Quality Assessment of Two Commercially Available Species of Entomopathogenic Nematodes: *Steinernema feltiae* and *Heterorhabditis indica*

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Summary. **The quality of entomopathogenic nematodes (EPN) is critical to their success as biological control agents, but it is difficult to evaluate quality because standard procedures are not available. Generally, the quality of biological control agents is determined by field performance because end users may have minimal knowledge pertaining to the condition of biological control agents before application. This study assessed the variability in quality of commercially available EPN products. The authors evaluated preapplication survival of five EPN formulations, *Steinernema feltiae* (NemaShield, Nemasys, Gnat Not, Horticultural Scanmask), and *Heterorhabditis indica* (GrubStake-Hi), based on eight shipments/samples of each EPN product received during a 5-month period (July to November). The estimated total number of EPN delivered per shipment (i.e., sample) was compared with the expected quantity listed on the label, and percent live EPN was determined for each shipment. One-half of the shipments of Gnat Not (four of eight) contained 40% to 70% of the number of EPN expected based on the label (25 million). The remaining shipments contained consistently higher numbers, with 99% of the expected quantity of EPN received. Entomopathogenic nematode mean percent survival was highest for Nemasys (98%) and lowest for Horticultural Scanmask (56%). The overall mean percent survival for Gnat Not and GrubStake-Hi, both from the same supplier, was more than 85%. Survival of EPN in the NemaShield product was as low as 50%, but was typically between 65% and 75%. NemaShield and Nemasys were the only two EPN products that provided return policy information if the product was damaged in any way. It is important for distributors and suppliers to ensure that EPN products are in quality condition before shipping to avoid performance failures and loss of customers. In addition, end users need to evaluate shipments upon receipt to determine the viability of EPN products.**

Entomopathogenic nematodes (EPN) are used as biological control agents to regulate a variety of insect pests (Chyzik et al., 1996; Georgis et al., 2006; Gouge and Hague, 1995; Hara et al., 1993; Oguzoglu and Ozer, 2003). 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 h (Burnell and Stock, 2000; Oguzoglu and Ozer, 2003). The use of EPN as biological control agents is challenging, and application techniques are still under development (Piggot and Wardlow, 2002). Effectiveness depends on the targeted host, and environmental conditions such as temperature and relative humidity (Choo et al., 1989; Hara et al., 1993; Lewis et al., 1992) as well as application technology (Gouge and Hague, 1995), because EPN are susceptible to desiccation (Ishibashi et al., 1987), temperature extremes, and ultraviolet radiation (Baur et al., 1995; Mason and Wright, 1997).

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* (Coleoptera: Curculionidae)], cranberry girdler [*Chrysoteuchia topiaria* (Lepidoptera: Pyralidae)], mint root borer [*Fumibotys fumalis* (Lepidoptera: Pyralidae)], citrus weevil [*Pachnaeus litus* (Coleoptera: Curculionidae)], mole crickets [*Scapteriscus* spp. (Orthoptera: Gryllotalpidae)], billbugs [*Sphenophorus* spp. (Coleoptera: Curculionidae)], white grubs (Coleoptera: Scarabaeidae), fungus gnats [*Bradysia* spp. (Diptera: Sciaridae)] (Georgis et al., 2006; Hom, 1994), western flower thrips [*Frankliniella occidentalis* (Thysanoptera: Thripidae)], and serpentine leafminer [*Liriomyza trifolii* (Diptera: Agromyzidae)] (Hara et al., 1993; Piggot and Wardlow, 2002).

The use of EPN as biological control agents was considered impractical nearly 30 years ago (Gaugler et al., 2000). In fact, the first attempt to commercialize EPN did not occur until 1983, when BioSys, a California-based company, developed an efficient in vitro production method, which was later discontinued (Friedman, 1990; Gaugler, 2000). Currently, several companies within the United States produce or distribute EPN. However, Gaugler (1997) noted that quality of EPN is a concern and is essential for the success of pest management programs. Parrella and Heinz (1998) indicate that quality is the major factor that affects the adoption of biological control by end users.

