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ABSTRACT

The long term field persistence of Steinernema carpocapsae Weiser, strain NY001, *S. feltiae* Filipjev, strain Valko and Heterorhabditis bacteriophora Poinar, strain Oswego was investigated in an alfalfa field infested by the alfalfa snout beetle, *Otiorhynchus ligustici* L. Nematodes were applied in single-species, two-species and three-species combinations at a total of 2.5 × 109 infective juveniles per hectare. Soil samples were taken approximately every two weeks from mid/late May to late October in 2004 and 2005. Two soil samplings were conducted in 2006 at the end of May and in early July. All nematodes persisted in the field at the time of the last sampling in July 2006, over two years after application suggesting long term persistence of these nematodes and the potential to coexist in combinations. *Steinernema feltiae* Valko was not detected in the three-species combination after June 8, 2005, approximately one year after nematode application suggesting that *S. feltiae* Valko cannot compete effectively when a specialized ambusher nematode (*S. carpocapsae* NY001) and a specialized cruiser nematode (*H. bacteriophora* Oswego) are present simultaneously. In 2006, two years after nematode application, a marked movement of nematodes into experimental plots where they were not applied was observed. *S. carpocapsae* NY001 was found in the highest number of plots where it wasn't applied. Given the ambusher behavior of *S. carpocapsae* NY001, it is suspected that its movement occurred via infected, but still live adult alfalfa snout beetles.

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* have been studied as potential biological control agents of soil-inhabiting insect pests (Gaugler 1988, Kaya 1990, Shapiro-Ilan et al. 2002, Grewal et al. 2005). These parasitic nematodes can kill hosts rapidly, are relatively easy to apply, and are exempt from federal and local registration requirements in most countries because of their safety to mammals and plants (Georgis et al. 1991). However, the use of entomopathogenic nematodes on large scale has been limited by their low persistence in field conditions (Lewis et al. 1995). Many factors such as soil moisture, texture and porosity (Barbercheck 1992, Glazer 2002, Stuart et al. 2006) can contribute to the persistence of nematodes. Lawrence et al. (2006) emphasized the importance of soil moisture as one of the most important factors associated with the presence and persistence of endemic nematodes. Besides abiotic factors, nematode strain selection can be also very important. Shields et al. (1999) compared *Heterorhabditis bacteriophora* Poinar (Oswego) to another strain, *H. bacteriophora* (NC), for biological control of the alfalfa snout beetle, *Otiorhynchus ligustici* L., the most severe alfalfa pest in

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Northern New York State. Shields et al. (1999) found that the NC strain would be more appropriate in situations where a biopesticide is desired, and the Oswego strain is more appropriate where long-term insect suppression is desired within more stable ecosystems. The NC strain was more aggressive in searching for prey than the Oswego strain but while the Oswego strain persisted in the field for >4 years, the NC strain did not persist for longer than one growing season under field conditions.

Recent research in the biological control of the alfalfa snout beetle has been focusing on a multi-species natural enemy approach using entomopathogenic nematodes with different foraging and dispersal strategies (Neumann and Shields 2006). Neumann and Shields (2008) conducted a field experiment in which *Steinernema carpocapsae* Weiser (NY001), an ambush nematode, *H. bacteriophora* (Oswego), a cruiser nematode, and *Steinernema feltiae* Filipjev (Valko), an intermediate nematode (both ambush and cruiser characteristics) (Grewal et al. 1994) were applied in single- and multiple-species combinations in alfalfa snout beetle infested fields. The objectives of that study were to control alfalfa snout beetle and to prevent root damage with nematode combinations. Besides alfalfa snout beetle larval control and root damage prevention, the persistence of the nematodes was also followed. Here we present the persistence data of these nematodes when exposed to interspecific competition.

