Reports

Increasing the Hatching of Eggs of Cyst and Rootknot Nematodes with Nabam

Abstract. Nabam in water solution retards hatching of *Meloidogyne* eggs. In soil nabam increases egg hatching of *Meloidogyne* and *Heterodera tabacum,* indicating that a decomposition product in soil is a hatching factor. Because of this attribute, combining nabam with a nematocide increases control of *Meloidogyne* by exposing more larvae to the nematocide while it is at maximum efficiency.

The hatching of eggs of cyst nematodes, genus *Heterodera,* and the rootknot nematodes, genus *Meloidogyne,* is increased by certain unidentified hatching factors released by roots of susceptible host plants. In addition, larval emergence may be increased by several synthetic organic and inorganic compounds (*1*).

We report here the effect of nabam (disodium ethylenebis dithiocarbamate) in the presence of soil on the hatching of the eggs of *Meloidogyne* sp. from *Stephanotis floribunda* and *Heterodera tabacum.*

Of more importance than the finding of another hatching factor is the discovery that combining nabam with a nematocide controls rootknot nematodes better than a nematocide alone.

The effect of nabam on hatching of eggs of *Meloidogyne* sp. is shown in the following experiment. The concentration of nabam in tap water was 0.55 g/lit. in this and subsequent experiments. Duplicate samples of eight egg masses were put for 5 days in (i) a water solution of nabam, (ii) a suspension containing 4 g of macerated tobacco roots per liter, and (iii) tap water alone. The average number of larvae recovered from each treatment was as follows: nabam, 12 larvae; tobacco root extract, 55 larvae; and tap water, 300 larvae. The nabam in tap water reduced the hatching of the eggs.

In order to test a possibility that nabam in the presence of soil might increase hatching of *Meloidogyne* eggs, the following experiments were made. One lot of infested soil was drenched with nabam solution, one lot was drenched with tap water, and another lot was left dry. Six replicates of 300 g each were made from each lot. The soil was kept moist at room temperature for 10 days. Then emerged larvae were removed by the modified sugar flotation method (*2*) and counted. The average number of larvae recovered was 587 larvae from each nabam replicate, 167 larvae from each tap water replicate, and 31 larvae from each dry sample.

To see whether results on infested roots were similar to those in soil, five replicates each of bare, infested roots of *Stephanotis floribunda* were immersed (i) in nabam solution with 10 g of uninfested soil added and (ii) in tap water with 10 g of uninfested soil added. The root-soil mixtures were left at room temperature for 10 days. The larvae were then removed by the modified sugar flotation method and counted. An average of 107 larvae were recovered from roots soaked in the nabam-soil mixture, and only 56 larvae were recovered from roots soaked in the tap water-soil mixture.

In the first experiment nabam in tap water depressed egg-hatching. In the two latter tests the same concentration of nabam in the presence of soil markedly increased egg-hatching both in infested soil and in *Stephanotis* roots.

In the suspensions of *Stephanotis* roots in nabam-plus-soil there were not only more larvae, but there were also more free, normal but unhatched eggs. More than 5000 eggs were recovered from each sample of roots soaked in nabam, and only 220 eggs were recovered from each sample of roots soaked in water. This would suggest that nabam or some of its decomposition products, in the presence of soil, causes a dissolution of the adhesive substance enveloping the eggs with the consequent release of the eggs. The release of the eggs from the egg masses may be an important factor in the increase of hatching, aside from a direct effect of the nabam decomposition products.

Soil drench treatments of infested soil with a nematocide, V-C 13 (O-2,4-dichlorophenyl-O,O-diethyl phosphorothioate) alone and in combination with nabam were made to determine the effect of nabam on control of root infestation by rootknot nematodes. V-C 13 was used at a concentration of 0.25 ml/lit. of tap water. Nabam was used alone and in mixtures containing 0.25 ml of V-C 13 per liter. Each treatment was replicated four times on infested soil. Four days after treatment tomato (*Lycopersicum esculentum* L.) plants were set in the treated soil. The plants were grown in the greenhouse for 36 days. Then the roots were washed free of soil, and an estimate was made of the percentage of infested roots (Table 1). It may be seen that the percentage of root infestation was markedly high with nabam alone and low in the combination of nabam with the nematocide. Nabam in soil causes dissolution of the protective covering of the egg masses and increases egg hatching, both effects exposing two stages of the nematode immediately to the maximum concentration of the nematocide.

