Evaluation of Cyst Selection as a Means of Reducing Variation in Sugarbeet Nematode Inocula[1]

ARNOLD E. STEELE[2]

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In this laboratory, cysts of *Heterodera schachtii* Schmidt, 1871, containing embryonated eggs and larvae are routinely used in bioassay tests and as inocula in studies of host-parasite interactions. Cysts are obtained by washing and screening nematode infested soil in which sugarbeets have been grown from 90-120 days. In order to reduce the variability of inocula due to differences in cyst maturity, and to hatching and emergence of larvae, only mature brown cysts, judged to be newly formed and full of eggs, are selected. White females and cysts which are damaged, or which have unusual shape, size, or which appear to be empty or partially evacuated are discarded. The selected cysts are refrigerated at 10 C for 10 days to reduce hatching and emergence of larvae until the cysts are used in a test.

Since whole cysts are selected for inocula, the numbers and condition of hatchable eggs are not known. Consequently, a study was undertaken to determine if present methods of selecting storing cysts at low temperatures effectively increase hatch potential.

Experiment #1. Hatching and emergence of larvae from cysts or precystic females.

White females and brown cysts of uniform size and shape, which appeared to be full of eggs, were stored at 10 C for 10 days and then treated with tap water or sugarbeet root diffusate at 24 C. Treatments were applied to four replicate batches of 40 females or cysts for four weeks. Cysts or females were transferred to fresh solutions each week and the numbers of emerged larvae counted.

When exposed to either tap water or root diffusate, sugarbeet nematode larvae hatched and emerged from both mature precystic females and from brown cysts. Hatching was increased by the use of the diffusate solution rather than tap water with the increase being much greater for brown cysts than for the precystic females. The interaction was significant (Table 1).

Experiment #2. Hatching and emergence of larvae from full vs. partially evacuated cysts.

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[2] Nematologist, Plant Science Research Division, Agricultural Research Service, U. S. Department of Agriculture, Salinas, California 93901.

Table 1.—Influence of treatment solutions on hatching and emergence of larvae from pre-cystic females or from cysts.[1]

Nematode development stage	Treatment solutions			
	Tap water	Root diffusate		Mean
Brown cysts	1,787	10,874		6,330
Mature females	909	2,937		1,923
Mean of solutions	1,348	6,906		
Source	d.f.	Mean square	F .95	
Solutions (A)	1	123,554,341	191.1	**
Nema stage (B)	1	77,695,411	120.2	**
Interaction (A × B)	1	49,836,539	77.1	**
Error	9	646,495		

[1] Mean larval hatch from four replications of 40 cysts maintained 4 weeks in treatment solutions at 24 C.

Mature brown cysts of uniform size, shape, and color were divided into two groups: one group included only whole cysts which were opaque and appeared to be full of eggs; the other group contained whole cysts with clear areas as seen by transmitted light, suggesting that some, but not all, of the larvae had hatched and emerged from the cysts. Eight replicate batches of 40 full cysts or 40 partially evacuated cysts were opened, and the numbers of hatched larvae which had not yet emerged from the cysts were counted. An equal number of each type of cyst was stored 10 days at 10 C and then treated with tap water or sugarbeet root diffusate for 4 weeks. Larvae that hatched and emerged from full or partially evacuated cysts in water or diffusate were also counted.

Significantly greater numbers of hatched but not·emerged larvae were found within "full" cysts than were found within "partially evacuated" cysts (Table 2). In addition, significantly greater numbers of larvae emerged from "full" than "partially evacuated" cysts treated with either tap water or diffusate. Although treatment solutions caused greater differences in larvae emergence than did cyst type, hatch was affected by the interaction of these conditions; there was a greater increase in hatch from full cysts than partially evacuated cysts by using diffusate in comparison to tap water. However, the magnitude of the interaction was less than the separate effects of solutions and cyst types. The nature of the interaction (stimulated or depression of hatch) could not be determined from the data.

