Northern NY Agricultural Development Program
2022 Project Report

Developing a Farmer/Applicator-Friendly Persistent Biocontrol Nematodes Formulation for Field Application

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Cooperating Producers:

- Murrock Farms, Jefferson County
- Double Dale Farm, Jefferson County
- River Haven Farm, Jefferson County
- Chambers Farm, St. Lawrence County
- Kelly Dairy, St. Lawrence County
- Greenwood Dairy, St. Lawrence County
- Mapleview Dairy, St. Lawrence County

Background:

Soil insects are economic pests across a wide array of plant production systems, impacting growers engaged in conventional agriculture, and organic crop production along with turf and ornamentals. The widespread adoption of soil-building cover crop practices often increases the soil insect pressure and damage on subsequent crops raised for production and sale.

Biocontrol nematodes (entomopathogenic nematodes, EPNs) are natural predators of soil insects in the soil environment. Commercial EPN strains have been bred/selected to only persist for a few weeks, requiring multiple applications and repetitive expense for producers. As a result, the use of commercial EPN strains requires accurate application timing and is expensive to utilize.

Over the past 30 years, with long-term support from the Northern New York Agricultural Development Program, the Shields Laboratory at Cornell University has conceptualized, developed, field-tested, proven, and implemented the concept of utilizing native-NY EPN strains as a viable biological control strategy against many soil insect pest species. The concept was first developed as a management tool against alfalfa snout beetle. The specialized rearing techniques developed by the Shields Lab allow these NY-native strains to retain their genetic ability to persist in the NY soil environment for multiple growing seasons. As a result, a single inoculation results in multiple growing seasons of soil insect suppression. The research initiated and first proven in northern New York expanded over time to show widespread successes in other field crops, organic vegetables, and small fruits. Trials funded by individual farmers, farmer groups, and grants organizations are now underwriting field trials elsewhere in New York State and multiple other states.

This NNYADP-funded research, and associated training workshops, prompted the startup of a woman-owned, northern New York-based new on-farm business enterprise that is rearing and custom applying persistent biocontrol nematodes in 2016 and one established in Austin, Texas, that is rearing and supplying persistent biocontrol nematodes in 2019.

In 2022, this NNYADP-funded research focused on the opportunity to extend the formulation for the "shelf-life" viability of the persistent native-NY biocontrol nematodes in holding solution prior to field application, and to refine the current biocontrol nematode low-tech mass rearing system that has provided biocontrol nematodes to farmer/applicators, who have inoculated more than 35,000 NY agricultural acres with the combination of two native-NY EPN strains.

Prior to this study, the production of persistent EPNs for field inoculation required holding the EPNs in inoculum for 14 days in controlled temperatures. With a shelf life of only 3-4 days after EPN production was complete, precise planning by supplier and farmer/applicator was required to have several factors in place for a timely successful field application. Rain delays that are a frequent occurrence in northern New York farming complicate this planning process. The shelf life of the current formulation at only 3-4 days created a limited window for ensuring that the biocontrol nematodes were applied successfully.

Furthermore, the current (as of 2021) persistent EPN formulation was inconvenient for the farmer/applicator to field apply, requiring labor intensive on-site flushing through fine screens just prior to application to remove the EPNs from the sawdust-base rearing medium, and to ensure living EPNs would be applied to the field.

Preliminary small-scale experimental results suggested that the EPN formulation concentration and shelf life might be increased to as much as 14-30 days with minimal technical difficulty.

Methods:

Once funding was secured, the Shields Lab was focused on:

I. laboratory trials to develop new liquid formulations and test them for EPN viability and shelf life;

II. refining the low-tech mass rearing system to improve the coordination of field application while reducing the farmer/applicator labor to prepare EPNs for field applications;

III. producing copious quantities of biocontrol nematodes for field application in 2022 for >350 acres using the revised mass rearing system, preparing EPNs for delivery by using the

new concentration rates, and using aeration to increase shelf-life on a larger scale then in laboratory trials; and

IV. deliveries of the concentrated EPNs to participating NY applicators, working with farm cooperators in field trials, and follow-up on applicator experience with the new methods.

