Quality *Does* Matter

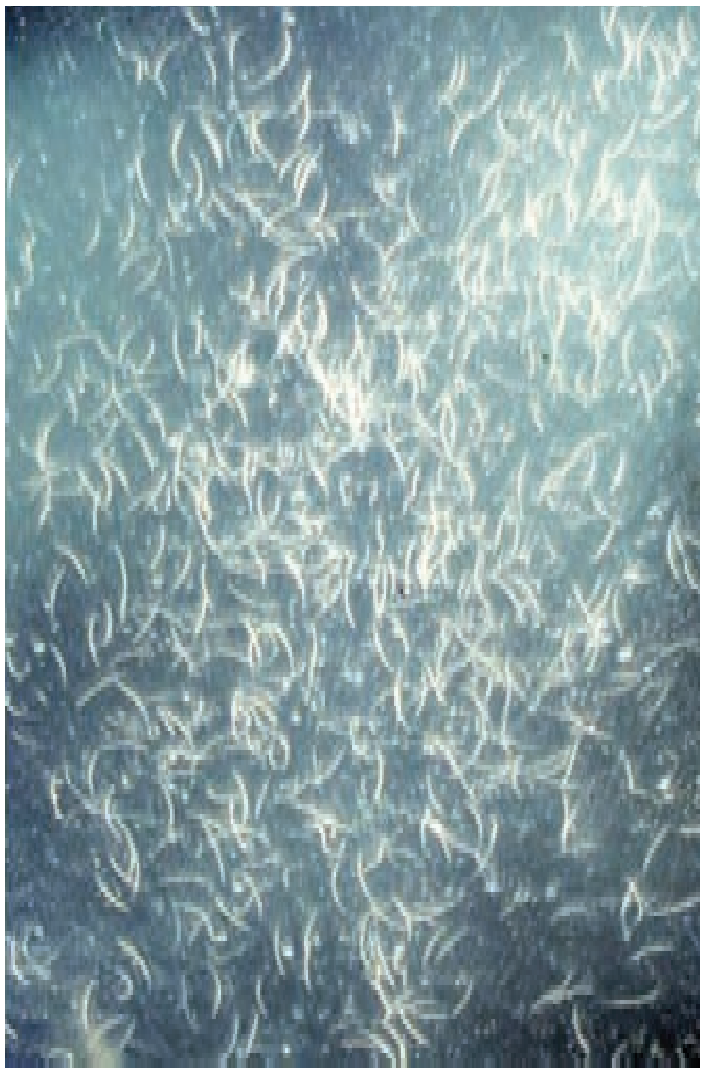
Not all commercially available entomopathogenic nematode products are the same!

By Erick X. Caamano, Raymond A. Cloyd, Leellen F. Solter and Declan J. Fallon

Entomopathogenic nematodes (EPN) are used as biological control agents to regulate a variety of insect pests. The two most commonly used and commercially available genera, *Steinernema* and *Heterorhabditis*, have a broad insect host range and can kill insect pests within 48 hours. The use of EPN as biological control agents is challenging, and application techniques are still under development. Effectiveness depends on the targeted host and environmental conditions, such as temperature and relative humidity, as well as application technology; EPN are susceptible to desiccation, temperature extremes and ultra-violet radiation.

Despite logistical issues, the use of EPN has been successful in field and greenhouse environments to manage certain insect pests, including the black vine weevil, *Otiorhynchus sulcatus*; fungus gnats, *Bradysia spp.*; western flower thrips, *Frankliniella occidentalis*; and the serpentine leaf miner, *Liriomyza trifolii*.

The use of EPN as biological control agents was considered impractical nearly 30 years ago. In fact, the first attempt to commercialize EPN did not occur until 1983, when California-based BioSys developed an efficient in-vitro production method, which was later discontinued. Currently, several companies within the United States produce or distribute EPN. However, quality of EPN is a concern and essential for the success of pest-management programs — and one of the major factors that affect the adoption of biological control by greenhouse producers.



EPN commercialization did not occur until 1983, when California-based BioSys developed an efficient in-vitro production method, which was later discontinued.

Commercial Name	Scientific Name	Company	Formulation[z]
Gnat Not	*Steinernema feltiae*	Integrated BioControl Systems, Inc. Greendale, Ind.	25 million IJs (sponge)
GrubStake-Hi	*Heterorhabditis indica*	Integrated BioControl Systems, Inc. Greendale, Ind.	25 million IJs (sponge)
Horticultural Scanmask	*Steinernema feltiae*	Biologic Company Willow Hill, Pa.	25 million IJs (sponge)
NemaShield	*Steinernema feltiae*	Biowork, Inc. Fairport, N.Y.	50 million IJs (gel)
Nemasys	*Steinernema feltiae*	Becker Underwood, Inc. Ames, Iowa	50 million IJs (gel)

[z] *IJs = Infective juveniles*

Table 1. *Commercially available entomopathogenic nematode strains, including commercial name, scientific, company information and formulation*