Materials and Methods

Study site. The experiment was conducted in an alfalfa (*Medicago sativa* L.) field infested with alfalfa snout beetle located in the township of Great Bend, Jefferson County, New York. The alfalfa field was in its first production year, therefore, the alfalfa snout beetle infestation of the experimental area occurred during the spring of the nematode application and establishment. Thirty two plots with dimensions of 3 m by 6 m were arranged in a randomized complete block design with seven different treatments and a control. Each treatment was replicated four times. The plots were separated by 3-m alleys. The site was surveyed for naturally occurring entomopathogenic nematodes before nematode application by taking five random soil samples per plot (30 cm deep, 2.5 cm diameter). The soil cores were placed in 350-ml plastic cups and were assayed for nematodes in the laboratory using last instar *Galleria mellonella* L. larvae as trap insects. *G. mellonella* were obtained from Webster's Waxie Ranch (5355 County Road A, Webster, WI 54893). Ten larvae were placed in each cup and were inspected for mortality after 2 and 7 days. Dead larvae were dissected to confirm nematode infection.

Nematode culture and application. *H. bacteriophora* Oswego, *S. carpocapsae* NY001 (both isolated in Oswego County, New York, in fall 1990), and *S. feltiae* Valko (isolated in Valko, Hungary, in spring 2002) were used in this study. The nematodes were mass produced in *G. mellonella* larvae (Flanders et al. 1996). Infective juveniles (IJs) were collected in White traps (Woodring and Kaya 1988) during a three-day period after the onset of IJ emergence from the *G. mellonella* cadavers. IJs were counted for each species by serial dilution. Subsequently, IJs to be applied to each plot were suspended in 100 ml deionized water in sterile 250-ml culture flasks and stored at 10oC for approximately 36 hours before application.

Nematodes were applied on 17 May 2004. The IJs for each plot from the culture flasks were suspended in 11.4 liters of water and applied using a 30-Psi CO2-powered backpack sprayer with 3.5-m handheld 2-person boom (Shields et al. 1999). Ten fertilizer nozzles (0006), with screens removed, were mounted on the boom 30 cm apart. All single-species and multiple-species combinations of nematodes were applied. Each treatment was 2.5 × 109 IJs/hectare (total of 4.5 × 106 IJs/plot). For combinations of nematodes, the total number of IJs was divided to equal proportions among the nematode species: 2.25 × 106 IJs/

species in two-species combinations and 1.5 × 106 IJs/species in three-species combinations. To avoid the contamination of nematodes with the sprayer among plots while minimizing the need for cleaning the sprayer, the treatments were applied in the following sequence: control plots (water only); *S. carpocapsae* NY001; combination of *S. carpocapsae* NY001 and *S. feltiae* Valko; combination of *S. carpocapsae* NY001, *S. feltiae* Valko and *H. bacteriophora* Oswego then the sprayer was washed. The next treatments were *H. bacteriophora* Oswego; combination of *H. bacteriophora* Oswego and *S. carpocapsae* NY001 then the sprayer was washed again. The final two treatments were *S. feltiae* Valko and the combination of *S. feltiae* Valko and *H. bacteriophora* Oswego.

Application of the nematodes began at ≈ 1900 hours EDT and finished at 2300 hours.

