Effect of Liquid Nutrient Culture, Vacuum Distillation and Dialysis on Hatching Activity of Sugar Beet Root Diffusate for *Heterodera schachtii*[1]

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Abstract: Roots of sugar beets grown in liquid culture excrete substances that stimulate egg hatch and emergence of larvae from cysts of *Heterodera schachtii*. Their hatching effect is comparable to that of sugar beet root diffusate leached from soil-grown sugar beet plants. Consequently, liquid culture provides a way of obtaining *H. schachtii* hatch-stimulant free of contaminants from soil. Root diffusate, concentrated 50-fold or dried by vacuum distillation, retained hatching activity. The active principle of diffusate is dialyzable with a diffusion rate between those of inorganic salts and compounds with molecular weights greater than 15,000.

Diffusible materials produced from germinating sugar beet seedlings, leaves or roots of young, rapidly-growing sugar beets stimulate egg hatch and emergence of larvae from cysts of *Heterodera schachtii* Schmidt (2, 3, 7). In quantitative measurements of hatching activity, diffusate leached from soil bearing young plants is commonly used as a standard. Our experience has shown that this method is of limited value because hatching activities are subject to seasonal variation and to decline during storage, even at 5 C.

The present study is a series of experiments in which techniques employed to standardize and preserve the "hatching factor" of various species of *Heterodera* were tested for their utility with exudates from sugar beet roots. The first of these involved the growing of plants in nutrient solution to eliminate massive contamination of the exudate by soil microorganisms (8) and thereby increase the storage life of the exudates released into the bathing solution.

Concentration of root diffusates of potato and turnip rape (*Brassica rapa oleifera* D.C.) by vacuum distillation has proven feasible (1, 4, 5, 9). Sugar beet root diffusate has also been concentrated 40-fold *in vacuo* at 35–40 C (10). However, pH and degree of concentration was critical for retention of hatching activity. Other reports indicate diffusate from sugar beets can be dried or frozen without loss of activity (6, 7).

Potato root diffusate has been successfully concentrated in vacuum at 5 C, using a condenser cooled with a mixture of dry ice and alcohol. In one of our tests we evaluated this procedure as a means of rapidly concentrating or drying sugar beet root diffusate at low temperatures.

The color of sugar beet root diffusate may vary from light straw to dark amber. In our experiments, darker diffusates exhibited greater hatching activity than lighter diffusates. In this study, we attempted to determine whether the colored materials and the active principle(s) in diffusate could be separated by dialysis from the proteins usually present in sugar beet root diffusate and, if so, to find whether or not the materials diffuse at the same rate thus suggesting similar molecular weights.

MATERIALS AND METHODS

Sugar beet root diffusate was leached from soil in 8-inch clay pots, each containing four

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vigorously growing sugar beet (*Beta vulgaris* L., Var. 'U. S. 75') plants. A volume of 200 ml was leached from each pot during 24 hr.

Sugar beet and wild beet (*B. patellaris* Moq.) were grown in the greenhouse in half-strength, aerated Hoagland's solution for a period of 41 days. The expended and fresh nutrient solutions were tested for hatching effect on larvae in cysts of *H. schachtii* for comparison with sugar beet root diffusate from soil. Each treatment was applied to four replicate groups each containing 40 cysts, a total of 160 cysts, for a period of 6 weeks.

In a second test, the diffusate was dried or concentrated to 2% of its original volume by vacuum distillation. Distillation was accomplished by using a Dewar condenser with a vacuum of about 0.5 mm Hg, and cooled to about –72 C with a mixture of dry ice and alcohol. The temperature of the diffusate in the evaporator flask was maintained at 5 C with a heated water bath. Under these conditions, water was removed from the diffusate at the rate of one liter per hour. The distilled condensate and untreated diffusates were stored 14 days at 5 C; then sufficient distilled water was added to the materials to obtain 1, 10, and 100% solutions of diffusate. Each solution was tested for hatching activity. The pH of fresh diffusate was 7.2.

