Nematode Cyst Hatch Rate as Influenced by Fractions of Beet Root Juice

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Introduction

The writers were interested in studying one phase of the relationship between sugar beets and the sugar beet nematode (*Heterodera schachtii*). The presence of certain plants near nematode cysts accelerates the rate of hatching of the eelworms, because of certain substances exuded from the plants into the soil. A relatively new method of separation, paper chromatography, was used to prepare fractions of sugar beet material, which fractions were tested for their influence on the hatching of the worms (cyst hatch rate). It was thought best to start with beet press juice because of its easy availability, in spite of the fact that the compounds in the soil may not be the same as or, in the same concentrations as the compounds found in the beet press juice. Moreover, there are certain disadvantages concerning root exudates, among others, variability resulting from various methods of collection, and the usual extreme dilution of such exudates.

Baunacke (1)[2], and Wood and Serro (2) have shown that the cyst hatch rate of the nematode, *Heterodera schactii* is greatly affected by the presence of certain stimuli. Rietberg (3) mentions that the cyst hatch rate is strongly stimulated by root exudates of certain tested plants, which were mostly host plants. Hijner and den Ouden (4) have studied the hatching of larvae from the cyst and also the substances which influence the hatching. They developed a more or less standardized method for the production of root exudates, and analyses were started on the root exudates. They also state that large numbers of substances were tested for their effect on hatching. Wood and Serro (2) have identified galactinol in the root exudate of sugar beets. This laboratory determined galactinol in the press juice from sugar beets grown in a field severely infested with nematode. Finkner and Swink (5) found a relation between the tons per acre yield and low galactinol.

In certain field trials, some beets seemed to display varying degrees of resistance to nematode attack. By chromatographing the beet press juice from selected beets, all substances including those that either enhance or inhibit the cyst hatch rate, were more or less separated from one another, and were studied as individuals or fractions. Separating compounds into fractions reduces the synergistic effects. To confirm the observed influence of fractionated beet press juice on cyst hatch rate, various concentrations of pure substances were similarly tested.

This entire series of tests was preliminary in nature and this paper is a report on the progress made to date.

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[2] Numbers in parentheses refer to literature cited.

Materials and Methods

Seven varieties of beets that differed in tons per acre yield and percent galactinol in the beets were rasped and juice was pressed from the pulp. Table 1 gives three characteristics of the varieties.

Table 1.—Yield Data of Varieties Used in Experiments.

Varieties	Tons Per Acre	Percent Galactinol	Percent Sugar
7-1	4.22	0.078	17.6
4-1	3.70	0.114	16.7
39-1	3.70	0.114	16.7
9-1	3.14	0.110	15.8
8-1	2.53	0.146	15.4
40-1	1.43	0.242	15.7
30-1	1.23	0.244	12.8
23-1	0.83	0.308	11.9
46-1	0.83	0.308	11.9

Fifteen microliters of the expressed juice, after two fold dilution, were spotted on a one-inch wide strip of Whatman No. 1 chromatography paper. A solvent was used, which consisted of seventy parts iso-propyl alcohol, ten parts n-butanol, twenty-five parts benzene and twenty-five parts water. The various constituents of the beet press juice were separated into fractions by allowing this solvent to flow down the strip for a distance of approximately twenty-one inches. Following the irrigation period the paper was dried and cut into thirteen sections. The first section contained the original spot and the last section contained the solvent front. Similar strips were spotted with amino acid, galactinol, inositol, raffinose, sucrose and saponin in order to locate the position of the constituents in the various fractions of the beet press juice chromatograms. The compounds, which compose the greatest percent of dry substance in the beet and which were determined by paper chromatography, are as follows:

Fraction	Constituents in Fraction
1	Original spot
2	Galactinol, raffinose and ninhydrin reactant (x)
3	Raffinose, inositol, kestose, glutamic acid, asparagin and aspartic acid
4	Sucrose and glutamine
5	Sucrose, dextrose, saponin, glycine and gamma amino butyric acid
6	Fructose and alanine
7	Valine
8	Unknown
9	Isoleucine
10	Ninhydrin reactant (y) and fats
11	Ninhydrin reactant (y) and fats
12	Unknown
13	Solvent front

Each section was placed in one milliliter of distilled water. A few drops of the resulting solution were placed in a cell containing three nematode cysts. Each cell was observed through a microscope and the number of eelworms hatched were counted every twenty-four hours.

