabinet. A new container is established each month in a similar manner until containers representing at least a 12 month period are available. To maintain the laboratory culture, 1–2 wax moth larvae are placed in each container spanning the entire time period (12–24 months). After death, cadavers are removed from each container and grouped together for IJ emergence, creating a mixture of IJs from across the stored soil time period. Soil containers are discarded once there are no more deaths from EPNs, usually 12–18 months after initial inoculation. Since

a new soil container is added each month, there is a continuous array of "aging" soil samples with IJs becoming infective at any point in time. This technique has been successful in retaining the delayed infectivity trait but does little to prevent adaptation to rearing under moderate temperatures.

## 6.4 Entomopathogenic Nematode Application Timing and Rate in the Inoculative Approach in NY State Program

Since the focus of the inoculative approach is to introduce or reintroduce persistent adapted EPNs into the agricultural system, the timing of the application is not very critical. Most native EPN species which have not lost their ability to persist in culture will maintain their presence in the soil profile for a minimum of a year without hosts to recycle. Figure 6.3 illustrates the results of a recent laboratory persistent study where two species of native NY EPNs retaining their ability to persist were inoculated into small cups containing moistened soil and stored on a darkened shelf at room temperatures without host available. After >300 days in storage, there is

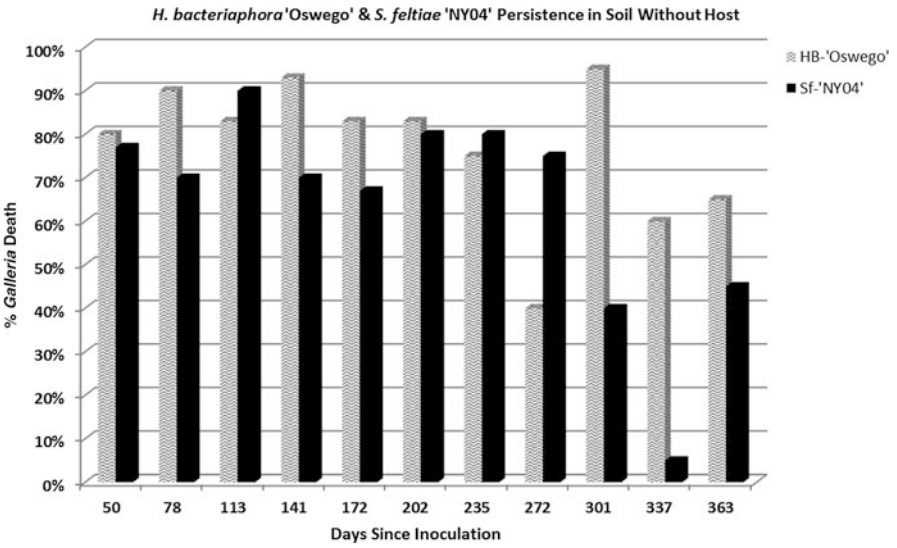


**Fig. 6.3** Persistence of two New York native nematode populations at laboratory temperature without hosts. Samples were removed from storage at room temperature (22–24 °C) on the date tested, bait larvae were introduced and mortality was recorded 7 days later. The test samples were then discarded. A new set of samples were then evaluated on the next test date. There was no recycling in the sample cups. No error bars are shown because there is no variation between reps. Decline over time is suggested to be reduction in viable infective juveniles (IJs) and/or a reduction in IJs activated to be infective.

little difference in EPN infectivity between soil samples tested after 30 days or 300 days. Field applications also support this conclusion. Native NY EPNs in our research have been applied late fall, early spring and throughout the summer with no significant differences in establishment. This application strategy is a conceptual difference to EPN inundative releases of commercial nematode populations which have poor long-term persistence in the soil and require accurate timing of releases to match susceptible host presence.

The required EPN rates for the inoculative approach with persistent EPNs is significantly lower than required for the inundative approach with non-persisting EPNs. With the latter approach, a high enough dose must be applied to effectively kill the majority of the host population within a short window while the applied EPNs remain viable. Typically, an inundative application consists of $2.5 \times 10^9$ IJs per ha (25 IJ/cm$^2$). In contrast, the inoculative approach with persistent EPNs relies on successful colonizing on host present in the soil, recycling in those hosts and persisting in the soil profile between periods of host availability. As illustrated in the previous section, EPNs populations, retaining the genetics for persistence, remain infective for more than 300 days at room temperature without host to recycle in. In the field where soil temperatures fall below EPN activity thresholds, the cold temperatures assist the adapted EPN with retaining its infectivity across periods of host unavailability. Our research has indicated that effective inoculation of the soil can be accomplished with $2.5 \times 10^8$ IJs per ha (2.5 IJ/cm$^2$), a ten-fold lower rate than the inundative rate. Perhaps future research will indicate an even lower rate may be also effective for uniform EPN establishment.

