RESEARCH

Biological Control of Alfalfa Snout Beetle with a multi-species application of locally adapted persistent entomopathogenic nematodes: The first success.

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Abstract: Alfalfa snout beetle (ASB), *Otiorhynchus ligustici* (L.), is a very severe pest of alfalfa, grown in areas of northern New York and southeastern Ontario, Canada, bordering Lake Ontario and the St. Lawrence River. ASB often kills out entire fields in a single year from larval root feeding when populations are high. This parthenogenetic insect was first reported in North America in 1896 in Oswego Co., NY, where it was introduced by the dumping of sailing ballast between 1848 and 1896. ASB was identified as an alfalfa pest when alfalfa was introduced into the area in the 1920's. With ASB adults and larvae spending all of their 2-year life cycle in close association with soil, a biological control strategy focused on the use of locally adapted persistent entomopathogenic nematodes (EPNs) appeared to be a logical research direction. Research was initiated in 1989 and these studies were conducted primarily on a single farm (the John Peck Farm, located in Great Bend, Jefferson Co., New York) that historically contained extremely high populations of ASB. In 2002, the Peck Farm experienced an unexpected and sudden collapse of the ASB infestation. Subsequent studies on the Peck Farm revealed that the combination of two locally adapted nematodes had dispersed throughout the entire Peck Farm. We believe that the coexistence and soil residence partitioning of *H. bacteriophora* 'Oswego' with the native *S. carpocapsae* 'NY001' together exposed a wider range of life stages of ASB to nematode attack and subsequently applied enough biological control pressure on snout beetle to reduce the population to sub-economic levels. This research suggests that using locally adapted EPNs in a multi-species application provides a more effective approach to the biological control of ASB and potentially that of other soil pests.

Introduction, Life History & Pest Status of Alfalfa Snout Beetle in North America

Alfalfa snout beetle, *Otiorhynchus ligustici* (L.), is a very severe pest of alfalfa in Northern New York state and Southeastern Ontario, Canada. Heavily infested fields are often killed out in a single year from larval root feeding. Alfalfa stand life in moderately infested fields is often reduced to 2-3 yr. Injury by ASB larvae shows characteristic deep scarring and girdling of the tap root (Fig 1A, Fig. 1B). Damage in alfalfa fields suffering from larval root feeding in the fall often mimics and is often misdiagnosed as root rot or boron deficiency. Stand loss in the spring is often blamed on winter kill (Fig. 2A). Flagged plants often show severe root feeding damage by ASB larvae (Fig 2B, Fig. 2C).

This parthenogenetic flightless weevil was first reported as a pest of alfalfa in North America in 1933 in Oswego Co., NY (Herrick 1933). Prior to this time, area farmers had observed their alfalfa dying out in spots, and blamed the alfalfa death on winter kill (Palm 1935a, 1935b). Claassen and Palm (1935) reported ASB larval densities of 250 larvae per 929 cm^2 of soil surface during their initial surveys between 1933 and 1935. At these larval densities, alfalfa roots were totally destroyed and fields were killed out. Shortly after the discovery of alfalfa snout beetle in Oswego, NY, a specimen was located in the National Museum in Washington, D.C. bearing the label "Oswego, New York, May 16, 1896, Wickham," indicating snout beetle had been present in the Oswego area for at least 40 years prior to being identified as a pest of alfalfa (Claassen and Palm 1935). Commercial alfalfa production in the Oswego area was initiated in the early 1920's, allowing ample time for the insect to shift its feeding from wild host plants to alfalfa and the buildup of the large populations observed (Claassen and Palm 1935). York (1974) and Lincoln and Palm (1941) report a list of 20 common plants as viable hosts to ASB larvae, ranging from the cultivated plants of alfalfa and red clover to wild plants including wild carrot, dandelion, and yarrow. This indicates that once ASB becomes established in an area, the area will be infested for a number of years.

The 1933-35 survey estimated that 235 hectares located in two counties were infested (Palm 1936). In 1941, Lincoln and Palm (1941) estimated the infested area to be 1200 hectares, and by 1970

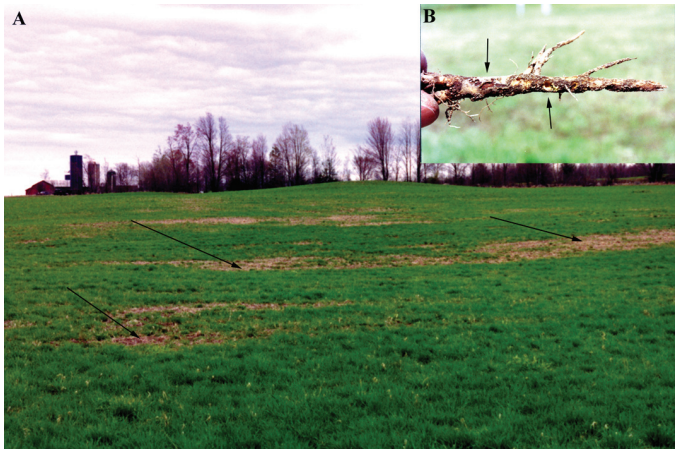

Figure 1. (A) Areas of dead alfalfa observed in the spring from fall larval feeding by alfalfa snout beetle, Otiorhynchus ligustici. (B) Characteristic larval feeding scars on the roots of alfalfa plants. Photos by P. C. Schroeder.

the infested area had grown to 6400 hectares in 3 counties (York et al. 1971). By 2006, the infested area had enlarged to include 200,000 hectares in nine different counties in Northern New York (Cayuga, Clinton, Essex, Franklin, Jefferson, Lewis, Oswego, St Lawrence, Wayne) (NNYADP 2006) and an area of Ontario, Canada across the St. Lawrence River around Prescott, Ontario, Canada (Loan et al. 1986) (Fig. 3).