EPN Product	Processing Date (2005)z (mean ± SE)y								Overall mean percent live EPNx
	July 29	Aug. 5	Aug. 22	Aug. 29	Sept. 6	Sept. 20	Oct. 26	Nov. 2	
Gnat Not	91.8 ± 0.5b	93.0 ± 0.5b	93.4 ± 0.5b	96.1 ± 0.3a	55.3 ± 1.3e	88.9 ± 0.3b	89.2 ± 1.4b	86.9 ± 1.1b	86.83 ± 0.041b
GrubStake-Hi	89.3 ± 0.6c	81.6 ± 0.3c	91.8 ± 0.5b	86.9 ± 0.8b	88.0 ± 0.4b	74.3 ± 0.3c	90.3 ± 0.4b	77.9 ± 0.4c	85.01 ± 0.02b
Horticultural Scanmask	6.6 ± 0.3e	45.2 ± 0.8e	72.9 ± 0.8d	70.1 ± 0.7c	60.3 ± 1.2d	30.9 ± 0.5e	84.3 ± 0.7c	79.7 ± 0.4c	56.25 ± 0.084c
NemaShield	72.5 ± 1.0d	63.6 ± 0.9d	88.4 ± 0.8c	66.7 ± 0.8d	72.3 ± 1.7c	49.9 ± 0.2d	72.7 ± 0.5d	55.9 ± 1.1d	67.74 ± 0.037c
Nemasys	98.1 ± 0.2a	98.9 ± 0.1a	99.0 ± 0.1a	97.0 ± 0.5a	98.6 ± 0.1a	95.1 ± 0.3a	100 ± 0.0a	99.5 ± 0.1a	98.27 ± 0.004a

y Means for each evaluation date within a column followed by common letters are not significantly different at P ≤ 0.05.
x Cumulative mean percent survival of infective juveniles (IJs) across all eight processing dates.

Table 2. *Live entomopathogenic nematodes (EPN) [mean ± SE (%)] observed in five different commercial products based on eight processing dates, and overall percent mean (± SE) live EPN per product; n = 10,000 EPN per product per processing date.*

Evaluating Quality

Quality assessment of any biological control agent, whether it be a parasitoid, predator or pathogen is important; however, no government agency regulates the quality of commercially produced natural enemies. In fact, evaluation of quality is basically self-regulated, and there are concerns regarding the lack of quality control standards. The quality of natural enemies impacts their performance, and standards would increase the potential success rate of augmentative biological control programs.

The quality of EPN is often determined by measuring viability or percentage of live, active infective juveniles (IJs) in an EPN suspension. One study evaluated the viability and pathogenicity of different commercial EPN products, which were shipped to three different locations within particular shipments. However, the researchers evaluated EPN quality from only one shipment per location. Greenhouse producers, however, typically order multiple shipments throughout the growing season, and quality may vary among batches in EPN production systems. Our study, therefore, focused on the pre-application survival of different commercially available EPN products received in multiple shipments throughout the summer and fall.

Materials and Methods

Entomopathogenic nematode strains used in this study were *Steinernema feltiae* Filipjev (Gnat Not; Integrated BioControl Systems, Inc.), *S. feltiae* (Horticultural Scanmask; Biologic Company), *S. feltiae* (NemaShield; Bioworks, Inc.), *S. feltiae* (Nemasys; Becker Underwood, Inc.) and *Heterorhabditis indica* Poinar, Karunakar and David (GrubStake-Hi; Integrated BioControl Systems, Inc.). Table 1 (page 26) provides specifics on the formulation of the different EPN products used in the study. Eight different shipments of each product were evaluated over a five-month period (July to November).

The number of live IJs and dead juveniles was recorded to obtain the proportion of live IJs per determination. To estimate the total number of juveniles delivered per shipment/sample, we calculated an average of the number of juveniles obtained from the 10 determinations (1,000 EPN per determination), multiplied it by the total amount of suspension and compared it to the total number of EPN stated on the label that were included in the shipment. Finally, we determined the estimated mean number of live IJs per product for each shipment by multiplying the mean percent of EPN received per shipment by the expected number of EPN stated on the label. This value was then multiplied by the mean percent of live IJs for each shipment date.

Understanding the Results

The quality of commercially produced EPN may vary among batches of particular EPN products and from season to season associated with a single producer. It is important to maintain quality during all stages of production; however, additional factors such as storage temperature, relative humidity or refrigeration may influence EPN quality before received by greenhouse producers. Although loss of quality during storage is common, the successful use of EPN is contingent on stability during shipping and storage, which may impact EPN survival and their effectiveness against target insect pests. In addition, confidence in the quality of a product is critical to the viability and success of using EPN as biological control agents.

