Effect of Nabam Solutions on the Emergence of Larvae from Cysts of Heterodera Schachtii Schmidt.

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Various attempts have been made to develop improved methods for controlling the sugar beet nematode. Studies by Franklin (4)[2] and Raski and Lear (9) suggest that reduction of the expense of chemical control would increase the economic feasibility of this practice. Certain workers maintain that the efficiency of control by crop rotations can be improved by the addition to the soil of a chemical which will induce hatching of larvae from cysts in the soil (1).

Miller and Stoddard (8) reported that disodium ethylene bisdithiocarbamate[3] (Nabam) in water solution retards hatching of eggs of root-knot nematodes (*Meloidogyne* sp.), but that in soil it increased hatching of eggs of both *Meloidogyne* sp. and *Heterodera tabacum* Lownsbery and Lownsbery, 1954. They also reported that better control of root-knot nematodes was obtained by combining Nabam and a nematocide than by either alone. These workers suggested that Nabam or a decomposition product is a "hatching factor" for *Meloidogyne* and *Heterodera*, but no acceleration of hatching of root-knot nematodes resulted when roots and soil were treated with ethylene thiuram monosulphide and ethylene thiuram polysulphide, which were reported by Ludwig and Thorne (7) to be breakdown products of Nabam. No information is available as to the effect of Nabam on hatching of the sugar beet nematode.

Materials and Methods

Two separate tests were conducted. In the first, the hatching effect of Nabam at 500, 1,000, or 2,000 parts per million (ppm) was compared with that of tap water and sugar beet-root diffusate.

The methods used to obtain beet-root diffusate treatment solutions and the conduct of hatching tests were essentially those described by Golden (5) but are briefly reviewed below.

Beet-root diffusate was obtained by intermittently adding tap water to a 4-inch pot containing three vigorously growing sugar

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[2] Numbers in parentheses refer to literature cited.

[3] The formulation of Nabam used by Miller and Stoddard and in the tests reported herein was Dithane D-14 (Rohm and Haas Co.) consisting of 22% Nabam.

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beet (*Beta vulgaris* L.) plants. In this way 200 ml of solution were leached from a single pot in a 24-hour period. Fresh leachate was obtained at weekly intervals throughout the six weeks of the test.

The Nabam solutions for the first test were prepared at the time the test was begun and stored under refrigeration at 1 to 3° C until needed.

The second test (Table 2) included treatments of tap water, beet root diffusate, 1,000 ppm Nabam (solution prepared at the beginning of the test), Maneb (1,000 ppm Nabam plus 1,197 ppm manganese sulfate), Zineb A (1,000 ppm Nabam plus 1,316 ppm zinc sulfate), or Zineb B (1,000 ppm Nabam plus 658

Table 1.—Total numbers of *Heterodera schachtii* larvae emerged from cysts exposed six weeks to various Nabam solutions, tap water, or sugar beet root diffusate.

Treatment	Concentration (ppm)	Replication 1	2	3	4	Total	Average	Percent of hatch in diffusate
Tap water		520	870	540	1,200	3,130	782.5	9.2
Nabam	500	5,240	2,600	2,890	3,840	14,570	3,642.5	42.7
Nabam	1,000	5,150	4,710	6,320	4,920	21,130	5,282.5	61.9
Beet diffusate		9,730	7,960	7,700	8,760	34,150	8,537.5	100.0
Significance							**	
LSD (.05 level)							1,825.0	42.0

Table 2.—Total number of *Heterodera schachtii* larvae emerged from 40 cysts exposed six weeks to various Nabam solutions, tap water, or sugar beet-root diffusate.

Treatment[1]	Replication 1	2	3	4	Total	Average	Percent of hatch in diffusate
Tap water	1,800	2,180	1,990	1,900	7,870	1,967.5	23.0
Maneb	1,810	2,370	2,630	2,110	8,920	2,230.0	26.1
Zineb A	2,390	1,780	2,020	1,520	7,710	1,927.5	22.5
Zineb B	3,880	5,390	3,790	4,090	17,150	4,287.5	50.5
Nabam A	4,980	6,180	4,340	5,190	20,690	5,172.5	60.5
Nabam B	2,830	2,340	2,810	2,470	10,450	2,612.5	30.6
Beet diffusate	10,140	6,820	8,940	8,300	34,200	8,550.0	100.0
Significance						**	
LSD (.05 level)						1,095.0	13.0

[1] Components of treatments:
Maneb—1,000 ppm Nabam + 1,197 ppm manganese sulfate; Zineb A—1,000 ppm Nabam + 1,316 ppm zinc sulfate; Zineb B—1,000 ppm Nabam + 658 ppm zinc sulfate; Nabam A—1,000 ppm Nabam; Nabam B—1,000 ppm Nabam prepared 6 weeks before preparation of all other treatments.

ppm zinc sulfate), and 1,000 ppm Nabam solution prepared six weeks before initiation of the test. Solutions for all other treatments were prepared at the time the test was initiated.