Lindroth (1957) provided strong evidence that soil-inhabiting insects introduced into North America in the 19th century were transported in sailing ship ballast consisting predominately of soil, sand, and construction debris. Multiple species of *Otiorhynchus*, including *O. ligustici*, were listed by Lindroth (1957) as being present at both European ballasting sites and North American ports. Prior to 1848, all trans-ocean ships were prevented from entering Lake Ontario by the Lachine Rapids on the St. Lawrence River, which produce the largest standing waves in the world. Goods bound for Lake Ontario had to be transferred at Montreal, Canada onto small boats capable of navigating the Lachine Rapids. In 1848, an enlarged canal around the Lachine Rapids at Montreal was opened, allowing trans-ocean ships of ca. 100 tons displacement to enter Lake Ontario and visit the Port of Oswego for the first time (Kingsford 1865). The Port of Oswego is believed to have been directly infested with ASB via shipping ballast

Figure 2. (A) Fall alfalfa plant symptoms caused by severe root feeding by alfalfa snout beetle larvae, *Otiorhynchus ligustici* and characteristic larval root feeding damage on alfalfa (B) and red clover (C). Notice the snout beetle larvae in Photo C. Photos by P. C. Schroeder.

because there are currently no known infestations of ASB around either Montreal or Quebec City, the previous terminal shipping point prior to the opening of the Lachine Canal. It is also unlikely that ASB was introduced during the French and Indian wars of 1754-63 or the Revolutionary War of 1776-83, as most military traffic utilized the Lake Champlain/Lake George water corridor, located ca. 250 km east of the Port of Oswego on the eastern border with Vermont (Eckert 1969, 1978). Alfalfa snout beetle was not detected on the shores of Lake Champlain until May 1991 (Shields, unpublished). Alfalfa snout beetle may have arrived via the Erie Canal, which opened in 1825 with a side canal (Oswego River) to Fulton and Oswego, NY. In the surveys of 1933-35, ASB was not reported around Fulton, NY, a local industrial center and terminal point for Erie canal shipping. The evidence is the strongest that alfalfa snout beetle was introduced to the Port of Oswego on sailing ballast between 1848 and 1896.

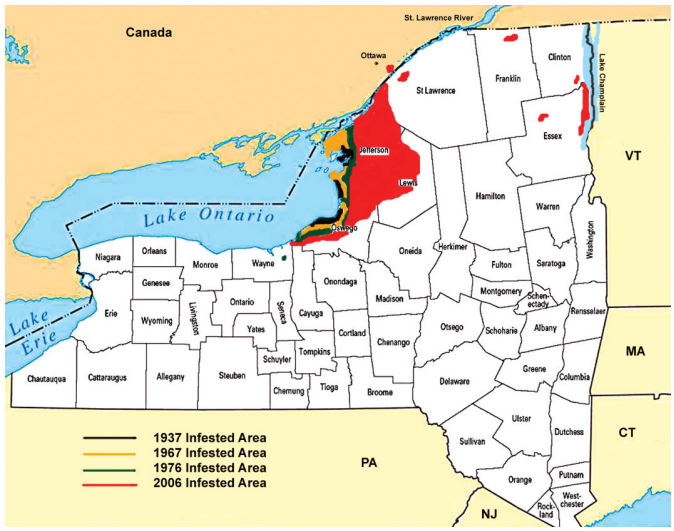

Figure 3. Current known infested area of alfalfa snout beetle, *Otiorhynchus ligustici*, in North America. Map drawn by A. Testa.

Natural spread of snout beetle is relatively slow, as the beetle is flightless and its dispersal seems to be predominantly from the Northwest to the Northeast (Nielsen 1969). The discovery of beetles on the Thousand Islands in the St. Lawrence River implies that water may also be an important means of transport (Nielsen 1969). Human-assisted movement is thought to be the mechanism contributing to the establishment of isolated infestations distant from the main infested area. Movement of adult beetles may be facilitated by hay harvesting equipment and the movement of hay bales (Harcourt and Bereza 1990). The movement of sand, gravel, and soil affiliated with road building, ditch cleaning, new construction, and tiling of fields for better drainage has been suspected as a means of moving ASB larvae and diapausing adults (Willson et al. 1976).

ASB adults diapause 45-60 cm deep in the soil. When soil temperatures at this depth warm to 3.5 °C in the spring, the overwintering adults begin to move to the soil surface (Hanuss 1958). Adult movement to the soil surface can be reliably estimated by monitoring soil temperatures after spring thaw at the 30 cm depth. Schroeder et al. (1995) observed that 90% of the adult beetles had moved to the upper layers of soil when 30 cumulative degree-days (CDD, base 7.0 °C) had accumulated.

