# Northern NY Agricultural Development Program
## 2007-2008 Project Report

### Biological Control of Alfalfa Snout Beetle:

### Developing a Farmer-Friendly Rearing and Application Method to Speed the Spread of the Biocontrol Nematodes

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**Background:**

Alfalfa snout beetle, within the infested counties, continues to be the single most contributing factor to alfalfa death and stand loss in NNY. Snout beetle related stand loss is often identified as winter kill because the majority of plant death occurs after the growing season during the fall and early winter. This insect was introduced into NNY at the port of Oswego between 1848 and 1896, when the first individual was collected and identified. Snout beetle became a major pest problem after alfalfa was introduced into NNY in the 1920s. Attempts were made to control this insect from the 1940s to 1972 with the widespread distribution of poison baits. During this time, snout beetle continued to spread. Widespread baiting ended in 1972, due to environmental concerns and the snout beetle population exploded in the early 1980s. Research focused on the use of biological control to suppress snout beetle was initiated in 1990 and has been supported in part by NNYADP since its initiation.

In 2002 and 2003, the snout beetle population on the Peck Farm (Great Bend, Jefferson Co.) crashed from about 1 million beetles per acre to an extremely low level. Subsequent

research has shown that the entomopathogenic (insect attacking) nematodes released on the farm in a series of small plots during 1993-98, have been moved throughout the farm by farming practices and caused the population crash of snout beetle. A small rebound of beetles was observed in a field bordering a neighbor's heavily infested farm in 2007 and 2008. John Peck's farm has progressed from having the alfalfa stands completely killed out during the first production year from snout beetle feeding to actually plowing alfalfa plants down at the end of an alfalfa stand's life (5 years). With the establishment of 6 different small test plots on the Peck farm, it required nearly 10 years for the nematodes to spread throughout the farm and control snout beetle.

The question is: "How can we move the biological control success on the Peck Farm to the rest of the infested farms in the NNY?"

Since the entomopathogenic nematodes used as the biological control for snout beetle are adapted to NNY, a farm or field only needs to be inoculated once for the establishment of the nematodes in the field. If only a single field per farm is inoculated, the farmer will move the nematodes around the farm with the movement of soil during normal farming operations, but it will take years for the nematodes to become established in all of the affected fields on the farm and control the population of snout beetle on the farm. However, if a farmer friendly method could be developed to rear the nematodes on the farm and a method of application be developed compatible with on-farm equipment to inoculate individual fields, then each farmer could more rapidly spread the biocontrol nematodes throughout their fields for faster control of snout beetle.

In 2007 and 2008, we shifted our research focus to address the problems associated with large-scale application of nematodes for area wide control of snout beetle. The focus and goal of the projects was shifted to develop the necessary components which would allow the ASB-Biocontrol nematodes to be inoculated into fields throughout the 9 county infested region by farmers themselves or by commercial applicators.

## Methods & Results:

**Nematode Field Application Techniques Compatible with Large-scale Agriculture.** In 2007, two field sites were established in southern Jefferson Co. on the Doug Shelmidine farm to test nematode field inoculation methods into established alfalfa fields compatible with current farming practices. At each site, four different soil inoculation techniques were tested. The inoculation methods tested were 1) nematode infected insect cadaver placed on the soil surface, 2) nematode infected insect cadavers placed four inches under the soil surface (a natural condition), 3) nematodes suspended in water and applied to the soil surface (a method used in experimental plots for more then 10 years) and 4) nematode infected soil placed on the surface.

Soil samples to bioassay for the presence of nematodes were collected five different times between June 2007 and October 2008 (22 days, 69 days, 109 days, 371 days & 420 days after inoculation). Soil samples were collected at 6 inch intervals from the initial inoculation site to document natural nematode spread from the single point of inoculation. The results indicated that the two best methods to inoculate fields with the biocontrol nematode were buried infected insect cadavers and nematodes suspended in

water. Since the application of a water suspension of nematodes is more compatible with agricultural practices than burying insect cadavers, all subsequent research utilized the water suspension method to inoculate fields. During the 420 day duration of this study, nematodes persisted in both locations and movement as far as 9.5 ft from the point of initial inoculation was recorded. The detected movement of the nematodes in this study shows that nematodes are capable of moving away from the point of inoculation without the movement of soil during tillage.