Quality assessment of any biological control agent, whether it be a

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

parasitoid, predator, or pathogen, is important; however, no government agency regulates the quality of commercially produced natural enemies (Waddington, 1993). Evaluation of quality is, in fact, basically self-regulated (Grewal and Peters, 2005), and there are concerns regarding the lack of quality control standards (Fernandez and Nentwig, 1997; Losey and Calvin, 1995). The quality of natural enemies impacts their performance, and standards would increase the potential success rate of augmentative biological control programs (Vasquez et al., 2004). However, in numerous studies, EPN quality or viability was not evaluated before conducting experiments (Arthurs and Heinz, 2006; Buitenhuis and Shipp, 2005; Head et al., 2004; Kim et al., 2004; Premachandra et al., 2003; Susurluk, 2006; Vanninen and Koskula, 2003).

The quality of EPN is often determined by measuring viability or percentage of live, active infective juveniles (IJs) in an EPN suspension (Grewal and Peters, 2005). Gaugler et al. (2000) evaluated the viability and pathogenicity of different commercial EPN products, which were shipped to three different locations within particular shipments. However, they 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 preapplication survival of different commercially available EPN products received in multiple shipments throughout the summer and fall.

Materials and methods

Entomopathogenic nematodes: Sources, ordering, and handling. Entomopathogenic nematode strains used in this study were *S. feltiae* (Gnat Not; Integrated BioControl Systems, Greendale, IN), *S. feltiae* (Horticultural Scanmask; Biologic Co., Willow Hill, PA), *S. feltiae* (NemaShield; Bioworks, Fairport, NY), *S. feltiae* (Nemasys; Becker Underwood, Ames, IA), and *H. indica* (GrubStake-Hi; Integrated BioControl Systems). Table 1 provides specifics on the formulation of the different EPN products used in the study.

Specific dates were chosen to begin the experiments, and suppliers were contacted to deliver the EPN products on those dates. All shipments (i.e., samples) were "blind orders," as suggested by O'Neil et al., (1998) to avoid biasing the quality of the shipments (samples) from the distributor or supplier. The EPN products that arrived before the expected dates were removed from the shipping package (when present) and refrigerated at 7 ± 2 °C until tested (1–3 d after receipt). This temperature was appropriate for EPN survival within the 3-d period (Strauch et al., 2000; S. Hove and R. Martin, pers. comm.). The processing date as indicated in Table 2 was when we evaluated the quality of the EPN products; this is not the date on which we received all the EPN products.

Assessment of commercially available entomopathogenic nematodes: Viability and numbers per package. The EPN were extracted from the packages and processed as follows. Each individual package, of 25 or 50 million EPN (Table 1), was diluted in 2500 or 5000 mL sterile tap water using a 5000-mL beaker. These EPN solutions were then diluted to obtain a final concentration of 100 EPN per milliliter. Ten milliliters of the suspension (1000 EPN) were pipetted into a glass Petri dish (100 × 15 mm), and the numbers of live IJs and dead juveniles were counted under 20× magnification using a dissecting microscope. A plastic grid was positioned underneath each Petri dish to assist in accurately counting the numbers of EPN. Juveniles that were not actively moving when inspected visually were probed with a dissecting needle to verify that they were dead rather than resting. Dead juveniles are completely extended, in contrast to live IJs, which have a slight "J" curvature at the end of the body or are actively moving (Kaya and Stock, 1997). For each of the five commercial EPN products, 10 10-mL determinations were assessed for each shipment (sample) of each product, which represented a repeated-measures design. Eight different shipments of each product were evaluated over a 5-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/shipment (sample), an average of the number of juveniles obtained from the 10 determinations (1000 EPN/determination) was calculated, multiplied by the total amount of suspension, and compared with the total number of EPN stated on the label that were included in the shipment. We used the PROC MIXED procedure (SAS version 8.2 for Windows; SAS Institute, Cary, NC) to perform a repeated-measures analysis of variance on the arcsine square root-transformed proportions. The model included EPN product as a fixed effect and shipment (sample) as a random effect, with the determinations within shipments (samples) as the repeated measures. A Fisher's protected LSD test was conducted to compare differences among the EPN products (SAS

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

Commercial name	EPN	Manufacturer	Formulation
Gnat Not	*Steinernema feltiae*	Integrated BioControl Systems, Inc., Greendale, IN	25 million IJs (sponge)
GrubStake-Hi	*Heterorhabdits indica*	Integrated BioControl Systems, Inc.	25 million IJs (sponge)
Horticultural Scanmask	*S. feltiae*	Biologic Co., Willow Hill, PA	25 million IJs (sponge)
NemaShield	*S. feltiae*	Bioworks, Inc., Fairport, NY	50 million IJs (gel)
Nemasys	*S. feltiae*	Becker Underwood, Inc., Ames, IA	50 million IJs (gel)

EPN, entomopathogenic nematode; IJs, infective juveniles.