Cysts of *H. schachtii* were removed from soil by washing and screening, transferred to Syracuse watch glasses containing tap water, and refrigerated for 10 days. To insure uniformity of cysts between replications and between treatments, the total mass of cysts was divided into four lots (replications) and each lot subdivided into groups having a number of cysts equal to the number of treatments of the test. The cysts included in a single group were similar in size, shape, color, and plumpness. Individual watch glasses, designated as the first replication of a randomly assigned treatment, continued to receive one cyst from each group until each eventually contained 40 cysts. The process was repeated three more times to give a total of four replications for each treatment.

Approximately 15 ml of each of the treatment solutions were added to individual watch glasses, which were kept in a dark, aerated cabinet in the laboratory during the 6-week test period. At weekly intervals the cysts were transferred to clean watch glasses containing fresh solutions and the emerged larvae preserved in 5% formalin until counted. Aliquot samples from replications which contained large numbers of nematodes were taken to expedite counting. Data for treatments and replications in this randomized complete block experiment were analysed for statistical significance by the "analysis of variance" method.

Results and Discussion

In the first experiment, Nabam at 2,000 ppm inhibited hatching (92 larvae emerged from 160 cysts), whereas hatching in 1,000 ppm was 62%, hatching in 500 ppm was 42%, and hatching in tap water was only 9% of that in beet-root diffusate. Results were erratic and the significance of some of the differences is doubtful.

However, the freshly prepared Nabam solution used in the second experiment gave results very similar to those obtained with a concentration of 1,000 ppm in the first experiment; so it can be concluded that hatching was increased by this material under the conditions of these experiments. The older solution of Nabam gave no better hatches than did tap water, indicating that the hatching effect of Nabam decreases with age, probably because of the decomposition of the Nabam in dilute solution.

The Zineb B treatment was about equal to the Nabam A treatment, but the Zineb A treatment and Maneb treatment were no better than tap water.

In the two experiments, there was a remarkable similarity in the numbers of larvae which emerged from the cysts in beet-root diffusate. The total number of larvae recovered (34,150 in the first and 34,200 in the second experiment) probably represents nearly the full potential number which could be hatched from these cysts. In similar experiments, Golden (6) reported weekly emergence of 7,520, 4,056, 1,292, 559, 171, and 80 larvae during the first to the sixth week, respectively. This is, 13,427 larvae of a total of 13,678, or more than 98%, hatched in the first four weeks, and less than 2% in the last two weeks.

The results can be summarized by stating that Nabam solutions containing 1,000 ppm increased hatching as compared with the tap water controls, but only about 60% as much as sugar beet-root diffusate. Addition of 1,197 ppm of manganese sulphate or 1,316 ppm of zinc sulphate reduced the action to about the same as tap water, whereas 658 ppm of zinc sulphate had no effect.

The increase in hatching in Nabam solutions is about the same as reported for ascorbic acid by Emanuelson (3), who considered the effect "not sufficiently pronounced to make it a suitable stimulus."

The reason for the increased hatching in Nabam solutions is not clear. It is highly unlikely that Nabam solutions contain the same "hatching factor" which occurs in beet-root diffusate or in ascorbic acid.

Dropkin et al. (2) have shown that hatching of nematode eggs can be delayed indefinitely by keeping them in solutions of high osmotic concentrations and that hatching proceeds when the osmotic stress is removed. These findings suggest that Nabam solutions and beet-root diffusate change the osmotic concentration in the egg in some way, perhaps by altering the permeability of the egg or larval membranes.

It can be concluded that while Nabam increases hatching, it is not highly efficient for the purpose in water solution. Its effect on *Heterodera* cysts in soil can only be determined by additional experiments, and its value for use in the field would depend on many factors including economic ones.

Summary

Laboratory tests were conducted to determine the effect of solutions of Nabam, Maneb, and Zineb on hatching of larvae from cysts of *Heterodera schachtii* as compared with those of tap water and beet-root diffusate during a 6-week period. In one test hatching in a solution containing 500 ppm of Nabam

was 42% of that in beet-root diffusate, 1,000 ppm 62%, 2,000 ppm 0.3% and in tap water 9%. In a second test, results were similar to these for 1,000 ppm of fresh Nabam solution and for 1,000 ppm of Nabam plus 658 parts zinc sulphate (Zineb B), but not different from the tap water controls for Maneb, 1,000 ppm of Nabam plus 1,316 ppm of zinc sulphate (Zineb A), or Nabam solution prepared six weeks before the beginning of the experiment.

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