I. Laboratory Trials to Develop New Liquid Formulation

The production of copious quantities of persistent EPN strains without the use of expensive fermenters is time, labor, and resource intensive. Until this research, the current best EPN production technology required:

1) the use of large volumes of water to extract EPNs and transport to crop locations,
2) significant labor to sieve out EPNs from rearing cups, and
3) equipment and short window of time to get EPNs applied before they were no longer viable.

We speculated that if water volume is reduced and we concentrated the EPNs, we could solve these challenges. However, this attempt came with risks such as the creation of an anoxic environment resulting in the death of the EPNs. So, we needed to determine thresholds to maximize EPN survival, including what concentrations could work best and how long the EPNs could survive.

Concentration of EPNs

To determine how nematode concentration affects survival over time we evaluated rates of 1x, 5x, 10x, and 25x cups of infective juveniles (IJs) per 1 gallon (gal). Biocontrol nematodes were produced following established protocols.

Fourteen days post-inoculation, rearing cups were flushed through fine screens to remove the EPNs from the sawdust-based rearing medium and washed with 1 gal of water that was collected. The EPNs were then poured through a third sieve to remove most of the residual water, leaving behind an infective juvenile (IJ) slime concentration.

Next, for the 1x, 5x, and 10x cup rates, we concentrated what we flushed out and transferred to 1-gal glass jars. Jars were filled to hold 1-gal of water. We repeated for the 25x rate, but only used 0.5-gal of water in each jar (25X equivalent). As a control, we used jars (1 gal each) of EPNs in the original rinsate.

Each jar had 1-2 aquarium bubbler stones connected to pumps to oxygenate the nematode suspensions. For the 1x and 5x rates we included a single bubbler stone, for the 10 and 25x rates we included two bubbler stones. The stones were held in place on the bottom of the jar using magnets: one glued to the bubbler stone, a second placed under the jar.

EPN Viability

Every 1-3 days (d) we removed a 1 ml aliquot (sample) of nematode suspension from each jar and diluted it to obtain countable number of IJs. Typically, we counted the nematodes in 5-10 pseudo replicated 100ul droplets and scored the number of live and dead nematodes in each aliquot. This continued thru 14d post-wash.

We believed the major factor influencing nematode survival would be oxygen deprivation A dissolved oxygen meter was to determine the concentration of dissolved oxygen in the jars at several time points.

II. Refine Low-tech EPN Rearing System

The ability to produce millions of EPNs is not difficult, but can be labor intensive. The Shields Lab explored the possibility of refining the current system of producing EPNS using standard individual media cups to a larger-scale system using proofing trays while striving to maintain waxworm death and subsequent IJ production.

Individual Cups vs Proofing Trays

To determine if we could match current waxworm mortality rate found with the individual cups to using proofing trays, the Shields Lab emptied the contents of multiple waxworm cups into multiple trays. Trays were then inoculated with EPNs to determine:

1) the appropriate rate of inoculum, and
2) the mortality rate compared to that of the individual cups used in the current system (Appendix Figure 1).

The initial test involved using 10 proofing trays split into two groups: 5 trays filled with 20 cups of fresh waxworms. the other 5 trays filled with 40 cups of fresh waxworms. Next, each of the 5 trays within the treatment group were inoculated with EPNs.

Inoculum treatment rates evaluated were 400 ml, 800 ml, 1 liter, ½ gal, and 1 gal of IJ inoculum per group. The rinsate was poured around the entire tray, mixed by using gloved hands to distribute the IJs, and then covered with a garbage bag taped down to the sides.

III. Produce Copious Quantities of Biocontrol Nematodes

Once an application rate (95-100% death) was established within 3d of inoculation we determined mass rearing could be achieved. However, during these trials we found that several unexpected issues needed to be addressed, and we wanted to evaluate how production within the proofing trays could be better improved by reducing the sawdust component.

Sawdust Reduction

The first step was to evaluate how production would be affected by removing all sawdust from the system. Second, we reduced the quantity of sawdust from the proofing tray system to evaluate how it impacted waxworm death and subsequent IJ production. To do this, we evaluated different ratios of cups with sawdust and cups without sawdust that were contained within the same proofing trays.