Soil containing cysts of the tobacco cyst nematode, *Heterodera tabacum,* was drenched with nabam or water to study effects of nabam on hatching of eggs of this cyst nematode. Eight samples of 300 g each were taken of soil 1, 3, 5, and 7 days after treatment of the soil with nabam. Control samples were from soil treated with water 7 days previous to use. The cysts were screened from the soil, rinsed in tap water, resuspended in tap water, and incubated at room temperature for 14 days. The numbers of larvae from the control and from the several periods of exposure to nabam in soil were as follows: control, 26; taken from treated soil after 1 day, 35; 3 days, 55; 5 days, 91; and 7 days, 131, [LSD ($P = 0.05$)–47]. These data show that

Table 1. Control of rootknot nematode (*Meloidogyne* sp.) on tomatoes with a combination of nabam and V-C 13.

Treatment and conc. (per liter)	Percentage galled roots		
	Test A	Test B	Av.
V-C 13 (0.25 ml)	41	25	33
Nabam (0.55 g)	81	40	60
Nabam (0.55 g) + V-C 13 (0.25 ml)	6	15	10
Untreated	72	28	50

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125,** 16 (1957)].

nabam in soil increases hatching of tobacco cyst nematode eggs as it did those of the rootknot nematode. It is also shown that the magnitude of the effect is a function of time because the 5- and 7-day exposures increased the hatch significantly. The data further indicate that the hatching factor or its effect is retained by the eggs and cysts after their removal from the soil, for the hatching took place in tap water after the cysts were removed from the treated soil and thoroughly washed in water.

In a preliminary attempt to determine which fraction of nabam acts as a hatching factor, lots of soil and roots infested with rootknot nematodes were treated with ethylene thiuram monosulfide and ethylene thiuram polysulfide, compounds known to be decomposition products of nabam (*3*). No acceleration of hatching resulted. The findings by van der Kerk *et al.* (*4*) that some dithiocarbamates have growth-promoting properties and Stoddard (*5*) that nabam, both in solution and in soil, has root-promoting properties suggested that growth-promoting substances might act as hatching factors. However, α-naphthaleneacetic acid and 3-indoleacetic acid did not increase hatching of rootknot nematode eggs.

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References and Notes

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5. E. M. Stoddard, unpublished data.

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An Infrared Study of the Hydrolysis of a Thiazolidine

Abstract. The hydrolysis in *aqueous* solution of L-4-carboxy-2,2-dimethylthiazolidine hydrochloride has been followed by infrared spectrophotometry. The absorption curve of the final reaction mixture had absorption characteristics which were also shown by a solution containing stoichiometric amounts of the expected reaction products.

The product obtained when L-cysteine hydrochloride is refluxed in acetone is L-4-carboxy-2,2-dimethylthiazolidine hydrochloride (CDMT) (*1*). Woodward and Schroeder (*2*) followed the hydrolysis of L-4-carboxy-2,2-dimethylthiazolidine polarimetrically and showed that the compound dissociates to form cysteine and acetone. We have examined the dissociation of CDMT in water, using infrared spectrophotometric techniques.

L-4-Carboxy-2,2-dimethylthiazolidine hydrochloride was prepared [MP, 166°C (lit., 163° to 165°C) (*1*)]. Eastman Spectro grade acetone and cysteine hydrochloride monohydrate from Mann Re-

