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Abstract

The black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), has a worldwide distribution and is a serious pest of many agricultural crops with a host plant species range of 140 plants. Common economic losses occur in small fruits, including strawberries, ornamental and nursery plants, caused primarily by the root feeding larvae resulting in reduced vigor and plant death.

The susceptibility of BVW to entomopathogenic nematodes (EPNs) is well established with numerous authors publishing papers using a wide array of EPN species from commercial sources and very high application rates for use as a biopesticide. The concept of using native EPN strains that are climate adapted and retain the genetic traits of phased infectivity to persist across multiple years was successfully developed and tested on a related species, *Otiorhynchus ligustici* (L.), alfalfa snout beetle.

In this study, a single application of climate adapted persistent EPN strains resulted in a reduction of an economically damaging BVW population in strawberries to sub-economic levels. Subsequently, the BVW population remained undetectable for four years while the EPN populations remained moderately high.

Keywords: Black vine weevil, *Otiorhynchus sulcatus*, persistent entomopathogenic nematodes, biological control

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The black vine weevil (BVW), *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), has a worldwide distribution (Willmott et al. 2002) with a host plant species range of 140 plants (Smith 1932), and is a serious pest of many agricultural crops. Common economic losses occur in small fruits, including strawberries, ornamental plantings and nursery plants (Fitters et al. 2001, van Tol et al. 2004). Economic damage is primarily caused by root feeding by the larvae (Smith 1932, Moorhouse et al. 1992), resulting in reduced vigor and plant death (Garth and Shanks 1978, LaMondia and Cowles 2005).

The susceptibility of BVW to entomopathogenic nematodes (EPN) is well established with numerous authors publishing papers using a wide array of EPN species from commercial sources. In these studies, EPN infective juveniles (IJ) were applied in water at very high rates and usually between 5 - 15 billion IJs per ha or 25,000 IJs per pot. If conditions are favorable and the IJs are alive, this biopesticide approach is effective at reducing BVW larval populations (e.g. Shanks and Agudelo-Silva 1990; Wilson et al. 1999; Fritters et al 2000, 2001; Georgis et al 2006; Lola-Luz and Downes 2007; Haukeland and Lola-Luz 2010).

The concept of using native EPN strains that are climate adapted and retain the genetic traits of phased infectivity to persist across multiple years was successfully developed and tested on a related species, *Otiorhynchus ligustici* (L.), alfalfa snout beetle (Shields et al 1999; Neumann and Shields 2006, 2008; Shields et al 2009; Shields and Testa 2017; Shields et al. 2018). These studies report the appropriate mix of EPN species from adapted strains, inoculated at a low rate to become established under field conditions, persisted for multiple growing season and suppress alfalfa snout beetle below economic levels. This research was the basis of an area wide biological control program against alfalfa snout beetle with over 8,000 ha inoculated to date (Shields and Testa 2017).

The focus of this study was to test the concept of biological control with persistent, climate adapted EPN strains against a related pest, BVW in the strawberry cropping system.

Materials and Methods

This study was conducted in a 4 ha strawberry planting of mixed ages with a high population of black vine weevil (BVW), *O. sulcatus* feeding on the roots and destroying the planting. The field was sandy loam and located east of Peru, NY, in Clinton Co. Preliminary larval sampling was conducted in June 2013, indicating a wide spread infestation across the entire 4 ha with an incidence of 50% of the plants being fed on by large larvae and many of the plants having multiple larvae feeding on their root system. The field was also sampled for the presence of naturally occurring entomopathogenic nematodes (EPNs). A replicated study was initiated in August 2013 with two treatments (Persistent EPNs and Untreated Check) with plots measuring 10 m × 10 m. Each treatment was replicated 4 times.

Nematode species and strains used. The EPN species/strains used in this study were *Steinernema feltiae* (Filipjev) 'NY 04' and *Heterorhabditis bacteriophora* Poinar 'Oswego'. *H. bacteriophora* 'Oswego' was initially isolated from soil samples collected in 1990 from Oswego County, NY and *S. feltiae* 'NY 04', was initially isolated from soil samples collected from Jefferson County, NY in 2004. To maintain the ability of these strains to persist under NY conditions, each species was re-isolated from the field every second year beginning in 2007, and used to reinitiate the laboratory culture (Shields and Testa 2015). The EPN strains used in this trial were re-isolated from NNY agricultural fields in 2013. Greater wax moth, *Galleria mellonella* (L.), 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 two years of laboratory culturing (Shields 2015). A Galleria based non-white trap rearing system (Testa and Shields 2017) was used for the production of IJs for field application.