Table 2.—The influence of sugarbeet root diffusate on hatching and emergence of larvae from full and partially evacuated cysts.[1]

Cyst type	Larvae within cysts[2]	Treatment solutions: Tap water	Root diffusate	Mean of cyst type
Full cysts	790[3]	3,295	11,274	7,285
Partially evacuated cysts	53[illegible]	1,750	7,223	4,484
Mean of solutions		2,520	9,248	
Source	d.f.	Mean square	F	
Solutions (A)	1	362,087,506	310.8	**
Cyst type (B)	1	62,742,403	53.9	**
Interaction (A × B)	1	12,517,505	10.7	**
Error	28	1,164,722		

[1] Hatching test conducted for a period of 4 weeks.

[2] Larvae hatched but not emerged from cysts before hatching test was initiated. Larvae forcefully extruded by accidental rupture of eggs were not included in counts. Difference between means highly significant.

[3] Each figure represents the mean of eight replications, each consisting of 40 cysts.

Experiment #3. Hatching and emergence of larvae from cysts or from eggs removed from cysts.

Mature brown cysts of uniform size, shape, and color were divided into two groups. Cysts of one group were opened and the eggs removed, examined, and assigned to either of two categories. One category consisted of eggs that remained clumped together after removal from cysts; the other category consisted of eggs which were not bound together in clumps. Any cyst which contained both clumped and loose eggs was discarded. Eight replicate batches of five whole cysts, clumped eggs from five cysts or loose eggs from five broken cysts were stored 10 days at 10 C, and then treated with tap water or sugarbeet root diffusate. The eggs and cysts were transferred to fresh solutions every 3rd day. Significantly fewer larvae hatched from loose eggs than from eggs which remained clumped after removal from cysts (Table 3). It was not determined, however, whether differences were due to fewer loose eggs, or to other factors, such as death of larvae within loose eggs. The emergence of larvae from clumped eggs and from whole cysts which contained both loose and clumped eggs were about the same.

Experiments #4 and 5. Influence of storage temperatures on subsequent hatching and emergence of larvae from cysts in diffusate or tap water.

Table 3.—Influence of sugarbeet root diffusate on hatching of larvae from eggs within cysts or eggs removed from cysts.[1]

Treatment solution	Larval hatch			
	Unsorted cysts	Clumped eggs	Loose eggs	Mean of solutions
Beet root diffusate	1,354[2]	1,391	1,027	1,278
Tap water	234	153	162	183
Mean of population	794	772	595	

Source	d.f.	Mean square	F .95	
Solutions (A)	1	6,927,312	113.50	**
Population (B)	2	382,904	6.28	*
Interaction (A × B)	2	145,584	2.39	N.S.
Error	35	61,020		

[1] Test conducted for a period of 15 days. Treatment solutions changed every 3 days.
[2] Mean of eight replications each consisting of five cysts.

H. schachtii cysts containing eggs and larvae were obtained from sugarbeets and stored in tap water 10 days at 6 C or 24 C and then treated for 15 days at 24 C with tap water or sugarbeet root diffusate diluted to 5% of its original concentration. Each treatment was replicated six times, and each replication included five cysts. In a similar test pre-treatment storage temperatures were 5 C or 24 C, and replications included 50 cysts treated 30 days with tap water or with full-strength sugarbeet root diffusate. Sugarbeet nematode cysts used in the later test were obtained from a population maintained 10 years on tomato (*Lycopersicon esculentum*, cultivar Pearson A-1).

Conflicting data were obtained from the two experiments (Tables 4 and 5). In one test, greater hatch of larvae occurred from cysts stored at 6 C, whereas in the other test, fewer larvae emerged from cysts stored at 5 C than from those stored at 24 C. In both tests only two storage temperatures were evaluated. Consequently, it could not be determined whether differences were due to stimulation or depression of hatching. In the 5th experiment, hatching was increased by the use of diffusate in place of tap water with the increase being much greater for cysts stored at 24 C rather than 5 C. There was a significant interaction.

