Effects of Sugar Beet Root Diffusates and Extracts, and Other Substances, on the Hatching of Eggs from the Cysts of the Sugar Beet Nematode, Heterodera Schachtii Schmidt

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The fact that the roots of host plants secrete substances which diffuse through the soil and stimulate and attract their nematode parasites was suggested by Marcinowski as early as 1909 (3)[2]. As early as 1900, Buerkel (2) had found that a marine nematode, *Oncholaimus vulgaris* Bastian, was attracted to edible mussels used as "bait." Baunacke (1) conceived the idea that this fact might be used to stimulate the hatching of eggs from the cysts of the sugar beet nematode, and demonstrated that excretions secured by washing beet roots did increase the hatching of eggs. Following the work of Baunake, Rensch (4) attempted to isolate the stimulating substance and synthetically produced two compounds, A and B, which greatly increased the rate of hatching. Compound A was a component part of vegetable roots, whereas B contained a substance found in the soil when plant material is breaking down. Although Rensch stated that field demonstrations were planned, he did not report them, indicating either that they were not made, or that they proved unsatisfactory.

Studying the emergence of the sugar beet nematode larvae under actual field conditions, the writer (6) reported on soil samples from 21 fields which had been in crop rotations for 1 to 12 years. A total of 38,128 cysts was examined and the data obtained showed that, even though the common host plants were not present, there was a continual emergence each year, with the bulk of the populations hatched by the end of the sixth year. Emergence was especially heavy the first year when it was estimated that more than half of the larvae left the cysts.

The problem of host stimulation and the manner in which nematodes respond to root diffusates were well discussed by Steiner (5). Citing a number of instances, he came to the conclusion that plant infecting nemas not only have the ability to recognize host plants, but are able to distinguish the preferred one. He postulated that the active parts of root secretions are of a rather simple chemical nature and that the element or elements which stimulate the larvae to hatch from eggs within the cysts are not the same as those which direct them to the roots. These elements are carried in soil water and recognized by the nemas, after which they proceed to the preferred roots.

In the same paper, Steiner discussed the possible methods by which nematodes perceive these stimuli, offering the very plausible explanation that they are detected by means of the amphids, a pair of organs located on or near the head. By means of these organs "the nema is led to its pre-

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[2] Numbers in parentheses refer to literature cited.

ferred host by some chemotaxic influence exerted by the plant." Apparently the young growing roots are the principal source of these secretions.

Wallace (9) found that 25° C. (77° F.) is the optimum temperature for hatching the larvae in the laboratory. But since field temperatures are not constant, he conducted a series of experiments in which temperature varied and found that 8 hours of 24° C. followed by 16 hours at 15° C. produced a marked increase in emergence. Other experiments demonstrated that low oxygen concentrations reduced emergence.

Procedures Followed in These Experiments

Experiments designed to study the effects of root diffusates or other substances on the hatching of sugar beet nematodes should follow natural conditions as nearly as possible. The nature of the work demands that most of it be done in the laboratory and immediately we introduce a series of unnatural conditions which may produce results entirely different from those existing under field conditions.

This work was conducted at the Salt Lake City, Utah, station during the years 1934 through 1939[8]. It will be noted that in these experiments we digressed from the usual procedures followed by other workers on the sugar beet nematode, and by those investigating the golden nematode of potatoes, *Heterodera rostochiensis* Woll., a relative of the sugar beet nematode:

1. Soil and small roots from severely infected sugar beets were collected in late October and stored in a cool basement. Portions were removed as needed and washed by the Cobb sifting and gravity methods. The cysts were picked directly from the screen residues. This method is in direct contrast to procedures used by other workers who air-dry the soil, immerse it in water and skim the floating cysts off the top. Since only a very small portion of the soil in a field ever becomes air-dried by lying on the surface, it is obvious that such a method introduces a condition entirely foreign to the usual habitat of the nemas. This is especially true of cysts which most investigators not only air-dry, but store under refrigeration for weeks or months before using them.

2. Water was obtained from a canyon stream which carried in solution many of the elements found in field soil.

3. Coarse sand grains from the stream bed were placed in the hatching tubes to simulate further natural conditions.

4. Light was excluded for obvious reasons.

5. Small sugar beets were carefully dug with the least possible injury to the roots, rinsed, and placed in the tubes to stimulate hatching.

6. Extracts were prepared by crushing the roots of a beet, about ½ inch in diameter, in 100 ml. of water and filtering the resultant liquid.

7. Cysts were opened and their contents recorded when the experiments were completed.

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[8] The assistance of Margaret Conder Deming in making many of the counts is gratefully acknowledged.

Selection of Brown Cysts for Experimental Purposes

Normally the female of *Heterodera schachtii* transforms to a brown cyst containing from 10 to more than 600 eggs in which the larvae are developed and become active if released. But in almost every lot, there were a few cysts in which the eggs were still in various stages of segmentation, even as late as mid-June. How much longer segmentation and larval development might have continued was not determined. Climatic conditions doubtless have considerable influence on larval development and these observations, under Utah conditions, may not preval in California and other states. In rare instances, the cysts did not contain viable eggs, perhaps because the female was not fertilized or the sugar beet was dug before the female was mature.