Nematode persistence. Soil samples were collected during 2004, 2005 and 2006 to track the persistence of the applied nematodes. In the year 2004, soil samples were taken on 25 May, 7 and 21 June, 7 and 21 July, 10 and 24 August, 13 and 29 September , and 18 October . In the year 2005, soil samples were taken on 17 and 26 May, 8 and 22 June, 7 and 21 July, 11 August, 10 and 28 September, and 17 October. In the year 2006, two samplings on 25 May and 7 July were conducted to detect nematode persistence and movement among the plots. On each sample date, ten, 30-cm-deep random samples were taken from each plot. In control and single-species plots, the soil cores were placed in 350-ml plastic cups. In multi-species plots, the soil cores were divided into two sections, 0-5 cm and 5-30 cm. The upper sections were placed in 118-ml cups while the lower sections were placed in 350-ml cups. All samples were taken to the laboratory and baited with *G. mellonella* larvae. Ten larvae were exposed to the samples in the 350-ml cups and five larvae were exposed to the samples in the 118-ml cups. This procedure was followed to facilitate the detection of nematodes in samples where more than one nematodes species with different vertical distribution in the sample were expected. The samples were incubated at 23°C and mortality was first recorded after five days. Dead larvae were replaced with live larvae to insure maximum detection of nematodes in the samples. Mortality was recorded every other day from this point until no more mortality was observed for 10 days. Dead larvae were dissected to confirm nematode infection and to verify the nematode species causing death. Nematode identification was based on adult morphological characteristics (Adams and Nguyen 2002). In 2004 and 2005, the mean percentages of positive soil cores for each species in all plots were determined as well as the percentages of positive soil cores containing two nematode species simultaneously in the two-species combination plots. In 2006, only the presence or absence of the nematodes in the different plots and the total percentages pooled across all plots were determined.

Results

Nematode persistence. The presence of naturally occurring nematodes was not detected during pre-sampling of the study site.

Eight days after nematode application, the percentages of positive soil cores (soil cores with nematodes detected) for each nematode species ranged between 37.5 ± 4.8% and 42.5 ± 4.8% in single-species treatments, between 25.0 ± 2.9% and 40.0 ± 4.1% in two-species treatments, and between 20.0 ± 11.5% and 27.5 ± 6.3% in the three-species treatment. One year after nematode application, the percentages of positive soil cores for each nematode species ranged between 2.5 ± 2.5% and 10.0 ± 4.1% in single-species treatments, between 0.0 ± 0.0% and 10.0 ± 4.1% in two-species treatments, and between 2.5 ± 2.5% and 7.5 ± 2.5% in the three-species treatment. With the exception of *S. feltiae* Valko in the three-species combination plots, all nematodes were present ~25.5 months after nematode application (Figs. 1 and 2). *S. feltiae* Valko was last detected in the three-species combination plots on 8 June 2005. In the third season, *S.*