## 6.5 Entomopathogenic Nematodes Movement and Application Strategies in Inoculative Approach in NY State Program

The ability of EPNs to move in the soil by themselves, move in infected insects before the insect dies or be redistributed in the field from soil movement with agricultural practices directly impacts application strategies in the inoculative approach. If movement is limited, then EPNs require a uniform application to the soil surface for effective soil inoculation and host suppression. However, if EPN movement is significant, application does not need to be completely uniform and areas between zones of application will naturally fill in with EPNs. As a result, areas of inoculation can be reduced, resulting in a less expensive application.

In the greenhouse, *H. bacteriophora* 'Oswego', moved 26 cm within 7 days after application (Schroeder, Villani, Ferguson, Nyrop, & Shields, 1993). Shanks and Agudelo-Silva (1990) reported movement of the nematode *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann) (Rhabditida: Heterorhabditidae) (syn. of

*H. bacteriophora*, Adams & Nguyen, 2002) into neighboring untreated plots within 3 weeks. This question has also been addressed in the NY alfalfa system indirectly and directly in several studies. Neuman and Shields (2011) reported a minimum of 3 m movement for all three native NY species detected by contamination of neighboring plots where the EPN species was not inoculated. Movement of the EPNs was detected after 12 months, suggesting the movement could have been the result of the movement of infected adult ASB before death as well as the physical movement of EPN infective juveniles (IJs) in the soil. EPN movement has also been reported in when *Steinernema scapterisci* Nguyen & Smart (Rhabditida: Steinernematidae) was used as a biocontrol agent against mole cricket (*Scapteriscus spp*.) in Florida. Parkman, Frank, Nguyen, and Smart (1993) report the nematode dispersed at least 150 m from the release point in three instances and the mean dispersal was 60 m within 21 months. Suggestions about the possible large scale movement of EPNs with the movement of soil during farming operations was reported by Shields, Testa, Neumann, Flanders, and Schroeder (2009). Two subsequent studies have documented unassisted EPN movement (all three species) of 1–1.5 m in a single growing season in the field and a longer distance movement of 25–45 m within soil when the field is plowed (Shields, unpublished) (Fig. 6.1).

This level of EPN movement and mechanical redistribution raises an interesting question regarding the interaction between application rate and the uniformity of the application. Would a better strategy be to inoculate a smaller area at a higher EPN rate to insure establishment and then have the EPNs move into the unapplied areas via natural movement or mechanical redistribution? Or, would it be a better strategy to treat the entire field with a reduced rate of EPNs risking poor establishment while gaining a uniform initial distribution? Research reported by Parkman et al. (1993) and Parkman, Frank, Nguyen, and Smart (1994) suggests that inoculating small areas with the persistent EPN and allow the EPN to spread naturally in infected insects is an inexpensive and viable way to introduce persistent EPNs into the desired host and ecosystem.

Our application/establishment research has been focused on the first option, thinking that EPN establishment is a restricting/limiting factor in the alfalfa ecosystem. With the previous documentation of a rather robust EPN movement in field situations, a completely uniform application of IJs to the soil surface is less important using an inoculative approach because the applied EPNs are persistent for many months without recycling, and will fill in the areas with low EPN density during efforts to locate hosts.

In addition, the focus of the inoculative approach is to establish a long-term EPN presence in the soil profile with the objective of long-term pest suppression. Active and passive EPN movement can then be utilized to reduce the cost of EPN application and still result in the effective inoculation of the soil profile. In semi-permanent agricultural ecosystems with minimum soil tillage and soil movement, EPN natural movement would be slower and a more uniform application would more quickly fill in. In these situations, the use of fertilizer stream nozzles (nozzle

type – 0008) on a commercial sprayer with the screens and filters removed would effectively apply the EPNs to the soil surface in continuous strips separated by ca. 55 cm. The area between the application strips would fill in with EPN within 30 days under field conditions. If the nozzles were separated by 1 m, EPN fill in would take a longer time period but EPNs would be found within the untreated areas within a growing season. In a more disturbed agricultural ecosystem where soil tillage occurs at least annually, EPNs will be redistributed with the movement of soil during soil tillage. For example, EPNs were applied in our initial large scale field research in concentrated zones at EPN rate of $2.5 \times 10^8$ IJs per ha (2.5 IJ/cm$^2$), perpendicular to the direction of soil movement during tillage operations. EPN sampling after field tillage indicated a movement-by-tillage of 45 m (Fig. 6.1).