With the exception of inositol, galactinol and glutamic acid, the concentration of the individual chemical substances tested is near that of the concentration in the chromatographed beet press juice. The three chemicals mentioned have more or less the concentrations found in root diffusates as prepared by Wood and Serro (2).

Cells 5 mm. in diameter were made in a paraffin layer about 3 to 4 mm. deep in a petri dish, according to the method of Wood and Serro (2). To retard the evaporation of the solutions from the cells a moist blotter was inserted in the top half of petri dishes. Each experiment was continued for a period of approximately thirty days. The experimental results are found in Tables 2, 3 and 4.

Nematode cysts were obtained for these tests by taking beets from a field severely infested with nematode and bringing the roots with adhering soil and cysts to the laboratory. Some soil was removed from the roots to expose the cysts, which were then removed from the small rootlets. This method of obtaining cysts may differ from the soil screening method in the amount of force necessary to disengage cysts from rootlets or soil. Cysts obtained by different methods may react differently. Cyst variability or other factors caused variable hatch rate to such an extent that study of replications indicates our results were not highly significant.

Table 2.—Number of Eelworms Hatched per cyst per Fraction in Solutions of Chromatographed Beet Press Juice. Average of Three Replications.

Fraction Number	Eelworms per Cyst					Over-all Fraction Average
	Variety 7-1	Variety 39-1	Variety 46-1	Variety 30-1	Variety 23-1	
1	15.4	8.6	5.0	0.0	2.0	6.2
2	25.5	6.0	0.0	10.2	5.6	10.1
3	6.8	0.0	5.5	5.9	13.8	6.4
4	22.0	9.6	4.8	4.1	4.0	9.1
5	0.1	1.5	3.3	0.3	15.2	3.6
6	13.3	3.2	11.6	0.1	6.1	6.9
7	0.4	6.8	0.8	0.1	17.9	5.0
8	9.4	0.8	5.6	0.0	4.8	4.1
9	2.9	20.4	0.8	0.7	1.8	5.3
10	8.4	1.1	13.3	8.7	4.9	7.3
11	4.8	9.8	4.4	5.8	11.0	7.2
12	2.8	22.6	8.4	2.2	1.4	7.3
13	3.8	6.7	0.2	0.0	2.3	2.5
Distilled H_2O	4.7	5.3	3.5	2.3	8.3	4.8

Table 3.—Number of Eelworms Hatched per Cyst per Fraction in Solutions of Chromatographed Beet Press Juice. Average of Two Replications.

Fraction Number	Eelworms per Cyst						Over-all Fraction Average
	Variety 7-1	Variety 4-1	Variety 9-1	Variety 8-1	Variety 40-1	Variety 23-1	
1	1.7	6.2	0.0	7.0	2.4	9.2	4.4
2	1.3	0.0	2.0	10.0	3.5	1.0	3.0
3	0.0	1.0	0.2	2.2	1.2	0.2	0.8
4	11.5	0.2	6.0	3.4	7.9	2.2	4.6
5	0.2	0.4	0.4	0.8	4.0	0.0	0.9
6	0.0	1.0	20.0	0.0	2.0	5.0	4.7
7	2.2	0.7	2.0	0.0	0.0	1.5	1.1
8	1.2	3.5	5.2	0.0	0.0	2.2	1.9
9	1.0	1.7	4.0	0.2	0.0	4.5	1.9
10	0.0	5.2	0.0	0.2	2.5	5.7	2.3
11	0.0	0.4	12.2	11.9	0.4	4.5	4.9
12	0.4	0.0	4.7	4.9	5.0	1.0	2.6
13	1.3	0.0	2.8	0.4	6.4	6.7	2.9
Distilled H_2O	2.3	0.0	2.0	2.2	3.5	2.0	2.0

Table 4.—Number of Eelworms per Cyst in Solutions of Twelve Chemicals and Distilled water.