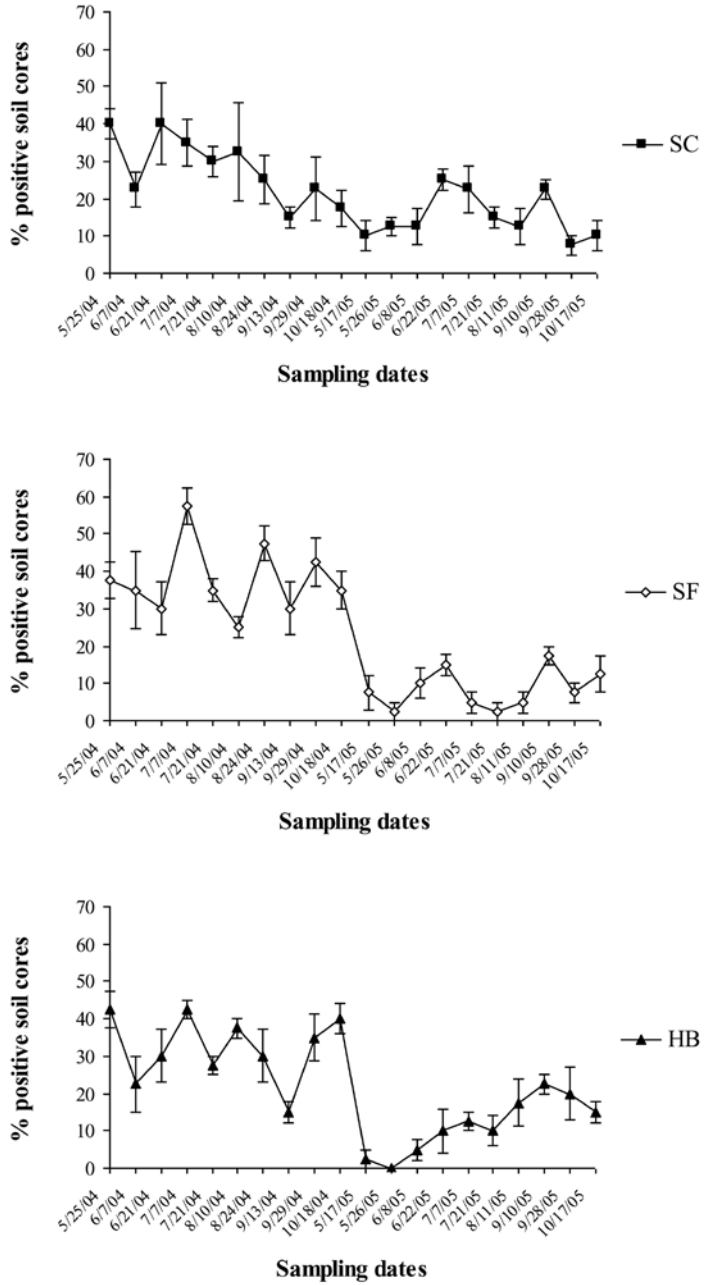

Fig.1. Mean (±SE) percent of positive soil cores in single-species nematode applications (2.5 × 109 IJs/ha application rate) through a more than 16-month period from application ("SC" – *S. carpocapsae* NY001, "SF" – *S. feltiae* Valko, "HB" – *H. bacteriophora* Oswego).