As innovative farmers, participating in an area wide EPN-root weevil pilot program, using *S. carpocapsae* 'NY 001' with *S. feltiae* 'NY04', listened and thought about EPN movement, they derived a more effective application strategy. In their commercial sprayers, nozzles are separated by 55 cm. By fitting the nozzle bodies with fertilizer stream nozzles (nozzle type – 0006–0015) and blocking some of the nozzles, only a portion of the field is treated with EPNs even though the sprayer is driven over the entire field. For example, if two out every three nozzles are blocked, only 33 % of the field is treated in higher concentrated EPN strips separated by 165 cm. EPNs moving inward from each application stream would need to move 82 cm for complete fill-in of the untreated area. Depending on the EPN species and their mobility, these untreated zones would be occupied with EPNs within 60–90 days and the cost of the EPNs for the application would be reduced by 66 %. Some farmers have taken the reduced application strategy a step further with only apply EPNs out of every 6th nozzle, blocking the 5 in between nozzles, and applying to only 17 % of the field while driving over the entire field, reducing the nematode cost by 83 %. With EPN application bands every 330 cm, EPN need to move (or be moved with soil movement) 165 cm for complete fill-in of the untreated area. Complete fill in of these untreated would be accomplished with 1–2 growing seasons or during a single major tillage operation. In these EPN inoculated fields, ASB damage declined to sub economic levels within 3–5 years depending on the application strategy and the intensity of the ASB population.

## 6.6 Nematode Persistence Results and Impact: Future Research

The utilization of these concepts have been successful in the development of an effective EPN-based biological control program against alfalfa snout beetle, *O. ligustici* and the full detail of the project is discussed under the case study section of this book (Chap. 11). Research projects are currently focused on the application of these concepts to other agricultural systems. Projects have been initiated in three other systems with semi-permanent ecosystems (Fig. 6.4).

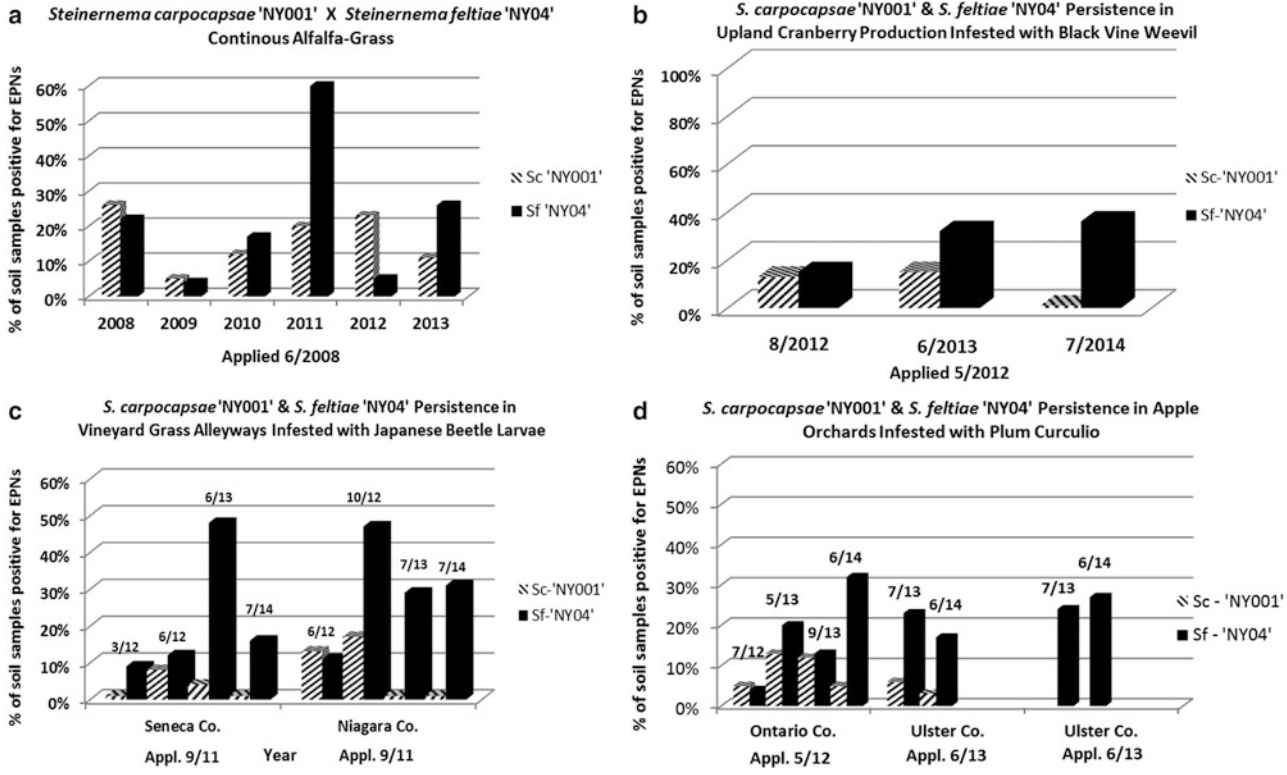


**Fig. 6.4** Four different cropping systems in NY where native EPNs were applied once. Entomopathogenic nematode (EPN) population frequency was measured one-two times per year during the growing season. See EPN rates in text. EPN recycling and persistence evident in each cropping system. No error bars because each column is a single value representing the percent of 200 soil cores positive for EPNs using a Galleria-based laboratory bioassay. (**a**) Continous alfalfa-grass. (**b**) Upland cranberry production. (**c**) Grape vineyard. (**d**) Apple orchards.