Table 2. Live entomopathogenic nematodes (EPN) observed in five different commercial products based on eight processing dates in 2005, and overall percent mean live EPN/product (n = 10,000 EPN/product/processing date).

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

[z]Gnat Not and GrubStake-Hi, Integrated BioControl Systems, Inc., Greendale, IN; Horticultural Scanmask, Biologic Co., Willow Hill, PA; NemaShield, Bioworks, Inc., Fairport, NY; Nemasys, Becker Underwood, Inc., Ames, IA.
[y]Means for each evaluation date within a column followed by common letters are not significantly different at $P \leq 0.05$ by Fisher's protected LSD test. Analysis of variance statistics: 29 July ($F = 4229.63$; df = 4, 29; $P < 0.0001$); 5 Aug. ($F = 1366.03$; df = 4, 29; $P < 0.0001$); 22 Aug. ($F = 258.09$; df = 4, 29; $P < 0.0001$); 29 Aug. ($F = 494.60$; df = 4, 29; $P < 0.0001$); 6 Sept. ($F = 268.29$; df = 4, 29; $P < 0.0001$); 20 Sept. ($F = 5225.46$; df = 4, 29; $P < 0.0001$); 26 Oct. ($F = 168.31$; df = 4, 29; $P < 0.0001$); 2 Nov. ($F = 452.17$; df = 4, 29; $P < 0.0001$).
[x]Cumulative mean percent survival of infective juveniles across all eight processing dates.
EPN, entomopathogenic nematode.

version 8.2 for Windows). All data presented are nontransformed.

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.

Results

Entomopathogenic nematode survival was significant for each processing date (Table 2) and differed significantly ($F = 15.06$; df = 4, 35; $P < 0.0001$) across the commercially available EPN products. Entomopathogenic nematode survival (based on percent live IJs) was highest for Nemasys, with the most consistent percent survival (95% to 100%) across the eight evaluation periods (Table 2). Mean percent IJ survival values for Gnat Not were between 87% and 97% except for one shipment, where nearly half (45%) the EPN were dead. GrubStake-Hi mean percent IJ survival values were between 74% and 92%, whereas, in most cases, mean percent IJ survival values of NemaShield were between 65% and 75%. Horticultural Scanmask had the lowest mean percent IJ survival values; one shipment contained only 7% live IJs (Table 2).

One-half of the Gnat Not shipments (four of eight) contained lower numbers of EPN than expected: 40% to 70% of the labeled quantity (25 million EPN). Several other EPN products contained lower numbers of IJs than indicated on the label; however, the number of IJs determined for each shipment was ≥90% of the label quantity (e.g., 45 million as opposed to 50 million). One shipment of GrubStake-Hi contained lower numbers than labeled (92%), as did Horticultural Scanmask (94%) and NemaShield (96%). All remaining shipments contained at least 99% of the number of EPN indicated on the label. The number of EPN delivered (live and dead) and estimated number of live EPN for seven shipments that provided less than 99% than expected, based on the label, is presented in Table 3.

Discussion

The quality of commercially produced EPN may vary among batches

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

Shipping date in 2005	EPN product[z]	EPN received (million)	Estimated live EPN (million)
29 July	Gnat Not	17.2	15.8
22 Aug.	Gnat Not	10.4	9.7
20 Sept.	Gnat Not	11.5	10.2
2 Nov.	Gnat Not	15.8	13.7
29 July	GrubStake-Hi	22.9	20.5
29 July	Horticultural Scanmask	23.6	1.5
6 Sept.	NemaShield	48.2	34.8

[z]Gnat Not and GrubStake-Hi, Integrated BioControl Systems, Inc., Greendale, IN; Horticultural Scanmask, Biologic Co., Willow Hill, PA; NemaShield, Bioworks, Inc., Fairport, NY.
EPN, entomopathogenic nematode.