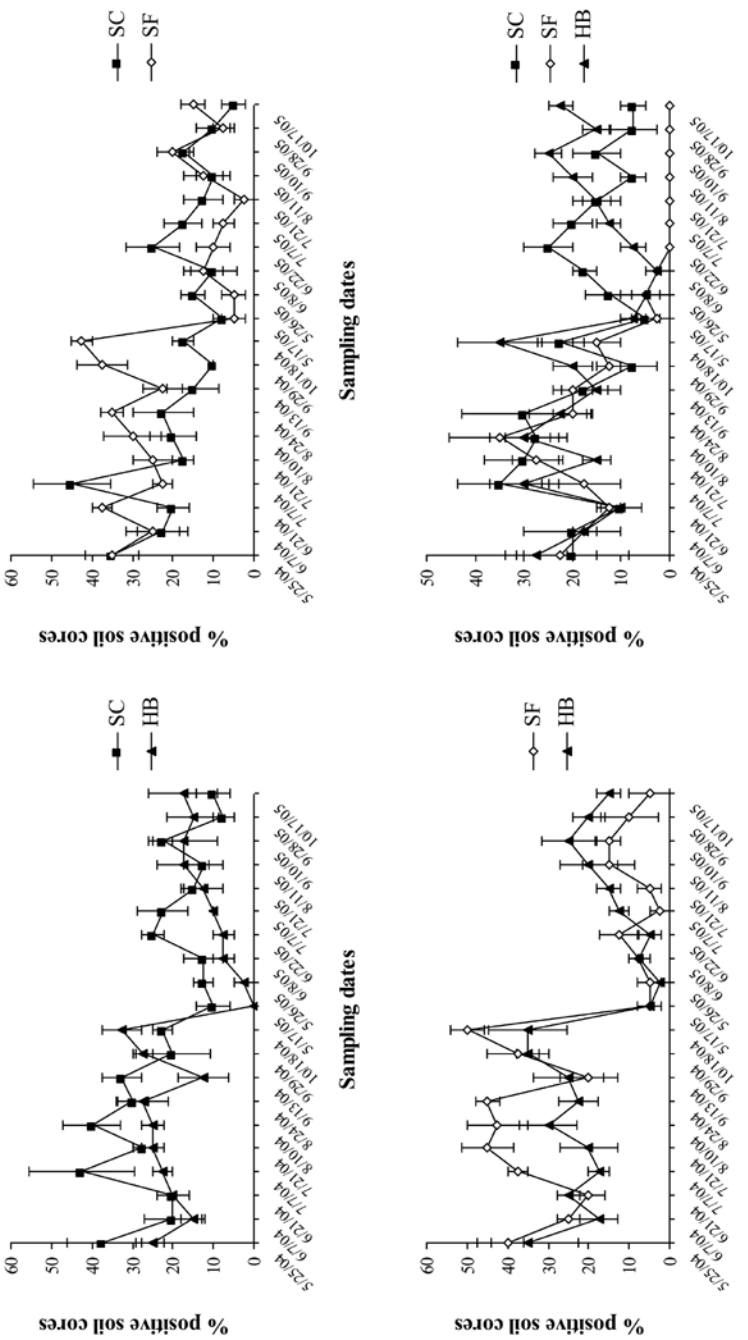

Fig. 2. Mean (±SE) percent of positive soil cores in multi-species nematode applications (2.5 billion/ha total application rate) through a more than 16-month period from application ("SC" – *S. carpocapsae* NY001, "SF" – *S. feltiae* Valko, "HB" – *H. bacteriophora* Oswego).

carpocapsae NY001 was found in 16.9% and 12.2% of soil samples on 25 May 2006 and on 7 July 2006, respectively. *S. feltiae* Valko was found in 4.4% and 3.4%, and *H. bacteriophora* Oswego was found in 8.1% and 12.8% of the soil samples on 25 May 2006 and on 7 July 2006.

Multi-species soil cores. In the *S. carpocapsae* NY001+ *S. feltiae* Valko combination plots, 28.6 ± 4.8% of positive soil cores contained both species eight days after nematode application (Fig. 3). The highest percentage of positive soil cores with both species (39.6 ± 21.3%) was observed on 21 July 2004. During the second season in 2005, the highest percentage of positive soil cores with both species was 13.3 ± 8.2% on 22 June. In the *S. carpocapsae* NY001+*H. bacteriophora* Oswego combination plots, 33.8 ± 11.1% of the positive soil cores contained both species eight days after nematode application. The highest percentage of positive soil cores with both species was 39.0 ± 9.9% on 24 August 2004. In 2005, the highest percentage of positive soil cores with both species was 12.5 ± 12.5% on 17 October. In the *S. feltiae* Valko + *H. bacteriophora* Oswego combination plots, 26.2 ± 9.2% of the positive soil cores contained both species eight days after nematode application. The highest percentage of positive soil cores with both species was 51.7 ± 21.2% on 10 August 2004. In 2005, the highest percentage of positive soil cores with both species was 13.3 ± 8.2% on 10 September. No soil cores contained both species between 17 May and 8 June 2006 and during the last two samplings on 28 September and 17 October 2005 in case of the *S. carpocapsae* NY001 + *S. feltiae* Valko combination. In case of the *S. carpocapsae* NY001 + *H. bacteriophora* Oswego combination, no soil cores contained both species between 13 September and 29 September, between 17 May and 8 June, and on 28 September 2005. In case of the *S. feltiae* Valko + *H. bacteriophora* Oswego combination, no multi-species soil cores contained both species between 17 May and 21 July, and on 17 October 2005 (Fig. 3).

Nematode presence and movement among experimental plots. *S. carpocapsae* NY001 was detected in 3 experimental plots in 2005 where it wasn't applied and in 12 additional plots in 2006. *S. feltiae* Valko was detected in one experimental plot in 2005 where it wasn't applied and in one additional plot in 2006. *H. bacteriophora* Oswego was found in four plots in 2006 where it wasn't applied (Fig. 4).