Adult emergence from the soil occurs shortly after the beetles move to the upper layers of soil. Emergence depends on soil surface

Figure 4. (A) Newly emerged alfalfa snout beetle adult; (B) mass migration by newly emerged alfalfa snout beetle adults; and (C) collecting newly emerged beetles for laboratory studies and cold storage. Photo A by P. C. Schroeder. Photo B by Dale Morse. Photo C by K. L. Flanders.

temperatures, and movement above ground occurs in a semi-synchronized manner, with thousands of adult beetles moving en masse at the same time (Fig. 4). Adults emerge from the soil reproductively immature, and must feed on available host plants before they can develop and lay eggs. During periods of adverse environmental conditions (cool, cloudy, rainy spring conditions), emerged beetles will shelter in the top 1 cm of soil around the base of the host plants. Pre-oviposition maturation rate is directly influenced by temperature and available food source (York 1974). Red clover and alfalfa are among the highest quality host plants, promoting the fastest egg maturation rates and the greatest fecundity (300-500 eggs per female) (Lincoln and Palm 1941, Hanuss 1958, York 1974).

In New York, oviposition begins during May and June and continues for 5-10 wks (Lincoln and Palm 1941, York 1974). Initiation of oviposition can be estimated using CDD (Schroeder et al. 1995). Eggs are laid 1-4 cm deep in the soil around the base of host plants (Pintér 1955, Hanuss 1958, Lehoczky and Reichart 1968) and hatch within 9-14 d depending on temperature and soil moisture (Lincoln and Palm 1941, York 1974). Small larvae feed on the lateral roots and the larger larvae move to the taproots of the host plant. Early in the summer, the small larvae are present in the top 4 cm of soil. As larvae become larger and soil moisture conditions become drier, the larvae move deeper in the soil profile. By mid-summer, many larvae are 45 cm deep in the soil, and as soil moisture increases in the fall, the late-instar larvae move back up from the deeper depths into the top 3-6 cm of soil to finish feeding and complete their larval development (Palm 1935a, 1935b, Mellors 1977). Larvae pass through 7 instars before pupation, but adverse conditions or poor-quality host plants can cause the insect to add 1-2 additional larval instars (Hanuss 1958). By early November, depending on soil conditions, most larvae mature and move 45-60 cm deep into the soil to overwinter. If soil temperatures drop to 5°C before sufficient feeding has been achieved to complete larval growth, the larvae stop feeding, move to the deeper soil to overwinter, then continue feeding the following spring (Hanuss 1958). The following summer, typically in July and/or August, diapausing larvae pupate and eclose as adults while remaining deep in the soil until the following spring to complete a 2-year lifecycle (i.e. Palm 1935a, 1935b, Lincoln and Palm 1941, York 1974)(Fig. 5).

History of ASB Management:

Poison baits composed of soybean flour, sugar, and toxicant sprayed on peanut shells were the primary tool for snout beetle control from the 1940's through the early 1970's. Prior to 1950, sodium fluosilicate was the toxicant of choice until replaced by heptachlor during the early 1950s. After heptachlor residues were discovered in milk in 1963, sodium fluosilicate was reused as a toxicant. The continued spread of snout beetle prompted the New York State Department of Agriculture and Markets to register heptachlor for use on non-forage cropland (Palm et al. 1941, Lincoln 1942, Muka et al. 1952, York et al. 1971). Area-wide baiting for adult control was discontinued in 1972 due to environmental concerns. Unfortunately, adult control had to be applied after the crop was heavily damaged or destroyed (York et al. 1971).

Chemical control of adults with foliar insecticides is difficult because only a small portion of the adult population is exposed and vulnerable at any one time during the season (Willson et al. 1976). Additional field trials in the early-mid 1990's confirmed the ineffectiveness of foliar applied insecticides in preventing the establishment of a damaging larval population in a field. The effectiveness of foliar insecticides is also complicated by the presence of an extended non-feeding dispersal period after oviposition begins. Fields that have been treated with foliar sprays are re-infested by dispersing beetles from neighboring fields (Shields, unpublished). The early area-wide baiting program was apparently effective in limiting or slowing the spread of ASB because the infested area expanded rapidly after the baiting program was halted (Fig 3).

Without a chemical option to control snout beetle, the only remaining management option was intensive crop rotation. Area farmers were advised to rotate alfalfa every 3-4 year with a non-host crop grown in the field for 2-3 years (Willson et al. 1976). However, to be effective, this approach requires a coordinated community effort, an action that was difficult to implement and maintain (York et al. 1971). Without a suitable and effective management strategy, the snout beetle area of infestation continued to enlarge from the original 2 counties in the 1930's to the nine counties in NY and a