Improving the system using proofing trays meant dealing with new obstacles, including preventing waxworms from escaping the trays, allowing for ventilation, and managing the increased heat being generated within the trays while maintaining humidity.

Ventilation, Waxworm Escape, and Heat and Humidity

Our first attempt was to drill holes for ventilation along the sides of the trays, then place each tray within a sealed garbage bag. We worked on preventing waxworm escape by evaluating three methods:

1) adding Vaseline™ to the interior of the trays surrounding the ventilation holes.
2) using Pam cooking spray in a similar effort, and

3) taking trays with no holes and nesting several proofing trays.

IV. EPN Delivery Preparation and Field Trial
Once we had determined the best course of action, combining all the knowledge learned, we were able to mass produce EPNs for a 100-acre field application as a demonstration at a farm in Jefferson County (Appendix Figure 2), and for seven participating farms.

To prepare for each delivery, the Shields Lab reared billions of infective juveniles (IJs) in proofing trays that were washed, then concentrated for 12-14d post inoculation. The EPNs were pumped into a transport tank that had either 75 gal or 150 gal of water determined by the EPN formulation under evaluation (10x and 25x). Once inside the storage tank, six 4" x 2" large aquarium air stones were added to allow continued oxygenation during storage and transport periods prior to field application.

Field Application of Concentrated EPNs
Two NNY-based commercial applicators (DeBeer Seed and Spraying and D&D Spray Service) with experience in the field application of EPNs agreed to evaluate the newly-developed EPN formulations and delivery method. Spring and summer 2022 dates were established for multiple EPN applications on seven cooperating NNY farms that had previously applied EPNs in the past. The farmers agreed to have their EPNs delivered and applied using the revised methods.

We reared and delivered biocontrol nematodes utilizing two of the researched EPN concentrations: 10x and 25x. The experimental formulations used EPNs reared using the revised low-tech rearing system and were delivered, at the appropriate field application timing, in an aqueous nematode solution for application to fields in a corn rotation so these EPN applications represented the preventive pest management strategies that the farms considered for possible corn rootworm infestations.

The Shields Lab prepared all the biocontrol nematodes for the field applications, rearing billions of infective juveniles (IJs) in proofing trays that were washed, then concentrated 12-14d post-inoculation. The slime concentration was pumped into a 200-gal transport tank that had either 75 gal or 150 gal of water determined by the formulation under evaluation (10x and 25x). Once inside the storage tank, six 4" x 2" large aquarium air stones were added to allow continued oxygenation during storage and transport periods prior to field application.

Each delivery of the concentrated EPNs had shown a longer shelf life using the new system in the laboratory then previously found by using individual cups.

Results:

Note: For this research project, only one native NY strain of EPNs, *Steinernema feltiae* (Sf), was reared at Cornell University following the revised low-tech rearing system and delivered to the commercial applicators. The applicators then delivered and applied the EPNs after coordinating with the farm owners.

I. Liquid Formulation
The evaluation of nematode concentrations resulted in each of the four concentration rates having survival of >90% over the 14d test period. Conducting an EPN viability check every 1-3d for each concentration allowed a real-time account of how well IJs could survive under these

conditions. Rates of survival over time as a function of the designated concentration using the EPN concentration was recorded. Excluding the standard rate (current field rate), results showed very little difference over the 14d evaluation period if the nematode suspensions were oxygenated. Levels of dissolved oxygen were close to what is expected in a fully saturated solution (~20.9%). When the aquarium bubblers were turned off for the 10x and 25X rates, the result was nematode survival stable over the first 24 hours (h), then rapidly declining along with the concentration of dissolved oxygen. This demonstrated that nematode survival is contingent on sufficient levels of available oxygen.

As theorized, this research trial increased the upper threshold of EPNSs survival with proper oxygenation in holding solution prior to field application.