Discussion

The nematode establishment in the field plots was lower than expected based on a similar study done by Shields et al. (1999). All nematodes were detected approximately 26 months after the application. Although the nematode populations were relatively low 357 days after application compared to the year of application, they still persisted. Neumann and Shields (2004) found similar percentage of naturally occurring *S. feltiae* in Hódmezővásárhely, Hungary, where the alfalfa snout beetle population was not at an economically important level. With the exception of *S. feltiae* Valko in the three-species combination plots, all nematodes showed an increasing frequency in the next three to five weeks suggesting nematode recycling. The patterns of nematode frequencies seemed to fluctuate to a lesser degree in the second season with less variability at the different sampling dates. Due to the high variability of percentages and the low positive percentages in the second season, only descriptive statistics were used in the analysis but some observations can still be made. We believe that the percentage data from the second season better represents a natural situation because the first season was less independent from the initial application of nematodes, even at the end of the season. In contrast, the nematodes went through several recycling periods by the second season which was heavily influenced by the density of the available hosts. Considering the data obtained in 2006 in single-, and two-species plots (Figs. 1 and 2), the positive soil core percentages of the *S. carpocapsae* NY001 and *S. feltiae* Valko appears to peak

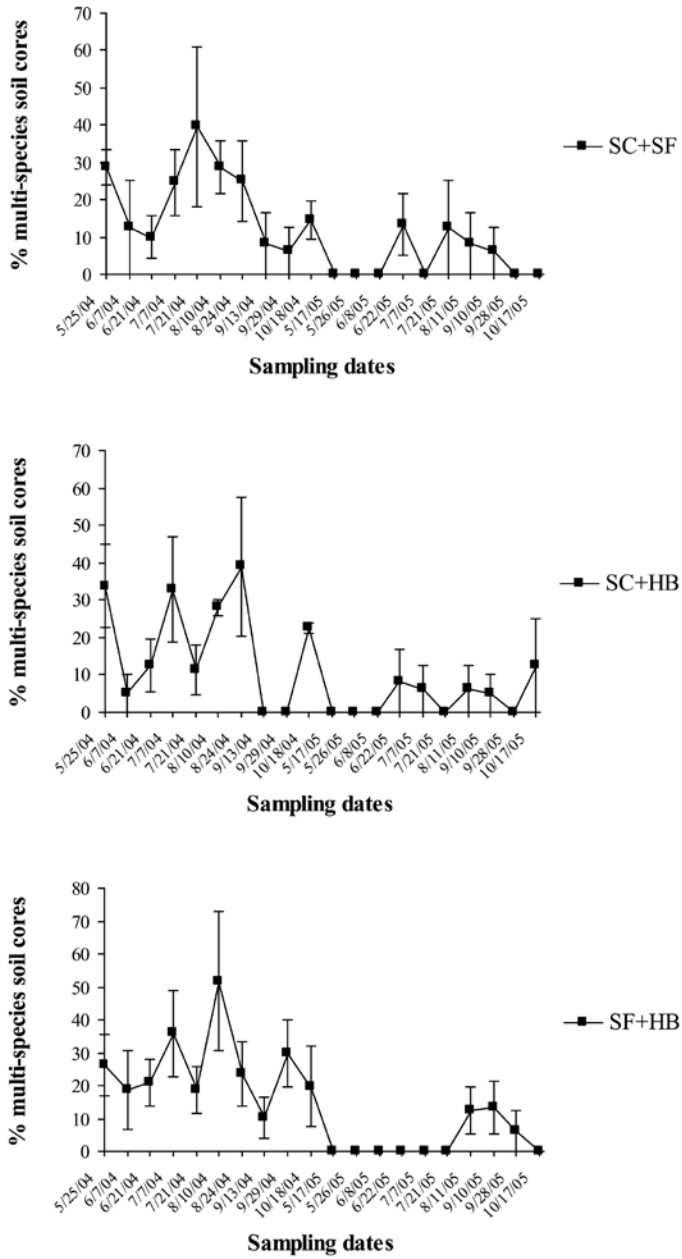

Fig. 3. Mean (±SE) percent of multi-species soil cores (multi-species soil cores/total positive soil cores) in two-species nematode applications (2.5 x 109 IJs/ha application rate) through a more than 16-month period from application ("SC" – *S. carpocapsae* NY001, "SF" – *S. feltiae* Valko, "HB" – *H. bacteriophora* Oswego).