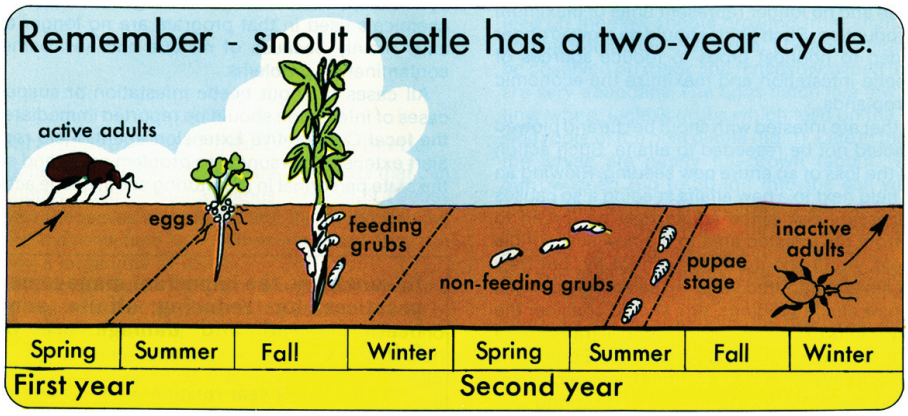

Figure 5. Life cycle of alfalfa snout beetle, *Otiorhynchus ligustici*. Reprinted from Willson et al. 1976. Used with permission from A. A. Muka, the sole surviving author.

small infestation in southern Ontario, Canada present today. Within this infested area, emerging adult populations frequently exceed 2 million beetles per hectare and affected fields are routinely totally destroyed from the larval feeding in a single season (Fig. 3).

Biological Control of ASB with Persistent Entomopathogenic Nematodes

All alfalfa snout beetle life stages are spent in the soil as larvae/pupae or in close contract with the soil surface as adults. This close soil contact makes ASB susceptible to attack by entomopathogenic nematodes (EPNs), and the use of EPNs for long-term biological control of snout beetle appeared to be a feasible management strategy and a logical research direction. The logic of this research direction was strengthened by the susceptibility of a close relative, the black vine weevil, *O. sulcatus,* to nematode attack (Shanks and Aqudelo-Selva 1990) and by studies indicating that EPNs can persist in soils for 250 d (Jansson et al. 1991) and 385 d (Klein and Georgis 1992). EPNs have also been shown to be effective and persistent in a variety of soil types (Kung et al. 1989). However, because alfalfa is a low-value crop, the high cost of inundatively releasing entomopathogenic nematodes for the field-wide control of alfalfa snout beetle prohibited the use of this management strategy. Our research instead focused toward the identification of native or cold-adapted species and strains of nematodes that were capable of natural long-term persistence in local soils and showed an effective pathogenicity to snout beetle and white grubs. Both insects can cause substantial losses in forage crops that commonly contain mixed alfalfa/grass plantings.

Isolation, Identification, and Evaluation of Locally-Adapted EPNs

During 1990, three entomopathogenic nematode species *(Steinernema carpocapsae* 'NY-001', *Steinernema sp.* 'NY 008-2E' (later identified as *S. feltiae*) and *Heterorhabditis bacteriophora* 'Oswego') were collected using traps baited with greater wax moth larvae from fields located in Oswego Co., New York that were currently or historically infested with alfalfa snout beetle. All three nematodes effectively attacked the larvae of alfalfa snout beetle and Japanese beetle in the laboratory and greenhouse (Schroeder et al. 1993, 1994).

Grewal et al. (1994) classified EPNs into two major categories by means of their dispersal and foraging strategies. *S. carpocapsae* is considered an ambush nematode, whereas *H. bacteriophora* is considered a cruising nematode. *S. feltiae* adopts an intermediate behavior between both strategies. These behaviors were reflected in a subsequent field study, which reported that *S. carpocapsae* 'NY001' preferred the top 5 cm of the soil profile, with a few individuals infecting hosts past 10 cm, and *S. feltiae* 'NY008-2E' preferred the top 15 cm, with a few individuals infecting hosts down to 20 cm. In contrast, *H. bacteriophora* (both 'Oswego' and a laboratory strain 'NC') was found 0–35 cm deep in the soil profile, thereby showing the expected tendency to move throughout the soil profile (Ferguson et al. 1995). All three of the NY native strains (NY001, NY008-2E, and Oswego) significantly persisted for at least 24 months in the field plots, while the population level of the laboratory strain (NC) declined during the same period. These data also suggested that different nematode species may partition the soil profile in a manner that would allow the coexistence of two or more nematode species for improved biological control of soil insects that move across the entire 35 cm soil profile.

With various life stages of alfalfa snout beetle occupying and passing through more than 35 cm of the soil profile, Shields et al. (1999) conducted a field trial to evaluate the efficacy and persistence of two strains of *H. bacteriophora* (NC & Oswego), a nematode species that occupies the top 35 cm of the soil profile. In the field study, two different inoculation levels (2.5 and 15 billion IJ/hectare) and two different application techniques (flat fan and stream nozzles) were compared in a field heavily infested with alfalfa snout beetle larvae. The laboratory strain 'NC' reduced the insect larval numbers to the lowest level, but could only be detected in the plots at a very low level (10% or less of the soil cores contained nematodes) 328 days after application and was completely absent 708 days after application. The 'Oswego' strain also reduced the larval snout beetle populations to a significantly lower level than the control, but persisted significantly better than the 'NC' strain (60-90% of the soil cores containing nematodes) 328 days after application and remained in 45-70% of the soil cores at 708 days after application. In the second year of the study, most of the surviving alfalfa plants died from secondary pathogen invasion through the snout beetle larval feeding wounds (Leath and Hower 1993). Measured levels of root feeding damage from this study suggested that snout beetle larval mortality from *H. bacteriophora* occurs late in the season after the root feeding damage has occurred. A natural and evenly distributed population of *S. carpocapsae* 'NY001' was detected across the entire plot area during the study. Although this nematode probably contributed to ASB mortality, its presence was not considered to have a significant impact on the results of the study. The continued presence of *S. carpocapsae* 'NY001' for the duration of the study suggested, however, that the coexistence of two nematode species can occur under field situations.