Table 4.—Influence of storage temperatures on subsequent hatching and emergence of larvae from cysts after 15 days in diffusate or tap water.

Treatment solution	Larval hatch		
	Storage temperature[1]		Mean of solutions
	6 C	24 C	
Beet root diffusate	450[2]	230	340
Tap water	223	78	151
Mean of temperatures	337	154	

Source	d.f.	Mean square	F .95	
Solutions (A)	1	215,272	10.29	**
Temperature (B)	1	199,655	9.54	**
Interaction (A × B)	1	8,626	0.41	N.S.
Error	20	20,931		

[1] Cysts stored 10 days at indicated temperature followed by treatment at 24 C.
[2] Mean of six replications each consisting of five cysts.

Table 5.—Influence of storage temperatures on subsequent hatching and emergence of larvae from cysts after 30 days in diffusate or tap water.

Treatment solution	Larval hatch		
	Storage temperature[1]		Mean of solutions
	5 C	24 C	
Beet root diffusate	3,122[2]	6,060	4,591
Tap water	777	2,614	1,695
Mean of temperatures	1,949	4,337	

Source	d.f.	Mean square	F .95	
Solutions (A)	1	50,320,896	121.16	**
Temperature (B)	1	34,210,488	82.37	**
Interaction (A × B)	1	18,172,200	43.75	**
Error	20	415,305		

[1] Cysts stored 10 days at indicated temperature followed by treatment at 24 C.
[2] Mean of six replications each consisting of 50 cysts.

Experiment #6. Hatching and emergence of larvae from single cysts.

The object of this test was to determine the distribution of larvae within cysts selected for increased hatchability of contents. Methods used in this test were similar to those described in the preceding studies except that larvae were collected from 405 individual cysts treated 10 weeks with sugarbeet root diffusate at 24 C after an initial 10 day storage period at 5 C. Data are tabulated in graphic form in Figure 1.

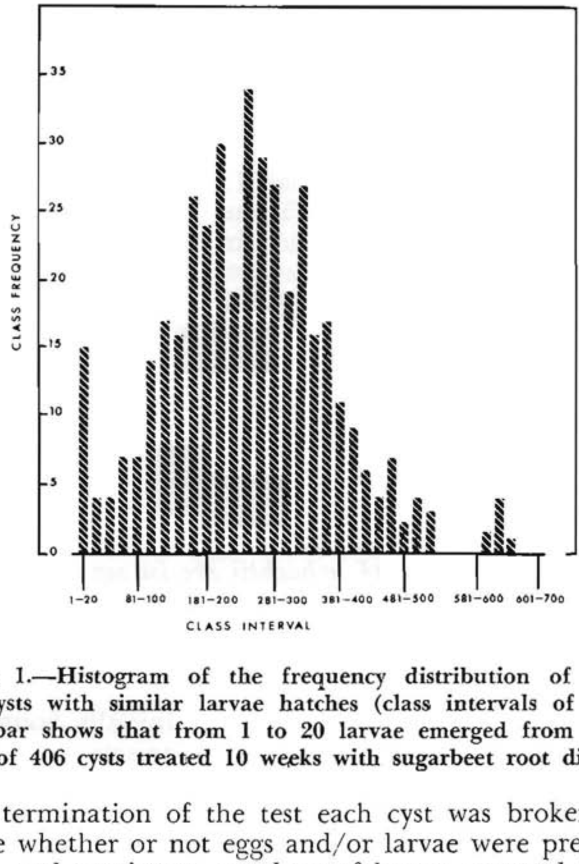

Figure 1.—Histogram of the frequency distribution of *Heterodera schachtii* cysts with similar larvae hatches (class intervals of 20 larvae). The first bar shows that from 1 to 20 larvae emerged from each of 15 of a total of 406 cysts treated 10 weeks with sugarbeet root diffusate.

