On the Nature of Hatching of Heterodera schachtii. II. Natural Sources of Hatching Stimulants[1]

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Introduction

The hatching response of cysts of *Heterodera schachtii* has received much attention since the report by Baunacke (1)[3] of the stimulatory effects of the leachings of sugar beet roots on larval emergence. If, as Shepherd (4) suggests, hatching is considered a starting point for the process of host infection and the life cycle of the nematode, the effort and attention devoted to this aspect of nematology in the past was almost inevitable. This line of work subsequently became extended to the effects of extracts of other plant organs and synthetic chemical agents. As a result the evidence is overwhelming that leachings of the appropriate plants contain active agents that stimulate significantly the emergence of larvae from cysts over the normal expected with water.

A great proportion of the research devoted to the phenomenon of hatching has been concerned with the description of the effect of physical or environmental factors on cysts or the bioassay systems (3,7,8,9). The research into the chemistry of the hatching process has resolved itself mainly into the testing of a number of diverse substances from natural and synthetic sources for hatching factor activity (10,12) and the identification of a naturally-occurring hatching factor -- as yet not achieved (2). Despite the research effort to date little is known about the nature or mechanism of hatching or the role of the naturally-occurring hatching stimulants in eclosion.

Materials and Methods

Plants used in these studies included dame's violet (*Hesperis matronalis*), tomato (*Lycopersicon esculentum*) rape (*Brassica napus*), sugar beet (*Beta vulgaris*), yellow milo (*Sorghum vulgare*), broccoli (*Brassica oleracea*, var. *botrytis*), alfalfa (*Medicago sativa*) barley (*Hordeum vulgare*) and onion (*Allium cepa*). Plants were grown under greenhouse conditions in 6-inch pots filled with sand and watered with modified Hoagland's nutrient solution (11). When the root systems had developed sufficiently

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[3] Numbers in parentheses refer to literature cited.

they were leached profusely with distilled water to remove soluble constituents from the sand culture. At 24-hour intervals thereafter each plant was leached with sufficient distilled water to make up 500 ml of leaching solution. The leachings from each plant were combined every 2 days and designated as a batch so that in a leach period there were obtained successively batch 1, 2, 3, etc. The leach period was arbitrary, but less than 3 weeks. To test the effect of lyophilization half of each batch was lyophilized and the remainder stored in a cold room as a solution. For assay purposes the dried material was resuspended in distilled water adjusted to pH 5 with H_2SO_4 or NaOH. The redissolved material was tested at 5 concentrations overlapping the concentration of the original leachate. The refrigerated solution was tested at natural strength as well as 2 dilutions.

Leachings from germinating seedlings were obtained by placing seeds on a stainless steel screen suspended over a funnel in a misting apparatus. The quantity of seed used for funnel leaching was determined primarily by seed size, i.e., previous experiments with sugar beet germ indicated that seed depths greater than about 4 to 5 seed diameters were unsatisfactory. The collection of the leachings was begun when stem or root growth became evident. The daily collection from each funnel was lyophilized and stored at −15°C.

The misting apparatus consisted of a box 3 feet on edge constructed of angle aluminum and aluminum sheet and a plexiglass door with a misting nozzle at the top center of the chamber. Hot distilled water was supplied to the nozzle intermittently from a hot water heater (with variable temperature controls) through an appropriate combination of solenoid valves controlled by a variable on-off interval timer. Fluorescent lights (6-40 watt tubes) were suspended just above the mist chamber to supply illumination to the germinating seedlings.

Cysts of *Heterodera schachtii* from sugar beets grown in sand under greenhouse conditions were selected, separated and stored by the method developed by Viglierchio (5). The hatching bioassay utilizing two sources of cysts was conducted as described by Viglierchio (6). The cysts were suspended at the surface of the test solution by stainless steel screens in plastic wells containing 0.5 ml of solution sample. The cysts and solutions (8 replicates/test solution of a concentration series) were incubated in a humidity chamber at 25°C. At 4-day intervals the emerged larvae together with test solution were withdrawn for counting and replaced with freshly prepared test solution. The bioassay was discontinued after three collections.

Results

Leachings from all the plants tested stimulated hatching of *H. schachtii* larvae to some degree (Figure 1, 2 and 3). It was evident that the stimulatory activity of the leachate varied as the leaching progressed. According to expectations the over-all activity of the leachate decreased to the level of water by the end of the leach period; however, two patterns of larval emergence in response to leachings were clearly resolved. The activity of milo, alfalfa, and dame's violet leachings decreased to the level of water hatching in a gradual fashion, whereas the activity of rape, tomato, broccoli, turnip and sugar beet did so erratically (Figure 1). The fluctuations were much too large to be accounted for by assay variability.