BVW larval sampling protocol. Individual plots were sampled for BVW larvae on 6/2013 (initial preliminary evaluation), 6/2014, 5/2015, 6/2015 (2×, early June and late June), 5/2016, 6/2016, 6/2017, 6/2018 and 6/2019. At each sampling date, 25 samples per plot were examined for the presence of BVW larvae. Each sample was taken centered over a strawberry plant with a Golf Cup Cutter (diameter 11 cm × 160 cm deep). The soil sample was removed, placed in a tray and examined for the presence of insect larvae. The number and instar of BVW larvae was recorded. Any insect cadavers infected with EPNs were also recorded. The percent of plants infested was calculated by dividing the number of infested plants found by the sample size (25). The number of larvae per plant was calculated by dividing the total number of larvae found by the number of infested plants per plot.

EPN sampling protocol. Individual plots were sampled for EPNs in 8/2013 (EPN application pre-sample), 10/2013 (40 days post inoculation), 5/2014, 9/2014, 5/2015, 9/2015, 5/2016, 9/2016, 5/2017, 9/2017, 5/2018 and 6/2019. At each sampling date, a total of 50 soil cores (2 cm × 20 cm) were collected from each plot and returned to the laboratory to be bioassayed for the presence of EPNs using Galleria larvae as indicator larvae. At the time of collection, the top 7 cm was placed in a 100 ml plastic cup with lid and the lower 13 cm was placed in a 240 ml cup with lid. Soil cores were divided in this manner to isolate *S. feltiae* in the upper layers from *H. bacteriophora* in the lower layers 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). Cadaver coloration between *S. feltiae* and *H. bacteriophora* is uniquely different and cannot be confused. Cadaver coloration suggesting possible *Steinernema carpocapsae* (Weiser), the most common wild EPN in NY were placed on moist plaster of Paris disks in Petri dishes (White 1927) ("White trapped"), and observed for IJ emergence. Isolated IJs were then used to infect *G. mellonella* larvae, dissecting out the adult males and verifying the EPN species with the shape of the male spicule head (Neumann 2007).

Initial EPN application. The initial application of EPNs was *S. feltiae* on 5 September 2013 and was scheduled to coincide with the presence of small instar BVW larvae in the planting. This species was originally selected because *S. feltiae* attack all size larvae including the smaller instars whereas *H. bacteriophora* prefers to attack the larger larvae after feeding damage has occurred (Neumann and Shields 2008). Approximately 3.6 million *S. feltiae* IJs were applied in 5 L of water (340 million IJs were applied in 500 L/ha) to each epn treated plot using an ATV mounted small plot sprayer equipped with fertilizer stream nozzles (TeeJet™ 0010, Springfield, IL). Application was made to the soil surface and was initiated late in the day (after 7 pm).

Subsequent EPN application. The spring 2014 BVW larval sampling indicated

Table 1. Percentages of strawberry plants infested with Black Vine Weevil, *Otiorhynchus sulcatus,* in the EPN treated plots over 6 years and the number of larvae per infested strawberry plant.

Date	% plants infested	Number of larvae per plant
6/2013	50 ± 0.7 a **	2.5 ± 0.37 a
6/2014	48 ± 0.07 a	2.0 ± 0.43 a
5/2015	48.7 ± 0.03 a	1.1 ± 0.09 b
6/2015 (early)	27.3 ± 0.03 a	1.0 ± 0.04 b
6/2015 (late)	17.3 ± 0.03 b	1.0 ± 0.03 b
5/2016	13.3 ± 0.03 b	1.0 ± 0.0 b
6/2016	7.0 ± 0.15 c	1.0 ± 0.0 b
6/2017	0 d	0 c
6/2018	0 d	0 c
6/2019	0 d	0 c

**Values within a column followed by the same letter are not significant different at the 0.01 level.

1) dead BVW larvae had been infected by *S. feltiae*, 2) *S. feltiae* had overwintered at a moderate level and 3) BVW population did not appear to be declining. The decision was made to add *H. bacteriophora* to the EPN population in the EPN treated plots. On 27 August 2014, approximately 4 million *H. bacteriophora* IJs were applied to each EPN treated plot using the previously described protocol (378 million IJs were applied in 500 L/ha).