Viability of the Multi-species EPN approach

A population bloom of the native *S. carpocapsae* 'NY001' was detected in numerous field studies during the mid-1990s, coinciding with the presence of small snout beetle larvae in the top 5 cm of soil. While *S. carpocapsae* 'NY001' evidently attacked and recycled in the snout beetle larvae, sufficient numbers of ASB larvae survived to kill out a large number of alfalfa plants. The emerging adult population, 18 months later, commonly exceeded 2 million beetles per hectare. Data reported by Ferguson et al. (1995) suggested that different species of nematodes may prefer different niches in the soil profile and data from Shields et al. (1999) suggested that the native strain of *S. carpocapsae* 'NY001' could co-exist with the native strain of *H. bacteriophora* 'Oswego.' Because snout beetle larvae and adults move through more than 35 cm of the soil profile, the presence of two different species of EPNs with different preferred soil niches may improve the biological control of ASB by increasing the time in which ASB is exposed to nematode attack. Additional information about the long-term co-existence of multiple nematode species and suggestions about improved biological control of ASB was provided by field observations in Hungary, where snout beetle is sub-economic when *S. carpocapsae* and *S. feltiae* are present in the field (Neumann 2003, Neumann and Shields 2004).

Alfalfa snout beetles are exposed to entomopathogenic nematodes at different stages of their lifecycle and at various depths within the soil profile. For example, adults are exposed to nematodes in the spring of the year, when soil warms to 3.5°C and the overwintering adults begin moving up to the soil surface from their overwintering site 45-60 cm deep. These pre-emerging adult snout beetles would be susceptible to nematode attack if the nematodes present in the soil

profile were able to successfully attack the host at soil temperatures ranging from 3.5 to 10°C. In addition, emerged adults are in frequent contact with the upper soil layers during the next 5-10 weeks as they deposit eggs around alfalfa roots in the top 4 cm of soil with soil temperatures increasing to 20°C.

Neumann (2003) investigated the susceptibility of ASB adults to nematode attack and the ability of *S. carpocapsae* (NY001 & Valko strains), *S. feltiae* (Valko & SF22 strains) and *H. bacteriophora* (Oswego & Szada strains) to infect beetles at temperatures ranging from 4°C to 10°C. For all the *Steinernema* sp. strains tested, infection of ASB adults started with a 10% mortality at 4°C and ranged from 80-100% mortality at 6°C. In contrast, *H. bacteriophora* (Oswego & Szada strains) started infecting adults at 8°C (25-45% mortality) and achieved 100% mortality at 10°C.

These data strongly suggest that adult beetles are susceptible to nematode attack during the long periods of time they spend in contact with the soil when resting or ovipositing in the upper soil layers. The early springtime attack of ASB adults by *Steinernema*, as suggested by these laboratory data, is supported by field collections of newly emerged adult snout beetles that were infested with entomopathogenic nematodes from areas of Hungary where snout beetle populations remain very low and sub-economic. Although the exact identity of these nematodes was not confirmed, they morphologically appeared to be *Steinernema sp.* (Neumann 2003). Nematode assays of soil samples collected the following spring from areas where nematode-infected beetles were found indicated that both *S. carpocapsae* and *S. feltiae* were present in the alfalfa fields.

Although many researchers consider *S. carpocapsae* to be an ineffective biological control organism in many systems, the presence of this nematode in the top 5 cm of soil where ASB adults are largely active and the ability of this nematode to attack adult beetles at relatively low temperatures (4-6 °C) promotes its significance as a natural mortality factor of ASB adults in the field and should not be discounted.

The susceptibility of ASB larvae to nematode attack begins when newly hatched larvae start to feed on roots in the upper soil layers where eggs are deposited, typically in the top 4 cm of soil. During this time, small larvae are particularly susceptible to attack from *S. carpocapsae*, which dominates the upper 5 cm of the soil profile. The annual "bloom" of *S. carpocapsae* in the snout beetle infested fields in June and July coincided with the presence of newly hatched snout beetle larvae in the top soil layers, suggesting that *S. carpocapsae* was attacking and recycling in the small snout beetle larvae before the larvae move deeper in the soil profile, below the zone of *S. carpocapsae* activity (Neumann 2007, Neumann and Shields 2007). Although larvae move below the soil layer inhabited by *S. carpocapsae*, they remain exposed to *S. feltiae* and *H. bacteriophora* down to 20 cm and 35 cm, respectively, if those EPN species are present in the soil profile (Ferguson et al. 1995). By mid-July, larger snout beetle larvae move deeper in the soil, often deeper than 35 cm. These larvae remain deep in the soil until soil moisture increases in the fall, typically early September, when they begin moving back into the upper soil levels and exposure to EPNs present at those depths.