II. Refine Low-tech Rearing System

Individual Cups vs. Proofing Trays

We quickly realized that treatment rates above 1 liter of rinsate were too much water for the tray system. The trays inoculated with ½ gal or 1 gal of IJ inoculum resulted in the waxworms being dead after 48h and black and putrid, unlike when they die by entomopathogenic nematodes. The trays inoculated with the lower rates of water had no non-IJ related deaths 3d post-inoculation.

Sawdust Reduction

Evaluating how production would be affected by removing all sawdust from the system produced little to no IJ production in either treatment and was not a viable option.

Evaluation of removing any amount of sawdust from the proofing tray system while maintaining IJ-induced waxworm death and subsequent IJ production coupled with determining the appropriate volume of water to add to trays was important based on what was learned during the inoculum trials. The result: optimal IJ production is found when using a 20:10 ratio: 20 waxworm cups with sawdust and 10 waxworm cups sieved to remove sawdust per tray, along with the 800 ml inoculum rinsate rate.

The result: optimal IJ production is found along with the optimal inoculum rinsate rate to achieve the desired waxworm kill rate (95-100% mortality). This improved mass-rearing system allows for reduction of sawdust and water usage throughout the system while gaining mass-scale production of EPNs.

Ventilation, Waxworm Escape, and Heat and Humidity

Improving the system using proofing trays included the challenges of preventing waxworms from escaping the trays, allowing for ventilation, and managing the increased heat being generated within the trays while maintaining humidity.

Trials to prevent waxworms from escaping out the sides of the proofing trays through the holes drilled for ventilation using the established waxworm ratio resulted in:

- within the nested trays, all the waxworms died from too much moisture, too little oxygen, or the inability to dissipate heat;
- the trays with Vaseline™ applied to the interior of the trays around the ventilation holes did not prove optimal since the smearing clogged the holes that were supposed to provide ventilation which resulted in many waxworms dying;

- the sprayable Pam cooking spray worked best, limiting waxworm escapes while still providing airflow thru the ventilation holes; and
- adding 1" x 1" wooden sticks as spacers to increase ventilation and cooling, and adding a fan to move airflow, helped keep heat down, humidity held at 50-55%, and we could stack the trays several feet high while reducing the space used to mass produce the EPNs.

We determined that the use of the Pam cooking spray along with adding spacers between trays resulted in limited waxworm escapes, and no excess heat buildup, while holding moisture levels for optimal EPN rearing conditions.

Covering the proofing trays with aluminum foil and adding small deli cups as spacers resulted in a method that prevented mass waxworm escapes and issues with humidity.

III. Produce Copious Quantities of Biocontrol Nematodes

We were able to mass produce EPNs for a 100-acre field application as a demonstration at a farm in Jefferson County (Appendix Figure 2), as noted earlier, and for field applications on a total of 3,450 acres by the two NNY-based cooperating commercial applicators. The Shields Lab prepared all the biocontrol nematodes for field application. We reared and delivered biocontrol nematodes utilizing two of the researched EPN concentrations: 10x and 25x.

IV. Delivery and Field Application

The experimental formulations used EPNs reared using the revised low-tech rearing system and were delivered, at the appropriate field application timing, in an the aerated aqueous nematode solution to meet farm interest in a management within corn and alfalfa. Each delivery of the concentrated EPNs using the new mass-rearing system had shown a longer shelf life in the laboratory then previously found by using the individual cups.

Field Application of Concentrated EPNs

Spring/May 2022:

EPNs were delivered to DeBeer Seed and Spraying by the Shields Lab in the aerated water on three different dates. EPNs were transferred into 275-gal caged totes with six 4" x 2" large aquarium air stones then added to allow continued oxygenation during storage prior to field application. EPNs were delivered in the aqueous nematode solution developed by the Shields Lab to DeBeer Seed & Spraying in mid-May once plant emergence had been reported. DeBeer Seed and Spraying applied persistent biocontrol nematodes to two farms in Jefferson County and three farms in St. Lawrence County (Appendix Figure 3).

Appendix Table 1 represents the farms, acreage treated, formulation rate evaluated, and time/viability (shelf-life) of aerated EPNs post-delivery in Spring 2022. Each delivery of the concentrated EPNs for application had shown a longer shelf life using the new system in the laboratory than previously found by using individual cups.