Statistical Analysis. The study was designed as a randomized complete block design with four replications using two treatments (EPN & untreated). Presence of BVW was recorded as the number of plants (cores) infested with larvae and the number of larvae per plant (core). The number of plants infested was converted to percent infested and normalized with Arcsine transformation before analysis. The number of larvae per plant was averaged across the plot. Significant differences in infestation levels between sampling periods was tested using analysis of variance for a Random Complete Block Design (ANOVA) with post-hoc t-test applying Bonferroni correction (Systat Software Inc. 2009).

EPN population levels expressed in percent of soil samples with a positive bioassay for the presence of EPNs were normalized with Arcsine transformation before analysis. Significant differences in populations between years was tested using analysis of variance for a Random Complete Block Design (ANOVA) with post-hoc t-test applying Bonferroni correction (Systat Software Inc. 2009).

Results

BVW Sampling. Initial sampling in June 2013 for BVW larvae indicated 50.0 ± 0.7% of the plants were infested with 2.5 ± 0.37 larvae per plant in the plots to be treated with EPNs and 52.0 ± 0.3% of the plants infested with 2.5 ± 0.51 larvae per plant in the untreated plots. After the application of the EPNs, the percent of infested plants declined over time to undetectable (2013–2019) and the population of EPNs increased over the 6 year duration of the study (15% to 45% of the samples positive for EPN IJ).

Sampling in June 2014 and the first sampling in May 2015 indicated that the percentage of infested plants remained statistically identical (2014 = 48.0 ± 0.07%, 2015 = 48.7 ± 0.03%). The percent of infested plants decreased significantly during the second sampling in early June 2015 (27.3 ± 0.03% plants infested) (F = 2.13; df = 8; P = 0.01) and the third sampling in late June (17.3 ± 0.03% plants infested) (F = 1.98; df = 8; P = 0.01). A significant level of decreased infested plants continued in May 2016 (13.3 ± 0.1%, F = 2.37, df = 8, P = 0.01) and June 2016 (7.0 ± 0.15%, F = 2.27, df = 8, P = 0.01). Sampling for BVW in June 2017, 2018, 2019 found no plants infested with BVW larvae with levels significantly lower than the final sampling in 2015. (F=2.57; df = 32; P = 0.01) (Table 1).

The mean number of larvae per infested plant decreased from 2 ± 0.43 (range 0 - 6) in the spring 2014 to 1.1 ± 0.09 in May 2015 and 1.0 ± 0.01 in May 2016, a statistically significant reduction (F = 2.31; df = 24; P = 0.01). All larval counts were statistically different from each other (F = 2.01; df = 11; P = 0.01) (Table 1).

In the untreated check plots, the BVW larvae initially infested 52 ± 0.3% of the plants in 2013. These levels were not statistically different from the initial levels in the plots treated with EPNs (50 ± 0.7). During the 2014, the percentage of infested plants increased to 86 ± 0.4% of the plants. A significant increase over the initial levels in 2013 (F = 2.03; df = 3; P = 0.01). The ini-

Table 2. Percentages of soil samples bioassayed positive for entomopathogenic nematode species *Steinernema feltiae* and *Heterorhabditis bacteriophora*.