In a laboratory study using multi-piece sand columns and *Galleria* larvae as nematode hosts, Neumann and Shields (2006) supported the suggestion that different nematode species have preferred niches in the soil profile. In this study, *S. carpocapsae* infected hosts throughout the 32.5 cm sand column when applied alone. However, when applied with either *S. feltiae* or *H. bacteriophora, S. carpocapsae* would completely dominate the upper layers of soil, leaving the lower soil profile to the second nematode species. When all three species were combined in the study, *S. carpocapsae* continued to dominate the upper 6.5 cm of the sand column, and *H. bacteriophora* dominated the 26-32.5 cm soil depth, allowing *S. feltiae* to maintain its population in the 13-19.5 cm levels. In the spring of 2004, a 30-month companion field study was initiated to investigate nematode species coexistence under field conditions. In this study, all two-species combinations were able to maintain their populations in the field while reducing the larval populations of alfalfa snout beetle. However, in the three-species combination, *S. feltiae* could not be recovered after 14 months following application, suggesting that *S. feltiae* could not successfully compete with the other two species (Neumann 2007, Neumann and Shields 2008).

Laboratory, greenhouse, and field data suggest that a combination of nematode species would provide the highest level of biological control for alfalfa snout beetle. The presence of *S. carpocapsae* in the upper layers of soil apparently provides an initial mortality factor for the newly emerged adult beetles, who remain in close contact with the soil surface beneath plants and duff to avoid harsh spring conditions (in the top 1 cm of soil) and to lay eggs (in the top 1-4 cm of soil). *S. carpocapsae* also seems to provide an initial mortality factor for the newly hatched larvae and small larvae feeding on lateral roots before moving down below the "carpocapsae layer" and feeding on the taproot of the plant. The presence of a second nematode species in the field exposes the snout beetle larvae that have escaped the "carpocapsae layer" to a second mortality factor. Root feeding injury observed in fields inhabited by both *S. carpocapsae* and *H. bacteriophora* suggests that larval mortality from *H. bacteriophora* occurs over a longer period of time, during the 3-4 months in July-October when larvae move deeper in the soil to avoid the hotter, drier upper layers of soil in the summer and then return to the upper soil layers in late summer and fall, when the soils again become cooler and more moist. Soil populations of *H. bacteriophora* also peak in the fall, supporting the suggestion of late-season larval mortality due to these nematodes. The absence of root feeding injury in ASB-infested fields that were simultaneously inhabited by *S. carpocapsae* and *S. feltiae* suggests that larval mortality from *S. feltiae* occurs during mid-summer, before the larvae become large enough to move to the tap root and cause deep feeding scars (Neumann and Shields 2008, Neumann 2007).

While the lack of deep root scarring in the *S. carpocapsae* X *S. feltiae* combination appears to be advantageous over the root scarring that occurs in the *S. carpocapsae* X *H. bacteriophora* combination, the relative effectiveness of each of the EPN combinations to exert and maintain effective ASB control in the field is difficult to determine, based on the current accumulated data. Further field experiments need to be designed and conducted to answer these questions.

ASB Population Crash on the Peck Farm

Field experiments testing the effectiveness and persistence of nematodes against ASB were conducted on the John Peck farm, located in Great Bend, Jefferson Co., New York from 1991 until 2006. ASB populations were consistently very high on the Peck farm since it was initially invaded in the mid-1980's. Even though many of the fields had large resident populations of *S. carpocapsae* ('NY001'), ASB populations estimated in individual fields during beetle emergence often exceeded five million adult beetles per hectare (Ferguson et al. 1995).

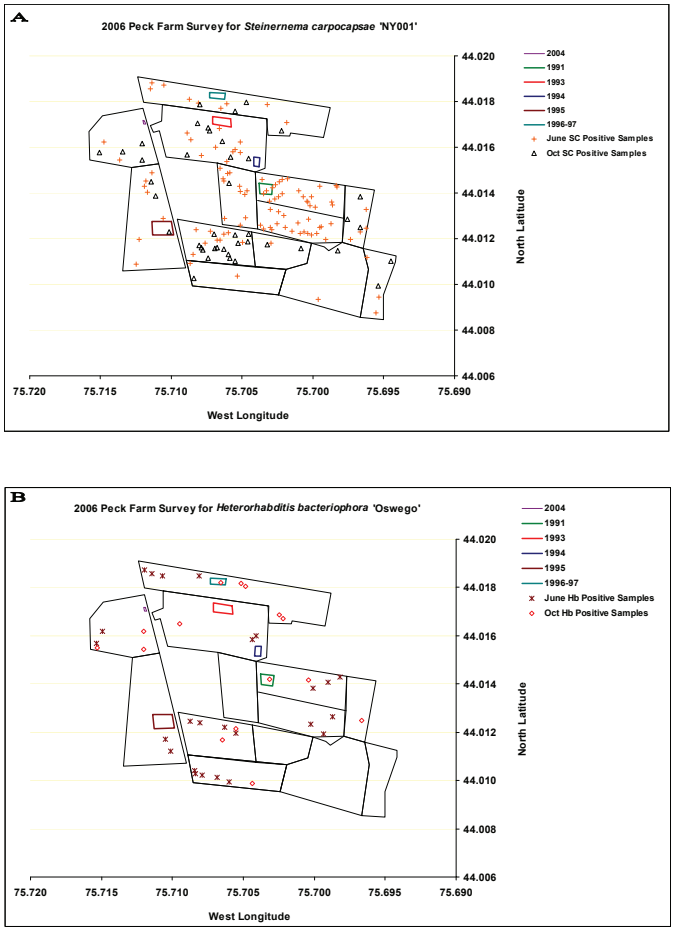

Figure 6. Detected locations of Steinernema carpocapsae 'NY001' (A) and Heterorhabditis bacteriophora 'Oswego' (B) during a 2006 farm-wide nematode survey on the Peck farm. The rectangular colored boxes were the location of the original 'Oswego' release sites during various field experiments. Figure by A. Testa.