### 6.6.1 Alfalfa-Grass

As baseline data for this discussion, the continuous alfalfa-grass field is considered a stable environment with a wide array of potential EPN hosts feeding on the roots of the plants. EPNs were applied in 2008 at $5.0 \times 10^8$ IJs per ha (5 IJ/cm$^2$) and the EPN population was monitored once per growing season (Fig. 6.4a). Several interesting points can be concluded from this graph. Native EPNs persisted and responded to insect invasion in a classical predator-prey response and using the "static" soil core bioassay sampling method, 15–20 % of the cores positive with EPNs appears to be a stable population capable of responding to insect invasion. Research is currently underway which indicates the soil core bioassay sampling technique significantly underestimates the actual potential for EPN host attack in the field.

### 6.6.2 Black Vine Weevil – Cranberries/Strawberries

Large areas within 80 ha of cranberries grown in a non-flooding culture in central NY were being killed out and large numbers of Black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae) were present in the areas surrounding the killed out plants. Since larvae of BVW are soil inhabiting, have a ca. 10 months of soil contact, and are reported in the literature to be susceptible to EPNs, native NY populations of *S. carpocapsae* 'NY 001' and *S. feltiae* 'NY 04' were applied to all 80 ha (Fig. 6.4b). EPNs were reared on the farm by the producer and self-applied at $2.5 \times 10^8$ IJs per ha (2.5 IJ/cm$^2$) per species (total $5.0 \times 10^8$ IJs per ha, 5 IJ/cm$^2$).

Within a single growing season, BVW was reduced to non-economic levels and EPN have persisted 2 years from a single application in May 2012. It appears that *S. feltiae* is becoming the dominate EPN in a sandy soil environment which requires frequent irrigation. In 2014, the grower noticed several small areas of BVW damage but subsequent EPN sampling of those areas indicated that the EPNs were responding to the insect presence. A similar project was initiated in response to a similar call regarding a severe economic outbreak of BVW in strawberries. Native EPNs were applied the fall 2013 and the insect population was declining during the 2014 growing season. Research is continuing with this system.

### 6.6.3 Japanese Beetle – Grape Vineyard Grass Roadways

Japanese beetle adults, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) causes leaf feeding damage on grape vines used for wine production in NY during the mid-summer months, requiring multiple applications of broad spectrum insecticides to minimize damage. Eggs are laid in grassy areas and the larvae feed on grass roots starting in late summer through the following spring and early summer.

In many vineyards, alleyways between the vine rows are frequently planted to a grass mixture and frequently, the entire vineyard is surrounded by grassy habitat. At five locations, the grassy areas between the vine rows and surrounding the vineyard were inoculated with native NY populations of *S. carpocapsae* and *S. feltiae*, targeting the beetle larvae. The focus was to determine that if the larvae population was suppressed within the vineyard, would there be a reduction in the adult population feeding on the grape foliage. Four of the five sites had very low Japanese beetle populations during the 3 years of the study and little adult feeding was observed in the check plot. However, in the fifth site, the area of the vineyard treated with EPNs recorded a 27 % reduction in adult Japanese beetle feeding on the grape foliage in 2014. Across all locations, the EPN persisted with the EPN population responding to insect invasion and two of the locations are illustrated in Fig. 6.4c.

### *6.6.4 Plum Curculio – Organic Apple Production*

Plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) is the single most limiting insect pest of organic apple production, causing fruit damage up to 100 %. In conventional production apple orchards, 1–3 broad spectrum insecticide applications are targeted toward controlling this pest. Adults overwinter either in the orchard floor or outside of the orchard in nearby sheltered areas. Adults enter the orchard after fruit set and lay eggs in the small developing fruit. If the apples remain on the tree, the eggs-young larvae is crushed by the growing apple leaving only the disfiguring oviposition scar(s). Many apples after oviposition abort and fall to the orchard floor where the larvae continue to development in the apple. Mature larvae leave dropped fruit on the orchard floor and burrow into the soil for pupation. This allows about a 30 day period which the mature larvae and pupa are susceptible to EPN attack. A project was initiated in 2012 and persistent native NY populations of *S. carpocapsae* and *S. feltiae* were applied to the orchard floor of several organic apple production orchards. EPNs are persisting in all locations at >20 % (Fig. 6.4d) within the grassy habitat on the orchard floor and the incidence of fruit damage has fallen within the treated areas 70–90 %. This research is currently in progress.

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