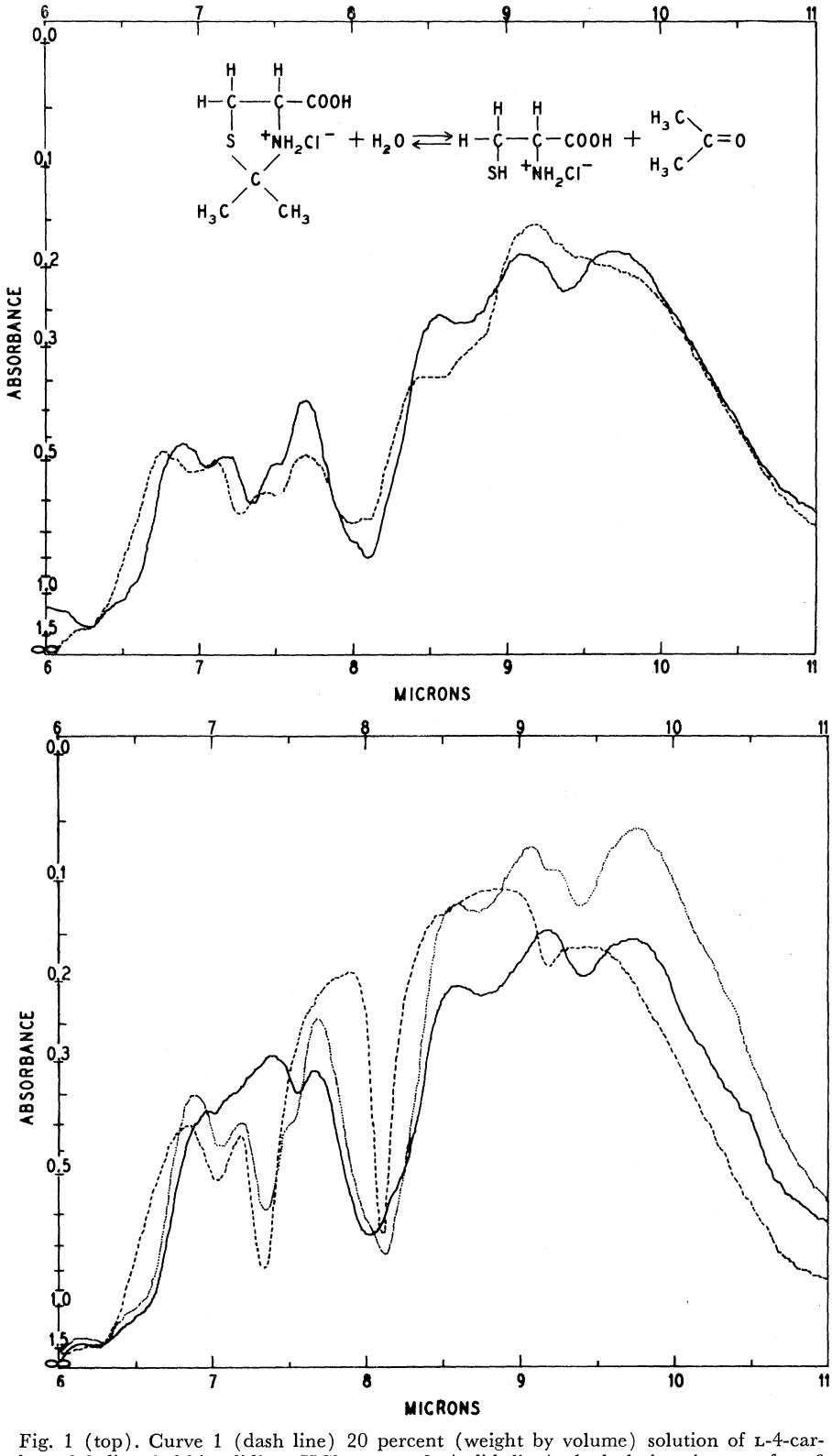

Fig. 1 (top). Curve 1 (dash line) 20 percent (weight by volume) solution of L-4-carboxy-2,2-dimethylthiazolidine · HCl; curve 2 (solid line), hydrolysis mixture after 6 hours. Fig. 2 (bottom). Curve 1 (solid line), 1.750 g of cysteine hydrochloride · H_2O in 10 ml of solution; curve 2 (dash line) 580 mg of acetone in 10 ml of solution; curve 3 (dotted line), cysteine hydrochloride · H_2O–acetone mixture containing the same amounts of solutes as for curves 1 and 2 per 10 milliliters of solution.