Cysts from beet dump dirt were not used because the heating and fermenting process during decay of the organic matter present, usually kill both larvae and eggs. Often cysts from this source are filled with fungi which thrive on the dead contents. Only rarely, if ever, are living larvae and eggs within the cyst attacked by fungi.

Cyst walls are very resistant to decay and they may remain in the soil for many years, accumulating in enormous numbers. Of the thousands present in a soil sample, only a few may have developed during the preceding season and contain a full complement of eggs. Partly, or entirely, empty cysts generally float during the washing process and are poured off and discarded. Selection of floating cysts for experimental purposes is therefore inadvisable.

Cysts for these experiments were selected from residues which passed through the 25-mesh per inch screen, and were collected on the 50-mesh, and settled to the bottom of the containers. These residues were thoroughly rinsed until clear, after which the cysts were picked out with a pair of sharp-pointed tweezers. When residues were properly prepared, it required only a few minutes to obtain a hundred cysts.

A minimum of 50 cysts was used, some experiments contained 500. Large numbers eliminate excessive errors from non-viable eggs. After the experiments were completed, cysts were opened and the contents examined. In some cysts every egg had hatched and the larvae emerged, while in a few none had hatched. In one cyst, 625 fully developed larvae were present which became active when the egg shells were ruptured. Occasional cysts contained two or three hundred hatched larvae which apparently had been unable to escape because there were no openings in the cyst walls. A few cysts contained dead eggs and larvae infected by fungi.

Saprophagous nematodes, *Acrobeloides butschii* (deMan), *Encephalobus oxyuroides* (deMan) and other species, often inhabit the cysts and the beginner should not confuse them with sugar beet nematode larvae (7).

Resume of Experiments

Data are here presented for only two of the many experiments conducted. These are typical of the results obtained, and the conclusions drawn were verified by repeated trials.

Equipment: Six carbon filter tube funnels, 35 mm in diameter, were fitted with drain tubes and stop cocks. (Figure 1B). These were mounted on a galvanized iron frame 150 mm wide by 200 mm long and 175 mm high (Figure 1A). Glass tubes 25 mm in diameter and about 100 mm long were covered on one end with coarse-mesh bolting silk and served as containers for the cysts and sand. Mailing tubes fitted about the funnel excluded most of the light and box covering the entire assembly made it completely dark.

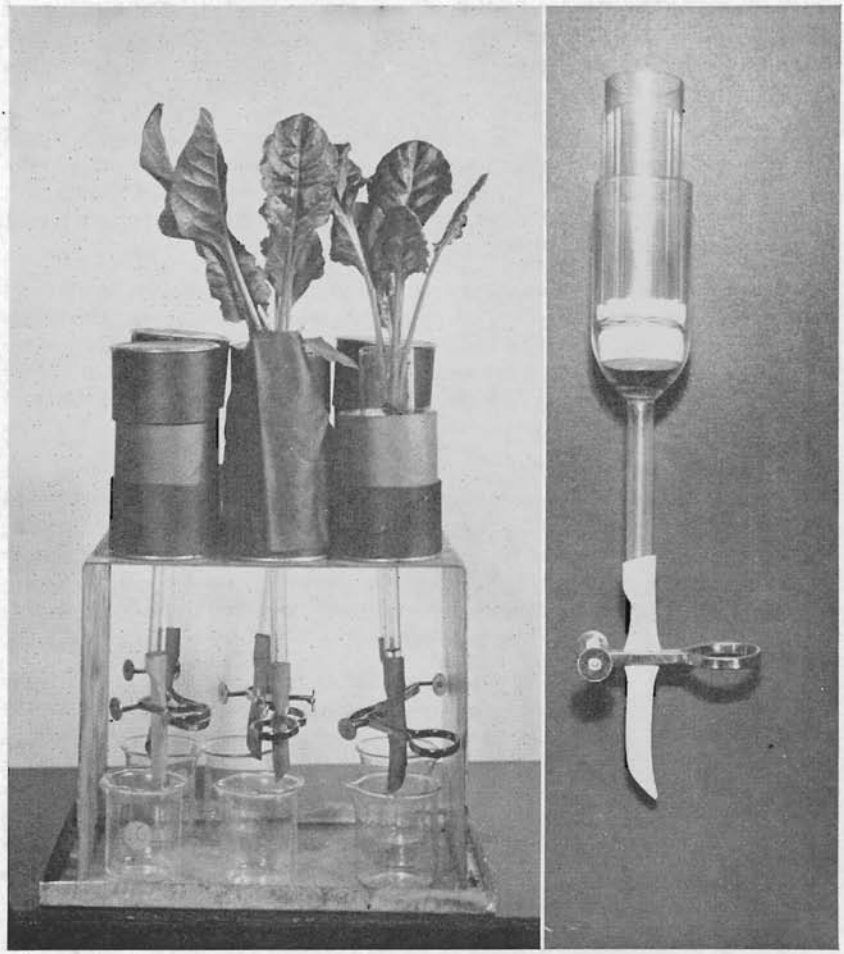

Figure 1-A (left). Frame holding funnels enclosed in mailing tubes, two small beets in place. 1-B (right). Funnel with tube in place.

