Journal of Nematology 18(3):415–418. 1986.

Methods for Reducing Experimental Variation in *Globodera rostochiensis*[1]

J. A. LaMondia, D. Rawsthorne, and B. B. Brodie[2]

Key words: cyst nematodes, *Globodera rostochiensis*, golden nematode, *Solanum tuberosum* ssp. *tuberosum*, potato, techniques, viability determination, vintage cysts.

Studies concerning the biology of cyst nematodes (*Globodera* and *Heterodera* spp.) have historically been performed using collections of cysts extracted from infested field soil or cysts produced in plots designed to facilitate extraction of cysts from soil (13,14). Attempts to standardize experimental conditions and thereby reduce variability in hatch or viability counts have involved either increasing the number of cysts hatched or crushed (5) or transforming hatch data to reduce the correlation between mean and standard deviation (3,6). Fenwick (5) stated that it is "absolutely imperative in order to secure a reasonable accuracy, that a sample of at least 50 and preferably 100 cysts be used."

The source of variation in viable second-stage juveniles (J2) in eggs in cysts lies in the nature of cysts extracted from field soils. Cysts may be produced and remain in infested soils over many years and are therefore of different ages and have different contents. Even under monoculture of susceptible potatoes, cysts varied greatly in content and only 35% of cysts contained viable J2 (8).

In an effort to reduce variation, *G. rostochiensis* cysts of known age (vintage cysts) were produced by inoculation of J2 on susceptible *Solanum tuberosum* L. 'Katahdin' in 200-cm^3 clay pots in a screenhouse in Prattsburg, New York. Cysts were extracted from soil with a USDA cyst extractor (10) and screened to produce batches of cysts of uniform size and age. Although many researchers have used identical or similar techniques to reduce variability,

Received for publication 9 August 1985.

[1] Cooperative investigations of U.S. Department of Agriculture Agricultural Research Service and Cornell University Experiment Station.

[2] Postdoctoral Associates, and Research Plant Pathologist, USDA ARS, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

TABLE 1. Viable *Globodera rostochiensis* second-stage juveniles (J2) per cyst.

		Cyst size categories (mm)			
		Vintage cysts		Field cysts*	
		0.45–0.84	0.35–0.45	0.45–0.84	0.35–0.45
1982	Mean†	370.4 ± 49.8	138.5 ± 35.5		
	Range‡	246–469	42–221		
1983	Mean†	468.1 ± 115.0	176.9 ± 55.3		
	Range‡	241–705	61–295		
1984	Mean†	347.9 ± 69.9	139.8 ± 46.3	57.5 ± 86.4	29.0 ± 52.8
	Range‡	185–443	98–258	0–396	0–208
Cysts (%) with viable contents		100.0	100.0	46.0	43.0

* Cysts collected in 1984 from naturally infested soil after 10 years monoculture of susceptible cultivars.
† Mean ± 1 standard deviation of 100 cysts crushed individually (1984, 0.45–0.84 mm, n = 50).
‡ Range of number of viable J2 per cyst.

published results comparing the viable contents of vintage and field cysts are lacking.

Vintage cysts produced in 1982, 1983, and 1984 were compared to field cysts extracted in 1984 from infested soil monocultured for 10 years to susceptible potatoes. Cysts were divided into two size categories. The mean, standard deviation, and range of cyst contents were determined by individually crushing 100 cysts of each type and size (Table 1). The vintage cysts within each size category were remarkably similar in viable J2 content, with all cysts examined containing viable juveniles. The number of J2 per cyst was positively correlated with cyst size and was similar over years. The standard deviation of vintage cyst content means ranged from 13.4 to 33.1% of the mean. The range of field cyst contents was great owing to the large percentage of empty cysts. The standard deviation of field cyst contents was 150.3 and 182.1% of the means for 0.35–0.45 mm and 0.45–0.84 mm cysts, respectively.

Hatching of J2 from eggs in sized vintage cysts in response to a range of potato root diffusate concentrations indicated that variation is minimal (7.4–26.2% of the mean) using only 10 or 20 cysts per replicate for five replications (Table 2). This finding contrasts with the 50–100 field cysts required by Fenwick to reduce variation to acceptable levels (5). Variation in hatching of *G. rostochiensis* eggs has been further reduced by maintaining the identity of cysts produced in different years and on different cultivars, because encysted juveniles from different sources (year or cultivar) respond differently to a hatching stimulus (1,2).

TABLE 2. *Globodera rostochiensis* second-stage juvenile emergence from vintage cysts.