Chemical Solution	Concentration p.p.m.	Eelworms per Cyst		
		Replication a	Replication b	Average a + b
Inositol	50-150	0.33	0.56	0.44
Galactinol	2-7	5.33	4.89	5.11
Glutamic acid	2-7	6.77	6.67	6.72
Glycine	2-6	7.33	5.33	6.33
Alanine	2-6	2.33	2.78	2.56
Valine	2-6	2.22	7.11	4.67
Isoleucine	2-6	6.89	0.44	3.67
Glutamine	10-20	6.22	4.00	5.11
GABA	2-6	1.78	0.44	1.11
Aspartic acid	2-6	3.56	1.33	2.45
Sucrose	500-1500	16.11	3.33	9.72
Saponin	8-12	6.00	1.56	3.78
Distilled water — Average of twelve tests				4.42

Conclusions

Certain fractions of the chromatographed beet press juice appear to enhance or inhibit the cyst hatch rate when compared with the hatch rate in distilled water. Figure 1, a bar graph, shows the average of the adjusted total number of eelworms hatched per cyst per fraction for all varieties and replications at the end of thirty days. The fractions that may inhibit are 5 and 7. The fractions that may enhance the hatch rate are 1, 2, 4, 6 and 11.

Table 5.—Comparison of Fraction Enhancing or Inhibiting Power with Individual Compound Reactions.

Fraction No.	Fraction Reaction	Known Compounds Present	Individual Compound Reaction
2	Enhance	Galactinol	Enhance
4	Enhance	Sucrose	Enhance
		Glutamine	Enhance
6	Enhance	Fructose	Enhance
		Alanine	Enhibit
11	Enhance	Ninhydrin Reactant	—
		Fats	—
3	Inhibit	Inositol	Inhibit
		Aspartic Acid	Inhibit
		Glutamic Acid	Enhance
		Kestose	—
		Raffinose	—
		Asparagine	—
5	Inhibit	Sucrose	Enhance
		Saponin	Inhibit
		Dextrose	Enhance
		Glycine	Enhance
		GABA	Inhibit
7	Inhibit	Valine	Enhance

The enhancing or inhibiting power of the fractions may be correlated to certain chemicals present in the fractions, Table 5. From the experiment conducted with pure substances, the following tentative conclusions were drawn:

1. Inositol, alanine, isoleucine, gamma amino butyric acid (GABA), aspartic acid and saponin in general tend to inhibit the hatching of eelworms from cysts when compared with distilled water.

2. Galactinol, glutamic acid, glycine, valine, glutamine and sucrose appear to enhance the emergence of eelworms from cysts when compared with distilled water.

Distilled water, which was used as a comparison, exhibited a variable pattern of cyst hatch rates. We confirm Rietberg's (3) observation that cyst hatch can occur in the absence of a stimulant, other than water.

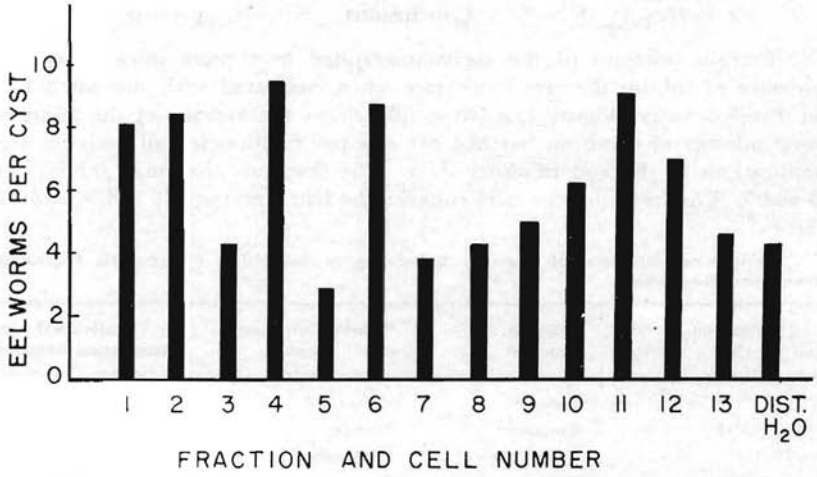

Figure 1.—Average of the adjusted total number of eelworms hatched per cyst per fraction for all varieties and replications at end of thirty days.

Saponin appears to have an abnormal effect on the eelworms that have hatched from the cysts. After a period of approximately three days the eelworms become immobile and unbending within the cells.

Because certain groups of beets reacted differently while growing in the field, it was thought possible that certain fractions would show differences between varieties. Even though there were great differences in the hatching rates, too few tests were run to obtain highly significant results.

Acknowledgment

The authors are indebted to J. F. Swink and R. E. Finkner for the beet roots and yield data used in these tests.

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