August 2022:

The fields designated for EPNs had prior infestations of alfalfa snout beetle and were in a corn rotation so the EPN applications represented the preventive pest management strategies that the farms considered for possible corn rootworm infestations. EPNS were delivered to both application services in the aerated water solution.

DeBeer Seed and Spraying applied persistent biocontrol nematodes for two farms in St. Lawrence County, and D&D Spray Service applied EPNs to one farm in Jefferson County. The EPNs for DeBeer Seed and Spraying for the farms in St. Lawrence County were delivered on two different dates. The EPNs produced and provided to D&D Spray Service were delivered on one day for the Jefferson County farm location. For each delivery, the EPNs were transferred into a 275-gal caged tote which then had six 4" x 2" large aquarium air stones added to allow continued oxygenation during storage prior to field application.

Appendix Table 2 represents the farms, acreage treated, formulation rate evaluated, and time/viability (shelf-life) of the aerated EPNs post-delivery in August 2022.

Application Service Feedback
After each application, the two applicators shared their evaluations and opinions about the formulations and application experience, with the consensus that all innovations developed led to an overall improved and positive application experience for applicator and farmer.

Each applicator expressed how the revised rearing-and-delivery method was a much-improved part of the entire process. No longer did they require hours on end to wash the single cups to collect the EPNs. One important takeaway is that having the ability to transfer a caged tote to the field with a generator allowing continued aeration of the EPNs resulted in less travel back and forth to farm to refill the tank.

Observations on the ease of delivery from the Shields Lab encouraged DeBeer Agri Service to adopt the same method of rearing in its on-farm lab and applying to improve efficiency and support adoption of farm applications of persistent biocontrol nematodes in NNY (~7,000 acres applied by the service in 2022).

Positive results were observed on the extended shelf life, despite weather challenges in 2022 due to drought.

The farms with >500 acres found they could spread out the time of application and not feel rushed to have it all done in a single event.

Conclusions/Outcomes/Impacts:

This research successfully:

- developed a new liquid-based formulation, concentration rate/s, and oxygenation rate to demonstrate that the survival of persistent EPN shelf-life in pre-application holding solution could be extended from 3-4 days to >14 days;
- improved the methods for the mass-rearing of persistent EPNs and delivery to farms or application services, overcoming issues with waxworm escape/retention, ventilation, heat and humidity;
- simplified the logistics for EPN rearing, including reducing labor and materials, and for applying persistent EPNs for farmers/applicators;
- reared persistent EPNs for a 100-acre demonstration and for application to seven cooperating farms in NNY;
- prompted adoption of the new rearing method at the New York State's only on-farm lab rearing persistent biocontrol nematodes as well as the new delivery/transport and application methods;

- created an extended EPN holding-in-solution timeframe that provides greater flexibility for farmer/applicators to plan applications of persistent biocontrol nematode applications as noted by this project's feedback from farmers and applicators. For farms with more than 500 acres the new methods provided breathing space not to rush EPN application; and
- received applicator feedback noting that the refined methods also accommodated northern New York extreme dry weather-dependent farming conditions in 2022.

Outreach:

The audience for this research includes producers and agribusiness professionals, an increasing number of woman farmers, NY's Amish agricultural communities, and the organic production community. Elson Shields provided dissemination of this research on multiple dates in the winter/spring of 2022 as follow:

- January 5, 2022: WNY Corn Congress, Zoominar
- February 4, 2022: NNY Corn Congress, Zoominar
- February 11, 2022: Chemung County Corn Congress, Zoominar
- February 16, 2022: Otsego County Corn Congress, Zoominar
- March 18, 2022: NNY Lewis County Corn Congress, Lowville, NY
- March 22, 2022: Cayuga County Corn Congress, Auburn, NY
- March 25, 2022: "How Did We Get Here, " NYFVI Webinar
- March 31, 2022: SWNY CCE Zoominar.