In another test, diffusate leached from soil was dialyzed for varying periods of time in either running tap water or distilled water, and then tested to compare the hatching effects of dialyzed and undialyzed diffusate (pH 7.5). "Cellophan"® brand dialysis tubing was used. The diameter of the tubing was 18 mm. The porosity of the tubing varied from 30–80 Angstrom units, permitting diffusion of materials of molecular weights below 15,000. The dialysis tubing was filled with diffusate and suspended in a cylinder of distilled water and gently agitated with a magnetic stirrer for 48 hr. At 12-hr intervals the dialyzate was replaced with distilled water. In this way, 400 ml of diffusate was dialyzed in a total of 14.4 liters of water. The salt-free dialyzed diffusate was tested for hatching activity (Treatment 3, Table 2). Dialyzate of the first 3 changes (10.8 liters) was concentrated to 400 ml by vacuum distillation, and again dialyzed 3 hr with running distilled water, to remove rapidly-diffusible salts. Subsequent tests showed that fresh diffusate leached from soil contained large amounts of chlorides whereas none were detectable in dialyzed diffusate. Aliquots of root diffusate were dialyzed 24 or 192 hr in running tap water. Fresh undiluted diffusate, fresh diffusate diluted to 10%, tap water, and the various fractions obtained by dialysis were tested for their effects on hatching. Treatments were replicated 4 times. Twenty cysts and 15 ml of treatment solution were placed in Syracuse watch glasses and the glasses kept in an incubator maintained at 24 C for 6 weeks. At weekly intervals the emerged larvae were counted and the cysts were transferred to fresh solutions of the same diffusate composition. Data were analyzed by standard analysis of variance.

In order to compare the diffusion rates of hatch factor and coloring matter during dialysis of sugar beet root diffusate, the latter was diluted to 0.1, 1.0, 10, and 20% and the absorbance of each of the dilutions was measured at 240 mμ. Absorbance values and solute concentration followed the Beer-Lambert Law. Absorbance values were determined for sugar beet root diffusate dialyzed in running tap water for various periods of time and these values converted to concentrations of coloring matter (Table 3).

Results and Discussion

The numbers of larvae hatched and emerged from cysts treated with sugar beet

® Dialysis Tubing, obtained from Kalle Aktiengesellschaft D-6202 Wiesbacken Biebrick. 9165, Germany.

TABLE 1. The influence of concentration by vacuum distillation on hatching activity of sugar beet diffusate.

Treatment	% Concentration		
	1	10	100
Tap water			280[b]
Concentrated[a]	506	4,134	4,513
Dried	466	3,486	4,639
Untreated	388	3,715	3,948
LSD .05 =	640	640	640

[a] Diffusate concentrated to 2% of its original volume then water added to obtain the indicated concentration. The original volume of diffusate was 500 ml.
[b] Average number of larvae emerged in 2 weeks from 4 replications of 20 cysts each.

root diffusate, or nutrient solutions in which sugar beet or wild beet were grown, amounted to 7.2, 6.8, and 6.5 times, respectively, the number emerged in tap water. Hatch in half-strength Hoagland's solution was similar to those in tap water. These findings indicate that roots of beets, grown in liquid nutrient cultures, excrete materials similar in effect to beet root diffusate leached from soil. Consequently, nutrient culture is viewed as a feasible means of obtaining quantities of root diffusates for bioassay or biochemical investigations.

TABLE 2. The influence of diffusate fractions separated by dialysis on emergence of larvae from cysts of *H. schachtii*.

Treatment No.	Treatment	Hatch[1]
1.	Tap water	95 a[11]
2.	Diffusate dialyzed 192 hr in tap water	104 a
3.	Diffusate dialyzed 48 hr in distilled water	1,641 b
4.	Diffusate dialyzed 24 hr in tap water	2,254 bc
5.	Dialysate of 36 hr dialysis[111]	2,617 c
6.	Untreated diffusate 10%	2,873 c
7.	Full strength untreated diffusate	4,043 d

[1] Average numbers of larvae emerged in 2 weeks from 4 replications of 20 cysts.
[11] Values with same letters not significantly different at 5% level.
[111] Redialyzed 3 hr.

TABLE 3. Influence of dialysis time on concentration of coloring materials in sugar beet root diffusate.

Dialysis time (hr)	Absorbance (at 240 mμ)	Concentration %
192	.08	13
48	.27	33
24	.31	37
4	.48	57
2	.60	72

Diffusate dried or concentrated by vacuum distillation retained its hatching activity (Table 1). Therefore, concentration of bulk quantities of diffusate for refrigerated storage can be accomplished by vacuum distillation.

The hatching activity of diffusate dialyzed 48 hr in distilled water was significantly lower than that of untreated diffusate diluted to 10% of its original concentration. More important, however, when the dialysate obtained after the first 36 hr of treatment was redialyzed for 3 hr, the fractionated solution gave a hatching effect similar to 10% dilution of diffusate (Table 2).

The loss of coloring materials in dialyzed diffusate was not associated with loss of hatching activity. Diffusate dialyzed 24 hr in tap water retained 37% of its color, but its hatching activity was less than that exhibited by 10% dilution of untreated diffusate. Diffusate, dialyzed 192 hr retained 13% of its color but did not increase hatching (Table 3).

These data show the active material in diffusate is dialyzable, that its diffusion rate is markedly lower than that of inorganic salts, and is not related to coloring materials in diffusate. Consequently, the hatch factor is probably not a protein or a simple salt, but an organic compound (or compounds) with a molecular weight of less than 15,000.

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