**Economical Mass-rearing of Biocontrol Nematodes (Farmer Friendly.)** Traditionally, the high cost of rearing nematodes was a major obstacle to using nematodes in a wide-spread biological control effort. These high rearing costs were mainly a result of the high labor costs and specialized laboratory supplies required for nematode rearing. By tossing out all of the published rearing procedures and starting over with a clean sheet of paper, we have been able to slash rearing costs by 350% and eliminate specialized laboratory equipment. To produce 100 million nematodes for a single field release, the old rearing technique required 8 hours of labor and $65 of laboratory materials for a total cost of $175. In contrast, the new rearing technique required 2 hours of labor and cost $50 to produce 100 million nematodes for field release. This $50 cost is broken down into $20 materials (insect larvae to rear nematodes) and $30 labor. With the new technique, the insect larvae used to produce the nematodes arrive in plastic tubs filled with fine wood chips. The lids are opened and 5000 infective nematodes suspended in 10 mls of water are applied to the wood chips. The nematodes then search out the larvae buried through the wood chips, infect the larvae and reproduce. After inoculating the tub, the lid is replaced and the tub is placed at room temperature for 15 days. By 15 days, the new nematodes have emerged from the insect cadavers and are dispersed throughout the wood chips. The tubs filled with wood chips, nematodes and insect cadavers are transported to the field where the tubs are dumped onto a fine screen over a 5 gal bucket. The wood chip mass is washed with a high pressure water stream and the nematodes are flushed out of the wood chips and into the bucket. The bucket of nematodes is then dumped into a spray tank for application in a field.

This new rearing technique was used to rear approximately 10 billion nematodes for field application during the 2008 growing season. Of the 10 billion nematodes produced 7.2 billion were applied to 36 fields distributed throughout the NNY snout beetle infected region. Below is the breakdown by species for the 2008 fields:

*Heterorhabditis bacteriophora* 'Oswego' – 2.8 Billion
*Steinernema carpocapsae* 'NY001' – 2.7 Billion
*Steinernema feltiae* 'Valko' – 1.7 Billion

**Field Application of Nematodes.**
Nematodes were applied to alfalfa fields using a custom built 8 ft spray boom which fit into a trailer hitch receiver mounted on a 2-wheel drive pickup. There were two sets of nozzles on the spray boom. The first set of nozzles was located on the front side of the boom and used only the nozzle bodies with the nozzles (and screens) removed. Nematodes suspended in water were applied through these nozzles bodies as a dribble of water. A second set of nozzles were mounted on the back side of the boom and utilized high volume flat fan nozzles. The purpose of this set of nozzles was to wash any nematodes off of the alfalfa foliage and provide extra wetting of the soil surface to

facilitate nematode entry into the soil. Two 50 gallon spray tanks were mounted in the truck bed with one tank used for nematode application and the second tank used for the "rain storm". Pressure to the two set of nozzles by four 12 V pressure pumps rated at 5 gpa. Two pumps were hooked in parallel to each tank to provide pressure to each set of nozzles. The spray system was powered by the pickup electrical system and was controlled by a switch panel located in the cab of the truck.

Nematodes (100 million) were applied to the field in four strips across the grain of the field. In this manner, any tillage of the field would help to distribute the nematodes from the strips along the length of the field. Each application strip was approximately 24 ft wide (3 truck passes) x 200 ft long. The spray system was calibrated to apply 50 gallons of nematode laden water and 50 gallons of supplemental water over this area (ca. 12,000 sq ft of the field)

Field application of nematodes required the alfalfa to be relatively short to allow the nematode laden stream of water from the spray nozzle to easily reach the soil surface. The nematodes once applied to the soil surface, needed to penetrate the soil before dying from desiccation or UV exposure. The ideal alfalfa field for application was a field with about 6" of re-growth after cutting. The ideal time of day for nematode application was late afternoon-evening which reduced nematode mortality from UV exposure and desiccation. Field applications were focused on the time intervals following 1st and 2nd cuttings. Project leaders spent most of the month of May setting up the contacts, scouting the field locations and trying to estimate the harvest dates for individual fields so nematodes could be produced in a timely manner for application in the field.

Field applications focused on the 1st harvest were initiated during the first week of June and continued for the entire month. A total of 19 fields were inoculated during the 1st harvest interval with the field located in Jefferson and Lewis counties. Field applications targeting the 2nd harvest interval were initiated in early July and were located primarily in Franklin Co and St. Lawrence Co. A total of 17 fields were inoculated with nematodes during the 2nd harvest interval.

Table 1: Time Table of Field Applications

| **Lewis County** | **Jefferson County** | **St. Lawrence County** | **Franklin County** |
|---|---|---|---|
| June 5 – 3 fields | June 12 – 1 field | July 30 – 6 fields | July 23 – 4 fields |
| June 11 – 4 fields | June 25 – 1 field | July 31 – 3 fields | July 24 – 4 fields |
| June 12 – 1 field | June 26 – 3 fields | | |
| June 18 – 3 fields | | | |
| June 19 – 3 fields | | | |
| June 25 – 1 field | | | |

Table 2: Breakdown of Producer Location and Number of Fields

| **Lewis County** | **Jefferson County** | **St. Lawrence County** | **Franklin County** |
|---|---|---|---|
| 15 fields | 5 fields | 9 fields | 8 fields |
| 7 Producers | 5 Producers | 4 Producers | 5 Producers |

Nematodes were successfully established in all fields in which they were applied. Experimental results from the Peck farm in past years, indicated that snout beetle could be effectively controlled using one of three different combinations of nematodes species. To test these results on a larger scale, different nematode combinations were selected for field sites in different counties. The three different nematode combinations were 1) *S. carpocapsae x H. bacteriophora,* 2) *S. carpocapsae x S. feltiae* and 3) *S. feltiae x H. bacteriophora.* In all cases, there were high levels of establishment of *S. carpocapsae* and *S. feltiae* regardless of the nematode combination and environmental conditions. In contrast, establishment of *H. bacteriophora* was variable across field sites and at a much lower level than desired. *H. bacteriophora* appears to be more sensitive to a wide array of environmental conditions during application in the field than the other two nematode species. Reasons for the variable establishment of *H. bacteriophora* is still being examined and analyzed.