In the first 13 yr of this study (1991 to 2004), a series of seven different field plots were established in five different fields on the farm (Fig. 6) to investigate nematode application methods, application rates, application timing, and nematode efficacy. These studies primarily focused on the effectiveness of the isolated native strain of *H. bacteriophora* (strain 'Oswego') in causing ASB larval mortality. Different locations were required for each of the series of experiments over the years because *H. bacteriophora* 'Oswego' is native to Northern NY and remained in the plot areas for more than 7 yr.

Populations of emerging of alfalfa snout beetle adults were consistently heavy, with only moderate variations on the Peck farm between 1986 and 2001. However, in 2002, the spring emergence of snout beetle on the Peck farm was extremely low, but still present in high numbers at other nearby locations. Adult beetle emergence has remained at extremely low to absent levels during 2003-2007, after more than 15 yr of consistently large emergences. The farmer reported during the winter of 2004-05 that he had plowed down the first surviving alfalfa plants in old hay fields since the mid-1980s, when snout beetle first invaded his farm from neighboring fields. A fall 2005 snout beetle larval survey of the entire farm indicated that snout beetle larvae were still present in very low numbers on the farm, but they were difficult to detect and the associated root feeding damage was absent in the farm's fields, despite large populations of snout beetle larvae and high levels of root feeding injury continuing on neighboring farms (Shields, unpublished). The question was: What happened to alfalfa snout beetle on the Peck Farm to cause the population crash?

To understand the population crash of ASB, soil samples were collected from fields throughout the Peck Farm and bioassayed in 2006 for the presence of EPNs. The sampling effort was divided into two sampling periods (June & September), each consisting of 1000 samples. The number of samples taken in each sampled field was proportioned to the acreage of the field. To provide a representative picture of the nematode population on the farm, sampling locations were distributed evenly throughout each field. A 2 cm diameter x 25 cm long soil core was taken at each sample location. The top 5 cm of the soil core was separated from the lower 22 cm of the soil core and each portion of the core was placed in a separate cup. Each sample cup was bioassayed separately for entomopathogenic nematodes. Since *S. carpocapsae* dominates the upper 5 cm of the soil profile, the upper and lower portions of the soil sample were bioassayed separately to prevent the presence of *S. carpocapsae* from masking the presence of other entomopathogenic nematode species in the soil sample. A set of GPS coordinates was taken with a Garmin™12 at each soil sampling location and recorded, then later mapped (Fig 6).

The survey results showed that the nematode native to the site, *S. carpocapsae* 'NY 001,' occurred throughout the farm, and *H. bacteriophora* 'Oswego,' which was introduced into small plots located in 5 different fields, had spread into most fields on the farm, including fields where the nematode was not released (Fig. 6). Data from Shields et al. (1999) indicates that *H. bacteriophora* 'Oswego' causes a ca. 70% mortality rate among the snout beetle larvae when present. These data coupled with the farm-wide nematode survey data suggest that *H. bacteriophora* 'Oswego' moved from the research plots where they were applied to throughout the farm over a 5-10 year window. These field data also suggest that *H. bacteriophora* 'Oswego' coexisted with the native *S. carpocapsae* 'NY001' and together applied enough biological control pressure on snout beetle to reduce the population to sub-economic levels.

During the 2006 farm-wide survey, the frequency of soil samples that tested positive for nematodes ranged from 2%-34% depending on the field sampled. Fields containing a previous research plot or fields immediately adjacent to those fields contained the highest percentage of positive soil cores. Fields the most distant from release sites contained the lowest frequency of positive soil cores. The spread of *H. bacteriophora* 'Oswego' throughout the farm may have occurred by natural movement of the nematodes in infected beetles before the beetles died and/or through the movement of nematode-inhabited soil throughout the farm by soil-laden farm machinery. The frequency of the nematode positive samples on the Peck Farm (2%-34%) was similar to sample frequencies in the areas of Hungary where snout beetle is native and held under effective biological control (3%-8%). The frequencies observed in Hungary are lower than the frequencies observed in New York, possibly because soil samples in Hungary were taken much earlier in the year (March) before nematode recycling had started and because snout beetle populations in Hungary were at a very low level, presumably due to the effective biological control (Neumann 2003, Neumann and Shields 2004).

Our research efforts will continue to monitor the presence and distribution of both nematode species along with the population levels of snout beetle to see if the presence, distribution, and frequency

of nematodes change over the next few years and if the population of snout beetle remains at sub-economic levels.