	Cysts per replication			
	20*	20*	20*	10†
	Potato root diffusate dilution			
Replication	1/1*	1/64*	1/1,024*	1/1†
1	7,374	1,543	270	799
2	8,145	1,225	226	834
3	7,405	1,828	198	1,024
4	6,977	1,438	297	904
5	8,331	1,408	384	878
$\bar{x}$	7,646	1,488	275	888
SD	570	222	72	86

* Twenty 0.45–0.84-mm cysts produced in 1983.
† Ten 0.35–0.45-mm cysts produced in 1981.

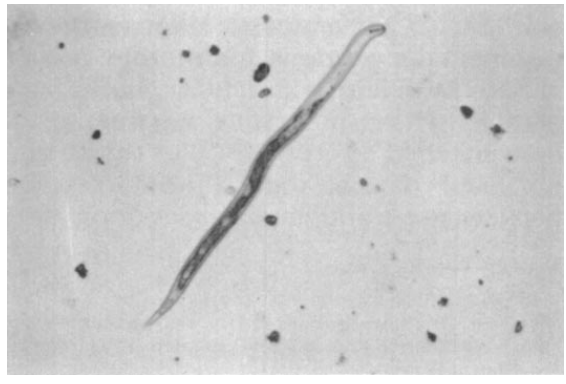

FIG. 1. Viable second-stage juvenile (J2) of *Globodera rostochiensis*.

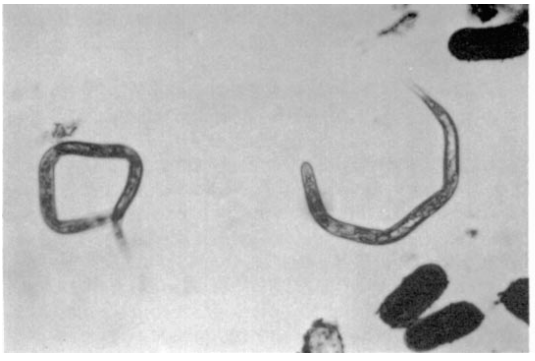

FIG. 2. Nonviable J2 and eggs of *Globodera rostochiensis.*

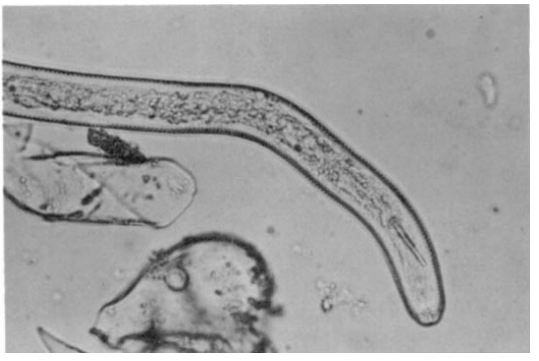

FIG. 4. Nonviable *Globodera rostochiensis* J2 with no border between the esophagus and intestine and a misplaced stylet.

In addition to egg hatch, parasitism and (or) chemical management may reduce the number of viable J2 per field cyst. Often, determination of J2 viability is difficult and subjective. Failure of nematodes to move is not a good indicator of nematode mortality. Other techniques investigated include forcibly removing J2 from eggs (12) and puncturing nematodes (7). Dyes and vital stains have been used, but determining if a partially stained nematode is alive or dead remains subjective (9,11).

To reduce variation in estimation of viable juveniles per cyst, a standardized procedure was developed for determining the viability of presoaked J2 in cysts by means of a couplet key. Viability of J2 in cysts was determined by correlating hatch in potato root diffusate with visual J2 characteristics. Viable J2 were distinguished by a clear esophageal region with a distinct border to the darker intestinal region (4); intestinal vacuolization was limited and organized. Nonviable J2 had no such border or difference between the esophagus and intestine. Vacuolization of the intestinal region was increased and irregular. The following key enabling visual determination of *G. rostochiensis* viability has proved useful both in research and USDA APHIS regulatory programs.

Preparation: Hydrate cyst in water for ca. 24 hours; crush cyst in a small drop of water on a microscope slide.

Compound microscope 100× to 450× required.