## Conclusions/Outcomes/Impacts.

The opportunity to blend funds and research/demonstration efforts from both the NNYADP and NYSFVI program together during 2008 allowed us to make tremendous strides toward the development of a production/application system which is technological feasible for adoption by commercial applicators and farmers themselves. These advances will allow the rearing and application of nematodes to move from the university research setting into the "real world" where biocontrol nematodes can be applied to infested fields across the infested counties at an intensity desired by individual farmers.

Our goal in the next two years is to work closely with both interested commercial applicators and interested individual farmers to teach them nematode rearing, and nematode application techniques. We hope to work with all interested individuals. In any biological system, quality control is an important issue. During our transition toward farmer/industry nematode rearing and application, we plan to use our soil bioassay procedure to verify successful field applications of nematodes by each individual. Our efforts to apply nematodes to 17 commercial fields reinforced the importance of three factors: 1) Nematode applications need to be applied during the evening hours due to their sensitivity to UV. 2) The amount of alfalfa regrowth in a field is important. A greater level of regrowth provides an increased level of soil surface shading and provides a higher level of protection for the nematodes while they are penetrating the soil. 3) Chlorinated water needs to be avoided at farms when filling water tanks for nematode applications.

There are still many unanswered questions about the long-term persistence of these biocontrol nematodes under the widespread soil conditions and various crop rotation plans common across the nine snout beetle infested counties. These questions need to be addressed in the near future for the biological control of snout beetle with entomopathogenic nematodes to be successful on a large scale.

## Outreach:

National-Regional News Coverage:

Due to the efforts of Kara Lynn Dunn, NNYADP publicist, the story of alfalfa snout beetle, its devastating impact on NNY alfalfa and promising research progress was

brought to the attention of an AP reporter who wrote an article for the AP wire. This article about snout beetle was widely distributed and printed in papers throughout the Northeastern region of the US and was even published in papers in Florida. The long-term support of NNYADP was mentioned in the article.

Professional Popular Journals:
With the infested area of alfalfa snout beetle in North America limited to nine New York counties and a small area in the providence of Ontario, Canada, many government officials in USDA and other entomologists are unaware or poorly informed about this serious insect. In an effort to educate these important groups of professionals, an article was written about the history of snout beetle along with current research progress and submitted for publication in the American Entomologist. This article with color pictures has been accepted for publication. The American Entomologist is a general interest publication widely read by entomologists, including individuals in USDA and APIS.

Extension Outreach:
March 2008: Update on the Biological Control of Alfalfa Snout Beetle and the Effort to Develop Snout Beetle Resistant Alfalfa. Presented in Madras and Carthage.
October 2008: Talk segment about Alfalfa Snout Beetle Damage, Biological Control and Resistant Alfalfa Development. Agribusiness Dealer Meetings and presented in four locations across the state.
March 2009: Snout Beetle Updates planned for Miner Institute, Madras and Carthage.

## Next steps.

The next logical steps are to expand the program into Clinton and Essex counties and to begin the movement of the program into the private sector. With a new snout beetle infestation identified in east-central Clinton Co. and the continued spread of the insect throughout Essex county, nematode establishment within infested fields within each of the two counties need to be implemented. To focus on these two steps, we would like to begin two pilot programs in 2009 and 2010 focused on working with interested commercial applicators and individual producers on the production and application of the biological control nematodes. In addition, we would like to expand our "key field" nematode application program into the infested areas of Clinton and Essex counties.

One area of research which has been neglected due to funding levels and priorities set by NNYADP is the longer term questions regarding the persistence on the biocontrol nematodes across a corn rotation. Most of our experience with these nematodes as successful persistent biocontrol agents for snout beetle has been under continuous alfalfa production conditions. We have little understanding about the persistent of the biocontrol nematode across a rotation of other crops. Insects which feed on the roots of the grass within the alfalfa field serve as alternate host for the nematodes to maintain the nematode populations in the field. However, the soil population of alternate insects declines when the field site is rotated to crops such as corn. Questions arise whether the soil population of nematodes remain high enough during the corn portion of the rotation to rebound during the alfalfa portion of the rotation to a level where invading snout beetle are controlled. If not, nematodes may have to be reapplied to the field after the corn portion of the rotation.

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**Reports and/or articles in which results of this project have been published.**
Since this project has been jointly funded by both NNYADP and NYSFVI, quarterly reports about the progress of this project has been filed with NYFVI.

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