Procedures: Cysts were placed in the tubes filled with the desired solutions. Each day, or at other specified intervals, about one-half of this solution was drawn off and immediately replaced by fresh solution poured into the tube in a small stream to insure aeration.

Cysts were examined by crushing on a cross-section microscope slide under a 25 mm square coverglass. Doubtless this crushing process released a few unhatched larvae from the eggs, but the numbers were negligible.

Larval counts were made under a low power binocular miscroscope. When numbers were near 500, two or more aliquots were selected, the larvae counted and the total was computed from these.

In the following described experiment, three lots of 500 cysts each, A, B, and C, were placed in canyon stream water on December 15. The first count of larvae was made December 18 and each day thereafter through the 27th. After the count was made on December 27, a young sugar beet was placed in A and extracts of young beet roots were added to C, while B continued in water only. During the following 10 days, the emergence was as recorded. These data indicate that young sugar beet roots stimulated hatching while the root extracts retarded it (Table 1).

Table 1.—Larvae Hatched from 3 Lots of 500 Cysts Each of *Heterodera Schachtii* Placed in Water from a Canyon Stream, December 15, 1934.

Date	A	B	C
Dec. 18	5,775	4,626	10,701
19	3,116	1,650	7,750
20	3,536	4,577	4,125
21	3,290	3,133	2,226
22	2,746	1,711	2,293
24	2,444	2,423	1,730
26	1,186	1,167	1,630
27	837	2,569	762
Sub-total	22,920	21,856	31,217
Dec. 29	365[1]	1,096	362[2]
31	865	675	283
Jan. 3	7,379[1]	1,668	348[2]
4	1,660	637	2,099
5	605	781	225
7	1,331[1]	1,127	1,209[2]
8	6,126	426	1,262
9	377	478	441
10	239	309	13
Sub-total	18,947	7,197	6,242
Grand Total	41,867	29,062	37,459

[1] Small sugar beet placed in tube when count was completed.
[2] Beet root extract added after count was made.

The important point revealed by this experiment is the fact that even in the presence of young sugar beet roots only about one-third of the larvae emerged from the cysts. This was determined by counting the larvae remaining in 50 cysts from each of the three lots after the experiment was concluded. When such a large portion of the larvae fail to respond to young beet roots, it seems as if there must be other factors which inhibit emergence.

Table 2.—Daily Hatching of Larvae from 50 Cysts of *Heterodera Schachtii* in Water and Solutions of Theelin.

Date	Water	Concentrations of Theelin		
		1/100,000,000	1/50,000,000	1/1,000,000
March 23	450	980	1,645	682
24	4,995	4,550	5,849	2,031
25	2,830	2,140	1,845	1,189
26	968	840	680	425
27	229	517	208	46
28	110	252	123	0
29	98	63	54	0
30	82	25	16	0
31	26	3	3	2
April 1	20	1	1	2
Total	9,808	9,377	10,424	4,377
Viable eggs and larvae remaining in cysts	1,716	2,608	2,148	7,242
Grand Total	11,524	11,985	12,572	11,619
Percent emergence	85.11	78.24	82.92	37.67

Since this experiment began December 15, the cysts were not sufficiently aged and a much greater emergence would have occurred had they been held 2 or 3 months longer. It appears obvious that the neophyte, testing materials for possible hatching stimulants, should not become excited if large numbers of larvae emerge during the first few days of an experiment.

Maturity of cysts had a decided influence on emergence of larvae. Four lots of 50 cysts each were placed in tubes on January 28, March 3, March 23, and May 18. At the end of 20 days the percentage of larvae hatched was 47.5, 69.4, 78.1, and 67.5, respectively. The low emergence from the May 18 lot was due to the fact that large numbers of the larvae had already hatched before the soil was washed to secure the cysts, many larvae being collected on the 200-mesh screen at that time.

Indol-3-acetic acid: A concentration of 1/50,000,000 of this chemical had a depressing effect on larval emergence; only 10.04 percent hatched as compared with 44.55 percent in the water check in early February.

Vitamin B_1: When Vitamin B_1 was used in concentrations of 1/100,000,000, 1/50,000,000 and 1/1,000,000, hatches of 70.4, 77.9, and 86.1 percent, respectively, were secured as compared with 76.0 percent in water in early February.

Theelin: Results of the theelin experiments are shown in Table 2. Little, if any, stimulation was secured from the 1/100,000,000 and 1/50,000,000 concentrations, and 1/1,000,000 strength apparently acted as a depressant. Emergence in water in this experiment was the highest recorded in the many tests conducted.

Summary

Experiments to determine the effect of root diffusates or other compounds on the hatching of the eggs of *Heterodera schachtii* should simulate natural conditions as nearly as possible. Even in plain water a large per-

cent of the larva emerge and it is that portion remaining within the cyst at the end of the experiment toward which all efforts should be directed, because these are responsible for continuing the infestation during crop rotations.

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