Date	*S. feltiae* $\bar{x}$% ± *SE*	*H. bacteriophora* $\bar{x}$% ± *SE*	Combined $\bar{x}$% ± *SE*	Days after application
10/2013	12.8 ± 3.0 a**	0 a	12.8 ± 3.0 a	35
6/2014	14.7 ± 2.5 a	0 a	14.7 ± 2.5 a	270
8/2014	15.0 ± 4.0 a	0 a	15.0 ± 3.0 a	330
5/2015	11.5 ± 2.0 a	3.9 ± 2.2 b	15.5 ± 1.9 a	600
9/2015	15.9 ± 1.3 a	9.0 ± 1.1 c	24.9 ± 2.1 b	720
5/2016	27.5 ± 4.2 b	10.0 ± 2.7 c	37.5 ± 3.4 c	960
9/2016	16.6 ± 6.5 a	13.0 ± 6.5 c	29.6 ± 6.5 bc	1,080
5/2017	15.8 ± 3.3 a	8.3 ± 3.2 c	24.1 ± 3.1 b	1,320
9/2017	24.8 ± 1.0 b	2.0 ± 2.0 b	26.8 ± 1.5 b	1,440
5/2018	24.0 ± 4.0 b	2.0 ± 1.0 b	25.8 ± 4.0 b	1,680
6/2019	45.0 ± 2.0 c	0 a	45.0 ± 2.0 d	2,075

**Values within a column followed by the same letter are not significant different at the 0.01 level.

tial mean number of larvae per plant in the untreated check plots (2.5 ± 0.51 larvae) was not significantly different than the initial larval levels in the plots treated with EPNs (2.0 ± 0.43 larvae).

The mean number of larvae per plant in the untreated plots significantly increased from 2.5 ± 0.51% of the plants (range 0–6) in May 2014 to 4.2 ± 0.23 larvae per plant in May of 2015 ($F = 2.13$; df = 3; $P = 0.01$). By July 2015, the plant stands in the untreated check plots were completely destroyed.

EPN sampling. Results from the EPN pre-treatment bioassay of soil cores indicated no native populations of *S. feltiae*. The presence of a native *H. bacteriophora* was discovered in less than 2% of the soil samples. Forty days after *S. feltiae* application (10/2013), soil core bioassay indicated 12.8 ± 3.0% of the cores with *S. feltiae* and 1.86 ± 1.0% of the cores with *H. bacteriophora* with a combined EPN positive cores of 14.7 ± 3%. In early June 2014, EPN sampling indicated 14.7 ± 2.48% *S. feltiae* and 3.6 ± 1.5% *H. bacteriophora* with a combined total of 18.2 ± 3% EPN positive soil cores. In August 2014, before the supplemental application of *H. bacteriophora*, the EPN levels were, 15 ± 4.0% *S. feltiae* , 0% *H. bacteriophora* and 15 ± 3.0% combined. In May 2015, EPN population levels were, 11.5 ± 2.0% *S. feltiae*, 3.9 ± 2.3% *H. bacteriophora* and 15.5 ± 1.9% combined. In September 2015, EPN population levels were 15.9 ± 1.3% *S. feltiae*, 9.0 ± 1.1% *H. bacteriophora* and 24.9 ± 2.1% EPN combined total. Spring sampling in May 2016 indicated, 27.5 ± 4.2% *S. feltiae*, 10 ± 2.7% *H. bacteriophora* and a combined EPN total of 37.5 ± 3.4%. Fall sampling in September 2016 indicated 16.6 ± 6.5% *S. feltiae*, 13.0 ± 6.5% *H. bacteriophora* and a combined total of 29.6 ± 6.5%. Spring sampling in May 2017 indicated, 15.8 ± 3.3% *S. feltiae*, 8.3 ± 3.2% *H. bacteriophora* and an EPN combined total of 24.1 ± 3.1%. Fall sampling in September 2017 indicated 24.8 ± 1.0% *S. feltiae*, 2.0 ± 2.0% *H. bacteriophora* with a EPN combined total of 26.8 ± 1.5%. May 2018 indicated 24.0 ± 4.0% *S. feltiae*, 2.0 ± 1.0 *H. bacteriophora* with a combined total of 25.8 ± 4.0%. Spring of 2019 indicated 45.0 ± 2.0% *S. feltiae* and 0% *H. bacteriophora* (Table 2).

The increase in total EPN populations (both species combined) was significant in Sept. 2015 and then again in June 2019. EPN population levels were not significantly different between Oct 2013 and May 2015 (16% of the soil samples positive for EPN). In Sept 2015, the EPN population increased significantly from the previous level and remained at the significantly higher level until May 2018 (32% of the soil samples positive for EPN). In June 2019, the EPN population increased to a significantly higher level (45% of the soil cores positive for EPN).