Summary and Conclusions

This study illustrates that entomopathogenic nematodes can be successfully used as a long-term biological control strategy against the alfalfa snout beetle. Although the success of this effort stemmed partly on the effective pathogenicity of nematodes, several key factors enhanced the success of using nematodes as a biocontrol agent against ASB:

1) The ability of nematodes to naturally recycle within the host, persist in the soil profile, and effectively disperse with the host, thereby exerting a sweeping, perpetual attack of the host insect, and

2) The tendency for different nematode species to coexist and partition their residence and activity within the soil profile, thereby broadening the exposure of the host insect and its various life stages to nematode attack.

The ability of *S. carpocapsae* to recycle in ASB adults and larvae was shown by the early-mid summer increases in the population of *S. carpocapsae* that coincided with the presence of ovipositing snout beetle adults and foraging small larvae in the upper soil layers (Neumann 2003, Neumann 2007, Neumann and Shields 2007). The ability of nematodes to disperse with their host was shown by the subsequent spread of *H. bacteriophora* 'Oswego' from experimental plots to areas where they were not released. These studies revealed how key nematode behaviors, namely the ability of the nematodes to recycle, persist, and disperse, enhanced their effectiveness as a biological control agent by permitting a sweeping, perpetual attack of ASB on the Peck Farm.

The tendency for different nematode species to partition and dominate different soil niches was shown by field studies conducted on the Peck Farm (Ferguson et al. 1995), soil column experiments performed in the laboratory (Neumann and Shields 2006, Neumann and Shields 2007), as well as field studies completed in Hungary (Neumann 2003). The continued high populations of ASB on the Peck Farm despite the persistent natural presence of *S. carpocapsae* 'NY001' showed that this nematode, despite its evident early seasonal attack of larval ASB, was not alone sufficient to reduce and then maintain the population of the snout beetles at sub-economic levels. The later introduction of *H. bacteriophora* 'Oswego' and the subsequent 70% increase in snout beetle larval mortality showed that these two nematode species together provided sufficient pressure on ASB larvae and adults to reduce ASB populations to a sub-economic level (Shields et al. 1999). These studies revealed how nematodes coexist in a soil profile and how a multi-species approach with nematodes enhanced their effectiveness as a biological control agent by broadening the exposure of ASB and its various life stages to nematode attack.

Spreading the success: Focus of future research

The success of this effort and the challenge of achieving similar successes in the nine-county region containing numerous farms infested with ASB raises a number of key questions and provides a focus for continued research. Evidence from the studies conducted on the Peck farm strongly suggest that only an inoculative release of each nematode species is required in each field for the nematodes to become established. Once established, the nematodes will likely spread through each nematode's natural movement, the movement of live but infected adult snout beetles, and by the movement of soil around the farm during farming operations. The remaining challenges are:

1) How can growers cost-effectively obtain and apply locally adapted EPNs? The high cost and weak field persistence of many commercially produced nematodes prohibits the broad scale use of commercially reared nematodes for ASB control in a low-value alfalfa crop. A more cost-effective source of efficacious nematodes is needed before this strategy can be welcomed and adopted by alfalfa growers. The use of locally isolated persistent EPN strains requires alfalfa growers to inoculate their fields only once for long term control, thereby greatly reducing the cost of application. Techniques need to be developed to allow individual farmers to rear and release their own EPNs.

2) What is the critical dose of EPNs (number per water volume per soil surface area) for inoculation and establishment? The field inoculation approach with persistent nematode strains for long-term biological control differs from using commercially produced nematodes. Commercially reared nematodes are generally adapted to the laboratory environment and require an inundative release or "biopesticide" approach to be effective. Further studies can reveal what dosages are necessary to effectively establish locally adapted species or strains of nematodes in the field.

3) What is the best inoculation strategy for establishment and spread? Additional studies are needed to determine which of two inoculation strategies is best for establishing nematodes after an inoculative release: 1) Applying high rates of nematodes in a relatively small area, thereby ensuring establishment, but then relying on natural nematode movement and normal farm activities to disperse the nematodes over a larger area; or 2) Applying low dosages of nematodes over much larger areas, thereby ensuring dispersal, but possibly compromising establishment.

4) What is the best nematode mix for long-term management? The success on the Peck farm was achieved by introducing *H. bacteriophor*a 'Oswego' to fields already naturally inhabited by *S. carpocapsae* 'NY 001.' Field data showed that this species combination was very effective in reducing snout beetle populations, but was slow in preventing plant root feeding injury, contributing to some level of sustained stand loss. However, after the established nematodes effectively reduced the snout beetle population over time, stand longevity was regained in subsequent plantings. When the two combinations of nematodes used in this study (*S. feltiae* 'NY 008-2E' + *H. bacteriophora* 'Oswego,' and *S. carpocapsae* 'NY001' + *S. feltiae* 'NY 008-2E') were compared, the first combination was just as effective in reducing ASB population over time as the second, but the first combination resulted in a higher sustained level of plant damage in the field (Neumann 2007, Neumann and Shields 2007). Large-scale field trials can determine the best species combinations for the long-term management of snout beetle.

5) What are the best cropping rotations to maintain and promote soil populations of EPNs in the ASB infested area? While EPNs recycle in a wide array of soil insects, additional studies need to be conducted that will identify and examine cropping systems and rotation durations that promote nematode persistence.

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