SC	*	*	SC	A
SC	+	SC	SC	
SC	*	SC	*	
*	SC	*	*	
SC	SC	+	SC	
SC	+	SC	SC	
SC		*	*	
*	*	SC	*	

		SF		B
SF	SF	SF		
		SF		
SF	SF		SF	
SF		SF	SF	
*	SF	SF		
SF		SF		
SF			+	

	HB			C
	HB	HB	HB	
HB			HB	
	HB	*	HB	
HB	HB	HB	*	
	*	HB		
	HB	*		
HB		HB	HB	

Fig. 4. Nematode (A: "SC" – *S. carpocapsae* NY001, B: "SF" – *S. feltiae* Valko, C: "HB" – *H. bacteriophora* Oswego) movement among the experimental plots. The letters indicate where a nematode was applied (either alone or in combination) in 2004. The + sign indicates plots where the given nematode was not applied but was detected in 2005 and the asterisk indicates plots where the given nematode was not applied but was detected in 2006. (The drawing shows the spatial arrangement of the plots but doesn't show the 3-m alleyways between the plots.)

early and late in the season with some decrease in percentages in mid-season. In contrast, *H. bacteriophora* Oswego shows a steady increase in positive soil core percentages and populations peaked late in the season. Shields et al. (1999) and Neumann and Shields (2008) showed that *H. bacteriophora* Oswego significantly reduced the number of surviving alfalfa snout beetle larvae in alfalfa while not preventing root damage, suggesting that this nematode maybe attacking the majority of alfalfa snout beetle larvae in its late instars. Neumann and Shields (2008) found that *S. carpocapsae* NY001 and *S. feltiae* Valko prevented root damage effectively suggesting that the adult beetles and/or early instar alfalfa snout beetle larvae may have been successfully attacked by these nematodes. This would explain the early increase of positive soil core percentages for *S. carpocapsae* NY001 and *S. feltiae* Valko. *S. feltiae* Valko also reduced the numbers of surviving late instar larvae similarly to *H. bacteriophora* Oswego which would explain the increase in positive soil core percentages of this nematode late in the season. However, the late-season peak of *S. carpocapsae* NY001 is not clear. There may be a host that is available for this nematode at this time of the year, or the alfalfa snout beetle larvae moving closer to the surface late in the season due to increasing soil moisture and larvae are exposed to *S. carpocapsae* NY001. These differences probably result in the spatial and temporal niche separation of the nematodes, allowing them to coexist, except in the scenario when all three species are present. In the three-species combination, *S. carpocapsae* NY001 and *H. bacteriophora* Oswego showed similar trends, but *S. feltiae* Valko was not detected after 8 June 2005. Since *S. feltiae* Valko persisted in the two-species combinations, its disappearance in the three-species combination may suggest that *S. feltiae* Valko cannot effectively compete when both a specialized ambusher (*S. carpocapsae* NY001) and a specialized cruiser (*H. bacteriophora* Oswego) are present in the same habitat.

The long term detection of positive soil cores containing multiple nematode species in two-species combination plots suggested that the long term coexistence of these nematode combinations is possible, although the proportion of multi-species soil cores was generally low and, on several sampling dates, zero. The data suggest that horizontal overlap, when more than one species of nematodes are found in a soil core, occurs between species to some extent and, therefore, distinct and distant pockets, where only one nematode is present, are less likely to form. This is an important factor in a multi-species natural enemy approach when targeting a host such as the alfalfa snout beetle that moves vertically during its larval stage through the different nematode niches.

The results of the sampling on 25 May and 7 July 2006 indicated that nematodes moved into several plots where they were not applied. The same descriptive analysis of the percentage data would have had limited value because a given plot may have contained positive soil cores but not the expected nematode species. The data summary method used in 2004 and 2005 was not used in 2006 instead all positive soil cores for a particular species were pooled for each sampling date. Of course, this method still limits the information gained, but it clearly shows that the nematodes still persisted well into the third growing season after the application.