In this study, we found variable numbers of live IJs shipped compared to product labels and variable numbers of live IJs across shipments and products per shipment over the five-month evaluation period. Gnat Not was the most inconsistent product based on the numbers of IJs provided compared to the label. For example, ➧

Loss of quality during storage is common, but EPN success is contingent on stability during shipping and storage, which may impact their effectiveness against target insect pests.

two shipments (Aug. 22 and Sept. 20, 2005) contained between 10 million and 12 million IJs, and two shipments (July 29 and Nov. 2, 2005) contained between 16 million and 23 million IJs instead of the 25 million expected. In contrast, only one shipment each of GrubStake-Hi, Horticultural Scanmask, and NemaShield was received with lower numbers of IJs than specified on the label; however, all three contained more than 90 percent of expected. Nemasys had the highest mean percent IJ survival (more than 95 percent) across the eight evaluation periods (Table 2, page 28). Mean percent IJ survival values for Gnat Not and GrubStake-Hi, both from the same supplier, were more than 85 percent. NemaShield and Horticultural Scanmask had the lowest overall mean percent IJ survival across all eight evaluation periods, although the range among shipments was greater for Horticultural Scanmask (6.6 percent to 84.3 percent) than NemaShield (49.9 percent to 88.4 percent).

Nemasys and NemaShield were the only two commercially available EPN products that offered a return policy indicating that if cold packages had melted, the product should not be used but instead be immediately returned to the provider for a replacement shipment. Two shipments of NemaShield (Sept. 20 and Nov. 2, 2005) arrived with melted ice packages. These shipments also emanated a putrid odor, possibly indicating microbial contamination, which probably affected EPN survival; these shipment dates had the lowest mean percent IJ survival values (49.9 percent and 55.9 percent, respectively) across all the shipments received (Table 3, below). Overall, Horticultural Scanmask had the lowest and most variable quality of all the EPN products tested. It is not known whether this is due to the processing of the EPN or issues associated with packaging, handling or shipping conditions.

Shipping Date (2005)	EPN Product	Number EPN Received (million)	Estimated Live EPN (million)
July 29	Gnat Not	17.2	15.8
Aug. 22	Gnat Not	10.4	9.7
Sept. 20	Gnat Not	11.5	10.2
Nov. 2	Gnat Not	15.8	13.7
July 29	Grub Stake-Hi	22.9	20.5
July 29	Horticultural Scanmask	23.6	1.5
Sept. 6	NemaShield	48.2	34.8

Table 3. Number of entomopathogenic nematodes (EPN) received (live and dead) and estimated number of live EPN for seven shipments that provided <99 percent than expected, based on the label.

Quality control is rarely considered after natural enemies have been handled in preparation for shipment. Maintaining EPN quality is essential and may be accomplished by implementing quality control programs that are designed to minimize variability in EPN viability and pathogenicity during production. Producers of EPN have made substantial progress in delivering and providing quality products. However, there are still no standards designed to define and/or evaluate quality of EPN products. Thus, it is critical for commercial suppliers or producers to ensure their EPN products are of the highest quality upon arrival so that greenhouse producers are confident that EPN products will effectively control the designated insect pest. A quality assessment should not only be based on laboratory evaluations but on the performance of EPN in the field.

Final Thoughts

The formulation and packaging of the different EPN products we ordered varied (see Table 1), which may have contributed to retention or loss of EPN quality. Complete contact information and explicit instructions for returning damaged products was provided only by the suppliers of Nemasys and NemaShield. This information would allow greenhouse producers to contact the companies regarding problem shipments and expedite receipt of new product.

We suggest when there are concerns

regarding temperature in transit, that suppliers or distributors of EPN products consider incorporating recording devices such as data loggers in the packages of each shipment. However, this cannot increase the cost so much that it discourages the use of EPN. The development of a rapid evaluation technique to determine the quality of EPN would allow greenhouse producers to quickly assess whether the product should be applied. Moreover, it is important to ensure quality before use via proper storage after receiving any EPN product, and check shipments to determine viability. Dead or inactive EPN in a product will reduce the number of viable units available for application and thus reduce total efficacy of the labeled product.

There are few published studies associated with quality assessment of EPN. However, as demonstrated in our study, the commercially available EPN products vary in their quality, based on survival of EPN. This is a concern because if EPNs fail to perform to the expectations of greenhouse producers, then they will likely use another pest control option, including insecticides, and it will be difficult to convince them to use EPNs again. However, attention to the issues we have elucidated may prevent failure of EPN products and ensure that expectations are met, which will enhance the use of biological control as a management strategy for insect pests in greenhouses.

Acknowledgements

We thank Amy Dickinson for providing technical support and Kenneth E. Kemp for assistance in analyzing the data. This research was funded in part by the American Floral Endowment, United States Department of Agriculture AD-421 Project No. 6204-22000-017-04S, the University of Illinois and Illinois Natural History Survey. GPN

Erick X. Caamano is research assistant in the University of Illinois' department of natural resources and environmental sciences in Urbana, Ill.; Raymond A. Cloyd is associate professor and extension entomologist in Kansas State University's department of entomology in Manhattan, Kan.; Leellen F. Solter is associate professional scientist with the Illinois Natural History Survey in Champaign, Ill.; and Declan J. Fallon is research scientist with the University of Hawaii's department of plant and environmental protection sciences in Honolulu. Cloyd can be reached at rcloyd@ksu.edu.

LearnMore
For more information related to this article, go to www.gpnmag.com/lm.cfm/gp100802