1. a) Movement of individual J2 (sinusoidal movement) ..... Viable
   b) No movement or movement attributable to liquid only 2
2. a) J2 in straight position or slightly curved (Fig. 1) 4
   b) J2 in "C" triangular position; bent at sharp angles (Fig. 2) 3

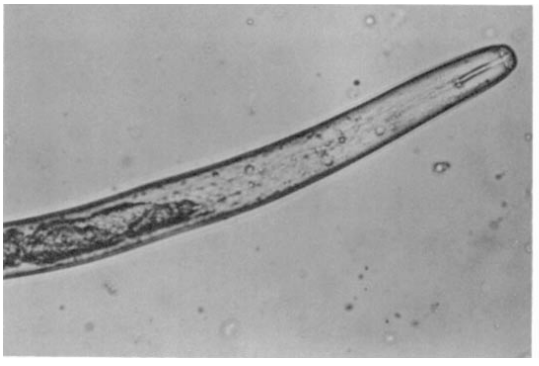

FIG. 3. Viable *Globodera rostochiensis* J2 with a distinct border between the esophagus and intestinal region.

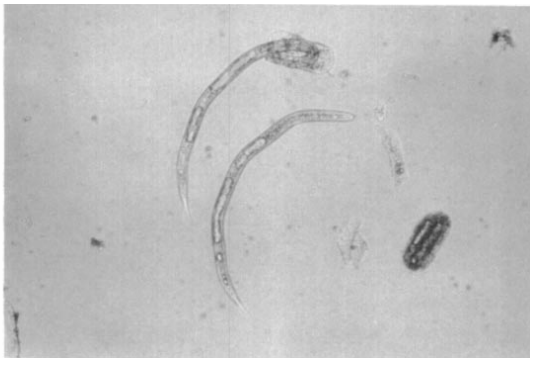

FIG. 5. Nonviable *Globodera rostochiensis* J2s with large irregular vacuoles present in the intestinal region.

3. a) Limited number of smooth, elliptical vacuoles in intestinal region (Fig. 1) 4
   b) Large irregular vacuoles in intestinal region and (or) intestine separated from cuticle (Fig. 5) ..... Nonviable
4. a) Stylet visible (Fig. 3) 5
   b) Stylet absent, misplaced, or knobs absent (Fig. 4) ..... Nonviable
5. a) J2 with clear esophageal region, with distinct border to darker intestinal region (Fig. 3) ..... Viable
   b) J2 either all transparent or all dark; no distinguishable border between esophagus and intestine. (Figs. 2, 4) ..... Nonviable

Utilization of sized vintage cysts and standardized determination of J2 viability has resulted in a reduction of both numbers of cysts and replications required to reduce experimental variation to acceptable levels without transformation of data.

Literature Cited

1. Ellenby, C. 1946. The influence of potato variety on the cyst of the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. Annals of Applied Biology 33:433–446.

2. Ellenby, C., and L. Smith. 1967. Emergence of larvae from new cysts of the potato-root eelworm, *Heterodera rostochiensis*. Nematologica 13:273–278.

3. Ellenby, C., and A. B. Gilbert. 1960. A convenient modification of the dilution hatching trial for the potato-root eelworm, *Heterodera rostochiensis* Wollenweber. Journal of Helminthology 34:99–102.

4. Endo, B. Y. 1962. Lethal time–temperature relationships for *Heterodera glycines*. Phytopathology 52: 992–997.

5. Fenwick, D. W. 1949. Investigations on the emergence of larvae from cysts of the potato-root eelworm, *Heterodera rostochiensis*. I. Technique and variability. Journal of Helminthology 23:157–170.

6. Fenwick, D. W., and E. Widdowson. 1958. The conduct of hatching tests on cysts of the potato root eelworm *Heterodera rostochiensis* (Woll.). Journal of Helminthology 32:125–134.

7. Fielding, M. J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proceedings of the Helminthological Society of Washington 18:110–112.

8. Mai, W. F., F. J. Spruyt, B. Lear, and J. Feldmesser. 1951. Yields of Green Mountain and Cobbler potato varieties grown on golden nematode infested soil. The American Potato Journal 28:617–625.

9. Shepherd, A. M. 1962. New Blue R, a stain that differentiates between living and dead nematodes. Nematologica 8:201–208.

10. Spears, J. M. 1968. The golden nematode handbook. USDA Handbook 353. Washington, D.C.: GPO.

11. Spivey, R. S. 1982. Hatching studies of the potato cyst nematode, *Globodera rostochiensis*. M.S. thesis, University of Virginia, Charlottesville.

12. Staniland, L. N., and L. E. W. Stone. 1953. Chlorphenol and related compounds as nematicides. Journal of Helminthology 27:41–74.

13. Viglierchio, D. R. 1959. Collection and selection of cysts of the sugar beet nematode, *Heterodera schachtii*. Journal of the American Society of Sugar Beet Technologists 10:318–329.

14. Winslow, R. D. 1955. A new method for the production and recovery of cysts of root eelworms (*Heterodera* spp.) for use in bio-assay. Journal of Helminthology 29:49–54.