BVW populations decreased over time with a corresponding increase of EPN levels. All of the dead larvae observed during soil sampling for larvae displayed symptoms of EPN infection. Larvae were not observed with any pathogenic fungi infection. Regular sampling of the untreated check plots for EPNs indicated no movement of EPNs into the untreated check areas during the duration of the experiments.

Discussion

During the duration of the study, no insecticides were used to suppress the BVW adult populations. The focus of the study was to see if persistent EPNs alone could reduce the economically damaging levels of BVW to a sub-economic level and maintain the BVW population levels below economic damaging levels for multiple growing seasons. This study suggests that EPNs can be utilized in a classical biocontrol strategy where the soil is inoculated with a relative low rate of EPNs which are climate adapted and retain the genetic ability to persist in the soil environment for multiple years including across months of frozen soil each winter. In addition, the EPN species mix was selected to overlap with the soil profile of the insect host to provide maximum opportunity for the EPNs to attack and recycle in the target host. The inoculation rate for both species combined was only 29% of the typical EPN application rate when EPNs are used as a biopesticide (720 million/ha vs. 2.5 billion/ha).

Steinernema feltiae 'NY04' was initially selected because it prefers small larvae which are attacked before significant root feeding occurs (Neumann and Shields 2008), its lower temperature threshold of host infection is 6°C (Neumann 2003) and it preferred soil profile niche was the top 20 cm of the soil. These characteristics were considered a better match to the temperature activity thresholds of black vine weevil larvae in the spring feeding on strawberry roots. In addition, *S. feltiae* 'NY04' has demonstrated its ability to persist for multiple years at a moderate population level (20–30% of the soil cores) in the NY agricultural system (Shields et al. 2018). The lack of host reduction 10 months after *S. feltiae* inoculation suggested that *S. feltiae* may not be able to reduce an economically damaging population of black vine weevil to sub-economic levels on a timely basis without help. At this point, *H. bacteriophora* 'Oswego' was applied to assist *S. feltiae* with the biocontrol of black vine weevil. *H. bacteriophora* 'Oswego' also was adapted to NY agricultural conditions, retained its genetics to persist for multiple season under NY conditions, has the lower temperature of infectivity at 8°C (Neumann 2003), soil profile niche of the top 30 cm of the soil and prefers sandy soils. The two less desirable characteristic were the higher temperature threshold of activity and the preference to attack larger larvae, allowing root feeding damage by the insect before being attacked by *H. bacteriophora*.

The trends of EPN populations was interesting. There appeared to be a significant lag period of 22 months before the EPNs were able to reduce the black vine weevil larval populations to a sub-economic level. In addition, it appeared to require a similar period before the EPN populations began to increase in the research plots. In 2016, peak EPN populations coincided with the significant decrease in black vine weevil populations. Starting in 2017, black vine weevil larvae were not detected in the EPN treated plots for the remainder of the study (3 years). With the absence of black vine weevil hosts, the population levels of *H. bacteriophora* declined to undetectable in 2019 while the population levels of *S. feltiae* increased. Interestingly, in 2019, the population of *S. feltiae* peaked at its highest level in 2019, suggesting an invasion of susceptible hosts even though black vine weevil larvae were not detected in a 2019 sampling. While strawberry yields were not recorded, the grower reported increased yields each year that the levels of BVW were reduced. This impact is also supported with the total destruction of the untreated check plots within 24 months. Subsequently, the grower has inoculated his entire strawberry and blueberry acreage against BVW.

Over 2,000 days after inoculation, a significant population of *S. feltiae* (45% of the soil cores) continues to be present in the treated plot areas ready to infect susceptible insect hosts which invade the area. Shields et al. (2018) indicates that this strain of *S. feltiae* will persist in the soil for multiple growing season going forward in time. A continuing questions is whether this persistent population of *S. feltiae* will prevent the buildup of an economic population of black vine weevils in future years.

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Literature Cited

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. Environmental Entomology 24: 149-158.

Fitters, P.F.L., R. Dunne, and C. Griffin, 2001. Vine weevil control in Ireland with entomopathogenic nematodes: optimal time and frequency of application. Irish Journal of Agricultural and Food Research, 40: 199–213.

Garth, G.S., and C. H. J. Shanks. 1978. Some factors affecting infestation of strawberry fields by the black vine weevil in western Washington. Journal Economic Entomology 71: 443–448.