Concentration hatch curves (Figure 2 and 3) for refrigerated leachate solution indicated that the plant leachings could be rated in activity from high to low: turnip, rape, dame's violet and broccoli. The leachings of the other plants were very low in activity, comparable to that of water.

When the plant leachings were lyophilized then resuspended for assay the hatching activity varied considerably. The response of dame's violet was very high; turnip and rape, high; sugar beet, onion and tomato, moderate; alfalfa, milo, broccoli and barley, very low—slightly above water. Comparison of the hatching curves of refrigerated solutions and lyophilized material indicated that drying had no effect on the activity of leachings from turnip, dame's violet, barley and milo but increased the activity of leachings from tomato, rape, onion and sugar beet. Drying decreased the activity of leaching of broccoli and possibly alfalfa.

The hatching response to dried leachings of germinating seedlings was remarkably similar in all plants tested. Larval emergence, moderately high, decreased with concentration of dry solids similarly with all plants tested.

The acidity of the leachings of all plants decreased to neutrality as the leach period progressed with the greatest change occurring between batches 2 and 3 (Figure 4). Preliminary experiments with sugar beet germ had indicated little change in pH between leach solution as collected and that lyophilized and redissolved. The leachings of the other germinating seedlings increased in pH and most of them became alkaline, pH 8-9. It became standard practice thereafter before lyophilizing to render the buffer capacity of the leachings acidic with H_2SO_4 until acidity began to increase rapidly as indicated by a pH meter.

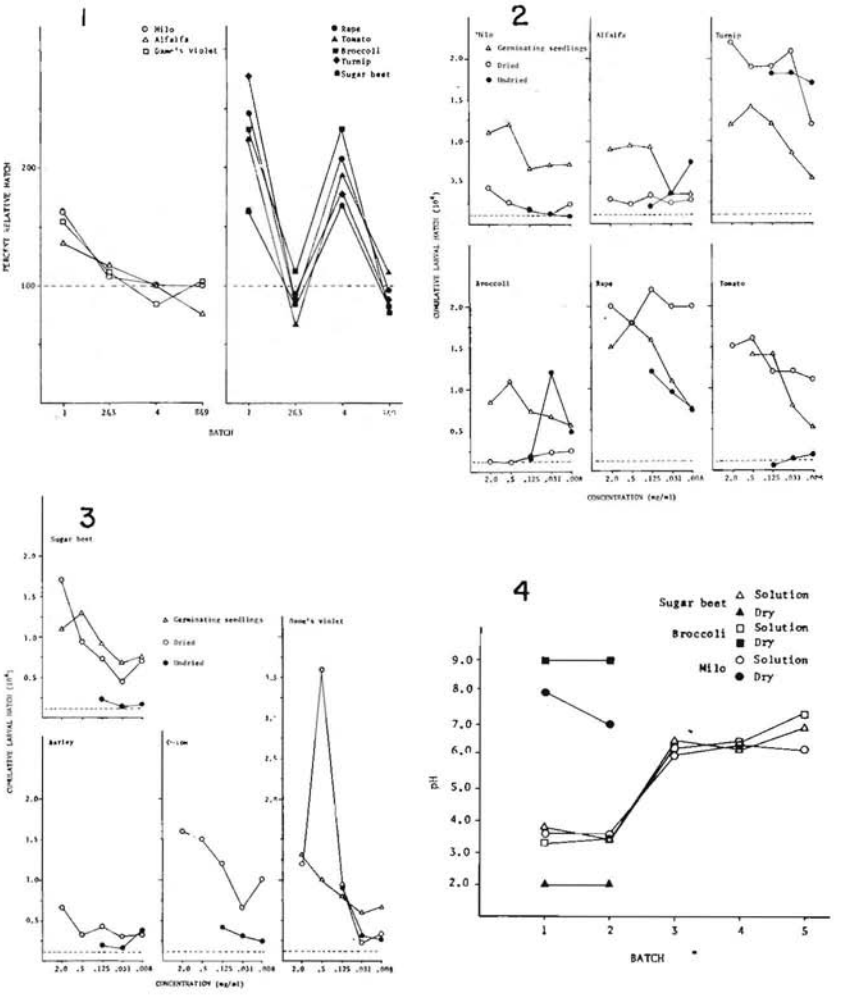

Figure 1.—The extraction of hatch factor materials from various plant roots with progressive leaching as determined by hatching tests of *H. schachtii* cysts with successive collections.

Figure 2 and 3.—The cumulative hatch from *H. schachtii* cysts obtained with several concentrations of hatch material from several sources prepared as indicated.