The movement of nematodes into adjacent experimental plots was rather surprising. We expected the ambusher *S. carpocapsae* NY001 to show little movement among the plots, and the cruiser *H. bacteriophora* Oswego to move into more plots. The results proved to be the opposite. It must be noted, however, that these results are not supported very strongly since they are based on one single observation per species where the observation is the whole experimental area. The movement of *S. carpocapsae* NY001 may be due to movement of infected hosts or could be phoretic, but there is no direct evidence for this. Due to the movement of nematodes into adjacent plots, the experiment could not be continued for more than two seasons. In future studies, the experimental plots should be placed much further from each other.

Acknowledgments

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Literature Cited

Adams, B. J., and K. B. Nguyen. 2002. Taxonomy and systematics, pp. 1-33. *In* R. Gaugler, (ed.), Entomopathogenic nematology. CABI Publishing, New York, New York.

Barbercheck, M. E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. Florida Entomologist. 75: 539–548.

Flanders, K. L., J. M. Miller, and E. J. Shields. 1996. In vivo production of *Heterorhabditis bacteriophora* Oswego (Rhabditida: Heterorhabditae), a potential biological control agent for soil-inhibiting insects in temperate regions. Journal of Economic Entomology 89(2): 373-380.

Gaugler, R. 1988. Ecological considerations in the biological control in soil-inhabiting insect pests with entomopathogenic nematodes. Agricultural Ecosystems and Environment. 24: 351.

Georgis, R., H.K. Kaya, and R. Gaugler. 1991. Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. Enviromental Entomology. 20: 815-822.

Glazer I. 2002. Survival biology, pp. 169–187. In R. Gaugler, (ed.), Entomopathogenic nematology. CABI Publishing, New York, New York.

Grewal, P. S. and A.Peters. 2005. Formulation and quality. In Grewal, P. S., R. U. Ehlers, and D. I. Shapiro-Ilan (eds.). Nematodes as Biocontrol Agents. CABI Publishing, Cambridge MA.

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

Kaya, H. K. 1990. Soil ecology, pp. 93-115. In Gaugler, R. and H. K. Kaya (eds.), Entomopathogenic nematodes in biological control. CRC Press, Inc., Boca Raton, Florida, USA.

Lawrence, J. L., C. W. Hoy and P. S. Grewal. 2006. Spatial and temporal distribution of endemic entomopathogenic nematodes in a heterogeneous vegetable production landscape. Biological Control. 37: 247-255.

Lewis, E. E., P. S. Grewal, and R. Gaugler. 1995. Hierarchical order of host cues in parasite foraging strategies. Parasitology. 110: 207-213.

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: 1533-1339.

Neumann, G., and E. J. Shields. 2006. Interspecific interactions among three entomopathogenic nematodes, *Steinernema carpocapsae* Weiser, *S. feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar, with different foraging strategies for hosts in multipiece sand columns. Environmental Entomology. 35(6): 1578-1583.

Neumann, G., and E. J. Shields. 2004. Survey for entomopathogenic nematodes and entomopathogenic fungi in alfalfa snout beetle, *Otiorhynchus ligustici* (L.) (Coleoptera: Curculionidae), infested fields in Hungary and in New York State. The Great Lakes Entomologist. 37(3-4): 152-158.

Shapiro-Ilan, D. I., D. H. Gouge, and A. M. Koppenhöfer. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus. In Gaugler, R. (ed.), Entomopathogenic nematology. CABI Publishing, New York, New York.

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

Stuart, R.J., M. E. Barbercheck, P. S. Grewal, R. A. J. Taylor, and C. W. Hoy. 2006. Population biology of entomopathogenic nematodes: Concepts, issues and models. Biological Control. 38: 80–102.

Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and heterorhabditid nematodes: a handbook of techniques. Arkansas Agricultural Experiment Station, Fayettville, AK, USA.