of particular EPN products (Westerman and Jung, 1992) 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 receipt by end users (Hom, 1994). Although loss of quality during storage is common (van Lenteren and Woets, 1988) the successful use of EPN is contingent on stability during shipping and storage, which may impact EPN survival and thus 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 with product labels and variable numbers of live IJs across shipments and products per shipment over the 5-month evaluation period. Gnat Not was the most inconsistent product based on the numbers of IJs provided compared with the label. For example, two shipments (22 Aug. and 20 Sept. 2005) contained between 10 and 12 million IJs, and two shipments (29 July and 2 Nov. 2005) contained between 16 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 that specified on the label; however, all three contained more than 90% of expected. Nemasys had the highest mean percent IJ survival (>95%) across the eight evaluation periods (Table 2). Mean percent IJ survival values for Gnat Not and GrubStake-Hi, both from the same supplier, were more than 85%. 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% to 84.3%) than NemaShield (49.9% to 88.4%).

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 should be immediately returned to the provider for a replacement shipment. Two shipments of NemaShield (20 Sept. and 2 Nov. 2005) arrived with melted ice packages. In addition, these shipments 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% and 55.9% respectively) across all shipments received (Table 2). Overall, Horticultural Scanmask had the lowest and most variable quality of all the EPN products tested. It is not known whether this is the result of the processing of the EPN or issues associated with packaging, handling, or shipping conditions.

Quality control is rarely considered after natural enemies have been handled in preparation for shipment (van Lenteren, 1986). Maintaining EPN quality is essential and may be accomplished by implementing quality control programs (Georgis, 1990) that are designed to minimize variability in EPN viability and pathogenicity during production (Georgis, 1992). Gaugler and Georgis (1991) indicated that the production process did not affect the quality of *S. carpocapsae* based on the results obtained in greenhouse and field tests. Production of *H. bacteriophora* in liquid culture, however, results in lower EPN quality than when this species is produced in solid culture or in vivo, possibly resulting from low lipid reserves (Abu Hatab and Gaugler, 1999). Producers of EPN have made substantial progress in delivering and providing quality products (Gaugler et al., 2000). However, there are still no standards designed to define 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 end users are confident that EPN products will effectively control the designated insect pest (van Lenteren and Woets, 1988). A quality assessment should not only be based on laboratory evaluations, but on the performance of EPN in the field (O'Neil et al., 1998).

Although Gaugler et al. (2000) found potential quality issues across different commercially available EPN products when evaluating only one EPN shipment for each of several locations, with lower than expected IJ numbers (based on the label) to be the most disconcerting, there are still few published studies that have addressed quality, based on the number of live, active IJs, of commercially available products. This is the primary reason that we conducted this study. In fact, the results obtained from our research will be valuable to practitioners of biological control in terms of EPN product selection and success in controlling insect pests. Our study differed from that of Gaugler et al. (2000) in that we evaluated EPN quality over time (5-month time period) and evaluated multiple shipments per product (n = 8).

The formulation and packaging of the different EPN products we ordered varied, which may have contributed to retention or loss of EPN quality. For example, Nemasys was delivered in sealed plastic containers (3.5 × 3.5 inches) in a gel matrix formulation, and NemaShield was shipped in a gel-type solution incorporated onto a moist, square piece

(≈5.5 × 5.5 inches) of sponge contained in a plastic bag. The other three EPN products were only prepared in a moist, square piece of sponge. It is possible that the gel matrix provided a more favorable packaging environment for survival than did the sponges, especially under potentially extreme conditions encountered during shipment. Quantitative research is needed to determine which formulation and packaging is likely to result in higher EPN percent survival.

Complete contact information and explicit instructions for returning damaged products was only provided by the suppliers of Nemasys and NemaShield. This type of information would allow end users to contact the companies regarding problem shipments and to expedite receipt of new product. In general, the service that end users receive from the supplier or distributor of biological control agents such as EPN may not be sufficient (van Lenteren et al., 1980a) and, when EPN are delivered via the postal service, poor shipping conditions may result in receiving dead or injured EPN (van Lenteren et al., 1980b).

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 (Grewal and Peters, 2005). 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 end users to assess quickly whether the product should be applied. Moreover, end users need to ensure quality before use via proper storage after receiving any EPN product, and to 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 EPN fail to perform to the end users' expectations, in all likelihood the end users will use another pest control option, including the use of insecticides, and it will be difficult to convince them to use EPN again. However, attention to the issues we have elucidated may prevent failure of EPN products and ensure that end user expectations are met, which will enhance the use of biological control as a management strategy to deal with insect pests in greenhouses.

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