Georgis, R., A. M. Koppenhofer, and L. A. Lacey. 2006. Successes and failures in the use of parasitic nematodes for pest control. Biological Control: 38, 103–123.

Haukeland, S., and T. Lola-Luz. 2010. Efficacy of the entomopathogenic nematodes *Steinernema kraussei* and *Heterorhabditis megidis* against the black vine weevil *Otiorhynchus sulcatus* in open field-grown strawberry plants. Agricultural and Forest Entomology 12: 363-369.

LaMondia, J.A., and R. S. Cowles. 2005. Prevalence and potential impact of soil dwelling pests and insect pathogenic nematodes in strawberry fields. HortScience 40: 1366–1370.

Lola-Luz, T., and M. Downes, 2007. Biological control of black vine weevil *Otiorhynchus sulcatus* in Ireland using *Heterorhabditis megidis*. Biological Control 40: 314-319.

Moorhouse, E.R., A. K. Charnley, and A. T. Gillespie. 1992. A review of the biology and control of *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Annals of Applied Biology 121: 431–454.

Neumann, G. 2003. Naturally occurring biological control of the alfalfa snout beetle, *Otiorhynchus ligustici* (L.) in Hungary and in New York State. M. S. Thesis. Cornell University. 84 pp.

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 113 pp.

Neumann, G., and E. J. Shields. 2006. Interspecific interactions among three entomopathogenic nematodes, *Steinernema carpocapsae*, *S. feltiae*, and *Heterorhabditis bacteriophora*, with different foraging strategies for hosts in multi-piece sand columns. Environmental Entomology 35: 1578-1583.

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. Journal of Economic Entomology 101: 1533-1539.

Poinar, G. O., Jr. 1984. On the nomenclature of the genus *Neoplectana* Steiner 1929 (Steiner nematidae: Rhabdita) and the species *N. carpocapsae* Weiser 1955. Revue de Nématologie 7(2): 199-200.

Shanks, C. H. Jr., and F. Agudelo-Silva. 1990. Field pathogenicity and persistence of Heterorhabditid and Steinernematid nematodes (Nematoda) infecting black vine weevil larvae (Coleoptera: Curculionidae) in cranberry bogs. Journal of Economic Entomology 83(1): 107-110.

Shields, E. J. 2015. Ch 6 - Utilizing persistent EPNs in a conservation or a more classical biological control approach. 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 (R. Campos-Herrera ed). Springer. 2015. Pp. 165-184.

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). Environmental Entomology 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. American Entomologist 55: 250-257.

Shields, E. J., and A. M. Testa. 2015. Ch 11—New York Case Study: Biological Control of *O. ligustici* with native persistent EPNs using a more classical approach. 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 (R. Campos-Herrera ed). Springer. 2015. pp 285-307.

Shields, E. J., and A. M. Testa. 2017. Biological Control of Alfalfa Snout Beetle with Persistent Entomopathogenic Nematodes: Expanding a single farm success to an area-wide biological control program. American Entomologist. Winter 2017: 216-223.

Shields, E. J., A. M. Testa, and W. J. O'Neil. 2018. Long-term persistence of native New York entomopathogenic nematode isolates across crop rotation. Journal of Economic Entomology 111: 2592-2598.

Smith, F. 1932. Biology and control of the black vine weevil. United States Department of Agriculture Technical Bulletin.325. Washington, DC, U.S. Department of Agriculture.

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. Biocontrol 106: 77-82.

van Tol, R.W.H.M., N. van Dijk, and M. W. Sabelis. 2004. Host plant preference and performance of the vine weevil *Otiorhynchus sulcatus*. Agricultural and Forest Entomology 6: 267–278.

Willmott, D.M., A. J. Hart, S. J. Long, R. N. Edmondson, and P. N. Richardson. 2002. Use of a cold-active entomopathogenic nematode *Steinernema kraussei* to control overwintering larvae of the black vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) in outdoor strawberry plants. Nematology 4: 925–932.

Wilson, M., P. Nitzsche, and P. W. Shearer. 1999. Entomopathogenic nematodes to control black vine weevil (Coleoptera: Curculionidae) on strawberry. Journal of Economic Entomology 92: 651–657.

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

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.