Additionally, press releases during 2022 reported on the persistent biocontrol research being conducted by the Shields Lab with continued support from NNYADP. Articles noted the continued success in NNY, the development of the use of persistent biocontrol nematodes over a 30-year history with the cooperation of northern NY farms, agribusinesses, and Extension, the value and benefits of using persistent biocontrol nematodes, as well as new applications focused on the persistent biocontrol formulations in other crops in other states (Figure 4).

Next Steps:

The persistent biocontrol nematode technologies and methods developed by the Shields Lab with the long-term funding support from NNYADP for developing the science behind the use of the native-NY nematodes (and other funders who have expanded the use of the EPNs to other areas of New York State, the U.S., and Canada) will be applied through the two companies noted earlier as birthed by this research and will continue to help farmers with pest management concerns in New York State and those growing multiple crops across the U.S.

Acknowledgments:

The seven farms cooperating on this project have consistently been the most receptive to trying the innovative technologies associated with biocontrol nematodes developed by the Shields Lab. Thanks are extended to the Northern New York Agricultural Development Program, the NY farmers, and agribusinesses for support of this research over the decades it took to develop, field test and prove the science and to Cornell Cooperative Extension for extending its value.

For More Information:

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APPENDIX: NNYADP 2022 Project:

Developing a Farmer/Applicator-Friendly Persistent Biocontrol Nematodes Formulation for Field Application

Table 1. Field Applications of Concentrated EPNs in May 2022; NNYADP Developing a Farmer/Applicator-Friendly Persistent Biocontrol Nematodes Formulation for Field Application.

May 2022	**DeBeer Spraying**				
Farm	**Location**	**Acreage**	**Crop**	**Formulation X**	**Shelf Life**
Murrock	Jefferson Co.	100	Corn	10x	4-days
Murrock	Jefferson Co.	100	Corn	10x	4-days
Riven Haven	Jefferson Co.	50	Corn	10x	2-days
Chambers	St. Lawrence Co.	650	Corn	25x	3-days
Kelly Dairy	St. Lawrence Co.	600	Corn	25x	1-day
Galilee	St. Lawrence Co.	100	Corn	10x	2-days

Table 2. Field Applications of Concentrated EPNs in August 2022; NNYADP Developing a Farmer/Applicator-Friendly Persistent Biocontrol Nematodes Formulation for Field Application.

August 2022	**D&D Spray Service**				
Farm	**Location**	**Acreage**	**Crop**	**Formulation X**	**Shelf Life**
Double Dale	Jefferson Co.	200	Alfalfa	10x	2-days
	DeBeer Spraying				
Greenwood Dairy	St. Lawrence Co.	1,150	Alfalfa	25x	3-5 days
Mapleview Dairy	St. Lawrence Co.	500	Alfalfa	25x	3-days



Left to right (photos: Tony Testa): Figure 1. Proofing tray with *Steinernema feltiae*-infected waxworm cadavers. **Figure 2.** Rearing room used to mass produce persistent biocontrol nematodes Figure 3: Field application of persistent EPNs **by DeBeer Seed & Spraying.**

Figure 4. 2022 NNYADP Biocontrol Nematode Media Hits 2022

NNYADP Annual Report, 09-27 Drainage Contractor, 09-26: Spot On New York, horticulturecourses.info, Plattsburgh Press Republican, 09-19: Country Folks, CCE St Lawrence County News; 09-16 NY Ag Connection, 05-19 Hoard's Dairyman e-News, 05-18 Lowville Journal, 05-14 Dairy Business Twitter, 05-11: NY Ag Connection, Morning Ag Clips; 05-10: Daily Advent, Spot On New York, Plattsburgh Press Republican; 05-09: CCE St Lawrence County News, CCE Franklin County Facebook; 02-28 North Country CCE Blog, 02-21 Country Folks, 02-12 Lancaster Farming Corn Talk, 02-02 Boonville Herald, 01-29 Hay and Forage Grower, 01-29 Linking Lewis County News, 01-28: Cornell Field Crops Blog, New York Ag Connection; 01-26 Linking Lewis County, 01-25 Cornell Field Crops Blog.