After termination of the test each cyst was broken open to determine whether or not eggs and/or larvae were present. The minimum and maximum numbers of larvae emerged from individual cysts were 1 and 656 larvae, respectively. The mean number of larvae emerged per cyst amounted to 255. Statistical analysis of the data revealed that under a similar set of conditions, the mean would fall between 216 and 294 with a probability of 95%. The prediction of the population mean to within 5% of its actual value would require the utilization of 20 hand-picked cysts. The minimum numbers of cysts routinely used at this laboratory amount to 4 replications of 20, or 80 cysts.

Examination of the cyst contents upon termination of the test revealed the following: 11 cysts contained only eggs; 20 cysts contained only larvae; 174 cysts contained eggs and larvae; 200 cysts were empty. One-hundred-ninety-four cysts (nearly half of the population) contained only 2,353 larvae or 2.3% of the total number of larvae emerged from cysts.

Discussions and Conclusions

In all tests greater hatching was obtained using sugarbeet root diffusate in comparison with tap water as the hatching media. Also, the greater the hatching potential of the cystic material, the greater was the response to the diffusate media. Results of these tests indicate that significantly higher egg hatch can be obtained from whole cysts if they are selected to exclude pre-cystic females and partially evacuated cysts. Since white females eventually turn to brown cysts, differences in hatch between populations were likely due to differences in maturity of cyst contents. Fewer eggs within females may have contained larvae sufficiently developed to respond to the hatching stimulus, resulting in a threshold effect and an interaction of treatment solutions and populations. Tests have shown that a dormancy develops in maturing *H. rostochiensis* cysts, resulting in low hatches from brown-harvested cysts compared with new yellow-harvested cysts (4)[3]. *H. rostochiensis* also exhibits a strong diapause during autumn (6) which is broken by pre-treatment of cysts with high temperatures (2).

The finding that similar hatches are obtained from eggs within or removed from cysts of *H. schachtii* are in agreement with results reported for *H. rostochiensis* (7). In the present tests, however, higher hatches were obtained from clumped eggs than loose eggs, suggesting that the latter category may have included older eggs with few larvae.

The majority of eggs within cysts are initially bound tightly together with an adhesive mucoid material secreted by the uterine wall of the gravid female (1). It has been suggested that hatching is initiated by enzymes that change the permeability of the cyst wall, the egg shell, or the matrix between the eggs, thereby allowing increased diffusion of root diffusate (3). The enveloping cyst structure, however, in addition to having moisture holding properties, may also serve as a crucible for the dissociation of the egg mass by the churning action of hatched larvae attempting to emerge from the cyst.

Pretreatments with low temperature had dissimilar effects on subsequent hatching and emergence of larvae from two groups of cysts. These cysts were obtained from the populations of *H. schachtii* differing in ability to successfully parasitize susceptible Pearson A-1 tomato (8) and resistant Nematex tomato (unpublished data). Additional tests are needed to determine if the observed responses to low storage temperatures are characteristic

[3] Numbers in parentheses refer to literature cited.

of the physiologically distinguishable isolates of the sugarbeet nematode.

The rate of accumulation of empty cysts in the root zones of host crops is highly variable and influenced by a number of interdependent factors. Routinely, from one-third to two-thirds of the numbers of cysts recovered from soil are judged to be undesirable for bioassay and are discarded. That selection of cysts effectively reduces the numbers of larvae with few or no hatchable larvae is shown by the histogram in Figure 1. Except for 15 cysts (3.7% of the population) which account for only .08% of the total hatch, the histogram closely approximates a normal frequency distribution. Similar studies on the emergence of larvae of *H. rostochiensis* Woll. from single cysts obtained by random sampling rather than by selection (5) resulted in histograms with the frequency distribution skewed to the right. Fewer than 201 larvae emerged from 53% of the cysts. In comparison, selection of *H. schachtii* cysts in the present study resulted in 32.3% of the cysts with fewer than 201 larvae.

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