Identification of Some Materials in Root Exudates of Nematode (Heterodera Schachtii, Schmidt) Host Plants

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Introduction

The problem of control of the sugar beet nematode *(H. schachtii,* Schmidt) appears to be almost universal wherever the crop is grown. The trouble was early rioted by Europeans on land having a history of many successive beet crops. They termed the land "beet sick." According to Stewart (1)[2], et al, in 1881-82 Julius Kuhn and others fixed the responsibility for the damage on the eelworm of the sugar beet nematode.

Baunacke (2) , in 1922 recognized the presence of some substance having a stimulating effect on the larvae in the drainage water from sugar beet plantings; also, that an infested plant ceases to send out chemical stimulii which would attract new larvae in the area. He does not state how he was sure the stimulii were of a chemical nature, but in the final paragraph of the article it is suggested that isolation of the chemical in the root secretion would, no doubt, facilitate activation of the larvae in the soil. Apparently attempts to isolate individual fractions in the root exudate had already proven unsuccessful by methods then available.

The principal control thus far employed has been crop rotation, using non-host crops. Baunacke (2) mentioned the possibility of use of ammonia as a soil fumigant and also the planting of a trap crop.

Thorne and Jensen (3) , 1946, reported successful use of two chemicals, "D-D," and "Dowfume W15" as soil fumigants in field scale experiments in 1944 and 1945. However, for extensive infestation and for all types of soil, these materials have not found wide acceptance with growers.

Materials and Methods

Plants of each of the following species were taken from the soil, care being observed in washing off soil particles adhering to the roots to leave as great a mass of the roots as possible. These plants were then placed each in a separate 500-cc. Erlenmeyer flask filled with distilled water and left for ten days.

1. Sugar beet *(Beta vulgaris L.)*
2. *Beta patellaris*
3. Alfalfa *(Medicago Sativa)*
4. Sweet clover *(Melilotus Alba)*
5. Cheat grass *(Bromus Tectorum)*

Five to seven plants of each species were used, and the root diffusate solutions resulting were composited for each species to form one sample for analysis.

[1] Agronomist, Agricultural Experiment Station, and Denver Research Laboratory, the Great Western Sugar Company, respectively.

[2] Numbers in parentheses refer to literature cited.

The combined root difiusates were evaporated under reduced pressure to about one thirty-fifth of their original volume (from 1,750 ml. to 50 ml. in most cases). The concentrated solutions were then qualitatively tested for ketose containing carbohydrates and reducing sugars by the standard oc-naphthol ring test, and for hydrolyzable carbohydrates containing reducing sugars by a modified Dische test (4).

Chromatographic analyses were then run on the concentrates for amino acids common sugars, and polyol-type carbohydrates.

In each case paper chromatography was used, with 160 micro-liters of concentrated diffusate per spot applied in 30 increments.

The amino acid chromatograms were run upflow under glass in the standard phenol solvent, dried, and developed with ninhydrin spray.

Common sugars were determined by the technique of Albon and Gross (5) using the propanol solvent and α-naphthol spray.

The polyols were determined by the use of chromatograms run in collidine solvent, dried and developed with silver nitrate spray (4).

Analytical Results

Table 1 gives information on the results of qualitative tests run on the concentrated root difiusates.

Table 1.—Qualitative Results of Color Tests and Paper Chromatography of Root Diffusates.

Plant	Solids in Concentrate	a-Naphthol Test	Dische Test	Amino Acids found	Carbohydrates found
Sugar beet	0.10	Negative	Trace	Glutamic acid	Galactinol[1] and Inositol
Beta patellaris	0.12	Negative	Trace	Glutamic acid	Galactinol[1] and Inositol
Alfalfa	0.24	Negative	Positive	None	Galactinol[3] and Inositol
Sweet clover	0.27	Negative	Positive	None	Galactinol[1] and Inositol
Cheat grass	0.12	Negative	Positive	None	Galactinol[1] and Inositol

[1] Galactinol is a new carbohydrate recently isolated by Brown and Serro (4) from beets and beet products. It is an α-galactoside of inositol.

The results of the qualitative tests indicate that no standard hexoses, sucrose or raffinose were present, but that there was a carbohydrate present with a reducing sugar available after the strong acid hydrolysis of the Dische test.