Figure 4.—The change in pH of extracts (original solution and reconstituted solution from dried solids) of several plant roots with successive leaching.

Root weights of the greenhouse leached plants were surprisingly uniform except for the coarse rooted milo and fine rooted dame's violet and alfalfa (Table 1). The physical seed dimensions of milo and tomato and dame's violet were not amenable to the use of uniform weights or numbers. Percent germination and germination times also varied as reported. The dry solid content of the leaching from germinating seedlings showed little correlation with either the seed number or seed weight. The host test for *H. schachtii* reported in Table 1 was for the indication of relative susceptibility and not necessarily absolute susceptibility. It was evident that sugar beet was the best host for *H. schachtii* but that the females were able to mature more quickly on rape.

Discussion

The abnormal environmental regime the plants experienced during the leaching process would effect mineral and nutritional imbalances resulting in the variable production of stimulatory agents and/or leaching of competitive inhibitors. It was of interest to note that milo, alfalfa and dame's violet were non-host plants whereas the others were hosts (Table 1). Tomato has been reported as a host of *H. schachtii;* however, the susceptibility of the Pearson variety was apparently too low to be detectable by the test method used in this experiment.

It was clear from the hatch curves of refrigerated leachings (Figure 2 and 3) that the stimulatory potential varied greatly from one plant to another. It was surprising to observe that lyophilization, normally considered a gentle procedure, had altered the hatch characteristics of the leachate of more than half the plants tested. The increase in hatch-factor activity obtained upon lyophilization of leachates could be explained by a loss of inhibitors either through volatilization or chemical inactivation. The change in pH of leachate upon drying was consistent with this notion. The loss of volatile organic acids or bases would explain a change in acidity.

It was unlikely that the environmental conditions during lyophilization would have been conducive to synthesis of hatch factors from precursors. The apparent inactivation of crude hatch material upon lyophilization as observed in the case of broccoli and to a lesser degree with alfalfa may have been a result of active factor volatilization or heat inactivation. In the process of freeze-drying water was removed from the frozen solution by sublimation. The dry solids remaining behind would have been at the temperature of the frozen solution if in contact

Table 1.—Various data for the plants used in this study.

	Sugar beet	Broccoli	Turnip	Alfalfa	Rape	Milo	Tomato	Dame's violet	Barley	Onion
Plants/pot	8	8	8	15	8	8	8	8		
Root Wt. (g)/pot	76	70	77	36	73	105	71	42		
Wt. seeds (g)/rep.	9	13	10	11	12	22	6	10		
No. seeds/rep.	4700	3700	4400	5100	3100	765	1500	5800		
Germination time (hrs.)	120	42	42	42	72	72	120	120		
Percent germination	20	96	94	85	93	96	85	44		
Dry solids (g) from seeds (10 days)	0.57	0.45	0.45	0.55	0.55	0.74	0.51	0.38		
Susceptibility										
H. schachtii females	1995	1127	958	0	679	0	0	0	0	0
H. schachtii cysts	31	15	9	0	556	0	0	0	0	0

with it. The dry solids in contact with their container would have been at the temperature of the room or the freeze-dryer warming plates usually 25-30°C in this case, while the residual ice was subliming. Since the lyophilization normally required some 24 hours, "inactivation" may have occurred. This observation was consistent with literature reports indicating the heat liability of hatch factor materials from root leachings. The "inactivation", presumably occurring with all leachings, would have been masked by a much larger inhibitor effect. It would be difficult to determine whether a lack of hatch factor activity was due to the absence of one or more hatch factor stimulants or the presence of an equivalent amount of inhibitor material.

Since the bioassays providing the data reported (Figure 2 and 3) were conducted within a few weeks of each other, it was reasonable to expect that differences in cyst response due to their increased age would be negligible. In that event one could conclude that the same hatch stimulatory substances in similar proportions would be present in hatch stimulating leachings. The diversity of seedlings with stimulatory potential would preclude specialization and suggest instead that the activity was due to common substances, perhaps products or constituents of metabolism. The similarity in response of germinating seedlings with respect to concentration of dry solids appears to be fortuitous. The procedures used in these experiments were not sufficiently sensitive to detect any correlation between dry weight of solids and weight of seeds or seed number.

If hatch stimulation were of value in control applications (4), these experiments would suggest that a number of economically profitable nonhost plants possess stimulatory activity. It would not be necessary to consider economically useless plants for such a purpose. The complexity of events occurring in soil, i.e., production, diffusion and decomposition of hatch inducing substances would necessitate confirmation of the notion by the appropriate experimentation.

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