The identity of the polyols found in the silver chromatograms was tentatively identified by chromatographic comparison with known compounds.

The concentrations of the two carbohydrates found were estimated by comparison with standard quantities of known inositol and galactinol. These estimates of concentrations in the original solutions are listed in Table 2.

While it is recognized that chromatographic evidence does not constitute proof of identity of the compounds listed, the tests do lead to the conclusion that the materials found are at least closely related to the postulated compounds, and more probably are the same.

Table 2—Estimated Concentrations of Organic Substances Found in Root Diffusate Solutions.

	i-Inositol	Galactinol	Glutamic acid
	ppm.	ppm.	ppm.
Sugar beet	50	5	Less than 2
Beta patellaris	26	2.6	Trace
Alfalfa	35	10	None
Sweet clover	26	5	None
Cheat grass	35	10	None

It is interesting to note that the other plants tested, alfala, sweet clover. and cheat grass, also appear to secrete the two substances, i-Inositol and Galactinol, while glutamic acid was absent. This suggests that reduction in nematode population numbers, as result of crop rotations, could be due in part to secretion of stimulatory substances by the roots of non-host plants.

Biological Materials and Methods

Distilled water solutions of the pure materials of each of the 3 substances described above were used at various concentrations in an attempt to stimulate the hatching of the eelworms from the encysted eggs. The following concentrations were employed:

1. i-Inositol at 100, 50, 25 and 12.5 parts per million.
2. Galactinol at 10, 5, 2.5 and 1.25 ppm.
3. Glutamic acid at 10, 5, 2.5 and 1.25 ppm.

In addition, diffusate solutions from sugar beet and *Beta patellaris*, with appropriate distilled water controls, were tested for stimulatory effect on the dormant cysts.

Cysts were collected from air-dry infested soil, using essentially the flotation technique described by Goodey (6) .

Cells of about 7 mm. in diameter were cut in paraffin layer about 3 to 4 mm. deep in a petri dish by the use of a cork borer. Within each cell was placed 3 cysts, care being taken to use fairly plump appearing cysts without any broken walls. A few drops of the material in solution to be tested was then pipetted into the cell. Four cells of each concentration of each chemical were used. The first attempts were made with the cells in the top half of the petri dish, with the solution and cysts somewhat as a suspended drop, the reason being to enhance the aeration of the solution containing the cysts. Later in the trial, the dishes were reversed to make easier observation with the binoculars. In all, two separate hatching experiments were conducted, and in the second trial the cells were always in the lower one-half of the petri dish. Each experiment comprised about one month's time.

[3] Private communication, Thorne, Gerald, Senior Nematologist, U. S. Department of Agriculture, Salt Lake City, Utah.

[4] Private communication, Steiner, G., U. S. Department of Agriculture, Beltsville, Maryland.

Experimental Results

In Table 3 are found the summarized results of the two trials.

Thorne[3] points out that under conditions of his experiments, considerable hatching may be induced by unchlorinated stream water. It was noted in the experiments reported here that only one case showed any hatching

Table 3.—Average Number of Eelworms per Cyst Hatched in Solutions of Three Chemicals at Various Concentrations.

Chemical Solution	Concentration ppm.	Eelworms per Cyst Average of 8 cells
i-Inositol	100	*1.6*
	50	0.04
	25	0.37
	12.5	0.09
Galactinol	10	1.6
	5	1.5
	2.5	3.3
	1.25	0.21
Glutamic acid	10	1.0
	5	0.04
	2.5	.00
	1.25	2.1
Beta patellaris diffusatc		0.12
Sugar beet difTusate		0.42
Distilled water		0.03

in the cells containing distilled water, and in that case there could have been some admixture of chemical from an adjoining cell. Many investigators[3 4] mention the difficulty of identifying cysts containing viable eggs. Some have overcome such a problem in their hatching experiments by the use of large numbers (50 or more) of cysts.

Summary

Three chemicals, i-inositol, galactinol and glutamic acid, appear from these experiments to constitute a major portion of the exudate from roots of nematode host and some non-host plants. The approximate concentration in the water solution in which the plants grew is given, but further work would be needed to determine whether the same concentration would be built up by secretion into the soil mass. The results of the hatching experiment reported here are no doubt limited by the numbers of cysts used, and should be considered only as sviggestive of a line of attack for further investigation.

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